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Physiologia—Pathophysiologia

THE ROLE OF MEMBRANE PROCESSES IN CONTROLLING SKELETAL MUSCLE FUNCTION*

By

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(Received May 30, 1980)

The role of membrane processes in the activation of skeletal muscle fibers has been investigated in details recently. It seems very probable that the intramembrane charge movement is the potential sensitive step of the excitation-contraction coupling. Considerable efforts were made to monitor the subsequent steps (SR function, Ca release) using optical methods. Experimental data were presented about the relations existing between charge movement process, Ca release and contraction threshold.

The action potentials of the surface membrane propagate along the transverse (T)-tubules in a regenerative manner. The T-tubule depolarization gives rise to calcium release from the sarcoplasmic reticulum (SR). The increase of intracellular Ca concentration removing the troponin inhibition leads to muscle contraction. The research of the membrane processes and especially the excitation-contraction coupling was facilitated by applying new methods, for example optical measurements on intact fibres, and by the great progress in electronics, making easier the signal and data processing.

As it is widely accepted, the membrane potential dependent changes of the sodium and potassium conductances underlie action potentials proceeding on the membrane of skeletal muscle fibres. The properties of these channels have been investigated in detail. Here I intend to refer to the importance of chemicals in studying membrane processes. Their application can modify the excitability or permeability of the channels. In the presence of veratrum alkaloids Dankó and Varga described a TTX sensitive, rhythmical oscillation of membrane potential on frog sartorius muscle [18]. The pacemaker-like activity lasting for hours has a half to one minute long repetition time with amplitudes of 30—60 mV. These parameters and the raising or falling phases of individual waves were significantly modified by phlorrhizin and monoiodoacetic acid treatment. From these results some connection between the chemically modified channels and the intracellular metabolism was suggested [17].

* Review lecture given at the Meeting of the Hungarian Physiological Society at Szeged on 6—8 September, 1979.

Besides the potential sensitive ionic conductances, the mechanical activation of muscle fibres also has a steep membrane potential dependence. Contraction can be evoked by different types of depolarization, not only by action potentials. Applying Ringer solution with increased potassium concentration, HODGKIN and HOROWICZ measured the mechanical movement of single fibres [24]. The contraction threshold was reached when the membrane had depolarized to —50 mV. The peak tension of the fibres was related to the membrane potential by a steep sigmoid curve. ADRIAN et al. studied the kinetics of mechanical activation by voltage-clamp depolarizing pulses [3]. The strength-duration curves for mechanical threshold obtained in the presence of TTX were suitable for quantitative analysis.

In the last few years great advances have been made in the knowledge of voltage dependence. Charge displacement currents were succesfully recorded which might reflect the movement of charged particles related to the opening of sodium channels due to depolarization [6, 26]. The intramembrane charge movement observed in skeletal muscle fibres by SCHNEIDER and CHANDLER [40] had a much slower time course, so it may not be linked to a gating of sodium channels. Its kinetic properties and voltage dependence make it probable that it is strongly related to the depolarization-contraction coupling [2, 15]. Permanent dipoles or charged particles would move reversibly between two different sites, at least according to the changes of membrane potential. The charge displacement current may reflect such movements.

It is suggested that this dipole movement in the T-tubule membrane would connect the T-tubule with the adjacent but separate SR. There is a gap between the T-tubule and the terminal cisternae of the SR, and small projections (the electrodense feet) extend between them [19]. The estimated density of charged groups and the feet is about the same. It is suggested that the intramembrane charged groups or dipoles might be the voltage sensor of the excitation-contraction coupling and their movement could determine the subsequent steps leading to the Ca release.

Examination of the relations of charge movement to contractile activation was severely limited by the use of internal microelectrodes for voltage clamping the fibres. To avoid the fibre damage only threshold movements were allowed or contraction was blocked by hypertonic solutions or by tetracaine. SCHNEIDER and HOROWICZ [41] have therefore recorded charge displacement currents during depolarizing pulses to and well beyond contraction thresholds using the simple gap method introduced by KOVÁCS and SCHNEIDER [29]. This procedure does not require internal microelectrodes during measurement, thus allowing the movement of the voltage clamped terminated fibre segment. In this way the critical durations necessary to reach the mechanical threshold were determined at different membrane potential levels. The amount of charge moving during the critical duration was found essentially

the same (10 nC/μF) at all membrane potential values. The results indicate that the threshold quantity of charge has to move to reach the contraction threshold, independently of the time course or amplitude of the charge displacement current.

The significance of the threshold charge movement was confirmed by experiments in which the effect of sub-rheobasic prepulses were studied [42]. Charge movement and critical durations were determined for test pulses alone and for test pulses preceded by a 50 ms prepulse to a membrane potential value below rheobase for mechanical activation. The prepulses, which moved a certain amount of charge, decreased the charge movement during the following test pulses. The total amount of charge which moved during each prepulse plus the critical duration of test pulse was about equal to the threshold amount of charge moved during the critical duration of the same test pulse alone. These results indicate that for reaching the mechanical threshold a certain amount of charge has to move independently of the way it had moved.

The charge displacement current has a complex time course in skeletal muscle. This indicates that there may be several species of mobile charge which would move during depolarization. At present it is not possible to verify whether these kinds of charges move independently and control separate membrane processes or their movements are sequential and related to one process [1, 5]. Considerable efforts were made to relate the movement of a certain species of charge to certain process.

ADRIAN and PERES [4] have suggested that one species of charge (Q_γ) may be connected with gating of the potassium current. This hypothesis reveals several problems. The gating currents of the sodium channels were separated on the basis of their time course by VERGARA and CAHALAN [46]. These authors found that this current was partially immobilized by sodium inactivation and had a maximum value which did not amount to 10% of the total charge displacement current.

In spite of the above mentioned complexity of charge movement in muscle fibres it is generally accepted that most of it is related to contractile activation. To confirm this hypothesis, direct comparisons of charge movement and the subsequent steps in the activation process (SR function, Ca-release) are essential.

The internal membrane system of muscle fibres, T-tubules and SR, are topologically inaccessible to microelectrode penetration. In the last few years intrinsic and extrinsic optical methods were used to study the changes in their electrical properties during activation. These investigations were facilitated by the favourable results obtained on axon membrane. Changes in light scattering or birefringence [16] and in extrinsic fluorescence [43] of axons were found during the action potentials. Later it was demonstrated that these

optical changes are linear functions of the membrane potential, therefore they are convenient for recording the changes of the membrane potential.

These methods seem to be specially convenient in case of the T-tubules or SR because they have large membrane areas. The surface area of the T-system is 8—10 times, that of the SR, 100—200 times greater than the surface membrane [32, 36]. For this reason the obtained optical signals are relatively large and easily detectable. On the other hand, the contractile activity can cause many difficulties as it is changing the optical properties of the fibre to a great extent.

There are strong evidences that the fluorescence [30, 44] and absorbance [33] signals obtained by the relatively impermeant dye, merocyanine, originate from the surface membrane and the T-system. The shape of these signals is similar to the surface action potential but its time course is slower. After detubulation the time course becomes faster and the amplitude is decreased by a factor of ten. The first component of the birefringence signal can be attributed mainly to the surface action potentials and less to the T-system [11].

There are optical signals which occur at about the time of latency relaxation, that is they follow the action potentials but precede the contraction. They may be intrinsic as the transparency [8, 21, 22] or the second component of birefringence changes [11, 12] and extrinsic as the fluorescence signals which are obtained by penetrating dyes, like Nile blue A [13], or indodicarbocyanine [34]. These optical transients have a similar shape and their time course is much slower than that of the previous signals. The transparency [28] and the Nile blue A signal [45] were analysed with the voltage clamp technique as well.

The time and voltage dependence of the transparency increase has been studied by KOVÁCS and SCHNEIDER [28] on voltage-clamped cut muscle fibres. A decrease in latency time of the signal and an increase in the maximum rate of rise were found on increasing the amplitude of depolarizing pulses. The minimum latency observed was 6—10 ms. The peak values of transparency changes were approximately the same for pulses of different durations with amplitudes 1—2 mV lower than that necessary to reach the mechanical threshold. In the case of long pulses, the relation of the peak values of the transparency signal to the membrane potential was characterized by a sigmoid curve. This relation was similar to the voltage dependence of the mechanical activation measured by potassium contractures on single fibres [24].

The kinetic analysis of Nile blue A fluorescence signals by VERGARA et al. [45] gave similar results in many respects but an exact comparison was not possible because of differences in experimental conditions. It seems that these optical signals may all monitor the same events most probably the voltage changes across the SR during activation. Alternatively, they may originate from the myofilament changes preceding the mechanical activity,

especially if the latency relaxation originates in the myofilaments and not in the SR [20, 23]. We cannot exclude the possibility that some optical changes are elicited by the increase in myoplasmic Ca concentration and not those processes are monitored by the optical changes which lead to Ca release. In this respect a direct comparison of the optical and the myoplasmic calcium signals would help to get more information concerning the mechanism [9].

The mechanical tension is regulated directly by the changes of myoplasmic calcium concentration (ΔCa). Therefore, exact measurement of this ΔCa signal is very important to know the relation between the different steps of the excitation-contraction coupling. Essentially, two types of indicators have been used to measure ΔCa: the bioluminescence protein aequorin and the metallochromic indicator dyes such as murexide, arsenazo III, and antipyrylazo III. Not penetrating the surface and SR membrane they are convenient to measure ΔCa after being taken into the intracellular space.

Using aequorin, ASHLEY and RIDGWAY [7] have measured ΔCa on giant barnacle muscle fibres. Later single fibres of the frogs were microinjected with aequorin by RÜDEL and TAYLOR [38], BLINKS et al. [14] and the ΔCa signal was recorded during twitches, tetani and potassium contractures. The use of aequorin is limited by the known difficulties: the relation between Ca concentration and light intensity is not linear, the kinetics of the luminescence reaction is slow, etc., but its application can be favourable for example to record ΔCa signals and mechanical activity simultaneously.

The metallochromic indicator dyes became increasingly popular in the last few years. They change their colour on Ca binding, therefore the changes in the absorbance of the fibre have to be measured to record ΔCa signals. These dyes differ from one another in their suitable differential wave length, relaxation time, dissociation constant, extinction coefficient, etc. [39].

Though its selectivity and response time are excellent, murexide is relatively unfavourable because of its low sensitivity to Ca. In spite of the problem JÖBSIS and O'CONNOR [25] on some occasions were succesful to measure ΔCa by murexide on frog muscle fibres. The features of arsenazo III are more advantageous. It has a high affinity to Ca resulting in large absorbance changes owing to small Ca concentration changes. On the other hand, its selectivity and response time are relatively poor which can cause some difficulties. MILEDI et al. [31], BAYLOR et al. [10], PALADE [35] have injected arsenazo III into the fibre by ionophoresis. ΔCa signals were measured in consequence of action potentials and depolarizing pulses. The antipyrylazo III (AP III) is a middle range Ca indicator presenting features which are between those of murexide and those of arsenazo III [39]. The sensitivity, selectivity and response time of AP III are very convenient to measure ΔCa signals in muscle fibres.

Using AP III, SCHNEIDER, KOVÁCS and RIOS have studied the time and voltage dependence of ΔCa and its relation to the charge movement. The

experimental procedure was similar to the one developed for cut fibre preparations [29]. Segments of single fibres were cut at both ends and mounted in a double vaseline gap chamber. The AP III containing a relaxing solution was applied to the two cut end pools and the dye was left to enter the myoplasmic space by diffusion. The disturbing effect of contractile activity was minimized by stretching the fibres, so the absorbance changes at 720 nm wave length were taken to be proportional to the ΔCa. The internal and external solutions were designed to block all ionic conductances without changing the excitation-contraction coupling. In this way, simultaneous measurements of ΔCa and charge movement were possible.

It was found that during a long depolarizing pulse the ΔCa transients increased to a peak after a latency period of some ms and then declined toward a steady level. On increasing the amplitude of depolarizing pulses, the latency time decreased and the rate of rise increased.

For quantitative analysis and interpretation, ΔCa signals were fitted with exponential functions. During depolarization, after a transition time (S_{ON}) each signal could closely be approximated by the sum of two exponential functions of time plus a constant. During repolarization after a transition time (S_{OFF}) the signal declined along a single exponential time course. All the rate coefficients determined by the fitting procedure and the S_{ON} transition times have shown membrane potential dependence (KOVÁCS et al. [27]).

This formal mathematical description can be interpreted by a closed three-compartment system. The myoplasm constitutes one compartment where the ΔCa transients are measured by the dye, the other two compartments are the release and uptake pools of the SR. After the S_{ON}, the ΔCa time course is consistent with Ca redistribution between the three compartments with membrane potential dependent rate coefficients which do not change in time. At a given membrane potential, 98% of the charge has moved during the S_{ON} (RIOS et al. [37]).

These data support the hypothesis that charge movement controls the rate coefficients for Ca movement across the SR membrane. During depolarization the rate coefficients could not attain their steady levels, characteristic of the given membrane potential, until the charge movement had been completed.

The role of charge movement in controlling Ca movement was further confirmed by SCHNEIDER et al. who have studied the effect of subrheobasic prepulses on ΔCa transients (unpublished observations). 50 ms prepulses, causing negligible ΔCa, did not change the time course of ΔCa during the immediately following test pulse, but caused it to occur earlier. As described previously [42], the prepulses have moved a significant amount of charge, the time course of the charge movement during the test-pulse was about the same with or without prepulse, but with prepulse it occurred earlier. The final level,

characteristic of the given membrane potential, was attained sooner with prepulse. The time shift of ΔCa and charge movement due to the prepulses, measured on the same fibre, was found to be about the same.

In conclusion it may be said that depolarization of the surface and of the T-tubule membrane results in the charge movement which will control the Ca release. Consequently, the intramembrane charge movement might be the potential sensitive step of the excitation-contraction coupling in the skeletal muscle fibres.

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SODIUM AND WATER TRANSPORT IN FROG SKIN: EFFECT OF INDOMETHACIN

By

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The transepithelial transport of sodium, rubidium and water was studied in the frog skin in the presence of the cyclic endoperoxidase inhibitor indomethacin. Sodium transport was studied by measuring short circuit current and by ^{24}Na radioactive isotope method; water transport was examined using the frog skin sac technique. Indomethacin produced the following changes.

1. The transepithelial potential difference and the intensity of short circuit current were decreased, while the resistance and conductance of the skin remained unaltered.

2. Passive transepithelial sodium transport from the inner to the outer side was enhanced.

3. Neither osmotic water transport nor passive rubidium transport were changed.

Conclusions:

a) Indomethacin causes a selective increase of sodium permeability in the frog skin;

b) The present results are in agreement with the view that the indomethacin may influence the rate of sodium transport in some tissues.

Indomethacin (IM) is the most widely used cyclic endoperoxidase inhibitor, its renal effects in the anaesthetised dog (depressed renal blood flow, the shift of intrarenal blood flow distribution toward the cortex, antidiuretic and antinatriuretic action) are well known [1, 2, 7, 9, 13, 14, 16, 18, 20, etc.]. As repeatedly shown, the decrease of salt and water excretion is associated with unaltered glomerular filtration [2, 5, 6, 8, 15].

Based on these findings it seems reasonable to assume that indomethacin may affect cellular sodium transport and thus saline output also by mechanisms independent of haemodynamic changes. Consistent with this view is our earlier observation demonstrating that indomethacin enhanced the ^{24}Na-efflux from kidney cortex slices saturated with ^{24}Na (BARTHA, [3]).

The present experiments were designed to further elucidate the effect on sodium transport of indomethacin. In order to circumvene the problem of interfering haemodynamic changes, the frog skin was chosen as an experimental model in which active unidirectional sodium transport is known to take place in two stages (outer side passive, inner side active) just as in the renal tubules [11, 21]. Water and rubidium transport characteristics were also studied in an attempt to reveal an eventual specific action of indomethacin on sodium transport.

Method

Abdominal and thigh skin samples were used, removed from frogs of both sexes weighing 30—50 g. Frogs were kept in a bath with continuous water exchange without food added. All studies were carried out at room temperature.

1. *Study of water transport* (n = 15)

The skin from the hind limbs was removed and from the thigh skin sacs were formed by ligatures. Two sacs were formed from the skin removed from each frog; one sac contained 1 ml Ringer's solution, while the other contained 100 μg (n = 15) or 1 mg IM (n = 20) in 1 ml Ringer's medium. Sacs were weighed on an analytical scale and incubated for 6 h at room temperature in tenfold diluted Ringer's solution permanently gassed with oxygen throughout the studies. Skin sacs were re-weighed at hourly intervals, and the rate of the osmotic gradient dependent, i.e. outer to inner water transport was calculated on the basis of the weight increment of sacs and expressed in μl water/h/g wet skin.

After termination of the studies the skin samples were dried at 105 °C until weight constancy, thereafter digested in 65% nitric acid, and sodium and potassium contents were estimated by flame photometry (Carl Zeiss, Jena).

2. *Study of ionic transport*

2.1. *Study of sodium transport by short circuit current technique*

2.1.1. Control group (n = 15)

An abdominal skin lobe was formed, rinsed with Ringer's solution and mounted over a hole between two plexiglass chambers. Both chambers contained 10 ml of Ringer's solution. The voltage developing between the two sides of the skin lobe was recorded using calomel electrodes immersed into 3 M KCl and attached to a voltmeter (Radelkis, Type 08-208). Electric connection between the KCl solution and the chambers was established by agar-agar bridge. The rate of sodium transport was measured by the short circuit current technique, i.e. the negative pole of a D.C. power supply unit was connected to the electrolyte solution being in contant with the inner (positive) surface of the skin lobe, whereas the positive pole was connected to the chamber fluid being on the outer (negative) side. Voltage generated by the skin and the intensity of short circuit current was recorded every 10 min. During the course of the studies the D.C. short circuit power supply unit was switched on at each recording and values recorded after 30 sec were considered as the intensity of short circuit current. Measurements were started following a 40 min preincubation period and continued until 180 min.

2.1.2. Indomethacin group (n = 14)

Experimental protocol was the same as in the control group with the exception that at the end of the preincubation period the Ringer's solution being in contact with the inner surface of the skin lobe was replaced for a Ringer's medium containing 4 μg/ml IM too.

Sodium transport was calculated from Coulomb and Avogadro values and expressed as the amount of ions transported through 1 cm^2 of skin within 1 sec (nmole $Na^+ \cdot s^{-1} \cdot cm^{-2}$).

2.2. *Study of sodium and rubidium transport using radioactive isotopes (^{24}Na and ^{86}Rb).*

In this series of studies different experimental models were used to examine the active (toward the inner surface) and passive (toward the outer surface) transepithelial transport processes.

2.2.1. Study of the active transport

The experimental model was the same as described in 2.1.1., i.e. Ringer's solution was put at both sides of the skin lobe, however the outer fluid contained 0.2—0.3 MBq ^{24}Na, and the amount ^{24}Na transported from the outer to the inner side was estimated.

2.2.2. Study of passive transport

The experimental setup was identical with that described in 2.1.1. with the exception that the fluid in contact with the outer surface of the skin lobe was tenfold diluted Ringer's

solution. Bathing fluid at the opposite (inner) side contained ^{24}Na (0.2—0.3 MBq) or ^{86}Rb (0.5 MBq) in normal Ringer's medium and the rate of downhill transport was estimated by measuring the amount of ^{24}Na and ^{86}Rb in the outer surface fluid.

The rate of ion transport in both studies (2.2.1. and 2.2.2.) was expressed as per cent of radioactivity transported from one side to the other within a given time. In the IM study the Ringer's solution at the inner side also contained 4 μg/ml IM. The number of observations was: (2.2.1.) control, n = 13; IM studies, n = 13; (2.2.2.) ^{24}Na control, n = 15; IM treatment, n = 14; ^{86}Rb control, n = 23; IM treatment, n = 13.

Statistical evaluation of data was done by Student's t test.

Results

1. *Effect of indomethacin on water transport and ionic content of the skin*

Indomethacin, even in very high concentration (100 μg per ml, or 1 mg per ml), failed to influence the transepithelial osmotic water transport in the frog skin (Fig. 1). Water, sodium and potassium contents of the skin also remained unaltered (Table I).

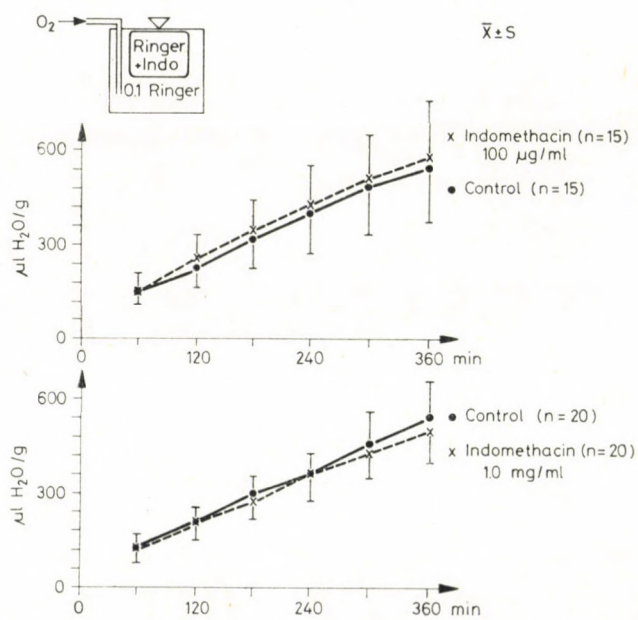

Fig. 1. Rate of transepithelial osmotic water transport in frog skin as a function of time in the presence of various concentrations of indomethacin. Frog skin sac contained Ringer's solution, external medium was tenfold diluted Ringer's solution. A cumulative plot, i.e. the amount of water passing across 1 g skin until a given time is plotted

Table I

Sodium, potassium and water content of frog skin in the presence of indomethacin

		Control n=15	Indomethacin 100 μg/ml n=15	Control n=20	Indomethacin 1 mg/ml n=20
Water content, %		79.2± 1.62	79.3± 2.00	79.2± 4.79	79.7± 6.05
Sodium,	μmol/g wet skin	53.6±12.4	54.5±12.4	62.6±10.3[a]	60.9±11.4
	μmol/g wet skin	258 ±60.4	256 ±42.7	315 ±64.5[b]	323 ±90.7[u]
Potassium,	μmol/g wet skin	19.9± 5.21	19.6± 4.48	21.5±2.87	18.3± 3.0
	μmol/g wet skin	95.8±20.9	95.3±20.9	105.7±19.6	98.3±37.9

[a, b]: significance between control groups: a: $p<0.05$; b: $p<0.02$.
[u]: significance between indomethacin groups; u: $p<0.02$.
Data are means ±S.D.

2. *Effect of indomethacin on ion transport*

2.1. *Effect on sodium transport*

In the control studies the voltage between the two sides of the skin lobe and the rate of sodium transport were slightly elevated during the 3-hour observation period. In the presence of indomethacin no such increase occurred, moreover, the potential difference as well as the amount of transported sodium were markedly decreased, the former from 25.00 ± 5.70 mV to 9.04 ± 0.89 mV ($p < 0.001$), the latter from 0.37 ± 0.06 nMol $Na^+ \cdot s^{-1} \cdot cm^{-2}$ to 0.059 ± 0.009 nMol $Na^+ \cdot s^{-1} \cdot cm^{-2}$ ($p < 0.001$) until the end of the studies. On the other hand, indomethacin did not influence either the electric resistance or conductance of the skin (Fig. 2).

Studies with ^{24}Na yielded similar results. In the presence of indomethacin the amount of ^{24}Na transported from the outer to inner side was considerably decreased with Ringer's medium of identical concentration on both sides (Fig. 3).

However, the amount of ^{24}Na transported from the inner to outer side was appreciably enhanced when a chemical concentration difference was produced between the two epithelial surfaces, by using Ringer's solution on the inner side and tenfold diluted Ringer's medium on the outer side (Fig. 4).

2.2. *Effect on rubidium transport*

In contrast to the effect on sodium transport, indomethacin did not influence the amount of rubidium transported from the inner to the outer side

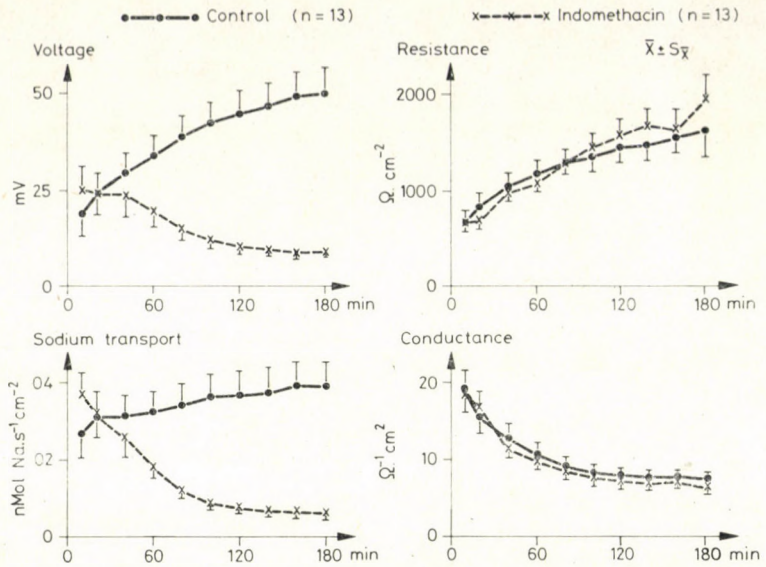

Fig. 2. Values for transepithelial potential difference, sodium transport, resistance and conductance in the frog skin in control study and in the presence of indomethacin (4 μg per ml)

even with Ringer's solution on the inner and diluted Ringer's medium on the outer side. As to passive sodium and rubidium transport, indomethacin increased the transport of sodium while not altering rubidium transport (Fig. 5).

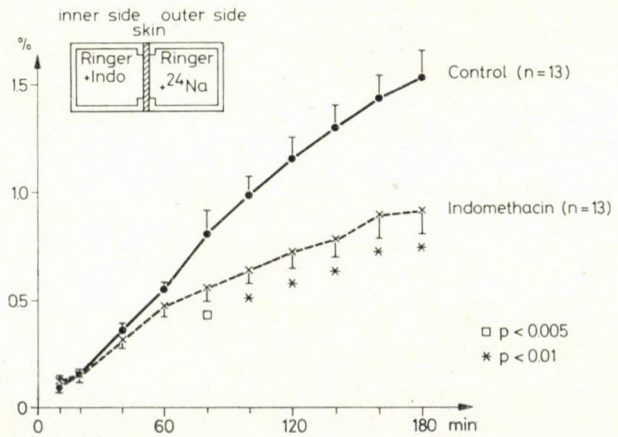

Fig. 3. Active transepithelial transport of Na^+ in frog skin in the presence of indomethacin. Figure demonstrates percentage of ^{24}Na transport from outer to inner side within a given time

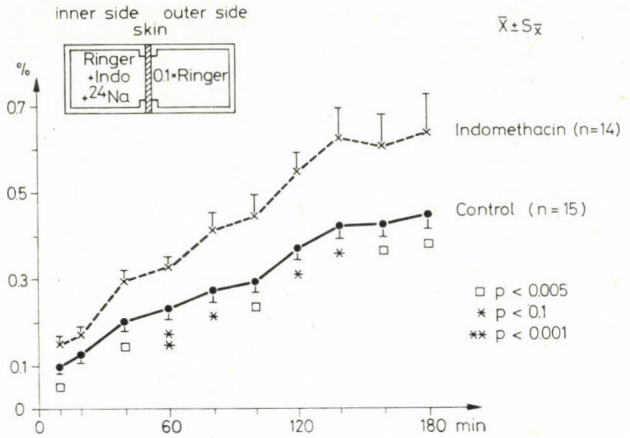

Fig. 4. Passive transepithelial transport of sodium in frog skin in the presence of indomethacin. Inner side contained Ringer's solution with ^{24}Na added. on the outer side tenfold diluted Ringer's solution was the medium. Figure shows percentage of ^{24}Na transported from the inner to the outer side down to the concentration gradient

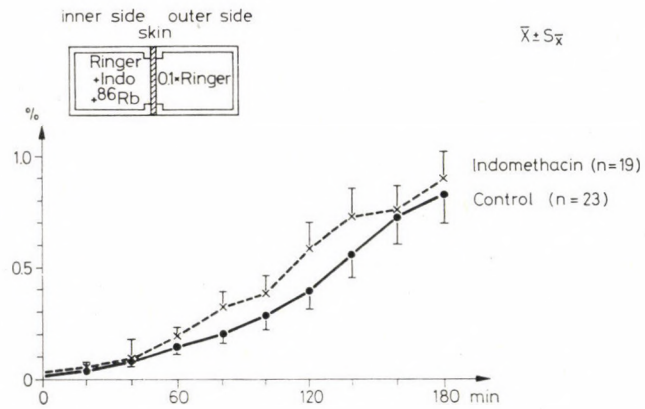

Fig. 5. Passive transepithelial transport of rubidium in frog skin in the presence of indomethacin. Inner side contained Ringer's solution with ^{86}Rb added, outer bathing fluid was tenfold diluted Ringer's medium. Figure demonstrates percentage of ^{86}Rb transported from the inner to the outer compartment along the concentration gradient

Discussion

The mechanism of the potential difference established between the two sides of the frog skin has been widely studied [10, 11]. According to the presently accepted view the epithelial cells on the inner side are capable of active sodium transport thereby generating the potential gradient. Opposite to this inward directed active Na transport is the passive Na movement limited

by the sodium concentration difference between the inner and outer side and by the permeability of the skin epithelium. The biological significance of the active Na transport is evident: the reaccumulation of sodium lost through passive transport.

In the present study indomethacin decreased the potential gradient between the two epithelial sides, the rate of inward sodium transport calculated on the basis of short circuit current (Fig. 2). The same effect could also be shown by an isotope method (Fig. 3). Theoretically, the drop of potential difference and the decrease of net sodium influx might result from

a) decreased active inward sodium transport and/or

b) the enhanced passive efflux from the inner to the outer side.

The first mechanism seems to be less probable in view of the fact that indomethacin exerts no effect on the activity of K-Na dependent ATPase which plays a key role in active transport [5]. However, DÜSING's [5] studies were conducted in a different species and tissue (rat renal cortex and medulla). ATPase activity was not measured in our study, still we suggest that if indomethacin had inhibited the active sodium transport then this should have caused a change of epithelial ion content (elevation of sodium concentration associated with potassium loss). Data in Table I, however, appear to demonstrate that indomethacin in a dose of 100 μg/ml exerted no influence on epithelial ion or water content and even with a very high dose (1 mg per ml) only a tendency (not significant statistically) of potassium loss and sodium accumulation was noted. Thus, the decrease of potential difference and sodium transport can not accounted for by the inhibition of active sodium transport.

If we accept that indomethacin exerts no influence on active sodium transport in a dose of 4 μg/ml, then its action to decrease potential difference and short circuit current can only be explained by an enhancement of epithelial permeability. The increased transepithelial sodium permeability from inner to outer side might counteract active sodium transport, thus net sodium flux will drop.

Consistent with this view is our result that passive ^{24}Na flux from inner to outer side was augmented in response to indomethacin, using a model with tenfold difference in initial sodium concentrations between the two sides. This appears to support the presumption of enhanced downhill transport of sodium. In contrast to the aforementioned the passive transport of rubidium from the inner to the outer side under similar conditions was not enhanced by indomethacin (Fig. 5). The rate of osmotic water transport also remained unaltered (Fig. 1). These findings suggest that the increasing effect of indomethacin on permeability is sodium specific.

The radii of sodium and rubidium ions are different (0.095 nm and 0.148 nm respectively). It is well known that the permeability of biological membranes to various ions does not correlate with ionic diameters. A larger

size ion might more rapidly penetrate the pores than a smaller one. This paradox behaviour can be explained by the different rate of hydratation of various ions. The smaller sodium ion has a higher hydratation energy (—388 kJ · mol^{-1}) than the larger rubidium ion (—280 kJ · mol^{-1}) [19]. Thus, the size of sodium ion with its hydrate shell is larger than that of the rubidium ion with its hydrate shell, i.e. the permeability of the membrane to sodium ion is less than to rubidium ion. In the present study indomethacin enhanced the permeability of the membrane to sodium, a solute with larger diameter ions, while it exerted no influence on the permeability of ^{86}Rb and water with smaller ionic size. This also suggests a selective action, possibly the opening of the specific sodium channel.

A further possibility for the decrease of potential difference is the enhancement of chloride ions due to electrostatic attraction resulting in diminished polarity between the two sides of the membrane. Chloride transport was not studied in this series of investigations, thus the possibility of such a mechanism can not be ruled out, however, this is independent of the enhancing effect of indomethacin on passive sodium transport.

It remains to be established whether indomethacin exerted its effect through the inhibition of endogenous PG-synthesis or by other mechanism. The present data are not sufficient to provide an answer to this question. Exogenous PGE_1 decreases the potential difference between the two sides of the frog skin, increasing at the same time the intensity of the short circuit current [17]. Changes observed in the frog skin under the effect of indomethacin are not, however, simply reversal of responses induced by exogenous PGE_1. Thus it appears reasonable to suggest that this effect of indomethacin, i.e. enhancement of sodium permeability, is independent of the inhibition of PG production.

The results of the present findings experiments are consistent with our previous devoted to ^{24}Na efflux in kidney cortex slices (BARTHA [3]) and provide further evidence for an effect of indomethacin on sodium transport independent of its haemodynamics action.

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EFFECT OF PROGESTERONE ON THE POST PARTUM RAT UTERUS

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The reactivity of the uterus to estradiol (E_2) and progesterone (P) has been examined in 44 post partum rats. Hormone treatment was initiated after surgical delivery at $\sim$ 24 hours before the expected onset of labor in 10 animals and immediately after spontaneous labor in 34 rats. At the time of steroid treatment each animal was equipped with a pressure sensor for the sequential recording of intrauterine pressure (IUP) before and after the oxytocin test.

Daily measurements during one week showed that post partum the IUP increases significantly in all animals not treated with P. The combined administration of E_2 + P significantly reduces the maximum IUP (IUPm) if treatment is initiated $\sim$ 24 hours before the spontaneous onset of labor. P treatment alone, initiated after spontaneous delivery, is ineffective, but it suppresses IUPm significantly if given in combination with E_2 and during 3 days or longer. Apparently, shortly before and during labor the reactivity of the rat uterus to P changes markedly, a process which is inadequately understood.

PORTER and CHALLIS [1] reported that 7 days post partum spontaneous myometrial activity is not diminished in the rat within 24 hours after progesterone (P) treatment, despite the high concentration of P in peripheral plasma and uterine tissue. This interesting observation led them to conclude that "in the rat, as in the guinea pig [2], progesterone may not be a myometrial-inhibiting factor". Recognizing the paradox between their conclusion and the well documented indispensability of P in pregnant rats [3, 4] they proposed that the P effect is mediated by: "*a)* an action inhibiting the release of oxytocic substances, e.g., neurohypophyseal hormones from mother or fetus; and *b)* a key role in maintaining the viability of the conceptus which in turn inhibits myometrial activity through an unknown mechanism." However, this interpretation does not explain the paradox. In the rat P treatment predictably prohibits parturition, not only when the fetuses are alive but even after prolonged pregnancy when they die *in utero* [5]. PORTER and CHALLIS considered [1] that the rat uterus is "sensitive to progesterone during pregnancy but becomes refractory following parturition", and noted the marked changes in the concentration of P receptors at around parturition [6] but

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rejected the former and were uncertain about the implications of the latter. Thus, the paradox remained unexplained.

In designing the present experiments we relied on the evidence that in the rat P is indispensable for the maintenance of pregnancy, since recent methods, which provoked P withdrawal (Pw) without the side effects of ovariectomy, unequivocally established this indispensability [7—9]. For example, treatment with anti-P serum (which binds P with immunological specificity) terminates pregnancy whenever it drastically reduces biologically active P [7]. The P synthesis inhibitor: Isoaxazol also terminates pregnancy [8] and induces labor [9] whenever it provokes a rapid Pw of critical degree. The additional demonstration that the prevention of Pw by P treatment prevents these actions of the anti-Ps [7—9], provides indisputable evidence that it is Pw and not some unrecognized side effect of the experimental procedure which intercepts pregnancy. These observations [3—9] and the additional finding that Pw suspendes and P-replacement sustains the suppression of myometrial activity in pregnant rats [10] firmly established the critical role of P in the control of myometrial function in pregnant rats. Assigning a similar role to other endogenous hormones would require substantiation by comparable evidence.

The present study was prompted by the recent observation in the rat that, while P treatment predictably prevents parturition if initiated 24 hours before the expected onset of labor, delayed P treatment fails to prevent normal delivery despite the excessive elevation of plasma and uterine tissue P levels [11]. These and the additional finding that a few hours before the onset of labor the rat uterus is depleted in P receptors [6, 12], suggests that near term the myometrium may lose its ability to "decode" the inhibitory signal of P. Apparently, the concentration and biological action of P cannot be equated whenever the decoding of the P signal is curtailed [11]. If so, P may not suppress uterine activity post partum until the decoding mechanism is restored by prolonged hormone treatment. The present experiments examined the validity of this premise.

Material and methods

A total of 44 Sprague-Dawly rats were studied. When 18 days pregnant (day of mating: day 0), the animals were shipped to the laboratory, where palpation excluded those rats which were non-pregnant or had low litter size. The 44 animals selected were kept in individual cages at constant temperature (22 °C), exposed to 14 hours of light and 10 hours of darkness and fed Purina diet.

All experiments begun immediately post partum but only 34 rats were allowed to deliver spontaneously, while in 10 the uterine contents were delivered by hysterotomy 24 hours before the expected onset of labor. In ether anesthesia and under strictly aseptic precaution all 44 animals were laparatomized. Through a small incision near the uterotubal junction of both horns the uterine contents were removed from the 10 pregnant rats. Only one incision was made in the 34 post partum animals. Before closing the incision by tobacco sutures, a balloon-tipped catheter has been inserted into that uterine horn which had at least 4 im-

plantation sites. Before insertion the air free rubber balloon of 5.5 ml capacity (when unstretched) was connected to a flexible polyethylene tube (O.d. = 1.5 mm). After the placement of the pressure sensor the extrauterine end of the catheter was tunnelled under the skin to exit at the dorsal end of the neck. The balloon was then filled to (5.5 ml) capacity, the catheter sealed by heat and the abdomen closed by suture.

In all 44 animals the intrauterine pressure (IUP) has been sequentially recorded, once daily for a week, starting 24 hours after surgery. At the time of surgery the animals were divided into 6 groups according to their treatment schedule (Table I). The steroids were administered every day in 0.1 ml oil, i.m., starting on the day of surgery. For the oxytocin test (OT) 5 mU oxytocin was administered intraperitoneally in 0.1 ml saline.

Table I

The Treatment Schedule of the Animals

(n)	PREGNANT
(3)	Estradiol 5 μg/Day
(7)	Estradiol 5 μg and Progesterone 5 mg/Day
	POST PARTUM
(4)	Vehicle Control
(5)	Progesterone 5 mg/Day
(8)	Estradiol 5 μg/Day
(17)	Estradiol 5 μg and Progesterone 5 mg/Day

At 24 hours after the placement of the recording system and at each consecutive day, the animals were anesthetized by 10 mg veterinary Diabutal (sodium pentobarbital) and placed in a restrain cage, to minimize movement-artifacts on the IUP tracings. In each animal spontaneous uterine activity has been recorded for $\sim$ 45 minutes and subsequently the response to 5 mU oxytocin for $\sim$ 60 minutes. From that 10 minutes portion of each tracing where IUP was maximal the maximum IUP was calculated by measuring each individual pressure cycle (in mm Hg) and by dividing their sum with the number of contractions. In each group the Mean $\pm$ S.E. of the maximum IUP (IUPm) was calculated at consecutive days and differences among these values were analyzed by the Student's t test for significant differences.

Results

Figure 1 presents two typical sets of original tracings of two rats which were surgically delivered 24 hours before their expected onset of labor. From the day of surgery animal A received estradiol (E_2) while B received E_2 + progesterone (P). It is apparent that in the E_2 rat maximum IUP (IUPm) gradually increases throughout the one week observation period while in the E_2 + P rat it is suppressed during the period of P-treatment. It should be noted also, that here and elsewhere the IUP is maximal and regular during the oxytocin test (OT) and, therefore, in the present study IUPm is measured during the height of oxytocin stimulation. In contrast, spontaneous activity is variable,

frequently showing long intervals without cyclic activity. In the Figures therefore, those portions of the (45 minutes) tracings are presented where spontaneous activity is maximal.

Figure 2 presents two sets of typical IUP tracings, obtained after spontaneous delivery in two post partum rats. One animal (A) is a vehicle control, while the other (B) is a P-treated rat. Figure 2 shows that P-treatment (without E_2) does not appreciably suppress the IUPm.

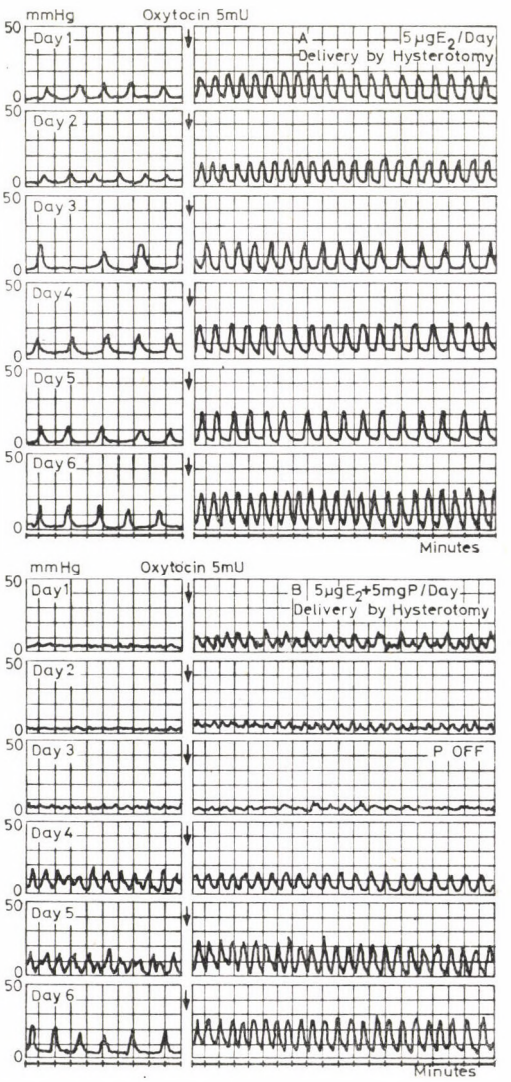

Fig. 1. The effects of estradiol and estradiol + progesterone on the intrauterine pressure of two post partum rats which were surgically delivered at ~ 24 hours before the expected onset of labor. For detailed description see text

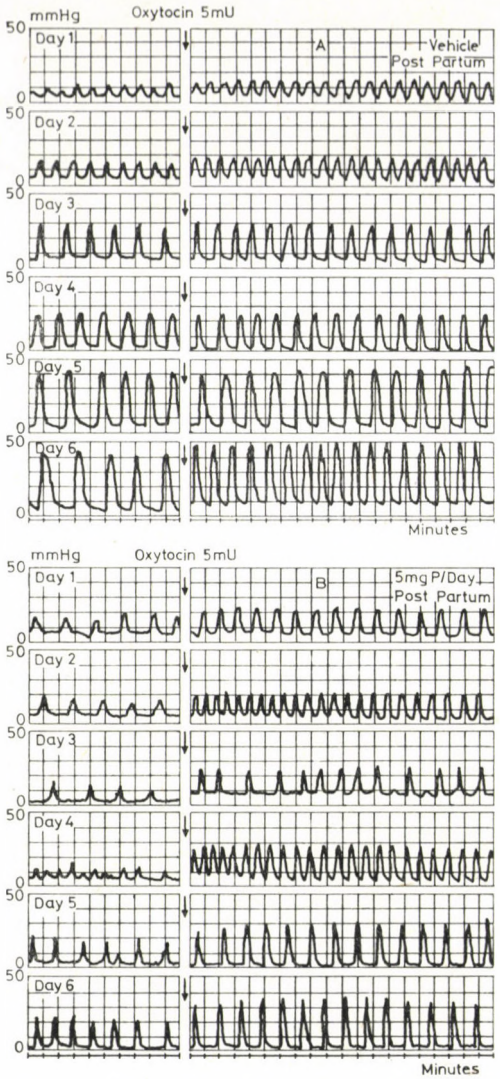

Fig. 2. The effects of vehicle and progesterone (without estradiol) on the intrauterine pressure of two post partum rats which delivered spontaneously at term. For detalied description see text

Figure 3 presents two sets of typical tracings, obtained after spontaneous delivery in two post partum rats. One animal (A) is an E_2, while the other (B) an $E_2 + P$ rat. Figure 3 shows that P moderately suppresses IUPm, an effect which is suspended by the discontinuation of P treatment.

Table II summarizes the results obtained in 44 post partum rats, 10 after surgical and 34 after spontaneous delivery. The analysis shows that in those

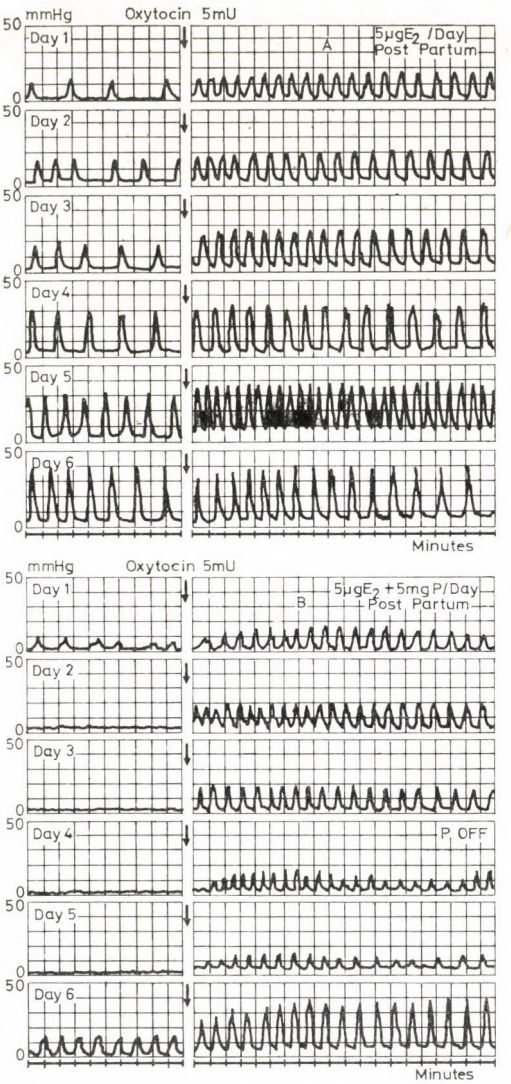

Fig. 3. The effects of estradiol and estradiol + progesterone on the intrauterine pressure of two post partum rats which delivered spontaneously at term. For detailed description see text

rats, which after their surgical delivery ~24 hours before term received E_2 during consecutive post partum days, the IUPm gradually increases during the one week observation period. In contrast, in those rats which received daily E_2 + P the IUPm decreases during the period of P treatment. The difference in IUPm between these two groups of animals is already significant at day 3 ($P<0.01$). Apparently, if E_2 + P treatment is initiated as late in

pregnancy as ~24 hours before the onset of labor, but before spontaneous delivery, then P is an effective suppressor of IUPm despite the surgical delivery of the uterine contents.

Table II

Effects of estradiol and progesterone on maximum intrauterine pressure in post partum rats

(n)	Schedule of treatment	IUP mm Hg at post partum days					
		1	2	3	4	5	6
	Surgical delivery 24 hours before term						
(3)	5 μg E_2/day	12±3	14±4	14±3	16±2	18±1	24±8
(7)	5 μg E_2+5 mg P/day	11±3	10±2	6±1↓ψ	7±2	12±1	17±2
	Spontaneous delivery						
(4)	Vehicle control	15±1	15±1	19±2	23±2*	29±4	31±5
(5)	5 mg P/day	15±2	18±3	21±1	22±1	23±2	30±4*
(8)	5 μg E_2/day	18±2	20±2	23±2	25±4	27±1*	30±2*
(17)	5 μg E_2+5 mg P/day	16±1	16±1	16±1ψ	14±1ψ	12±1*ψ	13±1ψ

All values are Means ± S.E. ↓ = P off. The maximum intrauterine pressure (IUPm) of the E_2 and E_2 + P rats is significantly different: ψ $P<0.01$. The IUPm within the same group of animals is significantly different from the initial value: * = $P<0.01$

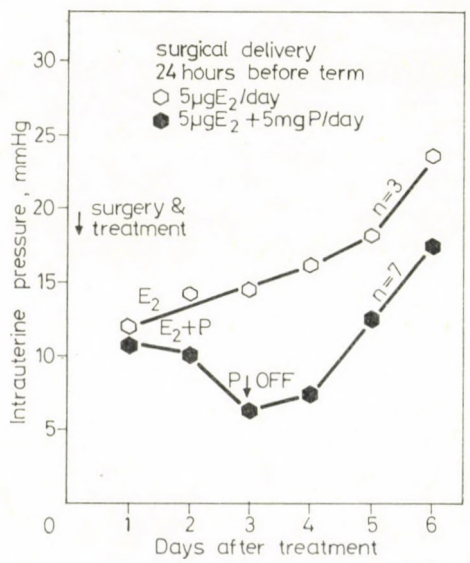

Fig. 4. The effects of estradiol and estradiol + progesterone on the intrauterine pressure of 10 post partum rats which were surgically delivere ~ 24 hours before the expected onset of labor. For detailed description see text

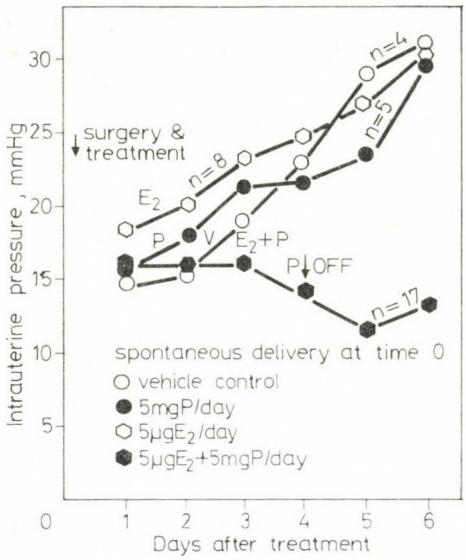

Fig. 5. The effects of vehicle, progesterone, estradiol and estradiol + progesterone on the intrauterine pressure of 33 post partum rats which delivered spontaneously at term. For detailed description see text

The analysis also shows that in those post partum rats which received daily treatment after spontaneous delivery, the IUPm increases gradually during the one week observation period regardless of whether they were treated with vehicle, E_2 or P (without E_2). The difference in IUPm at day 1 and 3 to 6 in these three groups of animals is significant ($P<0.01$). In contrast, the IUPm is suppressed in the E_2 + P rats during the period of P treatment. In this group the difference in IUPm at day 1 and day 5 is significant ($P<0.01$) and so is the difference in IUPm between the E_2 and E_2 + P rats at day 3 ($P<0.01$).

Figure 4 graphically illustrates the results obtained in those 10 post partum rats, which were surgically delivered and treated 24 hours before term. It shows, as does Table II, that while IUPm gradually increases in the E_2 rats, it is suppressed in the E_2 + P rats until P treatment is discontinued.

Figure 5 graphically illustrates the results obtained in those 34 post partum rats, which were treated after spontaneous delivery at term. It shows that during the one week observation period IUPm steadily increases in the vehicle controls, the E_2 and the P rats. Apparently, by itself (without E_2) P does not significantly suppress IUPm if the treatment is initiated after spontaneous delivery. However, if in addition to P the rats also receive E_2, their IUPm not only fails to increase (in comparison with E_2 rats) but is

suppressed after 3 days treatment. This finding indicates, that the post partum rat uterus can "decode" the P signal and respond to it if the animal is repeatedly exposed to effective E_2 + P treatment.

Discussion

The present study shows, that if 24 hours before the expected onset of labor surgically delivered rats are treated with estradiol (E_2) + progesterone (P), their maximum IUP (IUPm) is suppressed during the period of P treatment. This observation complements the demonstration that P treatment, initiated 24 hours before term, predictably prevents the onset of labor [11]. The present study also shows that if P treatment is initiated after spontaneous delivery it does not significantly suppress IUPm, unless it is combined with E_2 treatment. Furthermore, post partum the E_2 + P treatment has to be repeated for several days before its effect manifests. This observation complements the demonstration [11] that if P treatment is delayed until ~12 hours or less before the expected onset of labor, it does not prevent parturition despite the massive elevation of plasma and uterine tissue P-levels. These findings indicate that shortly before and during labor the rat uterus loses its ability to decode the P signal. They also complement the observation [6], that the preparatory action of estrogen is a prerequisite of the P effect.

Since the biological action of P near term is sharply dependent on the TIMING of P-treatment, it is apparent that the concentration and action of P cannot be equated [11]. Evidently, a few hours before the onset of labor the rat uterus (a classic model for the demonstration of P action [3—11], undergoes a variety of biologically significant changes which are incompletely understood. Alterations in receptors for progesterone [6, 12], oxytocin [13] and prostaglandin [14] and in gap junctions [15], have been proposed as preparatory for the onset of labor. However, these premises were not substantiated by evidence that the concentration of these receptors and uterine function change in a similar manner, for example when the onset of labor is predictably prevented by P treatment. The respective effects of E_2 and P on oxytocin receptors and on uterine function were only examined in the rabbit [16].

In view of these persistent uncertainties, regarding the biological meaning of those subcellular constituents and events which are implicated in the control of pregnancy and labor, the changing reactivity of the rat uterus to P at term cannot be adequately explained. Thus, in good agreement with earlier observations [11], the present study merely shows that at term the ability of the rat uterus to decode the P signal is drastically reduced — hereby its sensitivity against oxytocin is increased — and that post partum this organ requires several days long E_2 + P treatment to partly recover its reactivity to P.

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THE EFFECT OF ZINC IONS (Zn^{2+}) ON THE PROCOAGULANT ACTIVITY OF PMN LEUKOCYTES

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The effects of Zn^{2+}, Mg^{2+} and Mn^{2+} *in vitro* were studied on the procoagulant activity of peritoneal PMN leukocytes derived from endotoxin treated rabbits. The cells collected from the abdominal cavity were incubated in the presence of these divalent cations at 37° in Hank's solution followed by determination of the clotting activity of their supernatants in citrate treated rabbit plasma. When the cells were incubated in the presence of 50 μM/l $ZnCl_2$ the procoagulant activity of their supernatants decreased by 30%. Upon the application of 100 μM/l $ZnCl_2$ a 50—60% increase in clotting time was observed. Zinc ions inhibited in a concentration dependent manner the release of procoagulant material from leukocytes. Neither of the other cations used as controls has influenced clotting time if assayed in a similar system.

It has been established that zinc ions inhibited the liberation of tissue factor from endotoxin treated PMN leukocytes. The inhibitory effect was dose dependent and increased from 5 μM/l to 250 μM/l exponentially.

The inhibitory effect of zinc ions on tissue factor liberation might be explained by membrane stabilization.

In the suggested pathomechanism of endotoxin induced disseminated intravascular coagulation great importance has been attributed to the neutrophil leukocytes [6, 13]. In a previous study [18] we have been dealing with the generalized Shwartzman reaction which is a typical form of disseminated intravascular coagulation. In the state of granulocytopenia induced by nitrogen-mustard treatment of rabbits, the generalized Shwartzman phenomenon cannot be triggered by endotoxin [12] while it was possible to elicit this reaction with thrombin even in leukopenic animals [5]. It was also shown that, following endotoxin treatment, peritoneal leukocytes of rabbits acquired high procoagulant activity [16]. Leukocytes increasingly produce and release a procoagulant material of tissue factor nature upon the effect of endotoxin [10, 15]. The production of tissue factor can be inhibited by cycloheximide and puromycin, therefore, it is regarded as being the product of active protein synthesis [17]. It is released from the cells in the presence of Ca ions [14].

Recently a number of publications have pointed to a possible interrelations between zinc ions and biological membranes. Most of the authors have found that zinc ions in concentrations higher than the physiological range show membrane stabilizing properties and inhibit the release of certain biologically active materials [7, 8, 9, 19].

We know little about the exact nature of this process. Based on the above data it seemed worth studying whether zinc ions are capable of inhibiting the *in vitro* the release of tissue factor from polymorphonuclear leukocytes (PMN leukocytes).

Materials and methods

Chemicals used in the experiments

Casein according to Hammersten (Reanal, Hungary), Bacto Lypopolysaccharide W.E. coli 055:B_5 control 630903 (Difco Laboratories), $ZnCl_2$, $MgCl_2$, $MnCl_2$ (Merck) Trypan blue, silicone solution (Serva). Modified Hank's solution was prepared in our laboratory by omitting $MgSO_4 \times H_2O$.

Male albino *rabbits* of 2000—2500 g body weight were used throughout the experiments.

Plasma was obtained by drawing blood from the ear vein of untreated rabbits. A 3.8% solution of sodium citrate and blood were mixed 1 : 9, then centrifuged at 4 °C by $2000 \times g$ for 20 min. Plasma was sucked off with a siliconized Pasteur capillary and stored until use in polyethylene tubes at room temperature. For each experiment fresh plasma was obtained.

The preparation of PMN leukocytes

Following food withdrawal for 24 hrs, a 7% (w/w) casein solution in 40 ml of isotonic saline (pH = 7.4) and 100 μg/kg endotoxin dissolved in saline were given to the rabbits intraperitoneally. After 18 hrs, the rabbits were killed and after rinsing the peritoneal cavity with 80 ml of citrate containing saline (3.8% sodium citrate and 0.9% sodium chloride, 1 : 4) the exudate was collected quantitatively. After filtering it through 4 layers of gauze, the exudate was centrifuged at 300 *g* for 5 min. Supernatant was discarded and the sediment was washed twice. All steps of cell separation were carried out at 4 °C. Viability was detected by trypan blue uptake. At the end of cell preparation, 5—10% of the cells have taken up the dye. By repeating trypan blue uptake after incubation, the proportion of stained cells 10—15%. To study the exudate cells morphologically, smears were prepared and stained according to Pappenheim, then viewed at a magnification of 1000. Up to 100% of cells were leukocytes with no detectable morphological abnormality. A constant number of cells 10^7/ml was incubated with different amounts of zinc, magnesium or manganese containing Hank's solution at 37° for 1 h, applying gentle agitation every 5 min. In the controls the incubation was carried out in the absence of metal ions, while in another set of controls the zinc ions were added to the cells at the end of incubation.

The cellular suspensions were centrifuged at 1500*g* for 15 min, at 4 °C and the supernatant was carefully removed with siliconized Pasteur capillaries and stored until use at 4 °C.

The clotting potential of the supernatants was determined by a Clotek (Hyland) coagulometer: 0.1 ml of rabbit citrate plasma was preincubated and 0.1 ml of supernatant added, followed by recalcination of the system with preincubated 0.05 M/l $CaCl_2$ solution after standing for 2 min. In control experiments Zn^{2+}, Mg^{2+}, or Mn^{2+} containing Hank's solution was given to the preincubated plasma instead of the supernatants and clotting times were measured after recalcination. For statistical evaluation of the results Student's *t* test was used.

Results

Table I shows clotting times obtained from individual experiments in seconds. Since even suparnatants of control cells incubated without metal ions showed marked scattering of the values (57.2 ± 14.4 sec), for the purpose of a better evaluation the inhibitory effect of zinc ions was expressd as per cent changes in Fig. 1.

Figure 1 shows that, by applying a 50 μM/l final concentration of $ZnCl_2$, clotting could be increased significantly. In the presence of 100 μM/l of $ZnCl_2$

Table I

The clotting potential of leukocyte supernatants incubated in the presence of $ZnCl_2$, measured on normal rabbit plasma individual values in seconds

Treatment	Clotting times in seconds										Significance
Control	92.0	55.0	55.4	35.1	53.4	101.7	46.1	34.6	65.2	33.5	
$ZnCl_2$ 50 μMcl											
Before incubation	121.9	64.4	67.8	43.7	71.1	116.3	71.2	42.7	105.2	42.5	$p<0.001$
$ZnCl_2$ 100 μMol											
Before incubation	159.7	83.3	83.1	48.8	78.6	129.8	88.4	55.2	125.7	44.3	$p<0.001$
After incubation	90.5	54.2	57.1	34.3	55.3	104.6	—	—	—	—	$p>0.2$

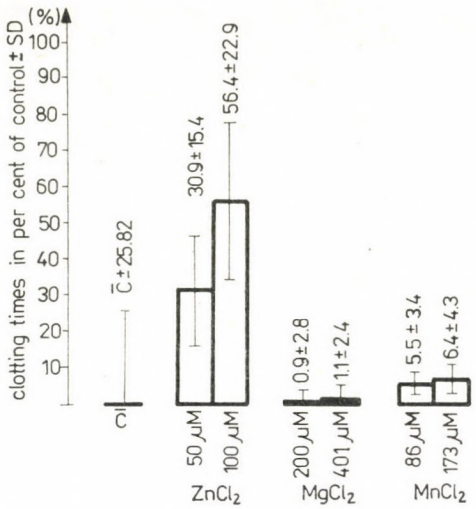

Fig. 1. The clotting potential of leukocyte supernatants incubated in the presence of $ZnCl_2$, $MgCl_2$ and $MnCl_2$ measured on normal rabbit plasma, expressed as per cent of the controls

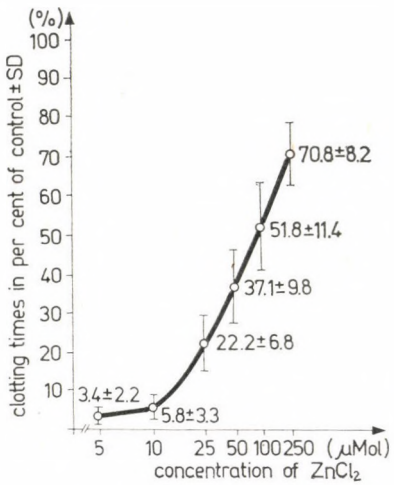

Fig. 2. The clotting potential of leukocyte supernatants incubated with different concentrations of $ZnCl_2$ measured on normal rabbit plasma, expressed as per cent of the controls

on the other hand, the procoagulant activity showed a marked decrease (values are averages of 10 independent experiments). In the experiments where 100 μM/l $ZnCl_2$ were added *after* incubation, clotting times were not different from the controls.

In subsequent experiments the changes in procoagulant activity i.e. the increase of clotting time, were studied in the same incubation system as

Table II

The clotting potential of leukocyte supernatants incubated in the presence of $MgCl_2$ and $MnCl_2$ (individual values in seconds)

Treatment		Clotting times in seconds				Significance
Control		46.1	34.6	65.2	33.5	
$MgCl_2$	200 μMol	45.5	34.9	68.4	33.2	$p>0.1$
	401 μMol	47.3	33.8	66.0	34.5	$p>0.2$
$MnCl_2$	86 μMol	50.3	35.9	70.1	34.1	$p>0.05$
	173 μMol	46.2	37.3	71.8	36.0	$p>0.05$

a function of Zn^{2+} concentration. As shown in Fig. 2, clotting times increased exponentially with increasing Zn^{2+} concentration, 25 μM/l $ZnCl_2$ already resulting in a 20% inhibition of clotting.

In another set of experiments we studied the effects of Mg^{2+} and Mn^{2+}. The final concentration of the above ions was chosen in a way that the number of metal ions was equal to that of zinc ions in the previous tests.

Table II and Fig. 1 show that, in the applied concentrations, neither Mg^{2+} nor Mn^{2+} have influenced the procoagulant activity. The differences were not significant statistically means of 4 experiments.

In control experiments — in a cell-free system — the effects of Zn^{2+}, Mg^{2+} and Mn^{2+} were studied on recalcification times. None of these metal ions influenced the process of clotting.

Discussion

The peritoneal leukocytes of endotoxin pretreated rabbits possess significant procoagulant activity which is due to increased production of tissue factor by the neutrophils [10, 15, 16]. In the course of incubation of citrate treated whole blood with endotoxin, the procoagulant activity develops *in vitro* within a few hours. The role of neutrophils is evident also in view of the observation that upon incubation with endotoxin the whole blood of a patient suffering from drug-induced granulocytopenia failed to exhibit procoagulant activity [11].

MUHLFELDER et al. [14] found that tissue factor is released from the cells in the presence of Ca^{2+} and other divalent cations (Co, Mn, Zn) would not substitute for Ca^{2+}. In our experiments the effect of a few divalent cations was studied on the liberation of tissue factor in the presence of Ca^{2+}. The present results show that zinc ions inhibited this process already in a concentration of 25 μM/l while two other cations, Mg^{2+} and Mn^{2+}, were ineffective.

This observation allows the conclusion to be made that the inhibition is due specifically to the presence of zinc. The increase of clotting time was not caused by a pH shift toward acidity since in control experiments, where $ZnCl_2$ was added after incubation to the cellular suspensions, the obtained values were similar to those found in zinc-free supernatants. MUHLFELDER et al. [14] were also unable to find any significant change in clotting time when studying the effects of pH shift actually not exceeding a few tenths of a unit. It is to be assumed that tissue factor is released into the surrounding media by destruction of the leukocytes. After incubation, repeated trypan blue exclusion tests revealed a cellular death rate of only 5—10% at all zinc concentrations applied, therefore, it is unlikely that the dose dependent effect would have been a consequence of cellular destruction.

The procoagulant inhibitory effect of zinc ions can be explained by their stabilizing effect exerted on biological membranes. CHVAPIL [2], in his review article, theorized that this membrane stabilization might be due to binding of Zn^{2+} to the free SH groups of membrane proteins, thus forming mercaptides. It has been also shown to inhibit the haemolysis of human erythrocytes by Streptolysin 0, while the effects of Vibriolysin and Triton X-100 are not interfered with [19]. Zn^{2+} inhibits also the histamine release from mast cells [7—9]. The aggregation of thrombocytes and their serotonin release is diminished on the action of Zn^{2+} [4]. CHVAPIL and co-workers [3] found that Zn^{2+} inhibited in a dose-dependent manner the oxygen consumption of leukocytes, their phagocytic activity and property of killing *E. coli*. There are relatively few data in the literature on the *in vivo* action of zinc in pathological systems. CHO et al. [1] allowed sensitized guinea pigs to inhale $ZnSO_4$ solution in a form of spray and found that Zn^{2+} inhibited *in vivo* histamine release from mast cells.

In view of these results and other data published in the literature it appears that the role of zinc ions in membrane processes is well established, however *in vitro* to clarify their role *in vivo* further experiments, are required.

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EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE SULPHATE ESTER ON BRAIN MONOAMINES IN THE RAT

By

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The effect of different doses of intracerebro-ventricularly administered cholecystokinin octapeptide sulphate ester (CCK-8-SE) was studied on dopamine (DA), norepinephrine (NE) and serotonin (5-HT) contents in the hypothalamus, mesencephalon, amygdala, septum and striatum, 10, 20 and 60 min following administration.

The DA and NE content increased and the 5-HT content decreased in the hypothalamus and mesencephalon. A biphasic action was observed in the amygdala of DA, NE and 5-HT depending upon the time and doses used. Similar action was seen on DA and NE in the septum.

In the striatum, the DA and 5-HT content decreased while the NE level first increased and then decreased.

The data indicate that the CCK-8-SE is able to modify the activity of DA, NE and 5-HT in different brain regions in a time and dose-dependent manner, with a local specific action.

The presence of cholecystokinin-like activity in the brain has been demonstrated by VANDERHAEGHEN et al. [26, 27], DOCKRAY [3, 4], MULLER et al. [16], REHFELD [19, 20, 21], HÖKFELT et al. [10], HOLMQUIST et al. [9], LARSSON and REHFELD [13], and LORÉN et al. [15]. Chemical identification of the peptide was carried out by DOCKRAY [3], DOCKRAY et al. [5, 6], REHFELD [19, 20, 21], and REHFELD and GOLTERMANN [23], in the brain and the CSF in humans (REHFELD and KRUSE-LARSEN [22]).

The physiological significance of cholecystokinin in the brain is incompletely understood (LOONEN and SOUDIJN [14]; HÖKFELT et al. [11]). The effects of cholecystokinin octapeptide sulphate ester (CCK-8-SE) (8 nmole) on brain monoamines following intracerebroventricular administration have been reported earlier (TELEGDY et al. [25]).

In the present paper the effect of different doses of CCK-8-SE was studied on the dopamine (DA), norepinephrine (NE) and serotonin (5-HT) content in the hypothalamus, mesencephalon, amygdala, septum and striatum, following intracerebroventricular administration in conscious, freely moving rats.

Methods

For the experiments CFY adult male rats weighing 200—250 g were used. The animals were kept under artificial light schedule (12 h light and 12 h dark), starting the light period at 6 a.m. Food and water was given *ad libitum*.

The test material was given via a chronic stainless-steel cannula implanted into the lateral cerebral ventricle by a surgical procedure (FIFKOVÁ and MARSALA [7]). The proper position of the cannula was checked by dissection of the brain. Experiments were began one week following the operation.

The test material used was CCK-8-SE synthetized by PENKE et al. [17]. The material was dissolved in 0.9% saline and the concentration given in the Tables was administered in 5 μl volume to freely moving animals. The control animals received the same volume of 0.9% saline. The animals were killed by decapitation at 0, 10, 20 and 60 min after the injection. The brain was rapidly removed, frozen, and the hypothalamus, mesencephalon, amygdala, septum and striatum were dissected according to BAUMGARTEN et al. [1]. Dopamine (DA), norepinephrine (NE) and serotonin (5-HT) content were measured by the spectrofluorimetric method of SHELLENBERGER and GORDON [24].

Each group consisted of 8—16 animals.

The results were evaluated statistically by analysis of variance. In the Tables mean values and standard error of the means are given.

Table I

Effects of different doses of cholecystokinin octapeptide sulphate ester on monoamine contents (μg/g tissue) of the hypothalamus

Group	0 min	10 min	20 min	60 min
DA				
1. Control	1.63±0.11	0.91±0.10	0.72±0.06	0.74±0.10
2. CCK-8-SE 80 fmole		1.17±0.18	1.00±0.07[b]	1.04±0.08
3. CCK-8-SE 800 fmole		1.35±0.17	1.36±0.14[a]	1.22±0.13
4. CCK-8-SE 80 pmole		2.87±0.22[c]	1.90±0.18[c]	1.76±0.23[c]
5. CCK-8-SE 8 nmole		1.66±0.15[b]	1.80±0.34[b]	1.65±0.28[b]
NE				
1. Control	2.25±0.08	1.79±0.17	1.26±0.11	1.22±0.09
2. CCK-8-SE 80 fmole		1.25±0.13	1.42±0.15	1.18±0.12
3. CCK-8-SE 800 fmole		1.58±0.14	1.52±0.10	1.40±0.11
4. CCK-8-SE 80 pmole		2.10±0.07	1.61±0.13	1.60±0.05[c]
5. CCK-8-SE 8 nmole		2.14±0.18	1.89±0.17	1.83±0.19[b]
5-HT				
1. Control	1.63±0.08	1.36±0.10	1.16±0.09	1.63±0.07
2. CCK-8-SE 80 fmole		1.28±0.13	1.12±0.10	1.51±0.14
3. CCK-8-SE 800 fmole		1.53±0.11	1.39±0.13	1.54±0.11
4. CCK-8-SE 80 pmole		1.57±0.17	1.24±0.10	1.19±0.07
5. CCK-8-SE 8 nmole		0.98±0.08	0.94±0.10	0.99±0.07[c]

Abbreviations: a = $p<0.05$; b = $p<0.01$; c = $p<0.001$ versus control

Results

The effect of different doses of CCK-8-SE on monoamine contents in the hypothalamus is shown in Table I.

For DA, the most effective dose was 80 pmole, which increased the DA content over the control level in 10 min; by 60 min the effect gradually decreased. All doses increased at 20 min, while at 60 min only 80 pmole and 8 nmole were effective.

The NE content increased following 80 pmole and 8 nmole versus the control at 60 min following injection.

On 5-HT content only the dose of 8 nmole was effective by decreasing the level at 60 min.

In the mesencephalon (Table II) 80 pmole increased the DA content in each time interval, while on the NE content a similar action was observed from 800 fmole. The 5-HT content decreased following 8 nmole at every point of time.

Table II

Effects of different doses of cholecystokinin octapeptide sulphate ester on monoamine contents (μg/g tissue) of the mesencephalon

Group	0 min	10 min	20 min	60 min
DA				
1. Control	0.47 ± 0.03	0.56 ± 0.07	0.45 ± 0.05	0.49 ± 0.05
2. CCK-8-SE 80 fmole		0.67 ± 0.06	0.67 ± 0.07[a]	0.65 ± 0.05
3. CCK-8-SE 800 fmole		0.61 ± 0.06	0.54 ± 0.07	0.59 ± 0.06
4. CCK-8-SE 80 pmole		0.91 ± 0.06[b]	0.81 ± 0.08[c]	1.10 ± 0.16[b]
5. CCK-8-SE 8 nmole		0.78 ± 0.14	0.69 ± 0.14	0.52 ± 0.09
NE				
1. Control	0.49 ± 0.02	0.44 ± 0.02	0.44 ± 0.04	0.42 ± 0.03
2. CCK-8-SE 80 fmole		0.36 ± 0.06	0.34 ± 0.06	0.33 ± 0.03
3. CCK-8-SE 800 fmole		0.60 ± 0.06[c]	0.53 ± 0.05[a]	0.57 ± 0.05[c]
4. CCK-8-SE 80 pmole		0.39 ± 0.04	0.39 ± 0.04	0.40 ± 0.03
5. CCK-8-SE 8 nmole		0.50 ± 0.03	0.51 ± 0.03	0.40 ± 0.04
5-HT				
1. Control	0.59 ± 0.03	0.64 ± 0.04	0.61 ± 0.04	0.57 ± 0.04
2. CCK-8-SE 80 fmole		0.82 ± 0.08	0.73 ± 0.06	0.86 ± 0.17
3. CCK-8-SE 800 fmole		0.79 ± 0.07	0.85 ± 0.07	0.94 ± 0.08[b]
4. CCK-8-SE 80 pmole		0.60 ± 0.04	0.62 ± 0.04	0.65 ± 0.07
5. CCK-8-SE 8 nmole		0.37 ± 0.03[c]	0.35 ± 0.03[b]	0.37 ± 0.02[c]

Abbreviations: a = $p<0.05$; b = $p<0.01$; c = $p<0.001$ versus control

Table III

Effects of different doses of cholecystokinin octapeptide sulphate ester on monoamine contents (μg/g tissue) of the amygdala

Group	0 min	10 min	20 min	60 min
DA				
1. Control	1.18±0.10	1.13±0.13	1.14±0.13	1.45±0.12
2. CCK-8-SE 80 fmole		0.67±0.04[b]	0.87±0.11	0.75±0.18
3. CCK-8-SE 800 fmole		1.06±0.12	0.98±0.12	0.74±0.04[a]
4. CCK-8-SE 80 pmole		2.01±0.23[c]	2.29±0.18[c]	1.86±0.24
5. CCK-8-SE 8 nmole		0.76±0.18	0.45±0.09[b]	0.71±0.21[a]
NE				
1. Control	0.45±0.05	0.45±0.09	0.34±0.04	0.42±0.04
2. CCK-8-SE 80 fmole		0.25±0.04[a]	0.37±0.09	0.33±0.04
3. CCK-8-SE 800 fmole		0.80±0.12[b]	0.65±0.10[b]	0.61±0.11[a]
4. CCK-8-SE 80 pmole		0.41±0.07	0.40±0.06	0.41±0.03
5. CCK-8-SE 8 nmole		0.49±0.06	0.28±0.06	0.31±0.07
5-HT				
1. Control	1.35± 0.07	1.34±0.09	1.21±0.09	1.34±0.08
2. CCK-8-SE 80 fmole		1.40±0.10	1.70±0.08[a]	1.81±0.21[a]
3. CCK-8-SE 800 fmole		1.65±0.25	1.54±0.16	1.35±0.17
4. CCK-8-SE 80 pmole		1.72±0.18	1.27±0.07	1.28±0.11
5. CCK-8-SE 8 nmole		0.82±0.09[a]	0.75±0.08[b]	0.92±0.11[a]

Abbreviations: a = $p<0.05$; b = $p<0.01$; c = $p<0.001$ versus control

In the amygdala (Table III), the DA content increased following 80 pmole at 10 and 20 min, and decreased following 80 fmole at 10 min, 800 fmole at 60 min, 8 nmole at 20 and 60 min. The NE content was increased by 800 fmole at all points of time and decreased at 10 min following 80 fmole. The 5-HT content increased after 80 fmole at 20 and 60 min and decreased at every point of time after 8 nmole.

In the septum (Table IV), the DA content increased at 10 min after the injection of 8 nmole and 800 fmole decreased it at all times studied, while 80 fmole decreased it at 60 min. The NE content increased after 800 fmole at 10 and 60 min, and after 8 nmole and 80 pmole at 60 min. 80 fmole decreased it in every time interval. There was no significant change in the 5-HT content.

In the striatum (Table V) the DA contents decreased after all doses and at all points of time, with the exception of 8 nmole, which was ineffective at 60 min. The NE content increased after 800 fmole at 10 min, and after

Table IV

Effects of different doses of cholecystokinin octapeptide sulphate ester on monoamine contents (μg/g tissue) of the septum

Group	0 min	10 min	20 min	60 min
DA				
1. Control	3.15±0.23	2.51±0 26	3.20±0.38	3.15±0.30
2. CCK-8-SE 80 fmole		2.27±0.32	2.07±0.30	2.02±0.29[a]
3. CCK-8-SE 800 fmole		1.67±0.25[a]	1.58±0.20[a]	1.93±0.15[c]
4. CCK-8-SE 80 pmole		3.36±0.19	3.58±0.32	3.81±0.24
5. CCK-8-SE 8 nmole		3.68±0.35[a]	3.66±0.45	3.69±0.51
NE				
1. Control	1.09±0.10	0.74±0.08	0.96±0.10	0.66±0.07
2. CCK-8-SE 80 fmole		0.48±0.05[b]	0.41±0.06[a]	0.46±0.03[a]
3. CCK-8-SE 800 fmole		1.06±0.11[b]	0.82±0.08	0.83±0.06[b]
4. CCK-8-SE 80 pmole		0.93±0.10	0.86±0.10	0.84±0.05[a]
5. CCK-8-SE 8 nmole		0.84±0.13	0.90±0.16	0.96±0.14[a]
5-HT				
1. Control	1.61±0.12	1.25±0.07	1.38±0.08	1.30±0.06
2. CCK-8-SE 80 fmole		1.30±0.12	1.11±0.09	1.34±0.13
3. CCK-8-SE 800 fmole		1.47±0.18	1.23±0.15	1.29±0.10
4. CCK-8-SE 80 pmole		1.49±0.24	1.49±0.13	1.43±0.17
5. CCK-8-SE 8 nmole		1.21±0.11	1.08±0.07	1.17±0.13

Abbreviations: a = $p<0.05$; b = $p<0.01$; c = $p<0.001$ versus control

80 pmole at 60 min. Eighty fmole decreased it in all time intervals while after 8 nmole at 60 min. The 5-HT content decreased with all doses at 60 min. At 20 min the 8 nmole and at 10 min the 80 pmole dose decreased it.

Some representative data of the dose-response action is shown on Figs 1, 2 and 3.

The action of CCK-8-SE on hypothalamic DA content showed a linear dose–response effect between 80 fmole and 80 pmole, by increasing the DA content at 20 min (Fig. 1).

CCK-8-SE increased the NE in the hypothalamus in a dose dependent manner between 80 fmole and 8 nmole at 60 min (Fig. 2).

The 5-HT content showed a dose–response linear decrease between 80 fmole and 8 nmole at 60 min (Fig. 3).

Table V

Effects of different doses of cholecystokinin octapeptide sulphate ester on monoamine contents (μg/g tissue) of striatum

Group	0 min	10 min	20 min	60 min
DA				
1. Control	6.76±0.32	6.19±0.36	6.98±0.47	7.11±0.41
2. CCK-8-SE 80 fmole		4.39±0.37[a]	4.43±0.37[b]	5.12±0.37[a]
3. CCK-8-SE 800 fmole		5.50±0.35[a]	5.32±0.26[c]	5.77±0.34[a]
4. CCK-8-SE 80 pmole		2.83±0.20[c]	3.44±0.23[c]	3.40±0.16[c]
5. CCK-8-SE 8 nmole		5.55±0.42[c]	5.53±0.38[a]	6.86±0.45
NE				
1. Control	0.13±0.01	0.16±0.02	0.18±.03	0.17±0.03
2. CCK-8-SE 80 fmole		0.09±0.01[a]	0.11±0.01[a]	0.11±0.01[a]
3. CCK-8-SE 800 fmole		0.21±0.03[a]	0.21±0.03	0.20±0.03
4. CCK-8-SE 80 pmole		0.19±0.01	0.18±0.01	0.20±0.02[a]
5. CCK-8-SE 8 nmole		0.14±0.03	0.13±0.03	0.13±0.05[a]
5-HT				
1. Control	0.44±0.04	0.44±0.03	0.47±0.04	0.56±0.06
2. CCK-8-SE 80 fmole		0.39±0.02	0.43±0.03	0.42±0.02[a]
3. CCK-8-SE 800 fmole		0.43±0.06	0.46±0.07	0.41±0.04[b]
4. CCK-8-SE 80 pmole		0.30±0.02[a]	0.39±0.02	0.36±0.02[b]
5. CCK-8-SE 8 nmole		0.29±0.02	0.28±0.03[b]	0.29±0.03[c]

Abbreviations: a = $p<0.05$; b = $p<0.01$; c = $p<0.001$ versus control

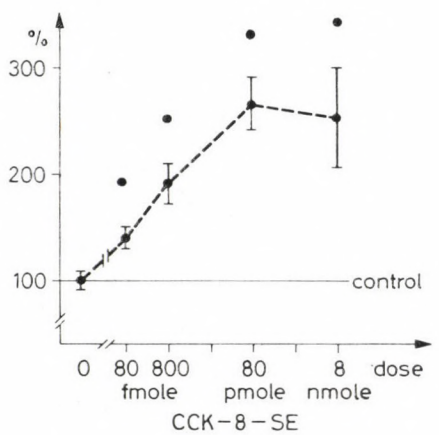

Fig. 1. Dose–response effect of CCK-8-SE on DA content of the hypothalamus at 20 min. Abbreviation: ● — significant difference between CCK-8-SE and control

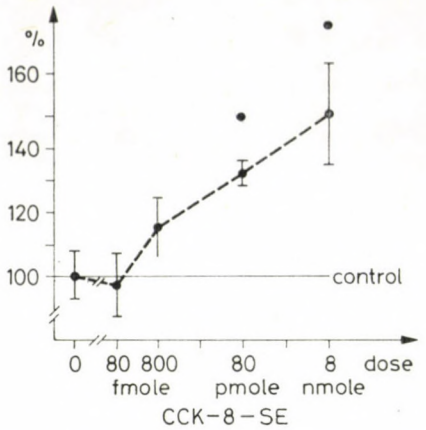

Fig. 2. Dose–response effect of CCK-8-SE on NE content of the hypothalamus at 60 min

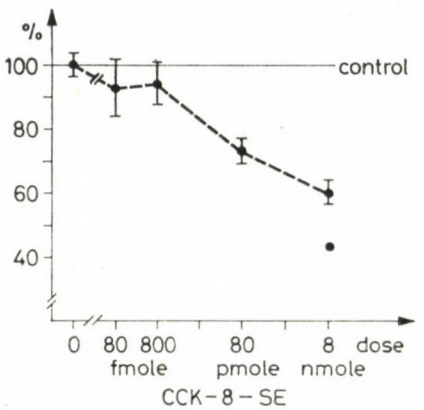

Fig. 3. Dose–responseeffect of CCK-8-SE on 5-HT content of the hypothalamus at 60 min

Discussion

The effect of CCK-8-SE on transmitters has been studied *in vitro* by Vizi et al. [28, 29, 30] who demonstrated acetylcholin release in the guinea pig Auerbach plexus. The effect of CCK-8-SE on brain transmitters (DA, NE and 5-HT) following intracerebroventricular administration was reported earlier (Telegdy et al. [25]) using 8 nmole (10 μg), a relatively high dose of CCK-8-SE.

In the present investigation smaller doses were used and the dose-response correlation was followed in order to study the physiological significance of the changes observed.

In general, on DA in the hypothalamus and mesencephalon an increasing, while in the septum and striatum a decreasing effect was observed following most of the doses used. One has to be aware of the fact that the intracerebroventricular administration of 0.9% saline in itself caused a decrease in the control, especially in the DA content in the hypothalamus, and all changes were correlated to these values. The smallest dose (80 fmole) affects all the brain areas studied. NE increased in all brain areas following 800 fmole, while 80 fmole decreased it in the amygdala, septum and striatum. The higher dose used (80 pmole) showed a similar action. The large dose decreased the 5-HT content in most of the brain areas studied, but in most of the cases the effect on this transmitter was less significant.

The dose response demonstrated in certain time intervals indicated a specific action on different transmitters. It seems that mainly the catecholamines had been affected. The action of CCK-8-SE on different transmitters was not only dose-dependent, but also showed a locus specificity.

One has to be aware of the fact that the measurement of the content of different transmitters in certain brain areas does not necessarily reflect the activity of a given transmitter system and therefore the data have to be complemented with turnover studies.

It seems that despite of the fact that the direct peptidergic transmission cannot be ruled out, the possibly modulatory role of CCK-8-SE on transmitters in the brain as specific physiological mechanism has also to be considered.

A number of reports indicated that CCK might be a physiological regulator of feeding behaviour in animals and man (GIBBS et al. [8], DELLA-FERA and BAILE [2], HSIAO et al. [12], PRANGE et al. [18]), although the mechanism of action is not clear. Our data offer a possible mechanism of the central action of CCK. Whether this action on transmitters is in correlation with the feeding behaviour remains to be seen.

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ENZYME INDUCER EFFECTS AFTER LIVER DENERVATION IN THE RAT

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The part of liver innervation was studied in the process of microsomal enzyme induction elicited by phenobarbital or muscular exercise. Enzyme induction was seen to develop: hexobarbital sleeping times became shorter after partial (vagotomy, coeliectomy) as well as total liver denervation in the rat. Though the present results did not preclude subtile differences, they demonstrated that the microsomal enzyme systems of the liver could be activated in the absence of innervation.

Upper median laparatomy, which was used as a sham operation, had the strange effect of elongating the sleeping time. This observation is a warning of a changed rate of drug elimination after explorative laparatomy.

In the last decade a number of compounds have been described to have an inducer effect upon the microsomal monooxygenase system of the liver [3, 4, 8, 9]. A similar phenomenon has been observed following regular muscular exercise in our previous experiments: the rate of drug metabolism in the liver was found to increase both in animal experiments and in human subjects [5, 6]. The phenomenon itself cannot be differentiated from the consequences of the (chemical) inducing effect of lipophilic xenobiotics and the mechanism by which it develops is also unclear.

The role of innervation in the process of enzyme induction was studied in the present work. The inducing effect of muscular is transmitted by endogenous routes and the part played by innervation may be different in the process elicited by drugs or by muscular work. Accordingly, the development of phenobarbital induction after liver denervation was studied first in rats. Next, animals subjected to liver denervation were studied to establish whether muscular work could effectively increase microsomal enzyme activity in this condition.

Material and methods

Female Wistar Wi: rg rats of 160 to 180 g body weight were used. Under superficial ether anaesthesia and after an upper median laparotomy three types of liver denervation were employed.

1. Vagotomy by transecting the nerve fibres immediately after their passage through the diaphragm.
2. Extirpation of the coeliac ganglion.
3. Total denervation: vagotomy and coeliectomy.

After sacrificing the animals every kind of denervation was thoroughly checked. The control animals were subjected to a sham operation in that after upper median laparotomy the abdominal viscera were explored and replaced.

The main part of the experiments began three weeks after denervation [2, 6]. To induce the microsomal enzyme system a single oral dose of phenobarbital 50 mg per kg b.w.) was given to 12 animals and 12 animals served as control. Enzyme induction was assessed by measuring the sleeping time [1, 3] after injecting 40 mg/kg of sodium hexobarbital. The daily exercise consisted of one hour of swimming with 4 g/100 g additional weight in water of 29 °C temperature. Results were compared with Student's t test.

Results

In the first series the effect of phenobarbital induction was studied following different types of liver denervation (Fig. 1). Owing to the inducing action, hexobarbital sleeping time became significantly shorter in every test group ($p<0.01$). Sleeping time in the non induced denervation group was not different from the control value — thus, the duration of hexobarbital sleep was not affected by any type of denervation. Sham operation, on the other hand, was associated by a very significant elongation of sleeping time ($p<0.01$). The inducing effect itself was not modified by the sham operation.

In view of these results, the sham operation was investigated in a separate experimental series. Twenty-one days after the sham operation and in weekly intervals thereafter, hexobarbital sleeping time was measured in groups of 12 animals each was consistently found to be prolonged. In the sixth post-operative week the sleeping time was similar as that of normal rats (Fig. 2).

In the third series, the different groups began swimming three weeks after denervation. Sleeping times were compared to the respective controls

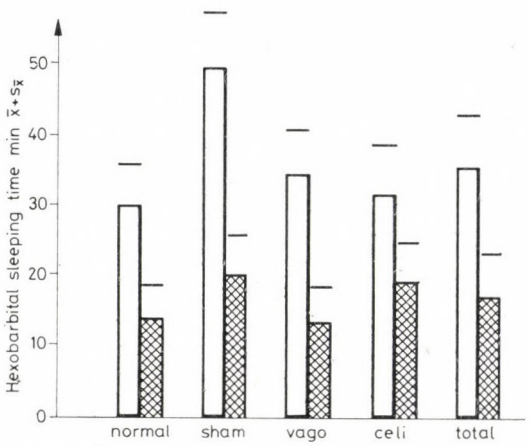

Fig. 1. Phenobarbital inducing effect after partial and total liver denervation in the rat as measured by hexobarbital sleeping time. Open bars: sleeping time in control animals; shaded bars: sleeping time one day after 50 mg/kg of phenobarbital treatment

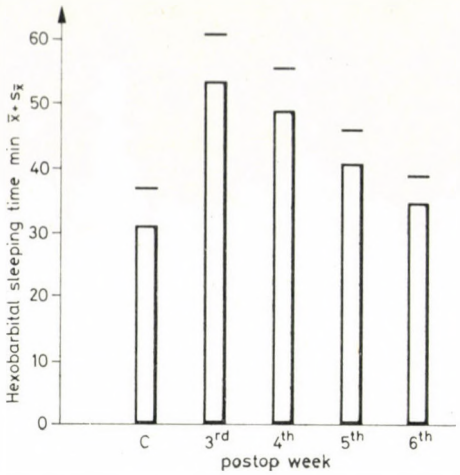

Fig. 2. Effect of sham operation on hexobarbital sleeping time

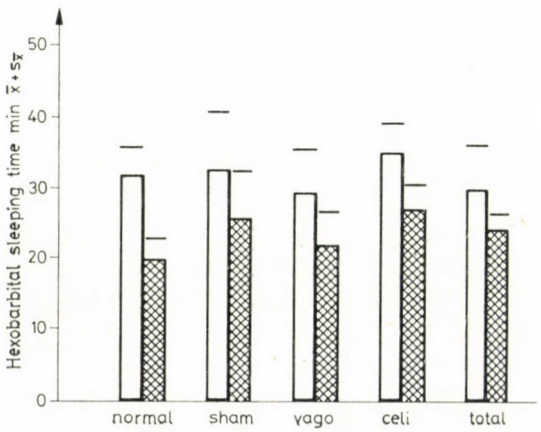

Fig. 3. Inducing effect of regular swimming exercise after partial and total liver denervation in the rat, as measured by hexobarbital sleeping time. Open bars: sleeping time in control animals; shaded bars: sleeping time in animals swimming regularly

highly significant in the normal, the sham-operated and the vagotomized groups ($p<0.01$) and significant in the coeliectomized and total denervation groups ($p<0.05$).

Discussion

The role of liver innervation in enzyme induction was studied after phenobarbital and exercise. It was found that neither partial nor total denervation brought about any appreciable change in the inducing effect of phenobarbital.

Denervation failed to effect the inducing action of exercise. The results do not however exclude the possibility that partial or total liver denervation may have some influence on the efficiency of inducing agents, but the present experiments could not bring to light subtile differences. Nevertheless, they were helpful in clarifying that the inducing effect of phenobarbital and regular physical exercise persisted in spite of liver denervation.

The inducing effect of muscular exercise was weaker than that of phenobarbital in both the intact and the denervated animals.

The protracted sleeping times after sham operations presented an unexpected problem. This effect was long-lasting as it took six weeks before the sleeping time had become normal again. Still, the effect was blocked by partial as well as total denervation, so the nervous system must have some part in it. This observation is a warning to consider the possibility of a changed rate of drug elimination following explorative laparotomies.

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GENERALIZED EPILEPSY WITH SPIKE-WAVE PAROXYSMS* AS AN EPILEPTIC DISORDER OF THE FUNCTION OF SLEEP PROMOTION

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A new hypothesis is offered regarding the pathomechanism of generalized epilepsy with spike-wave paroxysms (GESw) based on the pertaining literature and personal investigations.

The first section is devoted to a critical overview of the development of theories regarding GESw. The centrencephalic theory, the debate on subcortical versus cortical origin, the "corticoreticular" hypothesis of Gloor and, finally, the "dyshormic" concept of Niedermeyer are outlined.

In the next section it is shown that there is a particular optimum zone between sleep and wakefulness and between REM and slow wave sleep which highly favours the occurrence of spike-wave paroxysms. According to our investigations into the dynamics within this critical zone, the spike-wave paroxysms always appear with characteristic fluctuations of the level of consciousness where the changes towards awakening are always followed by rebounds towards sleep. Hence, the dynamic properties of this unstable border zone become especially interesting in the genesis of spikewave paroxysms.

It has been shown that even without epilepsy, a dynamics can be observed in the micro-oscillations in the depth of sleep which could be interpreted according to the reciprocal induction regulation model.

In our concept the process of falling asleep emerges from rebounds of the sleep promoting system in response to sensory inputs streaming in from the external environment. According to this model, arousal influences in sleep have a sleep promoting effect. We interpret in this way all synchronized EEG reactions elicited by sensory stimuli and we consider K-complex type synchronization reactions as a "building stone" of the process of falling asleep which contains the whole process in concentrated form.

The manifold similarities between the K-complex and the spike-wave pattern are demonstrated. On this basis spike-wave paroxysms can be regarded as an epileptic "caricature" of the sleep induction momentum reflected in the K-complex phenomenon. Hence, the GESw is the epileptic disorder of the sleep promotion function. This hypothesis resolves and explains many contradictory features of our knowledge about this mechanism and gives a new biologically oriented framework for further research. In the light of the hypothesis it has been attempted to interpret some of the characteristic features of the GESw: the genetic determination, the age dependency, the link with the sleep-waking cycle as well as the functional-anatomical characteristics and the symptoms of the seizures.

* Synonyms are: centrencephalic epilepsy of Penfield and Jasper; cortico-reticular epilepsy of Gloor; common generalized epilepsy of Niedermeyer.

1. Development of views regarding generalized epilepsy with spike-wave paroxysms (GESw)

Right from the beginning there has been a separate group of epilepsies which could not be adequately interpreted according to Jackson's focal-diagnostic, pathoanatomical principles. There are serious difficulties in defining topographically the brain focus of the epileptic malfunction in this particular epileptic mechanism. No phenomena in the clinical seizure symptoms indicate local brain malfunction; the EEG shows synchronous bilateral generalized phenomena in interictal periods and during seizures, generally in the special form of spike-wave pattern; interictal deficit symptoms indicating local brain damage responsible for the epileptic mechanism are generally absent, the illness is genetically determined, and the manifestations of the electroclinical symptoms are age dependent.

The concept of a midline pacemaker was supported by the date accumulated from 1940, since in several experiments the generalized cortical spike-wave pattern proved to be elicitable by electrical or chemical stimulation of certain parts of the thalamus [79, 58, 53, 5, 38, 114] and this effect was connected with behavioural signs similar to the human absence or myoclonic absence phenomena [52]. With the investigation of DEMPSEY and MORRISON in 1942 [23] the exploration of the thalamic non-specific system began. It came to light that thalamic nuclei generating a spike-wave response to stimulation belonged to this system.

These experimental findings served as basis for the Penfield and Jasper hypothesis according to which the regulating structures located in the diencephalic midline subcortical area in contact with both hemispheres, the "centrecephalon", is responsible for the basically generalized petit mal and grand mal seizures which do not represent any lateralized phenomena. According to this model, the GESw is determined by an epileptic focus in the midline structures by which the cortex is driven only passively.

Parallel with the "centrencephalic" concept, an other school sought the substrate of the mechanism not in the subcortical structures but in the cortex itself. The cortical origin was supported by hemispheral differences in the spike-wave pattern and focal features in clinical symptoms or even by local neuropathological findings in some of the operated or necropsied patients [20, 30, 60, 83, 102, 103, 106, 129]. Further evidence in favour of the cortical origin was supplied by human stereoelectroencephalographic investigations in which cortical trigger foci could frequently be explored in patients with GESw [6, 65, 90, 117].

Seeking a connection between the conflicting evidences of cortical and subcortical origin, Jasper et al. in the fifthies elaborated the secondary bilateral synchronization theory as an auxiliary hypothesis for the centren-

cephalic concept of GESw [56, 110, 131]. According to this view irregular asymmetrical forms of spike-wave synchronization showing focal features can be observed when the midline pacemaker is driven by a cortical focus (secondary synchronization), while in the symmetric forms without focal features the cortical spike-wave pattern is primarily of thalamic midline origin. The cortical trigger zone for the mechanism of secondary synchronization was supposed to be in the medial frontal region [7, 51, 78, 89, 135]. This view was supported later by human stereoelectroencephalic findings [1, 6, 9, 91]. Later several cortical areas became candidates for the trigger zone of the midline system [4, 8, 10, 29, 35, 85].

In the light of these findings it became more and more unlikely that an epileptic functional disorder of a certain, always identical, area may be sufficient for determining the whole mechanism. In the meantime there occurred important changes in views in both neurophysiology and neurology. The local diagnostic pathoanatomical approach gave way to concepts based on functional anatomy and system regulation. Knowledge concerning the function of the thalamic non-specific system increased considerably [2] and it was shown that the system contained closely cooperating thalamic and cortical elements. Thus, the debate between adherents of either cortical or thalamic origin became meaningless.

Animal experiments and human investigations provided further evidence of the cortical genesis of the spike-wave pattern and petit mal like seizures even without participation of mesodiencephalic structures [31, 32, 33, 34, 86, 87, 88]. On the other hand, the elicitability of the clinical and electrical symptoms of GESw by stimulation of the thalamic non-specific nuclei gained further support [34, 113, 114,]. In other words, the mechanism can be triggered from either the thalamic or the cortical pole of the non-specific thalamocortical system.

The above described changes in the views about the GESw mechanism are reflected by the "corticoreticular theory" of Gloor, published in the late sixties [31]. His concept determines the GESw as an abnormal interaction between the cortical and subcortical reticular structures.

A further important development was the discovery that not the excitation but the functional inactivation of certain central structures played a decisive role in the mechanism.

From the early 1960s, several authors concluded that the damage of the mesodiencephalic structures does not defend against but, on the contrary, facilitates the development of the spike-wave synchronization [86, 87, 94, 112, 134].

In the beginning of the 1970s, Gloor et al. could evoke spike-wave synchronization by intracarotideally administered pentetrazole without influence on the midline structures, in patients suffering from GESw, while pentetrazole

introduced into the vertebrobasilar circulation and thus exciting just the centrencephalic structures, decreased rather than enhanced the appearance of spike-wave paroxysms [33]. Similarly in our own investigations spike-wave synchronization could be elicited by some mg of intracarotidally administered hexobarbital in GESw patients, while higher doses abruptly eliminated [39]. The activation effect was interpreted as a differential inhibition of the reticulo-cortical synaptic transmission by small doses, while higher doses were possibly able to inhibit all cortical synaptic sites. So beside proving the "centrencephalic" or "centrenergic" [99] component, the existence of another "centrasthenic" component playing a role in GESw had been verified.

The holistic approach to the GESw was difficult because a suitable animal model was missing and therefore the different ablation and stimulation experiments could clarify the mechanism only from one or another particular aspect. Hence instead of the whole complex mechanism, only some of the elements, and mostly the anatomical ones were recognized. The discovery of generalized penicillin epilepsy in 1967 [116], which may bear the closes resemblance to its human counterpart, regarding both the electrographic and behavioural features, gave further impetus to the study of the GESw.

Experiments performed on this model again verified that the epileptic excitation of the thalamic non-specific system and at the same time a decrease in the activation of the arousal system are the two complementary factors which play the determining role in the development of GESw [34, 129]. Work on the same model then showed that the microneurophysiological basis of the cortical spike-wave pattern is the epileptic functional disorder of the mechanism playing a role in the production of recruitment and cortical spindles under normal circumstances.

On the bases of the above data, Gloor characterised the GESw to be determined by changes on three levels of the neuraxis. The basic condition for development of the mechanism is a relatively mild diffuse "epileptic state" affecting the neurones over wide areas of the cortex. The second factor is a depression of the activity of the upper brain stem arousal system. Where these two conditions exist, those thalamocortical impulses which under normal circumstances play a part in producing the cortical spindling, became potent trigger stimuli for precipitating the electroclinical symptoms.

Thus, the local diagnostic pathoanatomical views were gradually substituted by a functional approach. The merely structural approach became outworn and beside the question "where" emerged that of "how", or in other words, "which functional disorder is basically responsible for GESw" Nowadays the "local anatomic" and "functional" views can be compromised.

Those brain stem structures the functional inactivation of which are the important predisposing factor of thr GESw manifestation can be identified with the arousal system. On the other hand those thalamocortical structures

which are involved in the GESw mechanism in normal circumstances play a role in the organization of slow wave sleep.

At the 1953 symposium on "Brain mechanisms and consciousness" Jasper and Hess discussed the point that the site of successful stimulations in the thalamus and surrounding areas which produced the spike-wave pattern largely coincides with the diencephalic "sleep centre" identified by HESS [49]. Other studies produced further evidence of the relationship between the structures responsible for the electroclinical phenomena of spike-wave epilepsy and for the organization of slow-wave sleep. VILLABLANCA et al. [133] showed that the localized forebrain lesion which blocks the recruiting response in the region of the inferior thalamic peduncle (ITP) [123] is able to block the cortical spike-wave produced by chlorambucil. Damage to the same region produced insomnia and weakening of the EEG phenomena accompanying slow-wave sleep [62, 84, 92]. Similarly, FEENEY and GULLOTTA [25] found that destruction of the rostral portion of the thalamus of the cat inhibited the generalized epileptical discharges elicited by pentetrazole and the sleep spindles.

These experiments support the view that the cortical structures responsible for the electrical phenomena of spike-wave epilepsy may be precisely those which are "used" by the hypnogenic subcortical (thalamic and basal forebrain) structures.

Thinking either in terms of anatomical structures or in terms of functional systems, the functional factors playing a role in the GESw mechanism offer themselves within the sleep-waking system.

This idea led NIEDERMEYER to establish his concept of dyshormia [101]. Since he found that the spike-wave paroxysms frequently appear during sleep in response to arousal stimuli and very often in connection with the K-complexes which are interpreted as abortive arousal responses, he assumed that the GESw can be attributed to an error in the arousal mechanism. This notion somewhat recalls the "awakening epilepsy" concept of the German epileptology school [55] and at the same time *represents an extremely important new biologically oriented current approach.* It revived the general biological principle expressed by Speransky (quoted in MYSLOBODSKY [99]) as: "sickness is the heightening, weakening or change of normal functioning." Interpretation of the epileptic disorder as being some form of pathological change, a "caricature" of physiological brain function is readily compatible with the classical traditions of the Jacksonian approach to epilepsy. In the Niedermeyer approach, the GESw could thus be said to gain a biological meaning as it is linked to the arousal function and its disorder.

Due to the reciprocally antagonistic organization of the sleep-arousal system, a disorder of the arousal functions at the same time also brings the sleep promoting (hypnogenic) function to the fore and this is in line with the

above cited data which indicate a common substrate for the hypnogenic function and the spike-wave epileptic manifestations.

MYSLOBODSKY [99] was also guided by similar biological considerations when in seeking the precursors of spike-wave epilepsy in normal brain functions. He showed that the spike-wave discharge may emerge from the particular cerebral rhythm of the secondary evoked potential, and that conditions facilitating this metamorphosis may be created by lesioning the structures of the limbic-reticular system in mature animals or by irradiating them during a definite period of embriogeny. He reached the conclusion that the spike-wave pattern is not "centrenergetic" in nature but "centrasthenic": any inhibiting-synchronizing hypnogenic sub-system can set off the spike-wave synchronization if cortical activity escapes from the control of the limbic-reticular disinhibiting system. Attention was drawn to the fact that the EEG phenomena of spike-wave epileptic patients in wakefullness and the characteristics of their evoked potentials strikingly resemble those of sleeping normal subjects. The phenomenon was interpreted by Myslobodsky as a fragment of sleep or a partial sleep of the cortex. Together with Speransky he suggested that in petit mal epilepsy "the basic features of neural mechanisms of sleep and the epileptic fit are similar".

In the light of the above described facts the investigations concerning the connection between the GESw and the level of consciousness have became especially important.

2. Changes in the level of vigilance and generalized epilepsy with spike-wave paroxysms

Since the discovery of the generalized spike-wave pattern it has repeatedly been shown that the sleep-weaking cycle is an important determining factor in the appearance of the electroclinical symptoms of the GESw. As early as 1936, LENNOX and GIBBS [77] reported that spike-wave paroxysms occurred in light sleep and in the course of awakening even in patients in whom such formations could not be detected in the awake state, and they assumed that certain phases of sleep or the transition from one sleep phase to another could have an activating effect in certain seizure mechanisms. Modern polygraphic night sleep studies have confirmed that petit mal seizures similar to those in the wake state occurred almost exclusively on the borderline between sleep and wakefulness.

It has been shown that seizures occur in the brief period of falling asleep in the evening, in light sleep around the momentary awakenings during night, corresponding to a diminution of the level of vigilance in the course of transitional awakenings during the night just dropping down towards sleep, and also in intermediary sleep between REM and slow-wave sleep and follow-

ing awakening in the morning while still "drowsy" [44, 107, 122, 125, 130]. All this appears to indicate that the level of slightly reduced vigilance evokes the seizures. It was found that this factor can also be traced in the appearance of daytime seizures. Studies conducted with telemetry or by following certain psychophysiological parameters indicate that the daytime seizures occur or are concentrated precisely around stages marked by a slight transitional drop in the level of vigilance [14]. These are the periods of "sleepiness" following awakening, after lunch or in the evening. In the same way, monotonous, boring, wearsome activity demonstrably favours the occurrence of daytime seizures [95, 125, 132]. The highly activating effect of sleep deprivation in this form of epilepsy can be interpreted also in this sense [55], since an increase in "sleep pressure" must be taken into account during such periods.

The counter-check of the phenomenon has also proved to be true. Since 1936, descriptions can be found in the literature of how the arousing stimuli and the focussing of attention inhibit the spike-wave pattern and petit mal seizures [64, 68, 80]. Different attentive factors (sensory arousing stimuli) inhibited the seizures in the GESw models produced experimentally in animals and direct electrical stimulation of the RAS had a similar effect [37, 114]. In harmony with this, the temporary or lasting suspension of the reticular arousal system functions promotes the appearance of seizures [129]. (These studies have been described in detail in the previous chapter.)

It would thus appear that the seizures are evoked by a slight reduction of the level of vigilance. All influences which lower the tone of the arousal system and contribute to predominance of the sleep promoting system have an effect evoking or producing seizures and, on the other hand, those which improve the tone of the arousal system have an inhibiting effect. Further reduction in the level of vigilance in the course of the process of falling asleep again creates an unfavourable situation for the appearance of seizures. It would thus appear that there exists an optimum zone of superficially reduced vigilance which favours the appearance of seizures, and states of "extreme waking" and "deeper sleep" in comparison to this are unfavourable.

In the following sections we shall examine the characteristics of this zone favouring and evoking seizures and whether this is merely an incidental factor in the seizure mechanism or whether it is related in some way to the actual induction mechanism of the seizures.

There are many arguments in favour of the view that the decisive factor in inducing the seizure in this "critical zone" is an actual reduction of the level of vigilance that gives scope to the functions which probably simultaneously favour both falling asleep and the GESw mechanism. We have seen that really numerous identical anatomical and functional features can be discovered in the structures and functions producing sleep and the GESw seizure mechanism. It is, however, difficult to include within this concept those findings which

show that within the critical zone not only changes in the state towards sleep evoke seizures, but also "arousal" influences and spontaneous arousals which bring about a rise in the level of vigilance [41, 42, 50, 100, 121, 122].

It is on the basis of these observations that the German epileptology school defines the GESw mechanisms as "awakening epilepsy" [55]. NIEDERMEYER [101] too approached the question from this point of view, stressing that the GESw seizures appear to be linked to the K-complex, a phenomenon interpreted as abortive arousal in light sleep. He considers that the "common generalized epilepsy" (which is identical with GESw) is linked to some fault of the arousal mechanism termed by him as "dyshormia".

In view of this it is questionable whether the effect of the optimum zone lies simply in a slight reduction of the level of vigilance. It would appear that changes in the critical zone in the direction of both sleep and awakening promote the appearance of seizures. It follows from this that we must consider whether the decisive factor is not precisely some form of dynamic momentum, a change in the level of vigilance in one direction or another. In this case, the influence of the cortical zone lies precisely in that because of its transitional nature there is also a possibility for changes in the level of vigilance. Sleep-wakefulness and the REM-NREM borderline zone are an unstable intermediary state where it is only after numerous fluctuations of the level of vigilance that the process swings towards more superficial or deeper levels of vigilance.

Numerous observations indicate that there may be fine fluctuations of the level of vigilance in the region of spontaneous petit mal seizures occurring in the awake state. The observation of LEHMAN [75] and MELNITCHUK [93], for example, who reported that brief desynchronization of arousal reaction type can be observed in the background activity before petit mal seizures, can also be interpreted in this way as also the orientation behaviour before spike-wave seizures [54]. It has been shown that petit mal seizures are preceded by negative direct current shifts [16, 19] which are generally observed at the time of rises in the level of vigilance [15]. Another observation pointing to fluctuations of the level of vigilance accompanying petit mal seizures is that EDG discharges can always be observed after paroxysms occurring in a state of dozing [41], which indicates that there has been a rise in the level of vigilance. Longer stages of waking with successive seizures are frequent during night sleep of petit mal patients. In these stages too it can be seen that the paroxysms occurring while dozing awake, and then as soon as the sleeper dozes off again a further seizure with awakening follows, thus giving rise to a series of seizures. At the same time these events prolong the state of transitional waking and maintain continuous fine fluctuations between the state of waking and dozing [44].

3. Dynamic characteristics of the critical level of vigilance favouring generalized epilepsy with spike-wave paroxysms

3.1. The seizure activating effect of dozing induced by hexobarbital in the GESw mechanism

It has been known since the early 1950s that the electrical symptoms of different epileptic mechanisms can be activated by a slow intravenous administration of hexobarbital [25]. Following earlier studies by KAJTOR [68], we reported in 1971 [40], that induction of sleep by intravenous hexobarbital is a suitable method for activating the clinical and electrical phenomena of petit mal seizures in the GESw mechanism.

We found that after the patient has fallen asleep and administration of the hypnotic agent has been completed, it is principally the period of awakening that favours the appearance of seizure phenomena. In the course of a study of 72 patients suffering from generalized epilepsy with spike-wave paroxysms we found that during awakening from hexobarbital sleep we could evoke seizures practically at will by influencing the level of vigilance. Using polygraphy we were able to observe that the electrical spike-wave paroxysms are accompanied by somatic (e.g. rhythmic jerks of the eyelids) and vegetative phenomena (e.g. apnoea and changes in cardiac rhythm) corresponding to petit mal seizures in the awake state.

The hexobarbital was administered according to Kajtor's method at the rate of 20 mg/10 sec until the stage of deep sleep was reached (a total dose of 0.5—1.0 g). We then terminated the dosage and gradually awakened the patients by applying different sensory stimuli. In the period of awakening we observed the ability of orientation and to give verbal replies and with the aid of arithmetical problems and recording polygraphically the effect of sensory stimuli we checked continuously the level of vigilance. We used sensory stimuli, simultaneously observing the EEG and somatic reactions, in a similar way in the stages of falling asleep, too.

During the process of falling asleep the seizures occurred even before the first 0.1 g of of hexobarbital had been administered, in most cases after 0.15 g and always before the dose of 0.3 g had been reached. At this time the patients were generally still awake and their verbal contact was adequate. Most paroxysms occurred in the stage following beta synchronization, as the slow waves began to predominate. As sleep deepened, spike-wave discharges in series accompanied by behavioural seizure phenomena no longer occurred. At the same time, however, electrical activation of different other spike-wave patterns appeared. During awakening, the appearance of seizures went parallel with the appearance of those stages of sleep in which the slow wave activation had already ended, while at the same time the arousal stimuli produced

a response characterized by a synchronization of electrical activity, the appearance of slow waves of greater amplitude (Fig. 1). At this stage the verbal contact was reestablished, spontaneous movements and electrodermographic discharges of high amplitude appeared.

The sensorial stimuli either evoked or inhibited the seizures, depending on the actual level of vigilance. If the patient was close to full awakening and

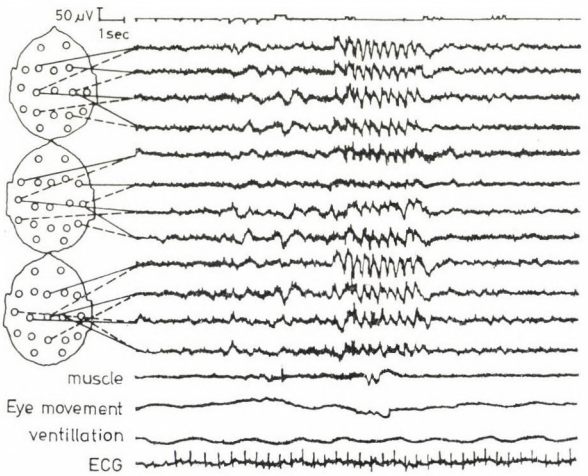

Fig. 1. Z. K. 12 years old girl. Spike-wave paroxysm after synchronization type arousal reaction evoked by nociceptive stimuli in awakening from hexobarbital sleep

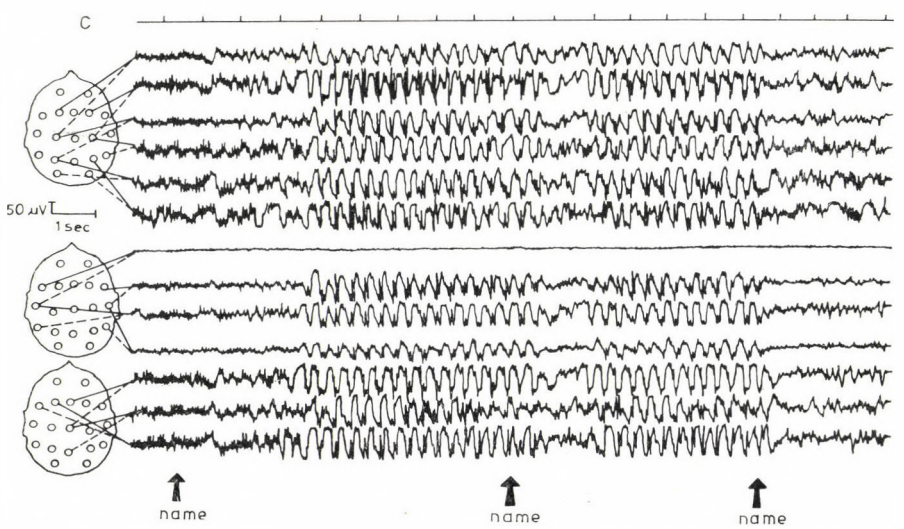

Fig. 2. Awakening from hexobarbital sleep. In the critical level of vigilance, calling by name first evokes a synchronization type arousal reaction and subsequently a seizure appears; later, the same stimulus inhibits the spike-wave paroxysm with a desynchronization reaction

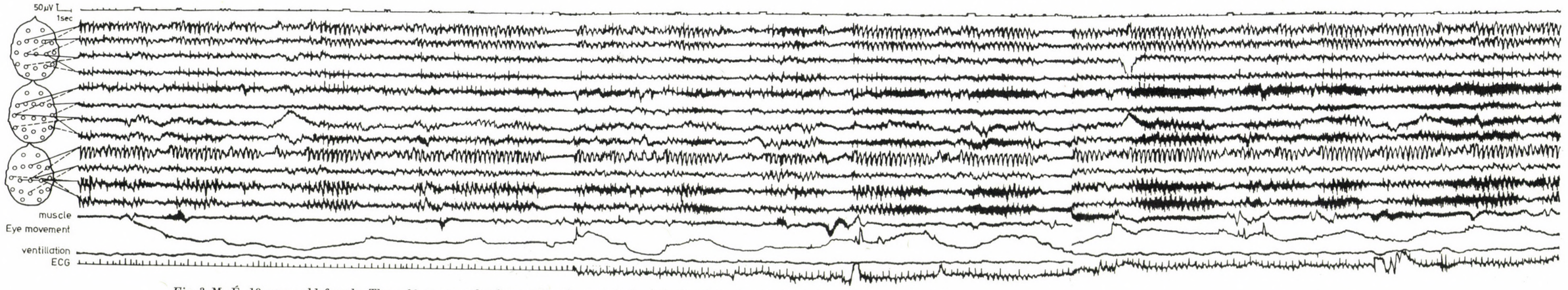

Fig. 3. M. É. 19 years old female. Three 20 sec samples from a 14 min period of awakening from hexobarbital sleep. Vigilance dependent activation of spike-wave paroxysms synchronously with clinical absences

the EEG desynchronization reaction had already reappeared in response to arousal stimuli, the stimulus did not evoke a seizure and even inhibited paroxysms already present. In contrast, while the subjects were still in slightly less awake when the sensorial stimuli produced an EEG synchronization reaction (drop in frequency and rise in amplitude), seizures very frequently followed the stimuli. We were able to observe in a number of cases that a seizure evoked in this way by a particular sensorial stimulus could, on the contrary, be inhibited by subsequent application of the same stimulus (Fig. 2). Thus, in the course of awakening it was always possible to identify a favourable zone where seizures appeared, whether it was approached from the direction of sleep or from that of wakefulness. If we did not rapidly awaken the patient but allowed him to sleep back repeatedly into a state of dozing, we were able to record as many as 30—40 seizures up to the point of complete arousal. In other words, when "floating" in the critical zone of slightly decreased consciousness, practically a state corresponding to status epilepticus (petit mal status) could be maintained (Fig. 3). The extent of seizure activation that could be induced with hexobarbital corresponded to the frequency of the patient's spontaneous seizures.

3.2. Dynamics of the appearance of petit mal paroxysms on the borderline between REM and slow wave sleep

Changes in the level of vigilance were studied in REM and in the intermediary sleep stages preceding and following them, and also the location of seizures occurring in this zone in 11 GESw epileptic patients over 12 nights and in 3 healthy subjects over 5 nights [44]. The stages studied were broken down into four domains which we considered to represent different levels of vigilance. These were: rapid sleep with eye movements, rapid sleep without eye movements, an intermediary stage of sleep with changes tending towards awakening (the appearance of alpha spindles) and an intermediary stage with changes tending towards sleep (sigma spindles and/or K-complexes). We scored the records at 20 sec intervals according to the four levels identified and in this way obtained microhypnograms with one sec resolution for each intermediary-REM-intermediary stage. We analysed a total of 50 intermediary-REM-intermediary stages of the patients and 23 such stages of the healthy subjects. The patients selected for the study were persons whose records showed seizure phenomena far more frequently than usual (Fig. 4).

The greatest proportion of seizures appeared at the level of intermediary sleep descending towards slow wave sleep and most particularly in the intermediary stages following REM (i.e. tending downwards). Here too, they were more frequent in the second than in tht first half of the night. Seizures appeared the least frequently in the stage of rapid sleep accompanied by rapid eye move-

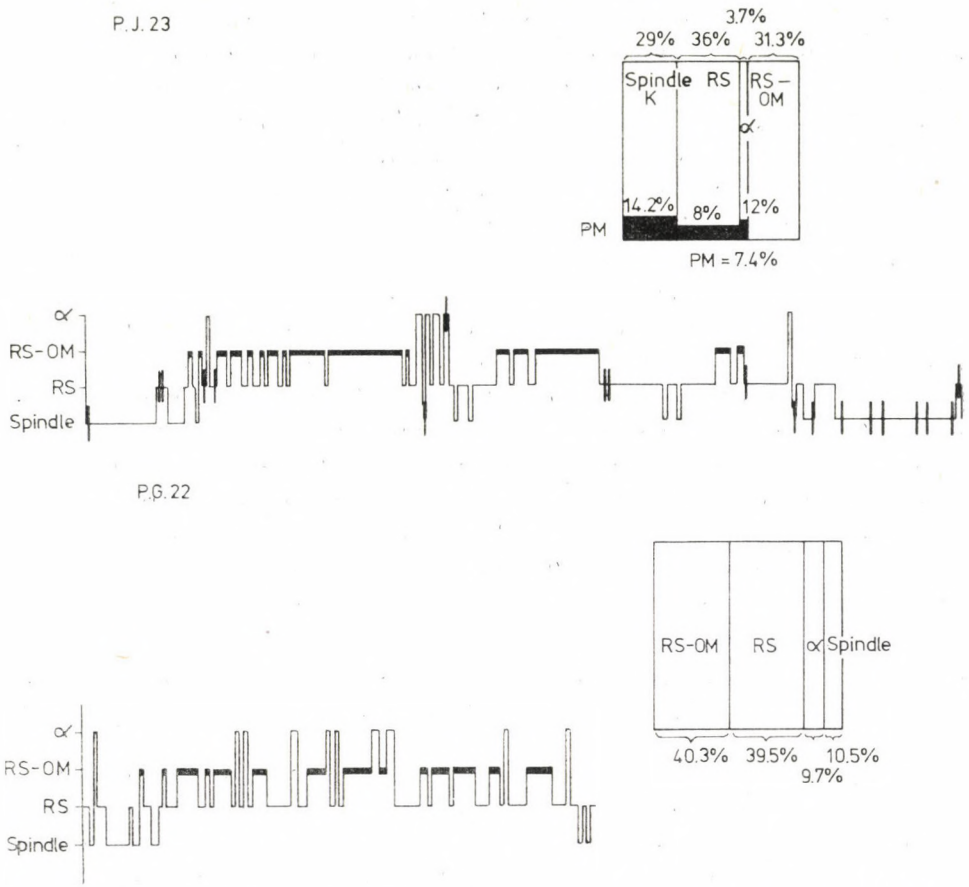

Fig. 4. Typical intermediary — REM — intermediary sleep periods in a control subject (below) and in a patient with GESw mechanism (above). α = intermediary stage of sleep with changes tending toward awakening (α spindles); RS-OM = rapid sleep with ocular movements; RS 2 rapid sleep without ocular movements; spindle = intermediary stage with changes tending toward sleep (sigma spindles). Each vertical heavy line represents a spike-wave paroxysm. PM = percent of those 20 sec which contain spike-wave seizures

ments (they practically never occurred here during eye movement clusters). Seizures were slightly more frequent in the stage of rapid sleep without eye movements and more frequent in the stage of intermediary sleep tending towards awakening but even here did not reach the values recorded in the intermediary stage tending towards slow wave sleep (Fig. 5).

There was a characteristic change in the structure of the intermediary-REM-intermediary stages in the patients in contrast with the healthy subjects. Whereas intermediary sleep before, after or during the REM stages represented an average of 5.5% of total sleep in the healthy subjects, it reached as much as 11.9% for the patients (Fig. 6). It was also characteristic that not only was

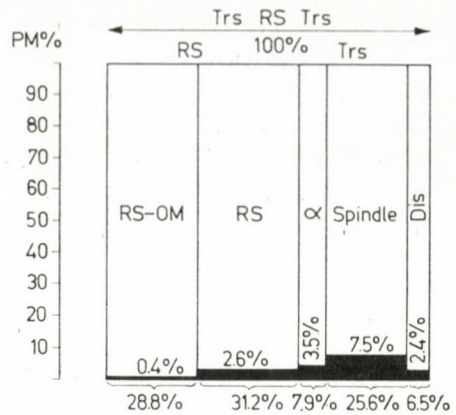

Fig. 5. Amount of spike-wave seizures at different levels of intermediary—REM—intermediary periods in 11 patients, distributed among 12 nights

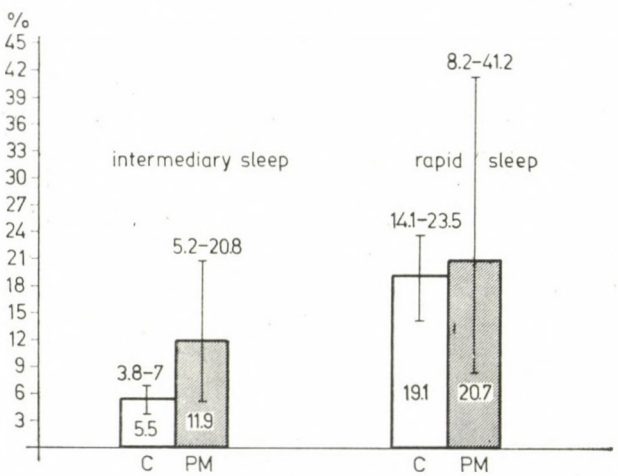

Fig. 6. Duration of intermediary and REM periods during 12 nights of 11 patients (PM) and 5 nights of 3 control subjects (C)

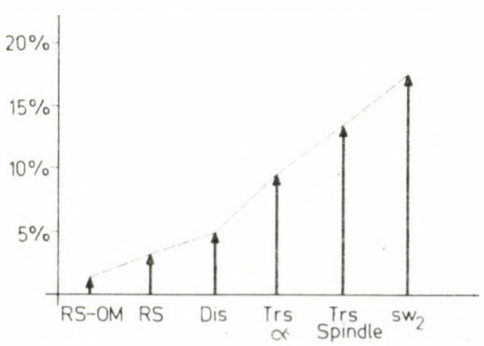

Fig. 7. Amount of spike-wave reactions to acoustic stimuli and body movements derived from the arousal effect evoked by 299 acoustic and 260 body movements (3 and 5 patients, respectively)

there an increase in the total amount of intermediary stages, but such stages occurred much more frequently within the periods of REM sleep. In other words, there appeared much greater and more irregular fluctuations of the level of vigilance in the patients than in the healthy subjects. There was therefore an increase in the patients in the proportion of precisely that stage of intermediary sleep which formed the "bed" of the seizures. The number of seizures appearing in the transitional stages was in proportion to the number of daytime seizures.

Abortive spike-wave series not accompanied by clinical seizure phenomena could be evoked by sensorial stimulation. This responsiveness was greatest in the superficial slow wave sleep stages preceding or following intermediary sleep, and declined with the reduction of the depth of sleep (Fig. 7).

3.3. Dynamics of seizures of generalized epilepsy with spike-wave paroxysms in the superficial stages of slow wave sleep

The level of vigilance was studied in four patients showing frequent seizures while dozing in the superficial stages of slow wave sleep (in stages 1 and superficial 2 according to the Rechtschaffen-Kales scale). These changes were studied for 110 spontaneous seizures and for 260 seizures evoked by sensory stimulation and in the region of 160 sensory stimuli not followed by seizures [72]. As in the previous study, changes in the level of vigilance were studied by the microhypnogram method, identifying more sublevels of stages 1 and 2 on the basis of electromorphological phenomena.

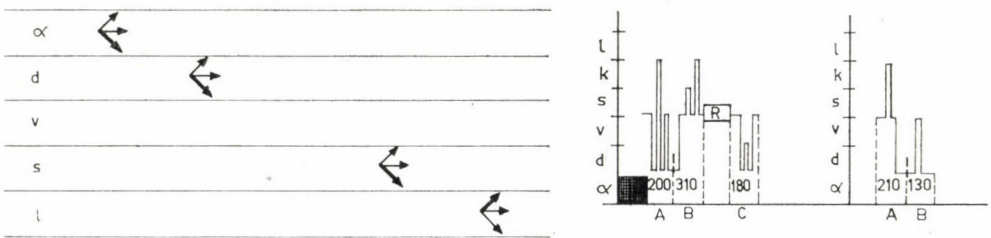

Fig. 8. Changes in the microhypnograms before and after stimuli A/B values in the case of seizures and without seizures, connected with stimulation at different levels of EEG activity. α = alpha spindles; d = flat without spindles; v = vertex spikes; s = sleep spindles; l = slow wave activity. R = stimuli followed by seizures; RN = stimuli not followed by seizures; nv = not suitable for statistical tests; ns = not significant; x = $p<0.001$. The insert right below shows the evaluation of the microhypnogram in the case of a successful (followed by a seizure (R) and an unsuccessful (RN, not followed by seizure) stimuli. On the vertical axis each layer characterised by an EEG phenomenon has 10 values. On the horizontal axis one value represents one sec. The numbers under the hypnograms are the territorial values expressed with the number of little squares under the relevant territories. Values higher than 1 determine fluctuations toward more superficial sleep or toward awakening while values below 1 determine tendencies toward deepening of sleep. Tendencies of fluctuation which are followed by seizures are underlined by heavy arrows in the insert left below

The sensory stimulus, a combined click and flash, was followed by a seizure if the stimulation had been followed by a parodoxical effect, a drop towards deeper sleep if the stimulation had reached the sleeper in the superficial layers and, turning towards awakening, if it had arrived in the deeper layers. The seizures, on the other hand, were always followed by a change acting towards awakening (a rise in the depth of sleep). Stimuli not followed by seizures, if the stimulation occurred in the superficial layers, resulted in no change or in a further rise in the sleep level and if the stimulation occurred in the deeper layers, it was followed by a further deepening or no change (Fig. 8).

We thus observed intensive fluctuations in the sleep level in the region of seizures. The sensory stimulation intensified these fluctuations and at the same time also increased the number of seizures.

3.4. Overall characteristics of the dynamics of fluctuations in the level of vigilance observed in the zone of vigilance critical for GESw seizures

It would appear from all three types of study described above that whether observed on the borderline between wakefulness and sleep or between REM and slow-wave sleep, the critical zone contains intensive fluctuations of the level of vigilance and these fluctuations promote the appearance of seizures. The fluctuations can be intensified by sensory stimulation and this again increases the frequency of seizures. A constant positive feedback effect develops between the appearance of seizures and the fluctuations of the level of consciousness favouring them and in this way the seizures can be said to perpetuate the transitional states.

It could be observed that the influence of both the sensory stimulation and the seizures on the dynamics of changes in the level of vigilance is such that each change, either towards sleep or awakening, is followed by a rebound in the opposite direction.

It therefore appears probable on the basis of the above observations that petit mal seizures appear on the unstable borderline between wakefulness and sleep and between REM and N-REM, and always appear with characteristic fluctuations of the level of vigilance where the changes towards awakening are always followed by rebounds towards sleep.

If we take into account the dynamic momentum analysed above which appears to be of decisive importance for the appearance of petit mal seizures, it is possible to resolve the contradiction that a reduction in the level of vigilance favours the appearance of seizures but despite this they frequently occur in response to arousal stimuli.

It follows from the above that in the induction of petit mal seizures we must attribute an important role to fluctuations in the level of vigilance

occurring at the borderline between sleep and wakefulness. We could thus hope that the study of dynamic characteristics of the fluctuations of the level of vigilance without epilepsy would throw some light on certain features which will be of value in the further understanding of the GESw pathomechanism.

4. *"Reciprocal induction" type regulation in the micro-oscillations of sleep*

Polygraphic night sleep record showed that there are constant fluctuations of the depth of sleep in all its phases. The smaller the scale on which this process is studied, the finer the fluctuations that can be detected. It would thus appear that the trends producing cyclical alternations in the depth of sleep (i.e. the deepening of sleep or its gradual change towards more superficial sleep, in other words on the descending or ascending limbs of the cycles) are asserted through "micro-oscillations" of the depth of sleep. It has been shown above that analysis of fluctuations of the depth of sleep around petit mal seizures suggested the action of a particular regulation principle. It appeared that a sudden change in any direction of sleep was followed by a correction in the opposite direction. All this raises the possibility that the fluctuations of the depth of sleep are determined by the balance of two forces acting in antagonistic directions, one towards arousal, the other towards sleep promotion.

The force behind the tendency acting in the direction towards sleep is obviously the sleep promoting system which, inhibiting the arousal system [11], sets off the process of falling asleep. Inhibition of the arousal system increasingly reinforces the sleep system previously counterbalanced by the RAS and in this way falling asleep could be explained by a self-reinforcing process [97]. But what explains the fact that at a certain point in deep sleep this process is reversed and a similar self-reinforcing awakening process takes place on the ascending limb of the sleep cycles. A similar problem is how to incorporate into the described "avalanche" like process of falling asleep the micro-oscillations which indicate the presence of trends acting towards arousal within the trend towards falling asleep. This kind of dynamics makes it likely that the arousal system probably plays a more active role in regulation than we had supposed previously and that it is not exclusively tonic influences but sudden abrupt phasic changes which determine the events in the regulation, that is, in the interaction of the sleep promoting and arousal systems.

4.1. The synchronization reaction

We thus sought phenomena in the sleep process which could be considered to represent sudden changes, particularly rises, in the depth of sleep and we studied the link between these phenomena and sudden rises in the depth of sleep in response to external stimuli. It became obvious that the

sleep process continually contains phasic phenomena which can be interpreted as sudden fluctuations of the depth of sleep, while at the same time all these phenomena can repeatedly be evoked by applying arousal stimuli, in exactly the same form as they appear in the spontaneous sleep process. These characteristically "Janus-faced" somnographic phenomena, partly spontaneous and partly evoked, are the vertex potential of dozing, the K-complex of superficial and medium deep sleep, the slow wave groups of deep sleep and the microarousals that can be observed throughout the whole of slow wave sleep and from the polygraphic point of view, the EDG discharge accompanying these phenomena can also belongs here. (In the same way, spontaneous phasic phenomena evoked by arousal stimuli, can be identified in the REM phase, too.) We considered these phasic phenomena in the course of the constant fine fluctuation of the sleep level to be "critical points", where the dynamics of fluctuations of the depth of sleep can be studied.

Let us now consider the effect of external, attention-raising stimuli acting through the arousal system, in the course of falling asleep. The non-specific arousal reaction, the "desynchronization response" of the waking state, changes in the course of falling asleep and characteristically assumes an opposed value, becoming "synchronizational" in nature. This begins with the stimuli recalling the alpha activity (paradoxical alpha reaction) in the course of light dozing, then in the most superficial stage of slow wave sleep vertex sharp potentials appear and subsequently in stages 2 and 3, K-complexes emerge. As the depth of sleep increases, the latter more and frequently become double and triple complexes and then take on the form of slow wave groups. Finally, as the slow waves gradually become predominant with the emergence of stage 4, the response cannot be followed since the reactive slow groups can no longer be distinguished from the background activity. (We shall see later that the responses can be detected even in this stage by using an averaging technique.) It would therefore appear that in the course of falling asleep and in all phases of slow wave sleep, the arousal stimuli produce responses of the same a morphology as that of the spontaneous phasic electrographic phenomena characteristic of that particular stage. It thus appears reasonable to assume that the "spontaneous" phasic electrographic phenomena also occur in response to phasic arousal influences which are constantly present, transmitted or physiologically produced by the RAS. In this case, however, we find the particular phenomenon that the arousal influence produces a reaction in the opposite direction, acting towards the deepening of sleep. We call this the "synchronization reaction".

Now we have reached for the second time the possibility that a regulation operates the fine fluctuations of sleep level in which sudden changes acting either toward arousal or towards falling asleep produce "rebounds" in the opposite direction. Regulation of this type is well known in the theory of bio-

logical systems and corresponds to the Selbach "reciprocal induction" regulation model [120].

According to this model, two mutually antagonistic half systems cooperate in such a way that a stimulus in one system causes a reciprocal induction (not inhibition) of the opposite halfcentre. Thus, stimulation of one half-system produces a rebound in the other half-system, again activating the first half-system, and so on. The "balance of forces" between the two half-systems regulates the trend and form of the oscillation produced in this way. Thus, in our case, the reciprocal inductional interaction of the sleep and arousal systems produces the micro-oscillations in sleep. (The balance of forces between the two systems is established by the cyclical changes influencing them for instance by the metabolism of biogenic amines.)

Application of the principle of reciprocal induction to the sleep process is much more readily compatible with the continous fluctuations of the sleep level and with the recurring phasic phenomena found in them, than is the reciprocal antagonism concept of MORUZZI [97] and BREMER [12]. In this way the reticular arousal system plays an important role in the establishment of sleep. Whereas under the antagonistic concept sleep occurs "despite" the reticular arousal system, according to our theory, it happens with the participation of the arousal system. If we accept that the sleep promotion and arousal system take part in the sleep process according to the principle of reciprocal induction, and that even the spontaneous phenomena of falling asleep are the products of rebounds arising as a result of phasic influences of the arousal system, then it becomes much easier to understand all the observations indicating that continuous sensorial stimulation has the effect of inducing sleep which had previously been difficult to explain [29, 67, 82, 96, 105, 115, 118, 127].

Assuming the reciprocal induction principle to be valid, the question may arise of the extent to which the actual "arousal effect" and that to which only the "rebound" can be followed in the electrographic phenomena in response to arousal stimuli. We would naturally expect to be able to follow both components. It seems, however, that this is not alway possible as it is probably determined by the state of balance between the two systems and the strength of the arousal influence. In a state where the sleep promoting system largely dominates the arousal system and the arousal stimulus is short and not too strong, we can observe only the rebound as for example, in the case of the K-complex. In a constellation where the sleep promoting and arousal systems are in relative equilibrium, or where the sleep promoting system predominates only slightly and at the same time the stimulus is comparatively strong and/or of longer duration, there is a possibility of detecting both phases. Thus, for example, in the case of awakening from hexobarbital sleep, while the arousal stimuli produce a "pure synchronization" response in a less awake state, in

the more superficial stage, we can observe "post-synchronization" only following initial "desynchronization" or, more rarely, desynchronization follows after transitional „synchronization", generally in cases where the stimulus acts for a longer period [69]. Similarly, in the microarousals described by SCHIEBER et al. [126] as "les phases d'activation transitoire spontanées", the transitory activation (arousal) is followed by disactivation (shift towards sleep).

Thus, in the course of falling asleep, the electrographic responses to arousal stimuli change and instead of the desynchronizational response of the awake state we find responses which, although of different morphology, are all "synchronizational". A uniform explanation can be supplied for the mechanisms giving rise to these "synchronizational responses" assuming that these are rebound phenomena acting in the direction of sleep and arising in the course of the reciprocal induction type interaction of the sleep promoting and arousal system. This explains why we treat them uniformly as "synchronization response". Now, as to the K-complex, the particular synchronization response to which many studies are devoted let us consider the extent to which its morphological and functional characteristics correspond to the above-outlined concepts.

4.2. The K-complex as the "building stone" of the sleep promoting process

Since the first descriptions of the K-complex only few studies approached the functional significance of the phenomenon. Its morphological characteristics, distribution, latency time and the different parameters under which it can be evoked have been described [22, 81]. Its connection with the nonspecific reticular system was recognized at an early stage [119]. One of the main trends in views concerning the K-complex is the link between the phenomenon and sensorial stimuli, and the assumption that it represents some form of abortive arousal reaction during sleep [61]. At the same time there was a trend right from the start that stresses the link between the elements of the K-complex and the spontaneous EEG phenomena of slow wave sleep and, contrary to the above view, attributed to the complex a sleep protecting function [36, 66].

Our own observations [48] are based on the night sleep at 5 different activation levels for each of 8 healthy young adult volunteers. We measured the average frequency per minute of the K-complexes in stage 2 on the descending and ascending limbs of the sleep cycles. There was no significant difference in the average frequency per minute of phase 2 on the descending and ascending limbs, only a characteristic deviation in the distribution of the K-complexes within the descending and ascending stages 2. While the number of K-complexes in the descending stages 2 steadily increased with progress

towards stages 3, in the ascending stages 2 no consistent trend could be found in the course of time, and the frequency of the K-complexes fluctuated from minute to minute. The extent of the steady increase found in the descending 2nd stages gradually declined from the beginning to the end of sleep. A similar step by step decrease was found in the density of the K-complexes in subsequent cycles from evening to morning (Fig. 9). It thus appeared that the beginning of sleep is accompanied by increasing K-complex formation. We therefore studied whether there was a connection between the extent of deepening of sleep and the origin of K-complex formation in a more general form, too. We compared the depth of the cycles and the frequency of K-complexes measured in the stages 2 of the given cycles, so we determined the "depth" of the cycle by the "depth" of the deepest sleep stage reached in the cycle. It was found that the number of K-complexes/time measured in the stages 2 rose in direct proportion with the depth of the cycle. In other words, the deeper the sleep reached in the given cycle, the more K-complexes were formed in a given time unit in the sleep stages 2 (Fig. 10).

In another study [47] we averaged the electrographic "synchronization responses" evoked by combined click-flash stimuli below the arousal threshold in the entire night sleep of 3 healthy young adult subjects. In agreement with the observations of CHURCH et al. [17], it was found that the K-complex type synchronization response could be followed even in the deepest sleep with the aid of an averaging technique. The amplitudes of the synchronization

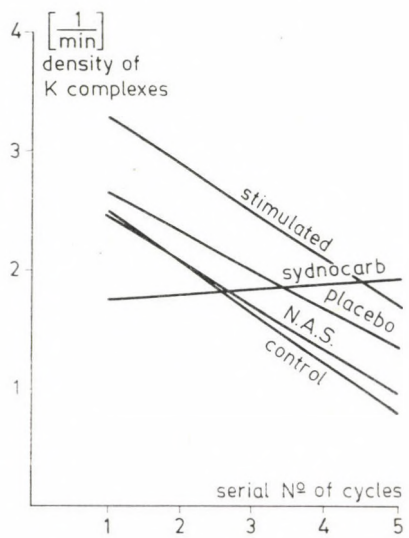

Fig. 9. Linear regression functions fitted to the K-densities measured in stages 2 from cycle to cycle in the five sleep groups. Except the group treated with psychostimulant (Sy) all sleep group show a decreasing tendency from evening to morning in the frequency of K-complexes

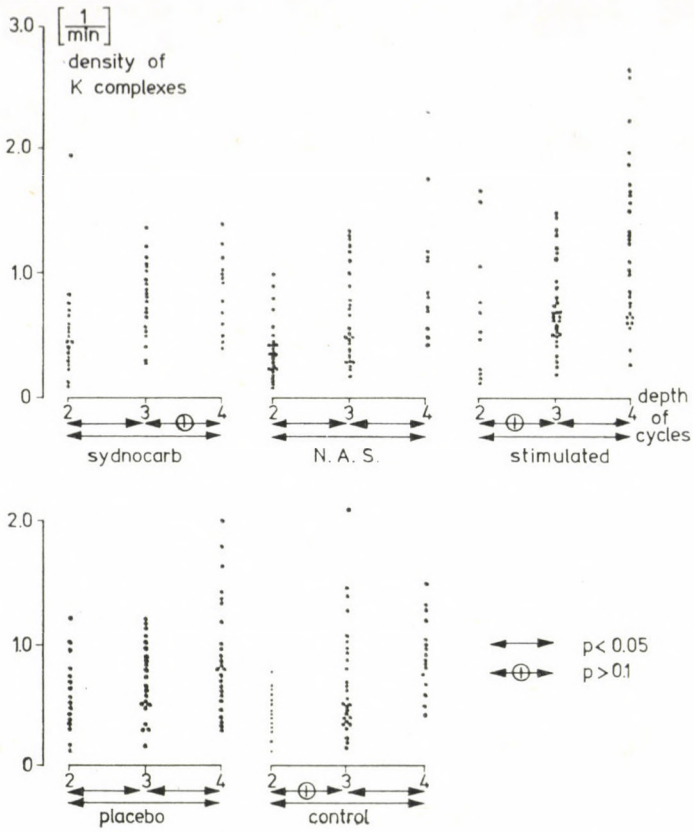

Fig. 10. Frequency of K-complexes of different sleep groups according to the depth of the sleep cycles. 2, 3 and 4: sleep cycles where the deepest stage was stage 2, 3 and 4, respectively. Double arrows show the result of Wilcoxon's test

responses followed very closely the fluctuation of the sleep level and were roughly proportionate to the depth of sleep in the sense that the more superficial the level of sleep where they were measured the lower and the deeper the level of sleep and the greater their amplitude (Fig. 11).

In a third (unpublished) study K-complexes were evoked by combined click-flash stimuli below the arousal threshold. One stimulus was given at a random rate every 20 seconds on the average. Stimulated and unstimulated periods were alternated every 5 minutes in the course of descending and ascending 2nd stages of the first two cycles of 6 nights of sleep in the same experimental subject. The proportion of spontaneous and evoked K-complexes was examined and also the comparative frequency of the spontaneous slow groups and K-complexes. It was found that considerably more K-complexes appeared in the stimulated periods than spontaneously, while the spontaneous K-complexes became much less frequent in the stimulated periods;

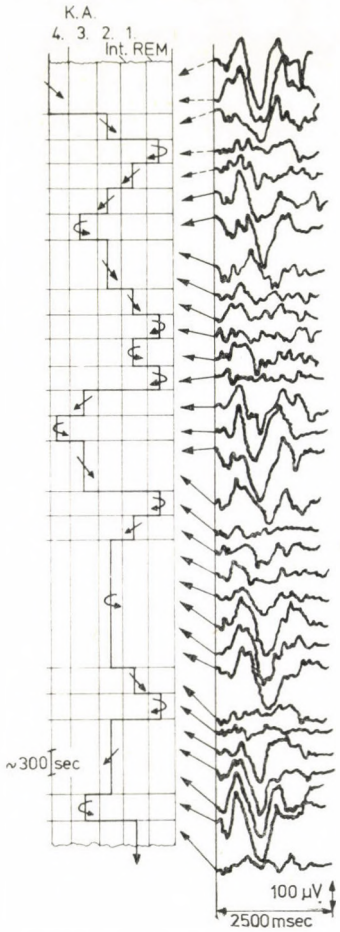

Fig. 11. Averaged electrographic synchronization responses during a whole night. On the left the hypnogram is shown. The actual trend of sleep is marked in all sleep stages. The arrows connecting the evoked responses with the hypnogram mean the places of sampling

in other words, the stimuli could be said to have exhausted the capacity of K-complex formation (Fig. 12). The same relationship was observed between the evoked K-complexes and the spontaneous slow wave groups. Spontaneous slow groups occurred only sporadically in the stimulated periods. All this indicated that spontaneous K-complexes could be identified with the evoked K-complexes and was compatible with the assumption that the spontaneous slow groups were also reactive elements similar as the K-complex.

It has been pointed out above that two apparently contradictory concepts exist in the explanation of K-complexes. According to one concept, the K-complexes represent abortive arousal responses and, according to the other, they reflect an inhibitory sleep protecting function. According to the

reciprocal inductional model, we shall attempt to combine the two concepts and we assume that the K-complexes are rebound reactions on the part of the sleep promoting process arising in response to sensorial impulses transmitted through the reticular arousal system. Our own experimental findings appear to be readily open to interpretation in the light of this concept.

If the K-complexes can be interpreted as arousal responses, a rise in the number of K-complexes would be expected as the night passes and as sleep becomes more superficial, with the increase of responsiveness to external stimuli. However, from evening to morning and from cycle to cycle, both the number of K-complexes measured in stage 2 and the extent of the gradual increase in frequency within the descending 2nd stage towards stage 3 steadily decline. At the same time, a phenomenon interpreted as an "arousal response" on the descending limbs while sleep is deepening in proportion to the reduction of "awakeness", could be expected to grow less frequent rather than more frequent. The frequency of K-complexes, however, increased in stage 2 as it approached towards stage 3. In other words, it would appear that the behaviour of the K-complex runs parallel to the sleep promoting rather than to the arousal process. Further evidence in favour of this is that the number of K-complexes measured in stage 2 indicated well beforehand the depth of the sleep cycle in which the stage 2 concerned is located. K-complexes are obvious determined not only by the extent of dominance of the sleep promoting system, since their number in the descending stage 2 of the cycles was the same as or greater than in the descending ones. The number of K-complexes fluctuated greatly from minute to minute and we did not detect any consistent tendency in the number of K-complexes per time unit similar as that for the descending

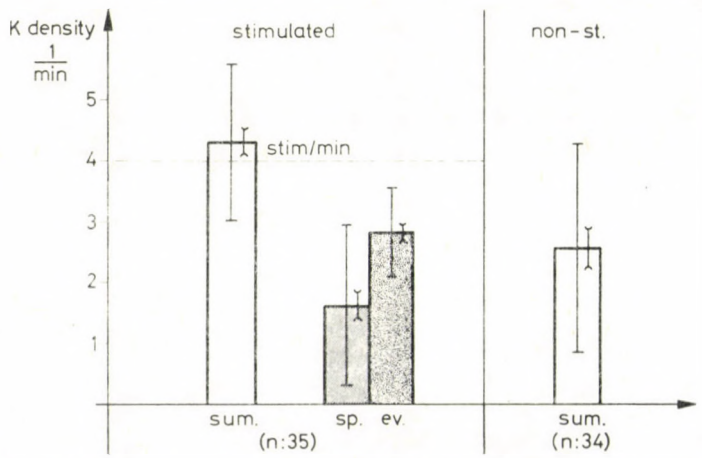

Fig. 12. Density of spontaneous (sp) and evoked (ev) K-complexes in stimulated (n: 34) and not stimulated (n: 35) stages 2 of the first two cycles of 6 nights of the same experimental subject. I = standard deviation; $\overline{\underline{\mathrm{I}}}$ = standard error

limb. This phenomenon is compatible with the conception of the K-complexes as a rebound reaction of the sleep-promoting system to arousal stimuli. Arousal influences can be effective increasingly frequently on the ascending limb and since the sleep promoting system still predominates in this period, all of them have a rebound evoking effect. Interpreted in this way, the steady increase in the frequency of K-complexes on the descending limb could indicate that the sleep promoting system becomes increasingly dominant thus giving rise to an ever greater rebound tendency and, together with it, a steadily growing reciprocal inductional activity of the arousal system which again results in increasingly frequent rebounds from the sleep promoting system. Thus, the dominance of the sleep promoting system on the descending limb determines the events, and the sensory stimuli through the rebound reactions resulting in a gradual mobilization of the sleep promoting system, push the process towards the deepening of sleep. In contrast, the appearance of K-complexes on the ascending limb is incidental and depends to a greater extent on the evoking influence of the sensorial impulses than does the behaviour of the sleep promoting system.

Thus, in our concept the process of falling asleep emerges from rebounds of the sleep promoting process in response to sensorial stimuli streaming in from the external environment.

This concept is in good agreement with the physiological changes in sensory transmission during sleep. There are several evidences in favour of the similarity between K-complex phenomena and the non-specific evoked response. The non-specific evoked response can be detected over the whole cortex transmitted by the non-specific thalamic reticular system [57], independently of the modality of the eliciting stimuli. In sleep the amplitude of the specific sensory responses connected with the specific sensory transmission decrease while the amplitude of the non-specific evoked responses increase. Contrariwise to the specific sensory evoked responses, the non-specific responses do not result in an increase of cortico-spinal motor excitability [13]. The specific sensory transmission is decreased during the recruitment originated by the thalamic non-specific system [3]. On the other hand, the amplitudes of the non-specific evoked responses remain unchanged during spindling [76]. The K-complex behaves similarly as the non-specific sensory response [17, 18, 70].

So the K-complex type synchronization reaction evoked by phasic sensorial input during sleep represents an operational change in the sensory transmission, as compared with the awake state. In the awake state, new sensory stimuli reach the specific cortical sensory areas through the specific, sensory relay nuclei of the thalamus and evoke a short latency cortical response, connected with a specific motor answer. In sleep the specific sensory transmission is depressed and the sensorial input, through another way, arrives into a common pool in the thalamic non-specific system. Through this system

they are feeding the slow wave sleep synchronization over the whole cortex. So the external sensory stimuli serve the sleep process.

In this way, inputs from the external environment assume a sleep promoting effect through the reciprocal inductional interaction of the arousal and sleep promoting systems. In this process the K-complexes behave as significant building elements of the "induction" process which can readily be studied. We could also say that in the antagonistic cooperation of the sleep promoting and arousal system the K-complexes are the tracer marks of the phasic interaction, the "clashes" of the two systems. The more balanced the "strength" of the sleep promoting and arousal systems, that is, the longer the duration of the fluctuations in the balance of forces through which one system gradually becomes predominant in the course of either falling asleep or awakening, the more possibility there is for the formation of K-complexes.

The K-complex can thus be considered as a "building stone" of the process of falling asleep which contains the whole sleep induction process in concentrated form and is an element of this process in which the whole is reflected like a model.

5. *Analogy of the K-complex type synchronization reaction and the generalized spike-wave pattern*

If we now recall the dynamics of the changes in the level of consciousness found in the emergence of petit mal seizures, we find a striking similarity between the K-complexes and the petit mal seizures. What we found valid for the spike-wave seizures may also apply to the K-complexes: this phenomenon too emerges following fine fluctuations of the level of consciousness in the course of falling asleep or awakening, when transitional phasic shifts towards awakening are followed by a rebound in the direction of falling asleep.

This similarity has already been noted. PASSOUANT et al. [108] pointed out earlier that there is a spiky distorsion of the K-complexes in certain epileptic mechanisms and he used the term "epileptic K-complexes". NIEDERMEYER [101] developed this idea further and recognized the link between the K-complexes and the generalized spike-wave discharges (his arguments will subsequently be used in detail). He showed that the K-complexes are the "indicators" or "vehicles" of the generalized spike-wave discharges and drew attention to the fact that all this frequently occurs in response to arousal stimuli. He built his arguments on the assumption that the K-complexes are arousal reactions. Starting from this assumption he considered that the petit mal seizures are linked to "faulty arousal reactions" and thus formed the concept that the GESw are of "dyshormic" origin. This would mean that some form of malfunction of the arousal mechanism plays a decisive role in establishment of the mechanisms accompanying the generalized spike-wave pattern.

In our concept the K-complexes figure not so much as arousal than as the rebound of the sleep promoting system to phasic arousal influences, it is thus regarded as a sleep induction factor. As to petit mal seizures, even if they appear in response to arousal stimuli, they arise as a rebound of the sleep promoting system. Even in this modified interpretation, the similarity between the K-complex phenomenon and the generalized seizures accompanied by spike-wave pattern has to be regarded as a very important question.

Let us now consider this similarity in greater detail.

— Both phenomena appear simultaneously over both hemispheres and their epicentres can be observed in the region of the vertex [111, 119].

— Electromorphological similarity can be demonstrated between the two phenomena [108].

— Both phenomena can be produced by sensory stimuli independently of sensorial modiality [21, 44].

— Both phenomena are connected with the secondary or non-specific evoked potentials [74, 99].

— The age determination of the generalized epileptic mechanisms with spike-wave pattern corresponds well to the "life history" of the K-complex: both phenomena appear mainly between the ages of 4 and 16 years [101].

— The role of the non-specific thalamic system can be demonstrated in the origin of both phenomena [34, 68, 119].

— The appearance of both phenomena is linked to a drop in the level of consciousness and the levels of consciousness anchoring them are overlapping [48, 72]. There appears to be a slight difference here in that the spike-wave seizures can also appear on a more superficial level than the zone in which the K-complexes appear. These, overlapping with the former, tend to be shifted slightly towards deeper layers.

— Both phenomena can be activated by sleep deprivation [48, 55].

— Experimental destruction of structures which inhibit the spike-wave seizures in animal experiments eliminates simultaneously the electrographic phenomena of slow wave sleep including the sleep spindles which form part of the K-complex [1, 4, 25, 84, 92].

— Both phenomena are rebounds on the part of the sleep promotion system to transitory arousals in fluctuations within a superficial level of sleep depth [44, 48].

We assume that these similarities give sufficient basis for regarding the spike-wave paroxysms as an epileptic "caricature" of the reciprocal sleep induction momentum reflected in the K-complex type synchronization reaction.

6. Generalized epilepsy with spike-wave paroxysms as an epileptic disorder of the function of sleep promotion

The possibility arives on the basis of the above arguments of forming a uniform hypothesis for the induction mechanism of the GESw, able to provide an explanation of all the facts available. This theory draws on the experimental findings and clinical experience which play a role in Gloor's corticoreticular concept and also in Niedermeyer's dyshormic theory. We amend the latter concept, as it were "by turning it over and setting it upright", that is, we consider that it is not a fault in the arousal mechanism, but an epileptic malfunction of the sleep promoting mechanism which is to blame. This is naturally "the other side" of the same thing since we assume that the sleep promoting and arousal systems are in reciprocal inductional interaction with each other which means that every "arousal" can result in a shift acting towards "falling asleep". With this, the genetically-determined GESw gains a "biological meaning", just as it does in Niedermeyer's interpretation, since with it we have finally found the brain function the epileptic disorder of which explains the mechanism of this type of epilepsy. In this interpretation, GESw is an epileptic disorder of the "sleep induction" function. Naturally, precisely because of the reciprocal inductional link, the antagonism between the sleep promoting and arousal systems means that all influences reducing the tone of the reticular arousal system are essential factors in provoking seizure phenomena since they "open the way" for operation of the epileptic malfunction located in the sleep promoting system.

The difference between the dyshormic concept and our own theory is not only that we replace the "disorder of the arousal process" with the "disorder of the sleep promoting system", but also that we interpret sleep induction as a dynamic process in which sleep occurs with the active participation of the arousal function and thus the "arousal" and the "sleep promoting" functions figure as two closely intertwined modes of operation of the same system which simultaneously contains the elements of both modes of operation in all manifestations.

All biological systems strive for independence from the environment (autonomy) and an adaptive link with the environment (adaptation) in dialectical unity. This means the simultaneous rejection (restoration of the original state) and use of stimuli from the external environment. An example of this is the reciprocal inductional interaction of the sleep promoting and arousal systems in which stimuli from the external environment are used in the process which ensures the temporary and partial independence of the organism from the environment, that is, sleep. The synchronizational rebound reaction produced by arousal stimuli is one of its elements. Thus a general biological reaction unfolds, which appears to be valid for the process of falling

asleep, too. In our concept, the spike-wave seizures are thus "overshootings" of this reaction.

Let us now consider the special characteristics of the GESw and attempt to evaluate them in the light of the above hypothesis.

7. *Evaluation of biological characteristics*

7.1. Genetic determination

If we accept the assumption that the GESw is an epileptic disorder of the sleep promoting mechanism, we must take into account that in certain patients, the malfunction is genetically determined. Definition of the responsible malfunction represents an intermediary link between the organically and genetically determined forms. If the epileptic mechanism did not have such a common functional core, it would be difficult to see how, in organic cases, an incidental acquired brain damage can produce epileptic symptoms similar to those in the genetically determined forms.

It is known that there is a continuous transition between manifest GESw epileptics and individuals in whom only the genetically determined EEG pattern exists, while seizure manifestations either never occur or occur only under the influence of certain external circumstances that promote or induce them. One such promoting circumstance can be a sleep deficit which may result in the appearance of seizures. In such cases the increased "sleep pressure" that arises as a result of sleep deprivation, the increased efficiency of the sleep promoting system, can turn into clinical seizures the sleep promotion system rebound phenomena appearing at the level of electrical spike-wave patterns. (We shall return later to the way in which the sleep function itself can produce an epileptic malfunction of the sleep promoting system.)

In the case of certain individuals suffering from episodic generalized epileptic seizures, the factor eliciting the seizures is sleep deprivation and the accumulation of a sleep deficit. Since these are produced on the basis of genetically determined characteristics, it can be assumed that the genetic determination is precisely a tendency to a shift towards epilepsy in the sleep inductional rebound momentum of the sleep promoting system.

Similarly, in the case of phenobarbital addition, spike-wave paroxysms appear in certain, probably genetically determined, cases, under the influence of withdrawal [71, 101].

Age dependence

In old age the operation of the sleep promoting system is known to decline and that of the arousal system comes to the fore. This is reflected in

the characteristic change in the hypnograms of elderly people which display a considerable decline in the proportion of deep sleep and the appearance of frequent intervening awake states [27]. It is also known that the EEG phenomena of slow wave sleep do not appear immediately after birth, but gradually emerge up to the age of 3—4 years, precisely at the age when the GESw phenomena appear.

Within the GESw, the strict age dependence of certain clinical forms probably runs parallel with the maturation process of the thalamo-cortical system and of the cortical functions linked to it, particularly the neural synaptic microstructures responsible for formation of the EPSP-IPSP sequences. With the advance of age, the effectiveness of the recurrent inhibition process steadily declines, multispike components become increasingly interspersed in the spike-wave pattern and first myoclonus and then tonic motor seizure phenomena appear [55]. The gradual repression with age of the recurrent inhibition reflected by the "wave" of the spike-wave pattern runs parallel with the gradual reduction of the spontaneous EEG synchronizational tendency in childhood.

7.3. Link with the sleep-waking biological rhythm

We have seen that the predominance of the sleep promoting system clearly favours the GESw symptoms, but all this only evokes seizures in the superficial region where the reciprocal inductional interplay of the sleep promoting and arousal systems is the most active. In harmony with this, sleep deprivation largerly promotes the emergence of seizure phenomena. Sleep induced in different ways is the most effective means to evoke the GESw phenomena. States which force the sleep promotion system into the background, including full wakefulness or simply the arousal reaction, and REM inhibit the GESw phenomena.

There is a possibility of seeking an explanation of the activating effect of sleep in an epileptic mechanism which we assume to arise from an epileptic disorder of the sleep induction function.

It is generally accepted that the epileptic disorder produces a state of hypersensitivity of some structures. Accordingly, the functional use of the structure concerned can result in epileptic manifestations. This phenomenon can be seen most clearly in the case of the so-called reflex epilepsies. In such cases the epileptic disorder in the sensory cortical areas appears in response to sensory stimuli of appropriate modality. This connection can probably be generalized and the physiological work of the system containing the epileptic seizure mechanism may set of seizures from these structures. This could also serve to explain the observations that point to the role of emotional seizure

activating factors in the case of temporo-limbic epileptic disorders. In the same way it is conceivable that precisely the act of falling asleep could activate the epileptic disorder of the sleep promoting mechanism.*

7.4. Functional anatomical characteristics

The findings of human studies and animal experiments indicate that the epileptic malfunction in the GESw mechanism is located in brain structures which have close operational links to the hypnogenic structures.

Experimental damage of the hypnogenic structures inhibits the phenomena of both sleep and the GESw.

Temporary or lasting, functional or anatomical, damage to the arousal system which operates in the opposite direction to the sleep promoting system clearly contributes to the GESw phenomena while the physiological, chemical or direct electrical stimulation of the system inhibits the GESw phenomena.

The bilateral simultaneous nature of the EEG phenomena related to the GESw can be explained by the diffuse cortical connections and hence the widespread effect of the sleep promoting system.

The microneurophysiological characteristics of the generalized spike-wave pattern can be deduced from the functional link with the hypnogenic structures, particularly with the thalamic reticular system.

7.5. Explanation of the seizure symptoms

Our views provide an effective explanation of the characteristics of the clinical seizure symptoms. The following main general factors determine the GESw seizure symptoms.

1. The epileptic malfunction of the sleep promotion mechanism means at the same time an epileptic heightening of rebound of the sleep promoting system and a reduction of the tone of the arousal system, that is an epileptic distortion of the reciprocal induction mechanism between the two systems.

2. The state of the hypnogenic, thalamocortical non-specific system and particularly of its cortical elements.

3. The possibilities for the spread of the epileptic excitation.

If we interpret the GESw as an epileptic disorder of the sleep promoting function, this effectively explains why the primary and decisive symptom of seizures is a loss of consciousness. The extensive limbic interconnections of the sleep promoting system can easily be blamed for both the accompanying vegetative and the psychomotor symptoms. The rhythmic periocular

* These relationships raise the possibility of a new functional classification of epilepsy in which the different seizure mechanisms could be classified according to the physiological functions the epileptic disorder of which forms the essence of the illness.

myoclonus symptoms that almost always appear with petit mal absences probably occur through the efferent interconnections of the brain stem. The extent and form of the motor symptoms can be linked to the characteristics of the cortical electrical phenomena. The fact that petit mal seizures are accompanied only by myoclonic motor phenomena where the spike component coincides with the myoclonic twitches, is probably due to the inhibitory feedback identified with the "wave" component of the spike-wave pattern. Tonic motor phenomena only appear if the inhibitory feedback is not operating adequately, for example in slow spike-wave encephalopathies involving an extensive cortical damage or, for example, if this mechanism is partially suspended with barbiturate [45]. Grand mal seizures also occur when this inhibitory feedback "breaks down"; the less effective this mechanism, the greater the possibility of the appearance of grand mal seizures [43]. The fact that in forms where mainly "pure absences" appear we find a regular 3 c/s spike-wave pattern, while in cases where myoclonic absences appear and grand mal seizures are also frequent we find a multi-spike-wave pattern and in cases where tonic motor seizures are frequent, "epileptic recruitment" occurs spontaneously or mainly during sleep (repetitive spike discharge), can also be effectively incorporated in this concept.

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Pharmacologia

THE SLEEP OF ARTIFICIALLY REARED NEWBORN RATS, EFFECT OF ALPHA-METHYL-DOPA TREATMENT ON PARADOXICAL SLEEP AND ON ADULT BEHAVIOUR[1]

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The development of paradoxical sleep was studied in newborn rats separated from their mothers immediately after birth and then fed artificially by an indwelling stomach tube. Development of PS in these animals was qualitatively similar but quantitatively it differed from that of animals remaining with their mother, the daily amount of PS being reduced. The PS depriving effect of α-methyl-dopa was studied after single and multiple doses. A 500 mg/kg dose daily led to a 75% continuous reduction.

Rats remaining with their mother were injected with α-methyl-dopa during the three postnatal weeks. Subsequent tests revealed an increased locomotor activity and a higher rate of acquisition but no disturbance in shuttle-box conditioning. The norepinephrine, dopamine and serotonin content of the whole brain was not affected.

One of the hypothetical functions of paradoxical sleep (PS) is to participate in the maturation of the CNS. Newborn animals display more PS than adults and the greatest amount occurs during intrauterin life [16], with the quantity decreasing during ontogenetic development. Studies on humans [27], chimpanzees [5], rats [20], kangaroo-rats [3], cats [19, 35], chickens [28, 33] have resulted in the same conclusion. Moreover, the more mature the animal at birth, the less PS it has [19, 23]. The CNS of the guinea pig is mature even at birth, and the newborn animals have as many PS as the adults [19]. But during intrauterine life it has considerably more PS, and it could be detected more than one week before the slow wave sleep (SWS) [4].

On the basis of these data it is supposed that the function of PS is to play a role in the maturation of the CNS: PS may provide the endogenous stimuli necessary to the functional maturation of the CNS [17, 18, 27].

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It seemed therefore of interest to deprive animals of PS after birth and examine the behavioural consequences. To accomplish the task we had to deprive newborn rats of PS continuously during the first three postnatal weeks. We have failed to find any studies concerning long-term deprivation of PS in early postnatal life. Since only pharmacological deprivation is practical for a long period, we have chosen alpha-methyl-dopa (α-MDOPA) because of its relative selective PS depriving action in adults cats [8, 10], mice [21] and rats [7].

To control the sleep deprivation, baby rats have to be observed permanently. As the mother-rat does not tolerate such a procedure she may neglect or devour her offspring. Thus, they have to be separated from their mother and reared artificially.

Materials and methods

1. *Artificial rearing*

The permanent stomach tube consisted of a polyethylene catheter 0.7 mm in diameter. To one end a 10—12 mm long, soft plastic catheter was attached to minimize traumatization of the gastric wall. The catheters were fixed to a Dacron plate. The other end of the soft catheter continued in a 3 mm long rigid piece rounded off to prevent its slipping out of the stomach.

Before use the catheters were sterilized in alcohol for 24 hours.

The muscle electrodes consisted of insulated copper wire 0.2 mm in diameter. The tip of the wire was freed from insulation then immersed into Atomex (Engelhard) solution for gilding. Three electrodes were fixed to a Dacron plate. This arrangement was suitable for recording ECG activity as well.

Implantation. Under light ether anaesthesia a lateral laparotomy was performed on the left side, and the skin of the nape was incised. The stomach was opened at the greater curvature, the catheter was placed into the stomach, then it was closed. The Dacron plate was fixed under the skin a few cm from the stomach, to reduce mechanical traumatization. The other end of the catheter was passed under the skin to the incision on the nape. The muscle electrodes were fixed into the deep neck muscles and the wires were led to the incision, which was then closed by adhesive.

The outer end of the catheter was attached to a swivel which allowed the baby rat to move freely without twisting the catheter. The electrodes were soldered to a ring (Fig. 1).

Rearing. After surgery the animals were placed into an incubator consisting of ten compartments. The temperature was kept at 32 °C, the relative humidity at 60—70%. The feedings were started after at least 8 hours following surgery. The baby rats were fed by means of an infusion pump (Sage) on Borden-Esbilac milk preparation for developing small rodents. The quantity of food did not exceed 1 ml daily during the first few days following surgery, then gradually more was supplied. The sleep pattern was registered by an 8 channel EEG (Polygraph Alvar). The records were read every minute. The ten compartments allowed to record several baby rats simultaneously. Continuous recording (24 hours/24 hours) were performed (Fig. 2). The light schedule was not standardized.

Nursing was fundamental to keep alive newborn rats in incubator, which had to be done at least twice daily: to wet the skin and to facilitate micturition by pressure proved to be most important [19].

2. *Behavioural studies on adult rats*

Locomotor activity was studied by the open field test, placing rats in a 123×135 cm floor divided into 36 equal squares. The number of squares crossed during 30 minutes was recorded. The light was kept constant.

The shuttle-box used for training the rats was a 20—40 cm chamber with a grid floor, divided into two equal parts by a 5 cm high barrier. After habituation, the conditioned stimulus, a shrill bell, was presented until the rats had crossed to the opposite compartment. If they

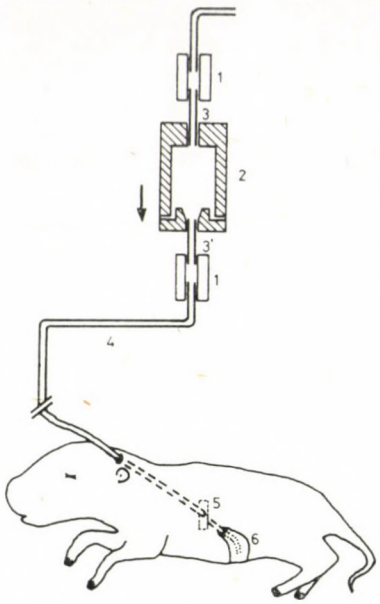

Fig. 1. Scheme of artificial feeding of newborn rats. 1: Attachment of catheter to swivel. 2: Fix part of swivel. 3: Turning parts of swivel. 4: Catheter. 5: Dacron plate. 6: Position of catheter in the stomach

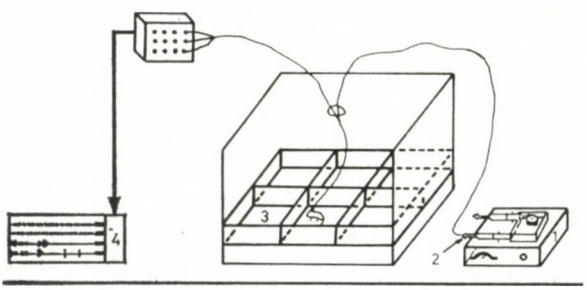

Fig. 2. Scheme of experimental set-up for rearing and feeding artificially and recording newborn. rats. 1: Infusion pump. 2: Syringe. 3: Incubator. 4: Polygraph

did not cross within 5 sec, an electric shock (110 V, 50 Hz) was given until the animals had escaped. One session consisted of 30 trials with 5—10 sec inter-trial intervals. The animals were tested throughout the day at random. Ten sessions were performed on consecutive days.

Biochemistry. Six treated and 6 control male rats were sacrificed and the serotonin (5-HT), dopamine (DA) and norepinephrine (NE) contents of the whole brain were determined spectrofluorometrically, by the methods of BOGDANSKY [6], FLEMING [12] and EULER and LISHAJKO [11], respectively.

3. *Animals and procedures*

Implantation was performed at the age of 2—3 days on the litters of OFA rats weighing 7—8 g. 36 rats were injected with d,l-α-MDOPA. Six of them served for dose finding studies for determining the optimal schedule and way of administration. 30 animals were treated continuously; they received 500 mg/kg d,l-α-MDOPA daily s.c. The daily dose was

divided into two parts: 200 mg/kg at 9 a.m. and 300 mg/kg at 5 p. m. 21 control animals were injected with 0.9% NaCl solution of the same volume (0.02 and 0.03 ml/kg respectively). Nursing in the incubator was continued for three weeks.

For studying adult behaviour, newborn rats were kept and treated in their original litter with their mothers. 38 (18 male and 20 female) rats from the age of 4—24 days received subcutaneous injections of 250 mg/kg l-α-MDOPA Calbiochem) daily for 21 days: 100 mg/kg at 9 a.m. and 150 mg/kg at 5 p.m. 13 control animals (7 males and 6 females) were injected with the same volume of saline. Littermates were used either as control or treated groups. The rats were 35 days old when tested in the open field tests, 41—50 days old at the time of shuttle-box conditioning and at the age of 52 days the animals were used for biochemical determinations.

Statistical evaluation. Means and S.E.M. values were calculated. Student's non-paired *t* test was applied.

Results

1. The sleep-waking cycle of newborn rats

1.1. *Spontaneous sleep patterns*

During the first postnatal week newborn rats reared in incubator and fed artificially had only PS, which appeared directly after the awake period. Slow wave sleep (SWS) appeared by the 10th postnatal day. During PS the electromyographic activity disappeared completely. Muscle atony was interrupted by muscle jerks. Considerable bradycardia appeared on the ECG during PS. The heart rate decreased from 400—460/min to 300—340/min. Three phases were observed in the evolution of PS (Fig. 3).

During the first ten days PS occupied about 50% of the registration time (TRT), with slight variations. The mean frequency of PS phases was

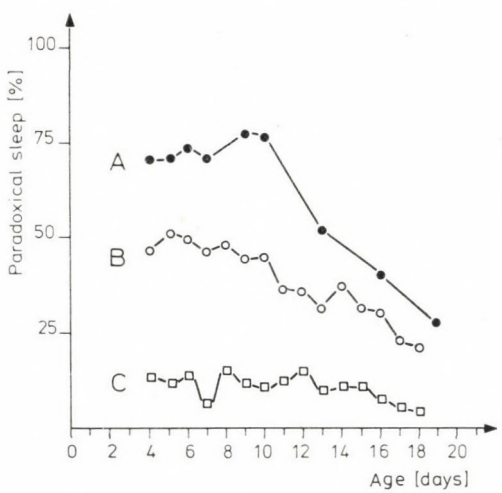

Fig. 3. Time course of paradoxical sleep in newborn rats. Ordinate: Paradoxical sleep (per cent of total registration time). Abscissa: age of rats (days). A: rats registered for 3—12 h, then returned to the litter (JOUVET—MOUNIER [19]); B: control rats reared in incubator; C: rats reared in incubator and injected with α-MDOPA

17/h on the 3rd day and 8/h on the 10th with a mean length of 2 min and 4 min phases, respectively. Then a transitional period followed, lasting generally for two days. During this period the amount of PS decreased quickly to 30% of TRT. In the 3rd phase a further slow decrease could be detected. At the end of the observation period PS amounted to 20—25% of TRT. The mean frequency of PS phases was reduced to 5/h with 3 min mean length and these figures then became stable.

Graph A on Fig. 3 originates from the paper of JOUVET-MOUNIER [19]. Her rats were reared more naturally, than our animals. Their EEG was registered for 3—12 hours by needleelectrodes, then they were replaced to their original litter. They had more PS than those kept by us but the PS curve was qualitatively similar in both experiments.

1.2. *Effects of α-MDOPA*

A marked reduction of PS could be observed after each dose of α-MDOPA, by whatever route it was applied but both the degree and the duration of the inhibition, depended on the dose and the route of administration. Sedation

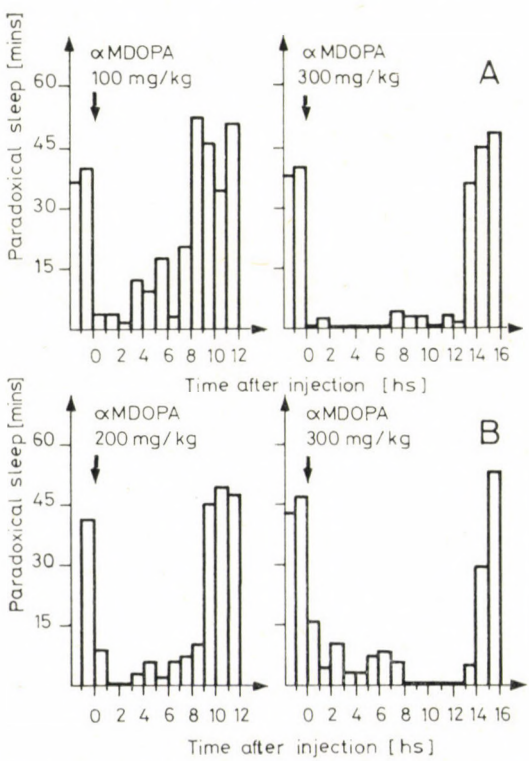

Fig. 4. Effect of single doses of α-MDOPA on PS of newborn rats reared in incubator. Abscissa: time after injection (hours); Ordinate: length of PS (min). A: intraperitoneal injection; B: subcutaneous injection

was elicited only after the tenth day. For that time a clearcut bradycardia appeared, too. The heart rate decreased from 420/min to 300/min following the administration of 300 mg/kg d,1 α-MDOPA

In the studies in baby rats, PS disappeared for 13—15 hours under the effect of 300 mg/kg d,1α-MDOPA. This occurred in 10 min after i.p. injec-

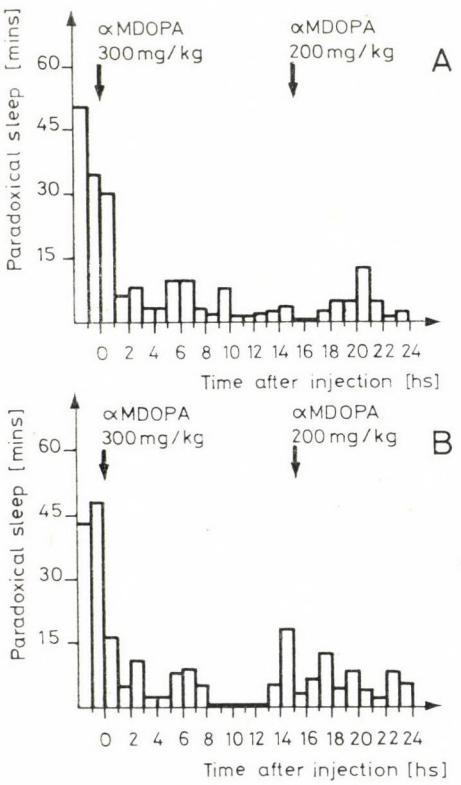

Fig. 5. Effect of repeated doses of α-MDOPA on PS of newborn rats reared in incubator. Abscissa: time after injection (hours): Ordinate: amount of PS (min); A: intraperitoneal injections; B: subcutaneous injections

tion, on one half hour after s.c. administration and in one hour when given by a gastric tube. Similar but less marked changes were observed after the administration of 100—200 mg/kg doses (Fig. 4).

When administered repeatedly, α-MDOPA produced a continuous and almost total PS depreviation (Fig. 5).

Continuous daily administration of 500 mg/kg d,1 α-MDOPA elicited a subtotal PS deprivation. PS amounted 10% of TRT during the whole period,

Table I

PS in per cent of total registration time in newborn rats reared in incubator. Control and α-MDOPA treated groups. Means ± S.E.

	Control	α-MDOPA per cent	Deprivation per cent
Whole period	39.65 ± 2.09	10.64 ± 0.74	73.19
3rd—10th days	47.18 ± 0.74	10.67 ± 1.12	77.93
11th— 20th days	31.75 ± 1.57	10.60 ± 1.08	66.61

its quantity did not change. These findings are represented in Graph C in Fig. 3.

Statistical evaluation of PS of control and treated rats is shown in Table I.

2. *Behaviour of adult rats*

The α-MDOPA-group showed an increased excitability. During the period of treatment their activity and responses to environmental stimuli were increased.

The body weight of the treated animals was significantly less than that of the controls, with a lag of 1—2 days. After treatment they regained their weight loss. There was a sex-related difference in body weight beginning from the third postnatal week but both male and female rats showed a decrease in body weight during drug treatment and displayed a similar recovery (Fig. 6).

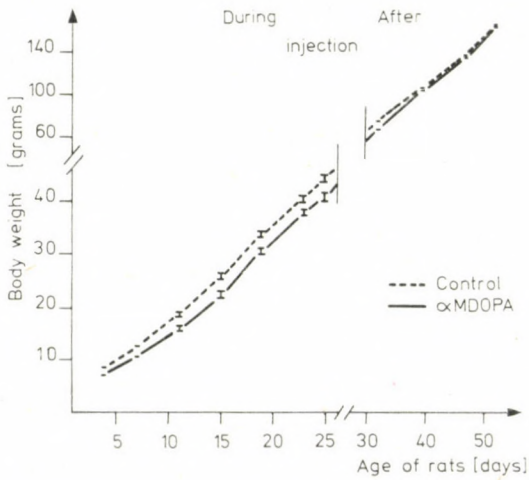

Fig. 6. Body weight of rats treated with 250 mg/kg l-α-MDOPA subcutaneously for three postnatal weeks; compared with saline-treated controls. Abscissa: age of rats (days); Ordinate: body weight (g)

In the open field test the treated animals were significantly more active than the control ones. Both groups exhibited habituation; they crossed less and less squares in the 10 min periods. Sex differences at this age proved to be negligible in this test (Fig. 7).

In shuttle-box conditioning marked differences were found. Females gave generally more avoidance responses than did the males, but the difference was significant only once, in the 4th session in the control group and never in the treated one. During the first half of the training, the α-MDOPA treated rats of both sexes learned the task more rapidly; the differences was strongly significant statistically. From the 7th session the difference decreased

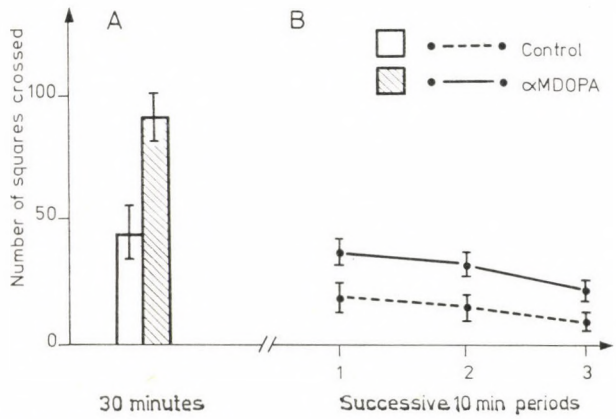

Fig. 7. Effect of α-MDOPA on locomotor activity. The number of squares crossed during the total session (30 min) is represented by the vertical bars in part A (means ± S.E.); the habituation in both groups in successive 10 min in part B

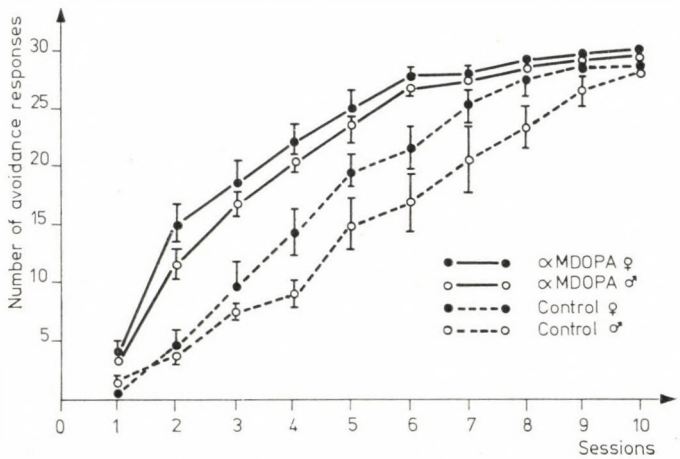

Fig. 8. Effect of α-MDOPA treatment on shuttle-box conditioning. Ordinate: Number of daily avoidance responses (mean ± S.E.)

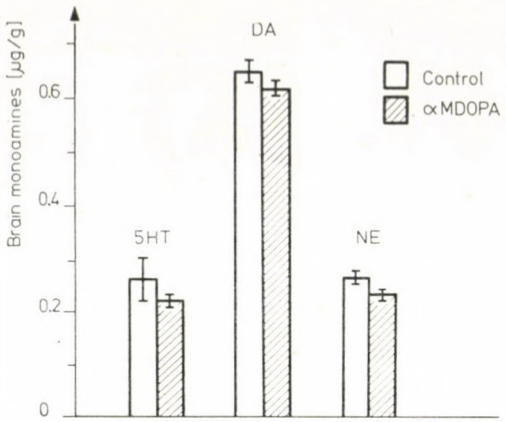

Fig. 9. Effect of α-MDOPA treatment on whole brain 5-HT, DA and NE content. Ordinate: brain monoamine content, µg/g; mean ± S.E.

gradually and finally the control animals displayed a performance similar to the treated ones (Fig. 8).

No marked differences could be detected in the brain 5-HT, DA and NE levels (Fig. 9).

Discussion

In our experiments early post-natal PS deprivation resulted in increased locomotor activity and an increased rate of acquisition. The latter is very likely a consequence of the increased activity.

The technique described in this paper allows to rear newborn rats artificially from the 2nd postnatal day, separated from their mothers, and to follow the evolution of their sleep-waking cycle in the three postnatal weeks. The development and maturation of a series of physiological mechanisms and regulatory processes could be studied by the method, and the immediate and late effects of some interventions carried out in early life could also be checked. The immediate mortality of surgery is about 20%. One of its causes is the failure of gastric motility. Only 30% of implanted animals have survived the 3 weeks' period.

The sleep of newborn rats reared in incubator is similar to that of animals left with their mother. The evolution of sleep, the changes in frequency and length of PS phases, are similar in both [19, 20, 35].

In contrast, the amount of PS was markedly less in the present study than in JOUVET-MOUNIER's experiments [19]. The cycle length was the same but the mean frequency was reduced, e.g. 17/h instead of 30/h during the first postnatal days. The difference may have been due to differences in feeding

which was continuous in our experiments. Less frequent and less adequate nursing and the impoverished environment may have also been responsible. Cats and rats reared in an isolated, impoverished environment have less PS than normally or in littermates reared in enriched environment [23, 34]. Decrease of PS was observed after 24 hours maternal separation [14].

In the presented study, α-MDOPA treatment resulted in subtotal PS deprivation. PS amounted to 10% of TRT and remained unchanged during the three weeks studied. PS of newborn rats seems to be more resistant to pharmacological deprivation than that of adults. In adult rats 100 mg/kg d,l-α-MDOPA elicits sedation for 2—5 hours followed by total PS deprivation for 11 hours [7].

Our results demonstrate an increased excitability and locomotor activity due to early postnatal α-MDOPA treatment. The superior performance in the shuttle-box does not reflect the capacity of learning but indicates a higher rate of acquisition which is very likely the consequence of increased locomotor activity and not of any specific process of learning. It is evident, however, that treatment did not cause any deficit in learning.

These results are in agreement with those of PAPPAS and SOBRIAN [25], and of SCHAEFER et al. [30]. These authors administered 6-hydroxydopamine (6-OHDA) and *p*-chlorophenylalanine (pCPA) to baby rats, two drugs which in adult rats cause selective or non-selective PS deprivation [22, 24] without any gross behavioural disturbance.

SMITH et al. [32] applied 6-OHDA in immature rats in a manner which preferentially reduced brain NE. They also observed an increased locomotor activity and shuttle-box acquisition. With other types of administration which reduced DA or both NE and DA, both the above mentioned activities were reduced. In our case, brain 5-HT, DA and NE levels were unchanged. It is though that the turnover of the biogenic amines might be affected, but their dynamics were not studied.

ISAACSON et al. [15] administered to newborn rats 5,6-dihydroxytryptamine intracisternally. In adult life a reduced brain 5-HT level was found, open field activity and FR conditioning and the effects of amphetamine on these two tests were unchanged.

ANDERSEN produced experimental phenylketonuria by pCPA and phenylalanine in rats [1, 2]; their body weight was similar as that seen in our animals. When tested as adults they were hyperactive in activity wheel and in the open field.

While active avoidance responses were unaffected in shuttle-box and in Y-maze, the treated rats were inferior in passive avoidance conditioning.

Undernutrition and handling may both affect adult behaviour [9, 13, 26, 31]. On the basis of these facts it is difficult to find the exact cause of our findings. The changes might have been due to PS deprivation but other specific

or non-specific actions of the drug and the procedure (e.g. hypotension, decreased heart rate, reduced body weight) may also have had a part.

The present finding might be explained as follows. Either the relationship between PS and the maturation of the CNS might not operate or at least not in this simple form, or the PS was not sufficiently deprived. Unfortunately, continuous recording of the newborn left with the mother has not been solved. If the newborn is separated from its mother, environmental conditions are unnatural, and the spontaneous sleep patterns are modified [7]. The pharmacological data obtained under such conditions may be extrapolated to less artificial ones with some reservations. Nevertheless, the experiment was repeated in newborn kittens reared in their original litters. 72% PS deprivation was found and only increased excitability and a slight retardation of co-ordination were detected but no gross behavioural changes have been found [29].

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PURINERGIC REFLEX ACTIVATED BY CATHARTICS IN THE RAT

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Phenlaxine and bisacodyl were shown to inhibit gastric emptying and motility by activating a reflex arising from the small intestine. This effect produced by the cathartics could not be prevented either by alpha or beta sympatholytic, or by parasympatholytic agents; further it was antagonized by quinine and quinidine, as well as by chloroquine and mepacrine in doses found to suppress gastric motility in untreated animals. The inhibition of gastric motility through cathartics does not appear to be due to an effect on adrenergic or cholinergic pathways but rather to involve a purinergic mechanism.

It is generally accepted that normal gastric motility is governed by the interaction of excitatory cholinergic and inhibitory adrenergic nervous impulses. Early in this century, the existence of a third, i.e. non-adrenergic, non-cholinergic, vagal inhibitory system was postulated in the innervation of the stomach [11, 12]. This was suggested by BURNSTOCK to be a purinergic system [4]. The preganglionic fibres of the inhibitory purinergic system are believed to run in the vagus nerve forming synapses on the ganglionic cells located at the myenteric plexus in the stomach. These neurons then supply the gastric smooth muscle cells. A number of experimental data is available showing that the putative transmitter of this inhibitory purinergic system, i.e. ATP, is capable of inducing relaxation of some parts of the smooth muscle elements in the gastrointestinal tract of various species, and during nerve stimulation ATP is released [2, 3, 6, 8, 20, 21]. The present paper does not concern the effect of putative transmitter but rather a reflex mechanism activated by drugs, suggesting a purinergic innervation of the rat stomach. As described earlier in two short communications [10, 15], some cathartics were found to inhibit gastric motility through reflex pathways, and the suppression of gastric motility was prevented by drugs, such as mepacrine, quinine, and quinidine which have been thought to be purinergic antagonists.

Methods

The experiments were carried out on female Sprague-Dawley CFY rats weighing 200—250 g after a 24 h starvation.

Gastric emptying was studied by Levine's phenol-red method [9] with an 0.1% dye concentration. Gastric motility was measured by using a method elaborated in our laboratory. In brief, the rats were anesthesized with 40 mg/kg pentobarbital intraperitoneally. After the abdominal wall had been opened, by the aid of an atraumatic needle a miniature strain gauge was fixed on the pyloric part of the stomach. The amplified movements of the stomach were recorded on a polygraph. Detailed description of the method has been published elsewhere [7].

For measuring the gastric emptying, phenol-red was given through a stomach tube in groups if at least 5 conscious rats. Drugs influencing gastric emptying were administered in part through a gastric tube or in part parenterally prior to the application of phenol-red. Thirty min after the administration of the dye, the animals were killed, their stomach was exposed, and the amount of the remaining phenol-red was determined photometrically, its quantity being expressed as per cent of the dye applied. The individual values were averaged, and the difference between various experimental groups was statistically evalued by Student's *t* test.

When cathartics were administered intraduodenally, the animals were given 40 mg/kg pentobarbital intraperitoneally, then their abdomen was opened, and a thin polyethylene tube was introduced by mouth through the stomach into the duodenum under visual control. The drugs were applied through the tube which was then removed.

The coeliac ganglion was excised under pentobarbital anesthesia. After median laparotomy, the coeliac artery was exposed the ganglion was carefully isolated from the surrounding tissues under a preparation microscope, and excised, then the abdominal wall was sutured. Measurement of gastric motility was undertaken 10 days after surgery.

Drugs used: phenlaxine (Richter), bisacodyl (Chinoin), chloroquine phosphate (Chinoin), mepacrine dihydrochloride (Sigma), quinine sulfate (Boehringer), quinidine sulfate (Boehringer), papaverine HCl (Chinoin), pentobarbital-Na (Abbott), phenol-red (Reanal), phentolamine (Ciba), pindolol (Sandoz), acetyl-beta-methylcholine chloride (Sigma).

Results

Phenlaxine and bisacodyl given by mouth to rats inhibited gastric motility causing a concomitant delay in emptying. These effects were dose-related (Fig. 1). Bisacodyl inhibited gastric motility within a few minutes after application, while the effect of phenlaxine developed with a latency and was sustained for 3 to 4 hr (Fig. 2). The inhibition did not occur when the cathartics were applied intraperitoneally or *per rectum*, while it was always demonstrable following intraduodenal application. It should be noted that the cathartic effect ensued after rectal, but not following intraperitoneal administration.

The initial observations put forward the hypothesis that the inhibition of gastric motility and emptying was due to a reflex activation of inhibitory adrenergic mechanisms or to an inhibition of stimulatory cholinergic innervation. Therefore, attempts were made to antagonize the cathartics induced inhibition of gastric motility by administration of phentolamine, pindolol, or acetyl-beta-methylcholine (ACBMC). It was found that neither 1.25 to 5.0 mg/kg subcutaneous phentolamine nor 0.125 to 0.5 mg/kg intraperitoneal pindolol prevented the reflectoric inhibition by cathartics of gastric motility (Table I), moreover both drugs were shown to somewhat increase the quantity

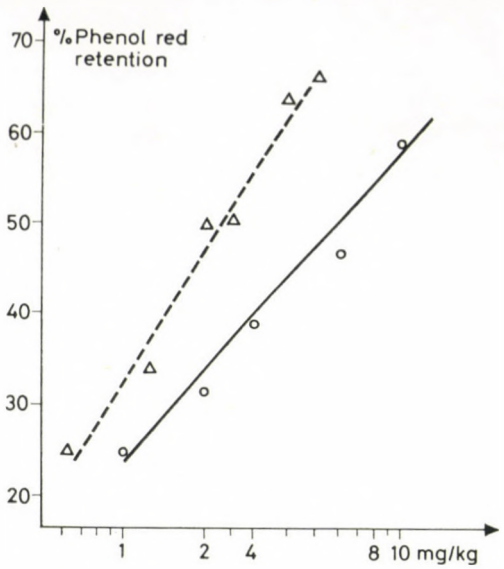

Fig. 1. Effect of phenlaxine (○—○) and bisacodyl (△ — — — △) on gastric emptying. Ordinate: the quantity of phenol-red retained in the stomach expressed as per cent of the dose administered; abscissa: dose of cathartics. Sixty min elapsed between the administration of phenlaxine and phenol-red; bisacodyl and phenol-red were given simultaneously. The animals were killed 30 min following the application of phenol-red. Each point represents the average obtained from groups of 5 rats

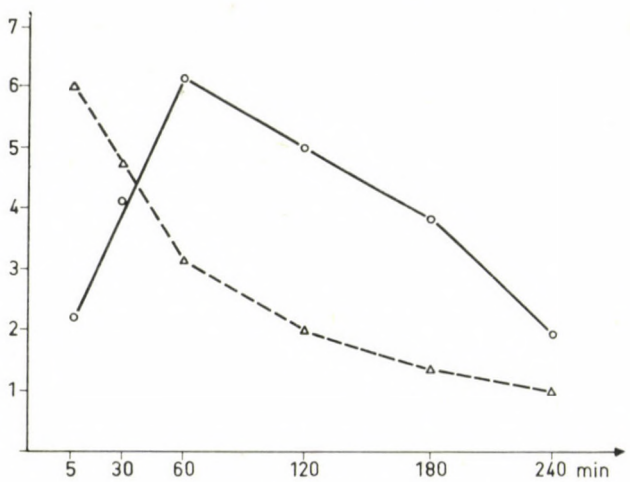

Fig. 2. 10 mg/kg phenlaxine (○—○) and 5 mg/kg bisacodyl (△ — — — △) induce inhibition of gastric emptying. Time-response relationship. Ordinate: increase in phenol-red retention expressed as percentage of the control value; abscissa: time elapsed between the administration of chatartics and phenol-red, min. Each value is average obtained in groups of 5 rats. The animals were sacrificed 30 min after phenol-red had been given

Table I

Effect of adrenergic antagonists on the inhibition by 10 mg/kg of phenlaxine of gastric emptying[1]

Drugs	Route of administration	Dose, mg/kg	Retention[2] of phenol-red; the control value was considered 1.0 (±S.D.)
Phentolamine	s.c.	5.00	1.59±0.40
		2.50	1.47±0.02
		1.25	1.32±0.07
Pindolol	i.p.	0.50	1.32±0.20
		0.25	1.50±0.05
		0.125	1.27±0.30

[1] A group of animals treated with 10 mg/kg phenlaxine orally served as control
[2] 30 min after phenol-red

Table II

Effect of phenlaxine, quinine, and their combination on the gastric emptying in the rat

Drugs	Route of administration	Dose, mg/kg	Retention of phenol-red[1]; the control value is considered as 1.0 (±S.D.)
Phenol-red	per os		1.00±0.3
Phenlaxine[2]	per os	10	2.08±0.5*
Quinine[3]	i.p.	25	1.53±0.6
Quinine[3]	i.p.	50	2.03±0.7*
Quinine[3]	i.p.	100	2.54±0.5*
Phenlaxine	per os	10	2.38±0.4
+quinine[4]	i.p.	25	
Phenlaxine	per os	10	1.54±0.6**
+quinine	i.p.	50	
Phenlaxine	per os	10	1.51±0.7**
+quinine	i.p.	100	

[1] 30 min after phenol-red
[2] Phenol-red given 60 min after phenlaxine
[3] Quinine and phenol-red given simultaneously
[4] Quinine and phenol-red administered 60 min after phenlaxine
* Significant difference as compared to the control
** Significant difference as compared to the group treated with 10 mg/kg phenlaxine

Table III

Effect of bisacodyl, quinine, and their combination of the gastric emptying in the rat

Drugs	Route of administration	Dose, mg/kg	Retention of phenol-red[1]; the control value is considered 1.0 (±S.D.)
Phenol-red	per os		1.00±0.3
Bisacodyl[2]	per os	5	2.54±0.4*
Quinine[3]	i.p.	25	1.43±0.5
Quinine	i.p.	50	1.90±0.4*
Quinine	i.p.	100	2.37±0.6*
Bisacodyl	per os	5	1.78±0.14
+quinine[4]	i.p.	50	
Bisacodyl	per os	5	1.59±0.62**
+quinine	i.p.	100	
Bisacodyl	per os	5	1.08±0.39**
+quinine	i.p.	150	

[1] 30 min after phenol-red
[2] Phenol-red given 5 min after bisacodyl
[3] Quinine and phenol-red given simultaneously
[4] Quinine and phenol-red administered 5 min after bisacodyl
* Significant difference as compared to the control
** Significant difference as compared to the group treated with 5 mg/kg bisacodyl

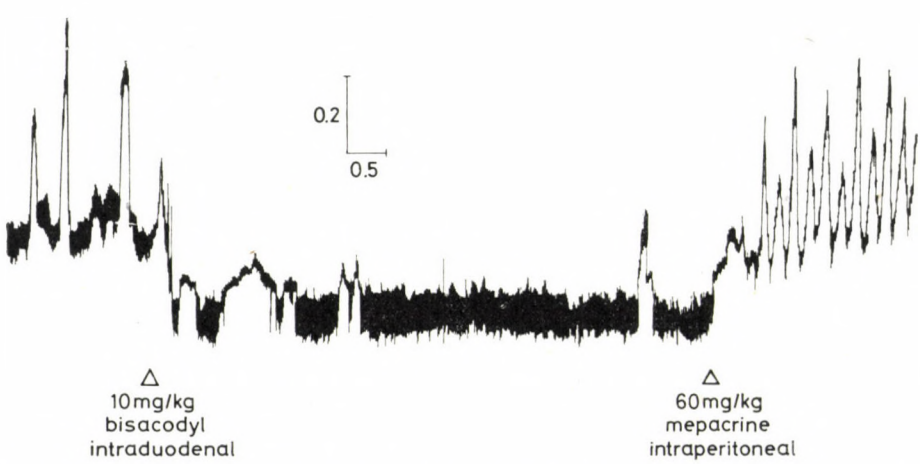

Fig. 3. Effect of mepacrine on gastric motility inhibited by bisacodyl. Pendular movements were recorded by strain gauge. Calibration: 0.2 g and 0.5 min

of phenol-red retained in the stomach. As compared to the control value a significant decrease in gastric emptying was already observed after 2.5 mg/kg phentolamine given subcutaneously or 0.25 mg/kg pindolol injected intraperitoneally. ACBMC, in a dose of 0.75 mg/kg given intraperitoneally, was unable to initiate gastric motility after the inhibition induced by chatartics.

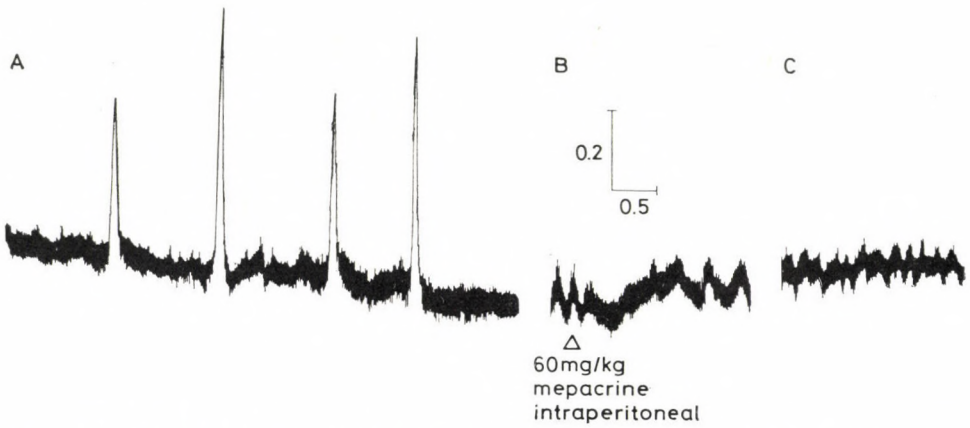

Fig. 4. Effect of mepacrine on the gastric motility of untreated rats. Recording was carried out by a strain gauge. Calibration: 0.2 g and 0.5 min. A: control; B: 15th min; C: 30th min

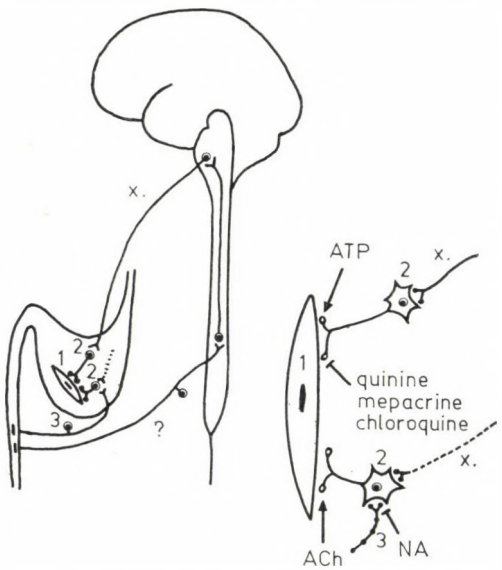

Fig. 5. The putative reflex arch through which chatartics induce inhibition of gastric motility. 1: smooth muscle cell, 2: ganglionic cell in the myenteric plexus, 3: adrenergic preganglionic fibres its cell body is located in the coeliac ganglion, X: preganglionic fibres running in the vagus nerve, ATP: adenosine triphosphate, ACh: acetylcholine, NA: noradrenaline, → activation, ⊣ inhibition

The cathartics were capable of inhibiting the pendular movement of the stomach in rats subjected to coeliac ganglionectomy. On the other hand, there is no inhibition in acute experiments when the vagus nerve was cut on the neck immediately before the administration of cathartic drugs. In such cases, the motility declined owing to vagotomy, it was however further increased when 1 mg/kg papaverine was injected intraperitoneally.

It appears from the obtained data that phenlaxine and bisacodyl inhibit gastric motility and emptying through a reflex in which afferentation is arising from the small intestine, and its efferent fibers are running in the vagus nerve. The reflex is not adrenergic and cholinergic in nature. A possible mechanism, i.e. the involvement of purinergic inhibitory pathways, was then postulated, and it was therefore suggested that the putative purinergic antagonists, such as mepacrine, quinine and quinidine, might have been able to prevent the inhibition of gastric motility induced by cathartics. As shown in Tables II and III, the retention of phenol-red in the stomach and the inhibition of gastric motility evoked by phenlaxine and bisacodyl were antagonized by quinine. Figure 3 demonstrates that gastric motility which had been stopped by bisacodyl returned to normal when 60 mg/kg mepacrine was injected. This dose was shown to abolish the pendular movement of the stomach in rats not receiving bisacodyl (Fig. 4).

It was also found that the inhibition of gastric motility induced by the tested cathartics was antagonized not only by quinine and mepacrine but also by quinidine (20 to 50 mg/kg intraperitoneally) and chloroquine (10 to 30 mg/kg intraperitoneally).

Discussion

Phenlaxine and bisacodyl, when exerting their effect in the small intestine, inhibit gastric motility and emptying in the rat. This may be due to an inhibitory gastroenteric reflex avoiding the coeliac ganglion and having efferent fibres in the vagus. Since the efferent pathways are not adrenergic or cholinergic, and in turn blocked by quinine, quinidine, and mepacrine, they are by all means purinergic in nature. The putative reflex pathways are shown in Fig. 5.

An ileo-gastric reflex passing through the coeliac ganglion, blocked by adrenolytic agents has been described by Rozsos et al. [17, 18, 19]. The existence of an oesophagal-gastric reflex has been suggested by Abrahamsson and Jansson [1], while a reflex, in which afferent pathways are derived from the abdominal region, and which induces gastric relaxation, has been suggested by Ohga et al. [16]. Moreover, gastroenteric reflexes activated by breakdown products of fatty acid and protein metabolism, or some acids as well as hyper-

osmotic solutions have also been postulated. Some of them can be interrupted by the removal of the coeliac ganglion, whereas others are blocked by vagotomy [5]. We assume that the inhibitory gastroenteric reflex activated by cathartics drugs belongs to the last group.

Bearing in mind our earlier results, it is remarkable that the inhibition of gastric motility by cathartics is antagonized by mepacrine and chloroquine. It has previously been established that chloroquine and mepacrine, owing to their parasympatholytic and spasmolytic character, are capable of inhibiting gastric motility and emptying in intact animals [13, 14]. It appears from the present findings that chloroquine and mepacrine in doses which are inhibitory on gastric motility under normal conditions, are able to initiate pendular movements in the stomach of the rat in which gastric motility is blocked by administration of some cathartics drugs. These different effects should involve different mechanisms.

Acknowledgements

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Recensiones

D. G. JONES

Some Current Concepts of Synaptic Organization

Vol. 55. Fasc. 4. Advances in Anatomy, Embryology and Cell Biology. Springer-Verlag, Berlin—Heidelberg—New York 1978. 69 pages with 21 figures. Price DM 34.—; US $ 18.70

Since the origin of the idea of synapse (FOSTER and SHERRINGTON, 1897) a very large literature has accumulated, dealing with the structure and function of synapses. The very different demands of neurobiology involve a detailed survey of synaptology and this makes the task of reviewing the synaptic organization a major one. This review presents only the synapses of limited areas of the CNS, especially of the cerebral cortex and discusses the presynaptic terminal, the enlarged termination of the axon.

The contents of the review are: 1. Introduction; 2. Outline of synaptic morphology (synaptic parameters, synaptic variations); 3. Issues in synaptic terminal organization (vesicle-membrane relationships, vesicle attachment sites, VAS and intramembraneous particles, VAS and synaptic activity, microfilamentous network, coated vesicles, protein composition of the junctional region, microtubular system, microtubules in presynaptic terminal microtubules and tubulin); 4. Models of the presynaptic terminal. The chapters are completed with abundant references and a subject index.

The review is an important source of information for the large field of neurobiology, in particular for synaptology.

K. LISSÁK

G. STERBA und F. SCHOBER

Topographie und Zytologie neurosekretorischer Systeme

Teil 1. Das klassische neurosekretorische System der Ratte. Gustav Fischer Verlag, Jena 1979. 119 pages with 118 figures. Price DM 76.00

This book is an atlas of the classical (supraoptic and paraventriculohypophysial) neurosecretory system of the rat. It deals with its organization, topography, myeloarchitecture, angioarchitecture and projections. A detailed picture is included of the course of fibres which arise in the supraoptic and paraventricular neurones and terminate in the infundibular process of the neurohypophysis or in extrahypothalamic regions, i.e. the subfornical organ, habenular region, rhombencephalon and the ependyma of the lateral ventricle. The neurosecretory elements have been visualized largely by the pseudoisocyanine technique and partly by immunochemistry of the neurophysins. Afferent connections, synaptology of the cell groups and immunohistochemistry of vasopressin and oxytocin are not encompassed. The first part (20 pages) of the book is textual, while the larger second part contains illustrations which are clear and of good quality. They consist partly of drawings based on the König-Klippel atlas and partly of microphotographs. Co-ordinates are given on each drawing.

The book can be recommended to all those who are interested in the detailed topography of the classical neurosecretory system of the rat.

B. HALÁSZ

W. BERGMANN

Niere und ableitende Harnwege

Handbuch der mikroskopischen Anatomie des Menschen. Bd. VII/5. Springer Verlag, Berlin—Heidelberg—New York 1978. 444 pages with 181 figures and 12. tables. Price DM 290.

The Volume VII/1 of MÖLLENDORF's monograph from 1930 gave a detailed description of the excretory system. Knowledge about the structure and function of the kidney and of urine transport has, however, been enlarged and fundamentally changed in the last decades. These facts prompted the editors of the monograph to edit a new volume which, beside the old data, contains the new results of recent morphological, physiological and biochemical researches.

Minor but important modifications of the old MÖLLENDORF volume were performed. Chapter 2 describes the architecture of the kidney (types, lobulation, vascular segments, etc.).

In Chapter 3, which deals with evolution, not only the development of the human kidney but also of that of some animal species is decribed. It is illustrated by excellent drawings and electron micrographs.

Two chapters (approximately 180 pages) describe the details of glomerular and tubular function. Data comparing human and animal kidney are also present. Almost 50 pages deal with the description of structure and function. Data about transtubular transport of proteins, fatty acid uptake, etc., are also mentioned. The macula densa is discussed in detail.

Chapter 6 describes the vascular system of the kidney. Very demonstrative histological pictures of granulated epithelial cells are given. The architecture of the vessels is discussed in parallel with that of the tubuli, providing a good morphological basis elucidation of for the countercurrent principle. This chapter describes the lymph vessels and interstitium as well.

The last part of the volume discusses the innervation of the kidney.

The 307 page part on the kidney is followed by Chapter 8 entitled "The urinary tract". Histological information about the cavity system, ureter and bladder are described on as few as 22 pages.

The author took upon himself a very difficult task, since being an anatomist, be tried to summarize the present state of knowledge concerning kidney structure and function, which is more or less out of the scope of his profession. The aim of the monographs was, however, successfully achieved. This fact indicates that the book is an excellent and valuable tool for both scientists and clinicians involved in kidney research.

F. RÉNYI-VÁMOS

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РЕЗЮМЕ

ВЛИЯНИЕ ИНДОМЕТАЦИНА НА ТРАНСПОРТ НАТРИЯ И ВОДЫ В КОЖЕ ЛЯГУШКИ

Р. БАРТА и ХАБЛИ

Авторы исследовали изменения транспорта натрия, рубидия и воды в коже лягушки, наступающие в присутствии индометацина, ингибирующего циклическую эндопероксидазу. Транспорт натрия изучали измерением короткозамыкательного тока, а также с помощью изотопа натрия (Na^{24}); транспорт воды изучали методикой мешочка, изготовленного из кожи лягушки.

Результаты исследования показали, что, под влиянием индометацина

1. уменьшается напряжение, измеряемое между двумя сторонами кожи лягушки, а также интенсивность замыкательного тока; сопротивление и проводимость не изменяются,
2. усиливается пассивный транспорт натрия, направленный изнутри кнаружи,
3. не изменяются ни транспорт воды, направленный в сторону осжотического градиента, ни пассивный транспорт рубидия.

На основании этих данных авторы приходят к выводу, что

а) под воздействием индометацина избирательно повышается проницаемость кожи по отношению к натрию, то есть

б) результаты настоящих экспериментов согласуются с предположением относительно того, что индометацин в некоторых тканях может оказывать влияние на интенсивность транспорта натрия.

СОН У ИСКУССТВЕННО ВСКАРМЛИВАЕМЫХ НОВОРОЖДЕННЫХ КРЫС. ВЛИЯНИЕ АЛФА-МЕТИЛ-ДОФАМИНА НА ПАРАДОКСАЛЬНЫЙ СОН И НА ПОВЕДЕНИЕ ВЗРОСЛЫХ КРЫС

П. ЮВАНЦ

Авторы разработали методику вскрымливания новорожденных крыс в инкубаторе. Исследовали эволюцию парадоксального сна на протяжении трех недель после рождения. Парадоксальный сон и временное изменение соотношение парадоксального сна у искусственно вскормленных крыс качественно соответствовали таковым у крыс, воспитанных матерями, количественно же пропорция парадоксального сна была на 20% ниже. Но кожновведение d, 1 альфаметил-дофамина по 500 мг/кг, ежедневно в течение трех недель, вызвало 10, 70—75% торможение парадоксального сна.

Крысы, вскормленные матерями, тоже в течение трех недель получали d, 1 альфаметил-дофамин, в день по 250 мг/нг. Исследования были выполнены позже на взрослых животных, получавших дофамин в новорожденном возрасте. У них была выявлена повышенная локомоторная активность и ***shuttle-box acquisition***, но не удалось показать нарушения процесса обучения. Содержание норадреналина, серотонина и дофамина в мозгу не изменилось.

ВЛИЯНИЕ ИОНОВ ЦИНКА НА ПРОКОАГУЛЯНТНУЮ АКТИВНОСТЬ PMN ЛЕЙКОЦИТОВ

Э. ГАЗДИ, Х. ЧЕРНЯНСКИ и Т. СИЛАДИ

Авторы изучали влияние ионов цинка, магния и марганца на прокоагулянтную активность перитонеальных PMN лейкоцитов кроликов, после обработки их в условиях ***in vitro*** эндотоксином. В присутствии этих катионов клетки, полученные из брюшной полости, инкубировали в растворе Ханкса при 37°С, после чего определяли свертывающую способность надосадочной жидкости в цитратной плазме крови кролика.

При инкубировании надосадка в присутствии 50 мкмоль хлористого цинка его свертывающая способность достоверно снижалась, при добавлении же 100 мкмоль хлористого цинка отмечали значительное удлинение времени свертывания. Ионы цинка в зависимости от концентрации подавляют выход прокоагулянтного вещества из лейкоцитов.

Взятые в качестве контроля два других двухвалентных катиона не оказывали влияния на время свертывания, определяемого в подобной же системе.

Было показано, что ионы цинка затормаживают высвобождение тканевого фактора из PMN лейкоцитов, обработанных предварительно эндотоксином. Ингибирующее действие цинка является дозозависимыми, экспоненциально возрастая от 5 мкмоль до 250 мкмоль.

Способность ионов цинка ингибировать освобождение тканевого фактора можно объяснить его стабилизирующим действием на мембрану.

АКТИВИРОВАННЫЙ СЛАБИТЕЛЬНЫМИ СРЕДСТВАМИ ПУРИНЕРГИЧЕСКИЙ РЕФЛЕКС У КРЫС

Э. МИНКЕР и Ж. МАТЕЙКА

Фенлаксин и бисакодил тормозят у крыс двигательную активность желудка и его опорожение посредством рефлекса с тонкого кишечника. Этот эффект слабительных средств нальзя предотворатить ни альфа- и бета-симпатолитиками, ни парасимпатиметиками, однако можно антагонизировать хинином и хинидином, а также такими дозами хлорохвина и мепакрина, которые у интактных (не получавших слабительных средств) животных и сами по себе подавляют моторику желудка.

Затормаживающий моторную активность желудка эффект слабительных осуществляется, по-видимому, через пуринергическую нейронную систему, а не через адренергическую или холинергическую.

ДЕЙСТВИЕ СУЛЬФАТНОГО ЭФИРА ОКТАПЕПТИДА-ХОЛЕЦИСТОКИНИНА НА МОНОАМИНЫ МОЗГА У КРЫС

ФЕКЕТЕ М., ВАРСЕГИ МАРИЯ, КАДАР Т., ПЕНКЕ Б., КОВАЧ К. и ТЕЛЕГДИ Г.

Изучали влияние разных доз сульфатного эфира октапептида-холецистокинина на (ХЦК — 8 — СЭ) на содержание допамина, норадреналина и серотонина гипоталамуса, мезенцефалона, миндального ядра, прозрачной перегородки и полосатого тела через 10, 20 и 60 минут после введения препарата.

В гипоталамусе и мезенцефалоне содержание допамина и норадреналина увеличивалось, а содержание серотонина уменьшалось. Содержание моноаминов миндального ядра изменялось двухфазно. Изменение зависело от дозы и срока действия препарата. Подобным образом изменялось содержание допамина и норадреналина прозрачной перегородке. В полосатом теле содержание допамина и серотонина уменьшалось, а содержание норадреналина в начале увеличивалось потом уменьшалось.

Наши результаты указывают что ХЦК — 8 СЭ изменяет содержание допамина, норадреналина и серотонина разных отделов мозга. Изменение зависит от дозы от срока действия от места действия препарата.

ИЗУЧЕНИЕ ДЕЙСТВИЯ ПРОГЕСТЕРОНА НА ПОСТРОДОВЫХ (post partum) МАТКАХ КРЫС

А. БЕРНАРД

У 44 крыс, после родов, изучали реактивность матки по отношению эстрадиола (E_2) и прогестерона (Р). У 34 животных со спонтанным родом введение гормонов началось непосредственно после родов, а у 24 животных у которых на 24 часа до спонтанных родов проводилось кесарево сечение, сразу после кесарева сечения. В матку животных ввели резиновый баллон, при помощи которого систематично измеряли интраутеральное давление до и после проведения окситоцинового теста.

По результатам измерений, проведённых ежедневно в течение недели, было установлено, что у всех нелечённых прогестероном животных пострадовое давление в матках значительно увеличивалось. Совместное введение E_2 + Р значительно уменьшало максимальное интраутериновое давление в том случае если лечение началось на 24 часа раньше родов. Введение одного прогестерона после спонтанных родов оказалось не эффективным, но применяя вместе с E_2 в течение 3 дней или дальше значительно уменьшало интраутеральное давление.

Само сабой разумеется, непосредственно перед родом и во время родов реактивность матки крыс по отношению прогестерона существенно изменялась. Механизм данного явления пока не известно.

ИЗУЧЕНИЕ ЭНЗИМНОЙ ИНДУКЦИИ В ПЕЧЕНИ ДЕНЕРВИРОВАННЫХ КРЫС

ФРЕНКЛ Р., СЕБЕРЕНИ С., ЧАКВАРИ Г.

Изучали роль иннервации печени в возникновении действия различных индуцирующих факторов (фенобарбитал, мыщечная работа).

Энзимная индукция, (укорочение гексобарбитвлого сна) — наблюдается и после частичной ваготомии (целиэктомии) и тотальной денервации печени. Хотья применяемые нами методы, мелкие изменения могут не выявлять, можно сделать вывод что индукцию микросомальных энзимных систем печени можно вызвать и после денервации.

Лапаратомия удлинняло время сне. Это действие продолжалось в течение 6 недель. Роль нервной системы в этом явлении не подлежит сомнению.

РОЛЬ МЕМБРАННЫХ ПРОЦЕССОВ В РЕГУЛЯЦИИ СКЕЛЕТНОЙ МУСКУЛАТУРЫ

ЛАСЛО КОВАЧ

В последнее время подробно изучали роль мембранных процессов в активировании поперечнополосатых мыщц. Вероятным кажется тот, что зависимость электромеханической связи от мембранного потенциала обусловлено движением зарядов внутри мембраны. Проводились усилия непосредственного измерения функциональных особенностей саркоплазматического ретикулума, и либерацни интрацеллулярного Ca^{++}. Приводятся экспериментальные данные о связи между снижением зарядов, либерацией Ca^{++} и пороговым движением.

ГЕНЕРАЛИЗОВАННАЯ ЭПИЛЕПСИЯ СО СПАЙКАМИ (GES_W) КАК ЭПИЛЕПТИЧЕСКОЕ НАРУШЕНИЕ ФУНКЦИИ СНА

ХАЛАС П.

На основании литературных данных и собственных исследований нами была выработана новая гипотеза, относительно патомеханизма генерализованной эпилепсии с волнами-спайк (GES_W).

В первой части, дается критический анализ взглядов на патомехнизм GES_w. Излагаются: возникновение «центренцефалической теории», дискуссии о кортикальных и субкортикальных причинах данного заболевания; кортико-ретикулярная теория Глора и наконец «дисгормическая» теория Нидермейера.

Авторы доказывают что приступы волн-спаек возникают на грани бодроствования и сна то есть на границе РЭМ-а и нон РЭМ-а при таких колебаниях уровня бодрости, в которых явления пробуждения меняются явлениями засыпания. Поэтому для понимания механизма приступов волн-спаек необходимо знание динимических свойств этой критической зоны.

На основании собственных исследований мы пришли к выводу, что микроосцилляции уровня глубины сна, и без эпилепсии показывают также динамические особенности, которые объясняются реципрокно-индукционным взаймоотношением системы сна и пробуждения.

По нашему представлению, процесс засыпания возникает в результате реципрокного-воздействия системы сна и засыпания в ответ на возбуждения органов чувств внешними раздражителями. Значит индукторами сна — через реципрокно индукционных взаймоотношений системы засыпания и пробуждения — являются внешние раздражители. Так мы объясняем всякую реакцию синхронизации ЭЭГ в ответ на внешние раздражители и реакцию синхронизации типа К- комплексов мы считаем таким фундаментальным явлением процесса сна, которая представляет собой суммацию всех индукционных явлений процесса сна.

Относительно механизма GES_w мы указываем на многосторонную сходность между комплексами-К и формой волн-спаек. На основании этого приступы волн-спаек, мы считаем своеобразными эпилептическими «карикатурами» индукционных явлений сна, выражающихся в виде К-комплексов, и GES_w считаем эпилептическим нарушением индукционных процессов системы сна и пробуждения.

Эта гипотеза дает возможность объяснить ряд противоречий относительно механизма GES_w, и служит биологической базой для дальнейших исследований.

В заключении на основании этой гипотезы мы попытаемся дать ответ на некоторые характерные свойства GES_w: на генетическую детерминированность, на зависимость от возраста, на связь с циклом сна — и бодрствования, на функциональные и анатомические особенности и наконец на электрические и клинические симптомы приступа.

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Physiologia—Pathophysiologia

CHARACTERIZATION OF ANISOTROPIC ELASTIC PROPERTIES OF THE ARTERIES BY EXPONENTIAL AND POLYNOMIAL STRAIN ENERGY FUNCTIONS

By

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(Received March 16, 1980)

Three-dimensional quasi-static mechanical measurements were carried out on cylindrical segments of the dog carotid and iliac arteries for determination of the passive anisotropic elastic properties of the vessel wall. On the basis of passive characteristics of outer diameter *vs.* intraluminal pressure, and axial extending force *vs.* intraluminal pressure, picked up at various fixed initial vascular length values, the incremental Young moduli and Poisson ratios of the vessel wall were calculated in the 0—33 kPa (0—250 mm Hg) pressure range. The strain energy function of the arteries was approximated by polynomial and exponential models. We found that an exponential energy function with 4-parameters gives more accurate results than the 7- or 12-parameter polynomial functions. According to the results the axial modulus reaches higher values than the tangential and radial moduli at a low tangential stretch level, while at high tangential stretch the tangential modulus is the highest in both carotid and iliac arteries. After elevation of the initial tangential stretch the increase in the tangential modulus is the most pronounced, while the values of radial and axial moduli increased less. A change in the initial axial stretch influences the axial and radial moduli to a similar extent, but has no substantial effect on the value of the tangential modulus. The values of corresponding Poisson ratios depend in a similar way on the initial deformation state. The different behaviour of the two Poisson ratios characterizing the mechanical coupling between tangential and axial directions, indicates that the structural coupling between the two main directions is asymmetrical. It is assumed that this property of the passive vascular structure can be explained by the network arrangement of collagen fibres in the vessel wall.

Knowledge of the biomechanical properties of the vessels is necessary for the understanding of the close connection between structure and physiological function of the arteries. Due to the viscoelastic properties and peculiar architecture of the active and passive vessel wall constituents, the arteries exhibit anisotropic and non-linear mechanical behaviour [10, 17, 22, 24, 30, 31, 32]. The non-linear elasticity of the anisotropic arterial wall can be characterized entirely by one single incremental modulus at constant vessel length [18], but for the description of its three-dimensional behaviour at least three independent parameters are necessary [30]. If the vessel wall is considered to be cylindrically orthotropic and incompressible [6, 27], the three-dimensional

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elastic stiffness and the strength of mechanical coupling between the normal strains can be characterized by three incremental Young moduli and six incremental Poisson ratios. PATEL and VAISHNAV [30] worked out useful methods for the experimental determination of incremental characteristics of straight cylindrical arteries. Still, only few studies have been engaged in the systematic evaluation of incremental moduli and Poisson ratios especially over a large deformation range [8]. In order to characterize the functional structure of the vessel wall and the mechanical interaction between the structural elements, the above mentioned incremental parameters should be known in a wide deformation range.

The aim of the present study was the determination of incremental Young moduli and incremental Poisson ratios in cylindrical artery segments in a wide (0—33 kPa $\sim$ 0—250 mm Hg) intraluminal pressure range and at various initial axial stretches. As a first step, the experiments and calculations were carried out on arteries with totally relaxed smooth muscle. Since the passive stress-strain characteristics of the arteries determine the effectiveness of vascular smooth muscle contraction [19, 25, 26], the information related to the passive vascular structure is very important for the elucidation of the mechanical properties of the vessel wall and its physiological function *in vivo*. The determination of incremental moduli is simplified by the fact that the viscoelastic hysteresis of the vessel wall can be neglected in the passive state and therefore the moduli can be determined from quasi-static measurements. Some of the results have already been published elsewhere [21].

Methods

Supposing that the vessel wall is homogeneous, cylindrically orthotropic [27] and incompressible [6], the definitions of incremental Young moduli (E_l) and of Poisson ratios (σ_{kl}) are as follows.

$$E_l = \frac{t_l}{e_l}, \qquad (k, l = r, \theta, z) \tag{1}$$

$$\sigma_{kl} = -\frac{e_k}{e_l}, \qquad (k \neq l) \tag{2}$$

if only t_l differs from zero. t_l and e_l are the principal stresses and principal strains in the r, θ, z cylindrical system of coordinates (Fig. 1). It should be noted that, due to the incompressibility of the vessel wall,

$$\sigma_{zr} = 1 - \sigma_{\theta r}, \quad \sigma_{r\theta} = 1 - \sigma_{z\theta}, \quad \sigma_{rz} = 1 - \sigma_{\theta z} \tag{3}$$

The deformation of the artery is almost reversible in the passive state and the vessel wall can be regarded as being purely elastic [13]. In this case the incremental moduli can be derived from the strain energy function of the vessel wall.

In the present work strain energy function of the vessel wall was approximated by 7 and 12 parameter polynomials — introduced by VAISHNAV et al. [37] — and by our exponential function containing 4 constants. This exponential model was formulated as follows.

$$W = W_1 \exp\left[A\varepsilon_\theta^2 + B\varepsilon_\theta\varepsilon_z + C\varepsilon_z^2\right] \tag{4}$$

where W is the strain energy density of the vessel wall, ε_θ and ε_z are the Lagrangian tangential and axial strains, A, B and C are elastic constants, and W_1 is a scale factor. Since the

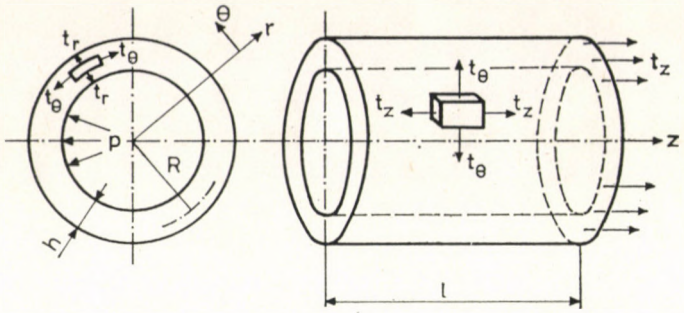

Fig. 1. Schematic diagram of the cylindrical vessel wall with the normal stresses; t_θ: tangential, t_r: radial, t_z: axial component; p: intraluminal pressure; R: mean radius; h: wall thickness

argument of the exponential function is the homogeneous quadratic form of the two principal strains and the radial strain component is lacking the above energy function allows cylindrically orthotropic and incompressible elastic properties.

The mechanical measurements were carried out on 5—5 cylindrical segments of the common carotid (CC) and iliac arteries (IA) isolated from mongrel dogs. A detailed description of the technique can be found in the literature [7, 17, 25], only the most main points will be mentioned.

After removal of the loose adventitial tissue, 10 to 30 mm long straight segments were excised from the isolated arteries, mounted in a tissue bath and stretched approximately to their *in vivo* length. Quasi-static mechanical test was performed on the vessel wall by slow (approximately 200 Pa s^{-1}) and cyclic changes of intraluminal pressure over the 7 to 33 kPa range. The outer vessel diameter, axial extending force and internal pressure were continuously recorded. The bath contained oxygenated physiological electrolyte-glucose solution at 37 °C.

Before recording the diameter-pressure and axial force-pressure curves, the arteries were mechanically conditioned by 10 to 20 pressure cycles which resulted in total smooth muscle relaxation and reproducible characteristics [13]. The measurements were repeated at 3 to 5 various vessel lengths, differing slightly from the *in vivo* axial extension. After this procedure the arteries were cut along the long axis and circumference, length and wet weight of the non-deformed vessel wall were measured.

Based on the mean values of the hysteresis loops of diameter-pressure and force-pressure curves and of other data measured, the mean radius, the wall thickness, the normal stretches and the mean normal stresses of the vessel wall were determined at each 1.33 kPa (10 mm Hg) pressure increments. The constants of the strain energy function were determined by regression analysis. The Hausholder transformation [5] was used for the fitting of the 12-parameter polynomial. For fitting the exponential model, the quasi-Newton method of Broyden–Fletcher–Schanno was used [14]. In this case, searching of minimum value was started from various initial points. Values of incremental Young moduli and Poisson ratios were derived from the material constants by standard equations [30].

Results

The results based on exponential strain energy function are demonstrated for 2—2 CC and IA arteries in Figs 2—5. The fitting of the model is illustrated at three different axial stretches. The changes of the three incremental Young moduli and of the three Poisson ratios are also demonstrated as the function of tangential strain (ε_θ).

The other three Poisson ratios are counterparts of those illustrated in the Figures (according to the notes given in Methods), so they are not demonstrated.

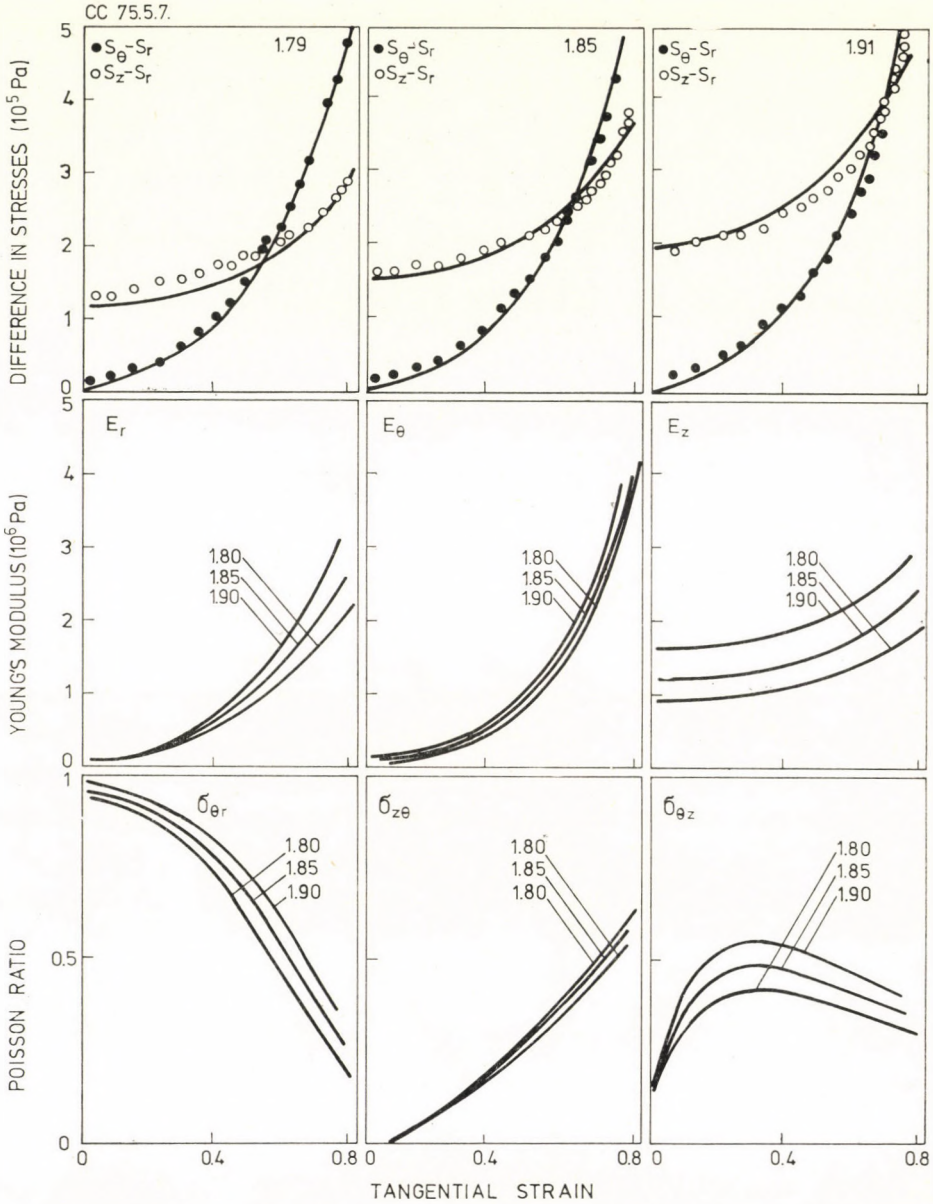

Fig. 2

Figs 2—5. Results of calculations by the use of the 4-parameter strain energy function based on mechanical measurements on the dog's isolated common carotid (CC) and iliac arteries (IA). The fitting of the exponential model is demonstrated in the three upper parts of the figure at three different initial vascular length values (axial stretch in the upper right corner). ● Measured tangential minus radial stress; ○ measured axial minus radial stress. Continuous

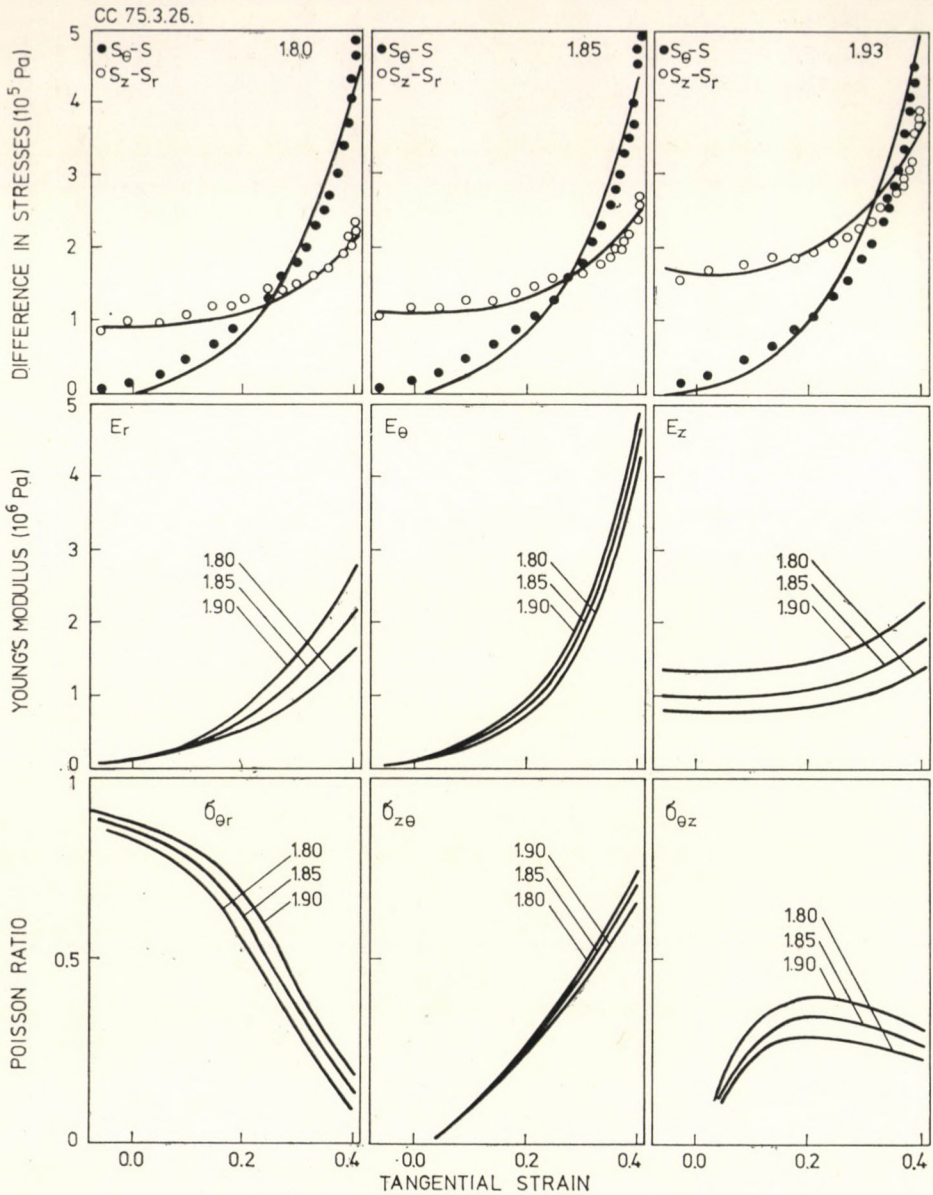

Fig. 3

line represents the corresponding values calculated from the exponential model. The calculated characteristics (at various axial stretches) of radial (E_r), tangential (E_θ) and axial (E_z) incremental Young moduli are illustrated in the medium part of the figure, while those of the corresponding incremental Poisson ratios are demonstrated in the lower part. Axial extension ratios are indicated besides the curves

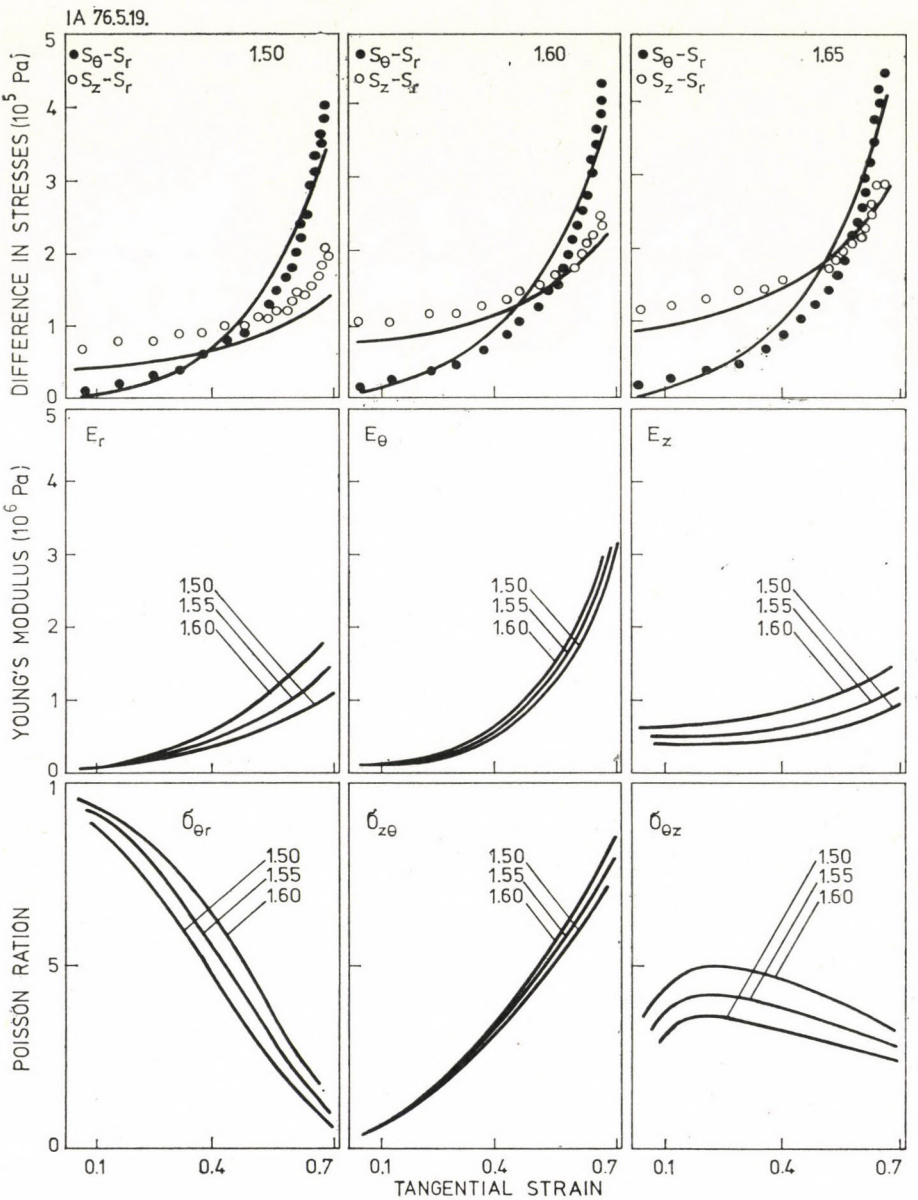

Fig. 4

The quality of the fitting was different in the individual arteries and generally they were better in the case of the carotid arteries. Individual variations were, however, more pronounced than the variations among the two types of arteries studied. Thus the carotid and iliac arteries have essentially

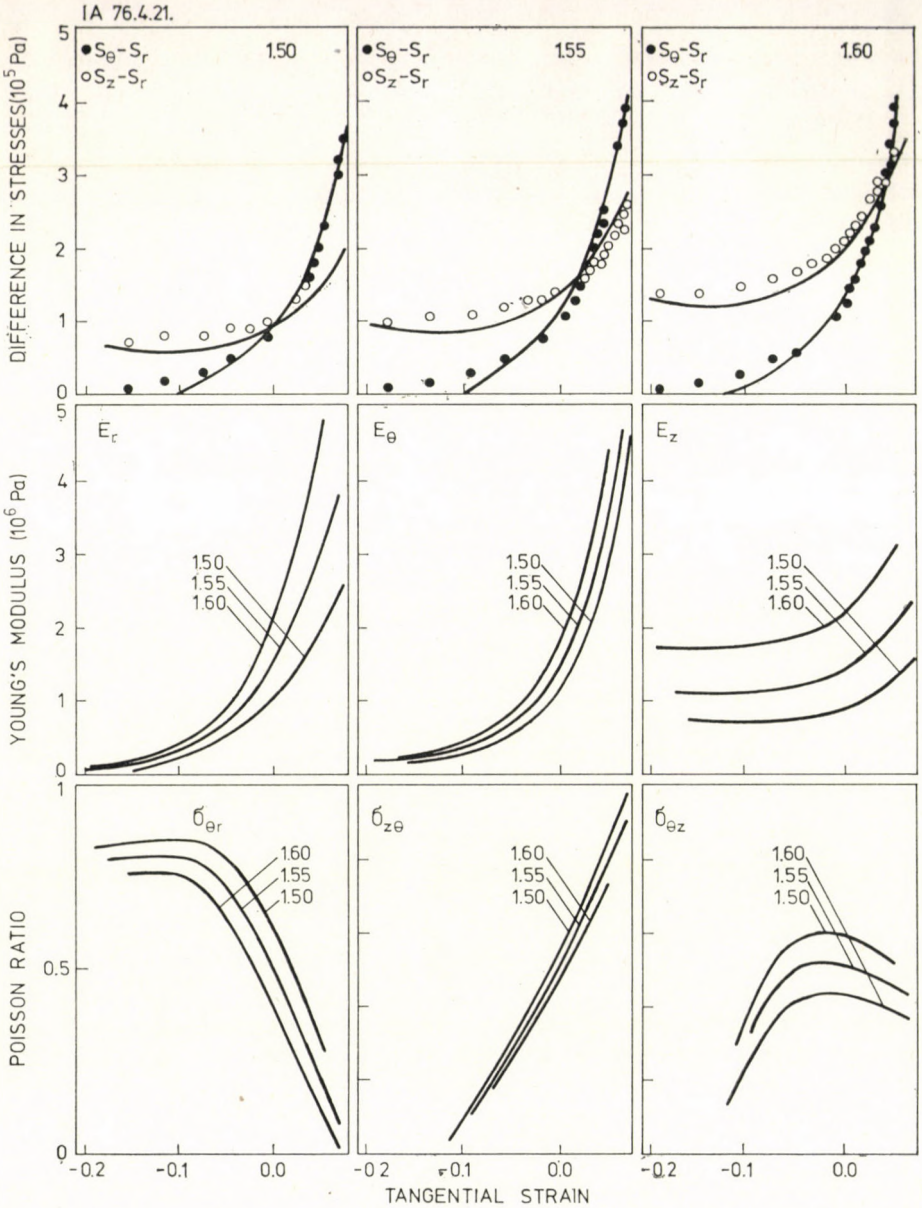

Fig. 5

similar incremental mechanical properties. The changes of both Young moduli and Poisson ratios indicate that the anisotropy of the vessels depends significantly on the initial deformation state. In the case of a small tangential strain $E_z > E_r \cong E_\theta$, i. e. axial elastic stiffness is maximum and the vessel wall is

approximately transversally isotropic in the θr plane. In the case of large tangential strain the tangential stiffness is dominating and generally $E_\theta > > E_r > E_z$: the artery is cylindrically orthotropic.

Poisson ratios represent the strength of mechanical coupling between incremental deformations in various directions. The values of $\sigma_{\theta r}$ demonstrate that in the case of small tangential strain the radial compression of the vessel wall induces mostly tangential stretch, that is an increase in vessel diameter. In the case of larger initial tangential strains radial compression induces axial stretch too, so that an increase in vessel length dominates if $\sigma_{\theta r} < 0.5$. The strength of coupling between tangential and axial directions is characterized by two Poisson ratios: $\sigma_{\theta z}$ and $\sigma_{z\theta}$, depending on the direction of the applied

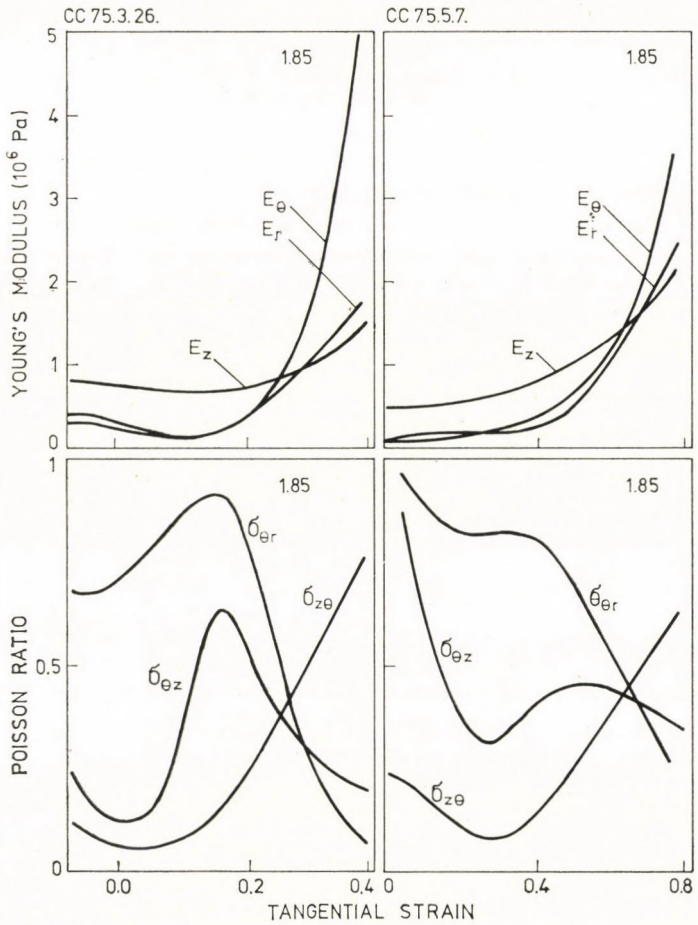

Fig. 6. Incremental Young moduli and Poisson ratios of two carotid arteries calculated by the 12-parameter polynomial strain energy function. The symbols are similar to those demonstrated in *Figs 2—5*

incremental stress. $\sigma_{z\theta}$ increases monotonically as the function of initial tangential strain, which indicates that changes of tangential deformation induce in an increasing proportion changes of the axial dimension rather than radial one.

In contrast, the changes of $\sigma_{\theta z}$ are markedly different, which means that the mechanical coupling between tangential and radial directions is not symmetrical.

A comparison of the computed curves at various initial axial stretches indicates further nonlinearities. The effect of changes in axial stretch influences primarily the values of radial and axial elastic stiffness. Interestingly, the influence on tangential stiffness is relatively small. The larger values of $\sigma_{\theta r}$ and $\sigma_{\theta z}$ at increasing axial stretch indicate the growing strength of mechanical coupling between the respective directions at a fixed initial tangential stretch. There are, however, no considerable differences between the various initial axial stretch levels if the $z\theta$ coupling ($\sigma_{z\theta}$), i. e. the axial influence of tangential deformation, is considered.

The models obtained by the application of polynomial strain energy functions did not prove to be so effective. In comparison to the 4-parameter exponential model, the fitting was considerably worse in the case of the 7-parameter polynomial model. Good fitting of the strains could be achieved by the 12-parameter polynomial model, but due to the large number of parameters, the elastic moduli, which could be expressed as the second derivatives of the strain energy function, became unstable. The instability was especially significant in the case of the Poisson ratios. This fact is demonstrated in Fig. 6 in the example of two carotid arteries. Due to these curve oscillations we do not deal with the polynomial models in details.

Discussion

The aim of the present study was to determine the incremental elastic moduli of the anisotropic vessel wall. Instead of direct calculations of the incremental moduli from the experimental data [28], they were determined by the use of strain energy functions, which generally gives more accurate results.

It was found that significantly poorer results were obtained by the use of polynomial strain energy functions [37] than by the application of the exponential energy function. Several authors have used models containing exponential function for the description of nonlinear elastic properties of vessels and other biological tissues [4, 9, 11, 12, 15, 23, 34, 35, 38]. In the majority of these studies, except [12], however, the cylindrical anisotropy of the arteries was not taken into consideration, the arteries were regarded as isotropic, and three-dimensional measurements were lacking. Thus, the three incremental Young moduli and the six Poisson ratios of the vessel wall could not be evaluated

simultaneously in any of the above mentioned studies. The exponential strain energy function, used by us, characterizes the elastic properties of the cylindrically orthotropic and incompressible vessel wall by a minimum number of elastic constants. A similar exponential model was proposed by FUNG [12] recently. Additionally, the generally good fitting of the exponential function to the three-dimensional experimental data allowed the evaluation of incremental moduli and Poisson ratios over a comparatively wide deformation range.

Our results can be compared to those obtained by direct incremental measurements or by the application of polynomial strain energy functions. According to our calculations, at tangential strain levels corresponding to the physiological or somewhat higher pressure range *in vivo*, the anisotropy of the vessel wall is characterized by $E_\theta > E_z$, which agrees well with previous data obtained on carotid arteries by various methods [1, 7, 10, 16]. The incremental moduli of the carotid and iliac arteries show similar values and similar changing tendencies. Other arteries, for example the aorta [2, 28, 29, 36] have anisotropic properties different from those of the carotid artery. Only COX [8] evaluated the incremental moduli of the vessel wall in such a wide pressure range which makes a relevant comparison possible. COX used the method of VAISHNAV et al. [37] for his calculations, and he found similar results to our ones on dog carotid arteries. One considerable difference, however, existed in the values and behaviour of the Poisson ratios $\sigma_{\theta z}$, since COX reported on a continuous decrease in the whole tangential strain range. The difference may originate from the differences between the exponential and polynomial energy functions. Since the second derivatives of the exponential strain energy functions are generally smoother than the derivatives of the polynomial functions (see the Poisson ratios in Fig. 6), we think that the results obtained by the exponential model are more reliable.

We found that the anisotropy of the examined arteries depends on the initial deformation state. Tangential stretch influences primarily the radial and tangential Young moduli and the values of the Poisson ratios $\sigma_{\theta r}$ and $\sigma_{\theta z}$. Axial stretch induces major changes in the axial and less in the radial modulus as well as in the corresponding Poisson ratios. From the physiological point of view it is worthwhile to mention that axial stretch exerts a minimum influence on the tangential incremental modulus of the vessel wall, which is one of the most important parameters determining arterial distensibility.

As mentioned in the Introduction, smooth muscle contraction has a considerable effect on the mechanical properties of arteries, so under conditions *in vivo* it may influence the results obtained *in vitro*. Due to the existing smooth muscle tone, mechanical hysteresis of the vessel wall cannot be neglected under these conditions and the use of dynamic methods is necessary for the determination of the frequency-dependent incremental moduli and

Poisson ratios [29]. The aim of the present study was, however, to obtain information about the passive structure of the vessel wall, which is most probably not influenced very much by the dynamics of the mechanical load.

Our results suggest the existence of a specific fibre structure, that allows an asymmetric coupling between the θ and z directions (see the different behaviour of $\sigma_{\theta z}$ and $\sigma_{\theta z}$). This finding agrees with the hypothesis that the collagen fibres of the vessel wall form a network-like structure [3]. Thus, in the case of increasing tangential strain, the fibres of the network come closer to the tangential direction and they may produce an asymmetric coupling depending on the initial deformation state. Taking into consideration that the number of stretched fibres is a function of the tangential strain [33], this network model may adequately reflect the passive, non-linear, anisotropic elastic properties of the vessel wall. A new model of vessel wall structure has been proposed by us to support the network hypothesis. Since the Poisson ratios determined by this model and the direction of their change as the function of tangential strain are similar to those calculated by the exponential strain energy function, the validity of the collagen network hypothesis is highly probable. The mathematical description of this network model and the results of the calculations will be published elsewhere [20].

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RENORENAL VASOMOTOR REFLEX

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The acetylcholine induced contralateral renorenal vasomotor reflex has been re-examined. In dogs under pentobarbital anaesthesia blood flow of the contralateral kidney as measured by electromagnetic flow probe did not change after an infusion or bolus injection of Ach. Basal sympathetic nervous activity of the contralateral kidney increased only slightly and these changes correlated with the decrease of arterial blood pressure. In conclusion, the renorenal vasomotor reflex could not be evoked under the present experimental conditions.

Several experimental data show that functional alterations in one of the kidneys, induced by some external factor(s), were soon followed by modifications of the function of the other kidney. Acute unilateral occlusion of the renal artery increases sodium excretion and diuresis in the contralateral kidney [9]. Denervation of the kidneys in volume expanded rats increased sodium and water excretion on the same side, but decreased these parameters in the contralateral kidney [1]. Similar results were observed by FRANCISCO et al. [4] who monitored the activity of sympathetic nerves innervating the contralateral kidney. They found that sympathetic activity was increased after denervation indicating the existence of a neural reflex connection between the two kidneys. This assumption was emphasized by the experiments of CALARESU et al. [2], who found that electrical stimulation of renal afferent nerves on the one side caused a reflex response of the efferent nerves on the other side. The short latency period indicated that the reflex was of spinal origin.

A renorenal vasomotor reflex was demonstrated by MACFARLANE [5.] Vasodilatation in one of the kidneys after intrarenal injection of acetylcholine (Ach) caused vasoconstriction in the contralateral kidney. The applied method (silicon rubber filling, fixation, visual examination and evaluation of microscopic sections), did not, however, allow a detailed analysis of blood flow values. Some authors could demonstrate [3, 8] but others could not verify, the existence of the renorenal reflex. This has prompted us to study the problem by various techniques.

Measuring renal blood flow by electromagnetic flow probes was designed to analyse whether the infusion of acetylcholine into one of the kidneys caused any change in blood flow or sympathetic activity of the contralateral kidney.

Methods

The experiments were carried out on mongrel dogs of both sexes. Renal blood flow was measured in six large dogs (12.5 to 24 kg body weight) and sympathetic activity was monitored in four smaller dogs (6 to 13 kg body weight). The animals were anaesthetized by an intravenous dose of 25 mg/kg b.w. pentobarbital (Serva, Heidelberg, GFR), with additional doses given as needed to maintain a constant level anaesthesia. The dogs were immobilized by 2 mg/kg b.w. gallamine (Flaxedyl, Specia, Paris, France) and ventilated artificially. Repeated doses of gallamine were injected when signs of spontaneous breathing were observed. The parameters of ventilation were adjusted so as to maintain the blood pressure observed during spontaneous breathing. After tracheotomy, a femoral artery and vein were cannulated for continuous monitoring of arterial blood pressure and for infusion. Renal arteries were approached retroperitoneally.

The experiments were divided into two groups. In group I, the blood flow of both kidneys was recorded, while in group II, renal sympathetic activity was monitored as well.

For blood flow measurements, 2 to 3 cm segments of both renal arteries were prepared and care was taken not to damage the nerves running next to them. Flow probes were placed around the arteries. Blood flow was measured by Nycotron Type 1603 flow probes and by a two-channel Nycotron equipment (Oslo, Norway).

Since the experiments were aimed at recording blood flow changes and not absolute values of renal blood flow, the flow probes were not calibrated *in situ* but rather the calibrating factors recommended by the makers were used in the calculations. Zero line was recorded during brief episodes of vessel occlusion.

A small needle was inserted into the left renal artery proximal to the flow probe. The needle was connected to a polyethylene cannula and it served for isolated perfusion of the left kidney without disturbing blood flow. A slow rate perfusion of Ach was accomplished by an Infamat pump (KUTESZ, Budapest, Hungary).

In the experiments where sympathetic activity was recorded, the needle was inserted into the right renal artery and a 2 to 3 cm long segment of a nerve running parallel with the left renal artery was prepared and placed on a bipolar platinum electrode. In order to avoid exsiccation the preparations and registrations were performed under paraffin oil. Nerve activity was gained by a 1623 C/D Type amplifier (Műszeripari Kutató Intézet, Budapest, Hungary), with 20 to 40 μV/V.

Evaluation of nerve activity was based on the time integral of the spike amplitudes. This value was derived by an electronic circuit developed in our institute, which bilaterally rectified the input signal, and the new signal was then passed through a low-pass filter having a time constant of 2 sec. This procedure resulted in a smooth signal suitable for recording.

Mean arterial blood pressure, renal blood flow and sympathetic nerve activity were recorded on a B 381 recorder (Rikadenki, Tokyo, Japan). In the majority of the experiments the parameters were stored by a digital data collector equipment (MIKI, Budapest, Hungary) and by the end of the experiments they were recorded on magnetic tape. Accuracy of the measurements was 8 bits data sampling was done every 5 seconds.

Evaluation of the data and statistical analysis (paired Student's t test, regression analysis) were carried out on a Cellatron C 8205 computer (Zella-Mehlis, GDR).

Experimental procedure

The left kidney in group I and the right kidney in group II (perfused kidneys) were perfused by a diluted solution of Ach. The infused fluid volume was less than 1% of the basal blood flow. Infusion of the same amount of physiological salt solution (no Ach) proved that infusion *per se* did not influence renal blood flow.

Infusion in group I lasted for 60 seconds, while in group II it lasted for 120 seconds. The longer perfusion time in the latter group was used because in the absence of blood flow measurement the time when the Ach had reached the kidney could not be detected directly. The infused Ach doses were, as recorded for the chloride salt of acetylcholine, 0.6—1.0—2.5——5.0 μg/kg body weight/min; and a bolus injection of 5.0 μg/kg body weight given in less than 5 sec. The sequence of the infused doses was randomized.

Mean blood pressure and blood flow values 1 min before beginning the infusion were regarded as controls and data obtained during and 2 min after perfusion were compared to

these values of 2—2 min periods before, during and after, the perfusion were compared and the values obtained before the beginning of perfusion were regarded as control.

The dependence of the increase of nervous activity on the decrease of blood pressure during infusion was analysed by linear regression: the relative change of nerve activity was illustrated as the function of the relative decrease of blood pressure. Calculation of the regression line was based on all corresponding data pairs irrespective of the Ach dose applied.

The mean and standard error of mean (SEM) are given in the Tables.

Results

Experimental results will be listed in the sequence of increasing Ach doses, i. e. 0.5—1.0—2.5—5.0 μg/kg body weight/min.

Figure 1 illustrates typical blood pressure and blood flow curves. After the infusion of the highest Ach dose, mean arterial blood pressure decreased slightly for a period of 10 to 20 seconds and then returned to the control level. Blood flow of the perfused kidney increased considerably during the infusion (in the individual experiment demonstrated in Fig. 1 blood flow increased to 165 % of the control) and returned to the control level 2 to 3 min after the infusion had been terminated.

The decline of flow consisted of two phases: a rapid phase having a time constant of 5 to 10 sec and a slower phase with a time constant of 1 to 2 min. Blood flow of the contralateral kidney did not change during perfusion of the left kidney. Small transient changes were observed due to blood pressure fluctuations, but these variations could not be detected in the one minute mean values.

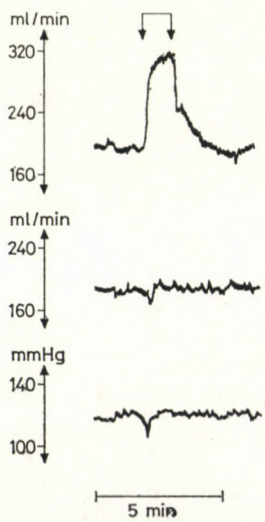

Fig. 1. Typical blood pressure and blood flow curves after intrarenal infusion of 5.0 μg/kg body weight/min of acetylcholine. Upper curve: left kidney; middle curve: right kidney; lower curve: blood pressure. Acetylcholine infusion is indicated by arrows

Table I demonstrates the experimental results obtained in group I. Mean arterial blood pressure did not change during infusion of the lowest Ach dose, but decreased slightly when higher Ach doses were injected (2.5—3.9—3.0 mm Hg, respectively). The change was not significant statistically in the case of 2.5 μg/kg body weight/min Ach, due to the large individual variation of the data. Acetylcholine infusion increased the blood flow of the perfused kidney significantly in a dose-dependent manner (147, 157, 177 and 186 %, respectively) but blood flow in the contralateral kidney did not change. Blood flow of the

Table I

Arterial blood pressure and renal blood flow as the function of injected acetylcholine doses and of time

Dose, μg/min	Time, min	Blood pressure, mm Hg	Left kidney, ml/min	Right kidney, ml/min	n
	C	129.5±5.6	6.19±0.71	6.67±0.62	
0.5	1	129.2±6.0	8.91±0.85 ♂ ○	6.68±0.58	9
	2	129.0±6.1	7.13±0.56 ○ ○	6.70±0.57	
	3	129.2±6.4	6.34±0.65	6.63±0.62	
	C	132.0±5.4	6.62±0.69	7.19±0.74	
1.0	1	129.4±5.0 ○	10.11±0.83 ♂ ♂	7.15±0.74	11
	2	129.7±5.1 ○	7.95±0.52 ○ ○	7.22±0.71	
	3	130.2±5.3	7.10±0.69 ○ ○	7.16±0.71	
	C	130.5±4.8	6.39±0.70	7.17±0.66	
2.5	1	126.6±5.1	11.01±0.96 ♂ ♂	7.27±0.72	10
	2	130.5±5.2	8.27±0.74 ○ ○	7.20±0.65	
	3	132.1±5.3	7.06±0.65 ○ ○	7.02±0.65	
	C	129.5±6.5	5.59±0.50	6.44±0.67	
5.0	1	126.5±7.2 ○	10.28±1.00 ♂ ♂	6.49±0.61	8
	2	127.5±7.4	7.91±0.45 ○ ○	7.03±0.59	
	3	128.0±6.9	6.70±0.57 ○ ○	6.59±0.61	
Bolus	C	119.8±6.0	5.95±0.40	5.61±0.55	
5.0	1	101.3±5.2 ○ ○	9.38±0.47 ♂ ♂	5.11±0.49	7
	2	119.1±5.9	6.65±0.69	5.47±0.62	

Acetylcholine doses and blood flow values are related to 1 kg body weight.
C = control values measured before the injection of Ach.
Symbols of significance: $p < 5\% < 1\%$
Left *vs.* right renal blood flow + ++
1 or 2 or 3 min *vs.* control (C) on the same side ○ ○ ○

left kidney returned to or near to the control level 2 min after the infusion had ended (103, 108, 113, 121 %).

The bolus injection of 5 μg/kg body weight of Ach decreased mean arterial blood pressure considerably (18 mm Hg) for a short period of time. The intensive vasodilatation in the left kidney (blood flow increased to 159 % of the control) was accompanied by a decrease of flow in the right kidney (91 %). After blood pressure has normalized, blood flow of the right kidney also returned to the control level.

Results obtained in group II are demonstrated in Table II and Fig. 2. Similarly as in group I, intrarenal infusion of Ach caused a slight but statistically significant decrease of mean arterial blood pressure. The extent of blood pressure drop in the function of the Ach dose was 0, 0.7, 3.8, 4.3 mm Hg. Blood pressure returned to the control level 2 min after the end of the infusion. Sympathetic activity changed only slightly (101.1, 103.4, 104.4, 107.3 %, respectively) and the increase was statistically significant only in the case of the lowest Ach dose.

In order to clarify whether the increase of sympathetic activity correlated with the decrease of blood pressure or else a reflex action independent of the

Table II

Arterial blood pressure and relative nerve activity as the function of injected Ach doses and of time

Dose, μg/min	Time, min	Blood pressure, mm Hg	Relative nervous activity, per cent	n
	C	122.8±1.1	100	
0.5	1—2	122.8±1.4	101.1±0.4+	7
	3—4	123.1±1.4	98.6±0.8	
	C	116.3±2.9	100	
1.0	1—2	115.2±2.8+	103.4±2.4	11
	3—4	116.4±3.0	101.5±1.4	
	C	118.1±3.4	100	
2.5	1—2	114.3±4.7+	104.4±2.7	10
	3—4	117.2±3.9	100.1±1.4	
	C	118.7±2.1	100	
5.0	1—2	114.3±2.2++	107.3±4.5	12
	3—4	117.2±2.2	99.0±2.0	

Ach doses are related to 1 kg body weight.
C = control values before injection
Symbols and levels of significance $p < \underset{+}{5\%} < \underset{++}{1\%}$
1—2, or 3—4, *vs.* control (C)

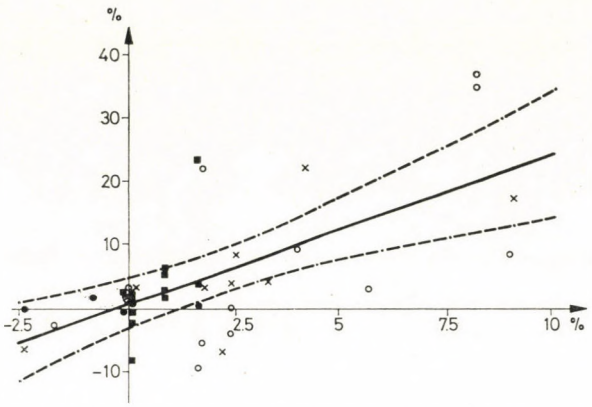

Fig. 2. Correlation between decrease of blood pressure and increase of nervous activity on the basis of data obtained in group II. The decrease of blood pressure corresponds to positive values on the abscissa. Symbols: 0.5 μg/kg b.w./min ⊙; 1.0 μg/kg b.w./min □; 2.5 μg/kg b.w./min; 5.0 μg/kg b.w./min ○

blood pressure also played a role in the changes observed, a regression line and correlation coefficient were calculated on the basis of the corresponding relative blood pressure drop and the increase in nerve activity (Fig. 2). All corresponding data pairs were used in the calculations irrespective of the applied dose of Ach.

The equation of the regression line was,

$$y = 0.42 + 2.41x \qquad r = 0.65 \qquad n = 40$$

where:

y = per cent increase of sympathetic nerve activity

x = per cent decrease of blood pressure

r = correlation coefficient

Correlation coefficient indicates a significant correlation between the two variables ($p < 0.01$) and the crossing point of the regression line on the y axis was not significantly different from zero.

Discussion

The aim of the present experiments was to re-examine by electromagnetic blood flow measurements the renorenal vasomotor reflex described by Mac Farlane [5] and verified later by Williams and Maines [8] and Chrysant and Lavender [3]. In the experiments done on anaesthetized dogs, beside measuring the blood flow of both kidneys, sympathetic nervous activity of the non-perfused kidney was also monitored. The renorenal reflex could not be demonstrated under the given experimental conditions: the vasodilatation in the Ach perfused kidney had no effect on the blood flow of the contralateral

kidney. Sympathetic nervous activity in the non-perfused kidney showed very slight and physiologically negligible changes, which correlated significantly with the decrease of mean arterial blood pressure.

The possible reflex connection between the two kidneys was studied by several authors. CALARESU et al. [2] demonstrated a reflex response of sympathetic efferent nerves in the contralateral kidney after electrical stimulation of the afferent nerves in the other kidney. The reflex response depended on the stimulus parameters, i. e. on the number and type (A and/or C) of stimulated afferent fibres. The physiological significance of this reflex is not known.

BELLO-REUSS et al. [1] performed unilateral kidney denervation in volume expanded rats. Denervation is well known to lead to increased sodium and water excretion in the denervated kidney, while these parameters show a decrease in the intact contralateral kidney. As a possible explanation it was suggested that the cessation of afferent nerve impulses due to denervation led to a decrease of the function of the contralateral kidney. These results were supported by the observations of FRANCISCO et al. [4] who monitored the sympathetic activity of the right kidney before and after denervation of the left kidney in volume expanded rats. Denervation of the left kidney led to a considerable increase of sympathetic activity in the right kidney with a concomitant decrease of sodium and water excretion without any change in GFR.

These data were indicative of a possibility of a reflex connection between the two kidneys. Our experimental results, however, showed that vasodilatation in the left kidney induced by intrarenal infusion of acetylcholine did not cause a reflex vasoconstriction in the contralateral kidney.

MACFARLANE [5] studied renal blood vessels after silicon rubber (Microfil) filling and examined the microscopic sections prepared after fixation. The bolus injection of 5 μg/kg body weight of acetylcholine (similar to that used in the present experiments) caused vasodilatation in the perfused kidney and a considerable vasoconstriction in the contralateral kidney. Since denervation of any of the kidneys eliminated this response, MACFARLANE [5] suggested that the reflex was of neural origin. Similar results were observed by CHRYSANT and LAVENDER [3] who measured renal blood flow by the PAH technique. The clearance method was used by WILLIAMS and MAINS as well [8], and they found that parallel with vasoconstriction a decrease of water, sodium and potassium excretion develops in the contralateral kidney.

In contrast with these observations and in agreement with our observations, ROBIE [6] reported on negative results. Intrarenal infusion of 50 μg/min Ach did not cause any change in the electromagnetically determined blood flow of the contralateral kidney.

Negative results were obtained by ROSIVALL et al. [7] too, who measured renal blood flow by electromagnetic flow probes and ^{133}Xe-washout and produced a 100 to 200 ng/ml Ach concentration in renal arterial blood.

The cause of the difference between the various experimental results is not known. In the case of MACFARLANE's [5] experiments, his interpretation might have been erroneous since the method used by him allowed to measure renal blood volume rather than blood flow. Blood volume may change without any decrease of the blood flow.

ROBIE [6] suggests that Ach induces changes in vascular and/or tubular PAH transport. This explanation may not be valid, since controversial changes occurred in the non-infused kidney.

On the basis of the present experiments we believe that a more specific stimulation of the renal afferent nerves is needed for the study of renorenal reflex connections.

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EFFECT OF RENAL DENERVATION ON FREE FLOW PROXIMAL TUBULAR POTENTIAL DIFFERENCE IN THE RAT

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Proximal transtubular potential difference (PD) was measured using the semi-microelectrode technique in control (C, n=10) and unilaterally denervated (D, n=10) rats. Acute renal sympathectomy resulted in a twofold and fourfold increase in diuresis and sodium excretion, respectively, with no change in GFR. PD (mean±S.E.M.) in the earliest accessible proximal convolutions (EPT) of group C was +0.27±0.08 mV (n=16), while in group D it was −0.16±0.07 mV (n=18) ($p < 0.01$). PD in mid (MPT) and late (LPT) proximal segments was unchanged by denervation (MPT: C = 0.94±0.05, n=21; D = 0.98±0.04, n=19 NS. LPT: C = 1.04±0.11, n=17; D = 0.95±0.06, n=18 NS.). The shift to the negative of PD in EPT caused by denervation lends support to our earlier suggestion that active transport of solutes and organic substances is depressed by sympathectomy in the proximal tubule. The unaltered PD in MPT and LPT seems to indicate that passive forces promoting reabsorption in this part of the nephron are probably preserved.

It is well documented that renal denervation in the anaesthetized dog and rat leads to a considerable reduction of proximal tubular volume absorption [4, 9]. However, the mechanism by which renal nerves affect the proximal reabsorption is not known. Since the urinary excretion of a number of actively reabsorbed solutes such as Na, HCO_3, glucose, PAH and inorganic phosphate (Pi) increases after denervation, a general decrease in the proximal tubular transport processes seems to be a plausible explanation [15, 16, 17, 18]. This possibility is supported by the finding that the ratio of sodium reabsorption to oxygen consumption was unchanged after denervation in the dog kidney [14]. However, there is no direct proof to yield unequivocal evidence that active transport is indeed affected by denervation.

The recent technical progress in electrophysiological methods has made possible the reliable measurement of potentials below 0.5 mV [1, 2, 6]. With this sensitivity of potential measurement FRÖMTER and GESNER were able to detect active transport potentials in the rat proximal tubule [8]. The electrophysiological approach offers a useful means to determine proximal transtubular free flow potential differences (PD), and to correlate the possible changes with the known transport alterations that take place after kidney denervation.

In the present experiments we measured the PD in proximal tubules of innervated and denervated rat kidneys. It was found that in the first accessible convolutions of the proximal tubule positive PD became negative on denervation, while no change in mid and late segments could be detected.

Methods

Experiments were carried out on 20 male CFY rats (LATI, Gödöllő, Hungary) anaesthetized with Inactin (100 mg/kg b.w. i.p.). The animals were placed on a heated table and body temperature was kept constant at 37 °C. After tracheotomy the right jugular vein, a femoral artery and both ureters were catheterized for infusion, blood sampling and blood pressure measurements and for urine collection. The left kidney was exposed according to the method of GOTTSCHALK and MYLLE [10] and was embedded in agar/saline. The kidney surface was bathed with physiological saline.

In one group of the animals (D, n=10) acute denervation of the left kidney was performed, another served as control (C, n=10). Denervation consisted of left splanchnicotomy and the adventitia from the left renal artery was stripped with a fine forceps.

After completion of surgery an infusion of physiological saline containing ^{3}H-Inulin was started at a rate of 20 μl/min. About the end of a 60 minute equilibration period early (EP), mid (MP) and late (LP) proximal tubular segments were selected by the i.v. injection of 50 μl of 5% lissamine green solution and measuring the transit time (TT). Early and late proximal segments were identified as those where the dye first appeared on and disappeared from the surface, respectively. Mid proximal convolutions were considered as those with a TT between 30 and 70% of endproximal TT. Experiments with more than 18 sec total proximal tubular TT were discarded.

After the equilibration two clearance periods of 20—30 minute duration were performed. During this time urine was collected, at the beginning and the end of the periods arterial blood samples were taken to determine urine flow, GFR and sodium excretion. During the clearance periods PD of the identified tubules was measured with the semi-microelectrode technique as described by BARRATT et al. [1]. In brief: conventional glass capillaries (OD, 1.0 mm) were pulled and grinded to have a tip OD of 3—5 μm. The electrodes were filled with 3 M KCl solution stained with 0.4% lissamine green. The lucite holder of the electrode was connected to a pump so that the pressure in the electrode could be controlled. The cut end of the rat's tail immersed into physiological saline served as reference potential. The connections to the electrometer (Type 2015, MIKI, Budapest) were made by Ag/AgCl electrodes. The resistance of the electrodes was in the range of 600—800 kOhm, and the asymmetry in the measuring system was usually less than 2 mV.

At the beginning of the measurement a small positive pressure was adjusted to the electrode so that KCl was continuously flowing out from the electrode and a stable "0" recording was made. Then the tubule was punctured. The proper position of the tip in the lumen was checked visually and was verified by a rapid inflow of tubular fluid into the electrode. By increasing the pressure in the electrode this inflow was immediately stopped, and the tubular fluid/KCl interface could be held in a nearly constant position inside the electrode. This was achieved by small pressure adjustments, and was controlled visually. The PD was recorded for 2—3 minutes, then the electrode was retrieved, tubular fluid was pushed out and a second "0" recording was made. The PD was accepted only when the differences between the two "0" recordings did not exceed 0.3 mV. For smaller differences the mean of the two recordings was regarded as "0". The electrode was replaced when its resistance increased or any visible material appeared in the tip.

Radioactivity of plasma and urine samples was measured in a Nuclear Chicago Isocap 300 liquid scintillation counter, sodium concentration was determined in a Flapho 4 (Zeiss, Jena, GDR) flame photometer. Statistical differences were evaluated by Student's paired and unpaired t test.

Results

Clearance data of both groups are presented in Table I. In the denervated kidneys of group D diuresis was increased twofold, sodium excretion fourfold while there was no difference in the GFR between the two sides. In the control

group no differences were found between the right and left kidneys in any of these parameters. The mean tubular transit time to early segments was 2.2 s and 2.0 s, to mid segments 6.7 s and 6.6 s for groups C and D, respectively, there being no difference between control and denervated kidneys. The total proximal transit time was somewhat shorter in denervated kidneys (14.1 *vs* 12.8 s). The PD in the early segments was +0.26 mV in the control group, and it was significantly decreased to —0.16 mV in the denervated kidneys. There was no difference between the mid proximal and late proximal values (Table II).

Table I

Urine flow (V), sodium excretion ($U_{Na}V$) and GFR in control (C) and unilaterally denervated (D) rats

	C (n=10)	D (n=10)
V, µl/min/g	R: 1.75±0.20 L: 1.86±0.20	Inn: 1.64±0.19 Den: 3.84±0.40+
GFR, µl/min/g	R: 1419±104 L: 1394±94	Inn: 1405±71 Den: 1455±118
$U_{Na}V$, nEq/min/g	R: 142±40 L: 140±46	Inn: 77±32 Den: 309±61+

Values are means ± SE; n = number of experiments; R = right, L = left kidney of control rats; Inn = innervated; Den = denervated kidney of denervated rats; + = $P < 0.05$ I *vs.* D

Table II

Proximal tubular potential differences (PD) and transit time (TT) in control (C) and denervated kidneys (D)

		EP	MP	LP
PD, mV	C:	0.27±0.08 (n=16)	0.94±0.05 (n=21)	1.04±0.11 (n=17)
	D:	−0.16±0.07 (n=18)	0.98±0.04 (n=19)	0.95±0.06 (n=18)
	P	0.01	NS	NS
TT, s	C:	2.2±0.1 (n=16)	6.7±0.4 (n=27)	14.1±0.4 (n=17)
	D:	2.0±0.1 (n=18)	6.6±0.3 (n=19)	12.8±0.3 (n=18)
	P	NS	NS	0.05

Values are means ± SE; n = number of measurements; EP = early proximal; MP = mid proximal; LP = late proximal tubules

Discussion

The results of the present experiments provide some new data about the proximal tubular effects of renal denervation. The well kown decreases in transport processes after denervation are in line with the observed negative shift of early PD, while that in the later proximal segments remains unaltered.

The free flow PD in the rat kidney proximal tubule shows a characteristic profile [7, 12]. Starting at values around 0 mV in the Bowman's capsule it becomes negative reaching —1.8 at a distance of 0.4 mm from the glomerulus, and after a rapid increase it reaches a nearly constant value of about +1.8 mV. The potential "profile" measured at only three points in our experiments fits well into this pattern. Since in our colony of rats the first convolutions of the proximal tubule are inaccessible to puncture, we could not measure at the negative peak. However, in the first accessible loops the PD was significantly lower than in the later ones. The PD value measured in the mid, and late proximal segments was about +1 mV. This is in the range of earlier measurements made by others (+0.5 to 2.8 mV) [1, 3, 7, 11, 12, 13]. On the other hand, some small potential shift could be caused at the diffusion surface of the rat's tail (extracellular fluid immersed into saline). The relatively wide range of PD value reported can be attributed to differences in the measuring techniques, and might also be ascribed to strain differences. An explanation of the existing potential profile along the proximal tubule was given by FRÖMTER and GESNER [8] and SEELY and CHIRITO [12]. According to them the negativity in the EP segments is the result of a prevailing Na^+ reabsorption coupled to amino acid and glucose transport [8, 12]. Also the H^+ secretion/HCO_3^- reabsorption may have measurable influence on the potential in this segment [8]. With decreasing amino acid, glucose and HCO_3^- and increasing Cl^- concentration the tubular PD becomes more and more positive, and the positivity in the later segments is solely attributed to a Cl^- diffusion potential [8, 12]. Our earlier studies on dogs proved that both glucose transport maximum and glucose threshold were depressed in the denervated kidney [17]. Based on these results it seems probable that the negative shift after denervation in our experiments is caused by the diminished glucose and HCO_3^- reabsorption. Since glucose is almost completely reabsorbed in the first 30 % of the PT, the depression by denervation in this portion of the PT would increase the load to later segments [5]. The excess glucose that escaped reabsorption in the early loops was probably reabsorbed in the later segments causing a negative shift in PD.

A depression of H^+/HCO_3^- transport was also demonstrated in our previous experiments on dog by SZALAY et al., (unpublished results) and also by others [18]. This change can delay the increase in the TF/P ratio of Cl^-, and might cause a negative shift, too. Since we found no change in the PD of mid and late PT segments after denervation, our results suggest that the Cl^- diffusion potential was unaltered. Although a decreased LP Cl^- concentration with an increased Cl^- permeability could establish an unaltered PD, a change in the LP Cl^- concentration seems unlikely in the light of our preliminary experiments showing an unaltered TF/P chloride ratio after denervation (innervated, 1.25 ± 0.03 n $= 10$; denervated, 1.25 ± 0.03, n $= 10$).

In conclusion, our results seem to support previous data about depression of the transport of organic substances and probably H^+ secretion in the early proximal tubular segments after denervation. As there is no change in PD and Cl^- concentrations in the later segments, passive forces promoting reabsorption of salt and water are probably preserved.

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PULMONARY AND SYSTEMIC CIRCULATORY RESPONSES ELICITED BY HYPEROSMOTIC SOLUTIONS INJECTED INTO THE BRONCHIAL ARTERY

By

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In open chest anaesthetized dogs the haemodynamic effects of solutions of equal hyperosmolarity (*viz.* $NaHCO_3$ 8%, NaCl 5.6%, and glucose 34.3%, solutions) given into the bronchial artery were studied. Administration of any of these solutions directly into the bronchial artery resulted in increased cardiac output, stroke volume, bronchial blood flow, and bronchial fraction of the cardiac output, and decreased heart rate and bronchial as well as pulmonary vascular resistances. When given into the pulmonary circulation, the same solutions evoked similar reactions of smaller magnitude. To exclude the effect of major surgical trauma and the open-chest condition, another experimental model closer to the physiological situation was also developed. In this preparation $NaHCO_3$ failed to produce the above haemodynamic response even when given into the bronchial artery. After a one-hour bleeding period resulting in a drop of arterial blood pressure to 40 mmHg, while using the same preparation, the administration of $NaHCO_3$ solution into the bronchial artery caused a significant rise in blood pressure in both the systemic and pulmonary arteries. In these experiments a correlation was found between arterial oxygen tension and the extent of change in blood pressure. The exact mechanism of action of the observed haemodynamic changes is still not clear. However, it is likely that receptors localized in the area of the bronchial circulation and sensitive to hypoxia might have played a role in the development of the haemodynamic effects described.

In previous experiments devoted to the bronchial circulation it was found that the injection of an 8 % solution of $NaHCO_3$ into the bronchial artery resulted in marked increases in systemic and pulmonary arterial blood pressure and cardiac output [38]. These effects were much less marked than those evoked by similar injections given into the pulmonary artery. In addition, the effects of NaCl and glucose solutions of identical osmolarity given into the bronchial artery were significantly smaller when compared to those of the $NaHCO_3$ solution. Injection of TRIS buffer, used as a control to exclude pH effects, given either into the bronchial or pulmonary circulation was without any significant effect on haemodynamics.

Since $NaHCO_3$ is frequently applied for the correction of acidosis and we are aware of no similar haemodynamic studies, it seemed worth to study the effects of $NaHCO_3$ and other hypertonic solutions on several haemodynamic parameters in an attempt to assess the role of bronchial circulation in these effects, thus gaining more definite information on their mechanism of action.

Methods

The present experiments were performed in two series. In the first one (*Group 1*) dogs with open chest were studied (direct investigation of the bronchial circulation). In the second series (*Group 2*) dogs were studied with closed chest (indirect investigation of the bronchial circulation) under conditions of a) normovolaemia and b) haemorrhagic hypovolaemia.

In *Group 1*, 12 mongrel dogs of either sex with a body weight of 8 to 17 kg were used. To study the bronchial circulation, the dogs were anaesthetized (30 mg/kg^{-1} pentobarbital intravenously) and injected with heparin (500 IU kg^{-1}). During intermittent positive pressure breathing (ensuring a tidal volume and breathing frequency of 300—350 ml and 16—18 min^{-1}, respectively) the preparation described [37] earlier was made (Fig. 1). In brief, part of the aorta corresponding to the origin of the broncho-oesophageal artery was ligated

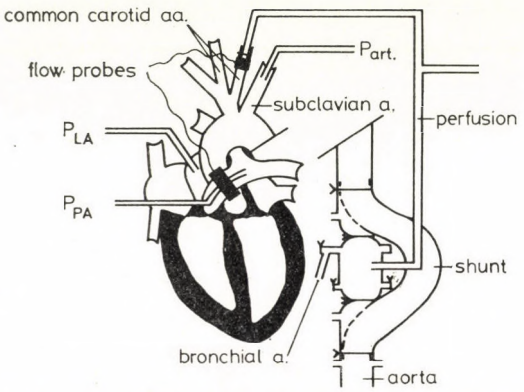

Fig. 1. A schematic diagram of the experimental preparation used for injections into the bronchial circulation. For detailed description see text. Abbreviations: P_{art} = arterial blood pressure; P_{LA} = left atrial pressure; P_{PA} = pulmonary arterial pressure

and the potency of the blood flow was re-established by preparing a shunt from a plastic tubing of the size of the ligated aorta. Branches arising from the ligated aortic segment as well as the oesophageal branches of the broncho-oesophageal artery were also ligated to restrict the blood flow to the bronchial artery [24, 37]. The bronchial circulatory system was perfused through a cannula introduced into the ligated aortic sac with blood from the left common carotid artery by using a plastic tubing. By means of an attached T piece, this cannula served also for the injection of various solutions directly into the bronchial artery.

Injections given into the pulmonary artery served as control. For this purpose, a thick puncture needle was introduced into the common pulmonary trunk and used as a guide for a fine gauge catheter pushed up to the branching point of the pulmonary artery. This catheter was used also for the measurement of pulmonary arterial pressure (Statham transducer P23 Db). Another catheter was introduced through the left auricle into the left atrium to measure pressure at this site, using a Statham transducer P23 BB. Electromagnetic flow-sensors placed on the common pulmonary trunk and the left common carotid artery and connected to CME 501 electromagnetic flowmeters were used to measure cardiac output and bronchial arterial blood flow, respectively. Heart rate was monitored by a cardiotachometer constructed in this laboratory, which was driven by the blood pressure waves. All these parameters were recorded on a 6-channel Beckman R Dynograph.

The calculated parameters were as follows:

a) stroke volume: $\dfrac{\text{cardiac output}}{\text{heart rate}}$

b) bronchial fraction of the cardiac output:

$$\frac{\text{flow in the bronchial artery}}{\text{flow in the pulmonary artery}} \times 100$$

c) total systemic vascular resistance:

$$\frac{\text{systemic blood pressure (mmHg)}}{\text{flow in the pulmonary artery (L min}^{-1})}$$

d) pulmonary vascular resistance:

$$\frac{\text{(pulmonary arterial pressure (mmHg) — left atrial pressure (mmHg)}}{\text{pulmonary arterial blood flow (L min}^{-1})}$$

e) bronchial vascular resistance according to HORISBERGER and RODBARD [24]:

$$\frac{\text{systemic blood pressure (mmHg) — left atrial pressure (mmHg)}}{\text{flow in the bronchial artery (ml min}^{-1})}$$

The right femoral artery and vein were cannulated for withdrawal of blood samples and administration of maintenance doses of the anaesthetic.

A volume of 0.5 ml/kg^{-1} was injected (8% $NaHCO_3$, 5.6% NaCl or 34.3% glucose solution) randomly either into the bronchial or the pulmonary circulation, the injection itself lasting for 1.5 min. During injection into the pulmonary artery the bronchial perfusion was stopped.

Three min after each injection a blood sample was taken for measurement of osmolarity (Knauer osmometer) and pH. Other blood parameters occasionally determined were packed cell volume, arterial pO_2 and pCO_2. The pH, pO_2 and pCO_2 were measured by a Radelkis Type OP 210/2 biological microanalyser.

In *Group 2a* 5 mongrel dogs of either sex (mean body weight of 14 kg) were used. After an intravenous injection of pentobarbital (30 mg/kg^{-1}) the left femoral artery was exposed and a double-lumen catheter was introduced with the end of the proximal lumen lying in the thoracic aorta at the level of the origin of the bronchial artery and the end of the distal lumen lying 15 cm more distally. A balloon catheter was also introduced into the same artery with the balloon being placed half-way between the two lumen endings of the other catheter. The proper position of the catheters was checked at necropsy after the experiment. The right jugular vein was also exposed and a silastic catheter was floated into the pulmonary artery, the position of the catheter tip being checked by oscilloscopic pressure signals (Statham transducer P23 Db). A shunt was made between the right common carotid artery and the femoral artery to prevent a drastic drop in blood flow to the lower part of the body in case of inflating the aortic balloon. Close to the carotid artery, the shunt was supplied with a ramification for the measurement of systemic blood pressure (Statham transducer P23 Db). The anaesthetic and heparin were administered through a catheter introduced into the right femoral artery. Heart rate was determined as in the experiments of Group 1.

An 8% solution of $NaHCO_3$ was injected into the aorta either at the level of the bronchial artery or as a control into the abdominal aorta in a volume of 1 ml/kg^{-1} with the aortic balloon inflated, the injection lasting for 1.5 min. The packed cell volume, pH, pO_2 and pCO_2 were measured as described for Group 1.

In *Group 2b* 15 mongrel dogs weighing 9 to 20 kg were used. The methods were similar to those described for Group 2b with the modification that one hour before the injection of $NaHCO_3$ the dogs were bled into an open reservoire to reach a mean blood pressure of 40 mmHg.

In each experiment, the respective values measured before and after injection were compared and evaluated. The scattering of the data were characterized by the 20th and 80th percentiles [23]. For statistical evaluation of the effects the one-sample and the two-sample Wilcoxon tests were applied [48].

Results

Group 1

All solutions caused an increase in *cardiac output*, the largest rise having been evoked by $NaHCO_3$ when injected into the bronchial artery (Fig. 2). The rise in cardiac output reached maximum (25 ml/kg^{-1}. min^{-1}) at the 2nd

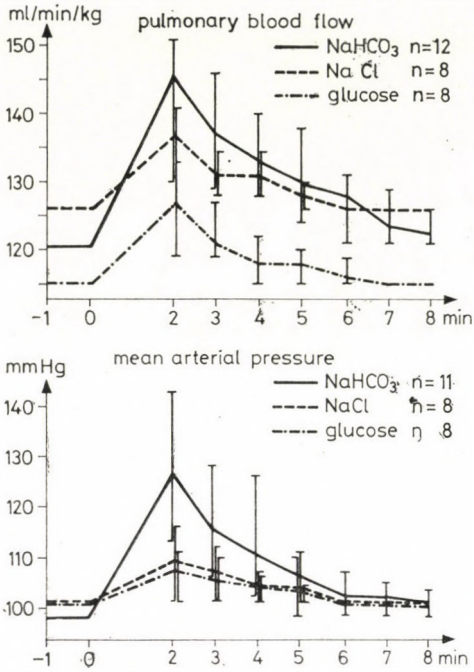

Fig. 2. Changes in cardiac output and mean arterial blood pressure after injection of $NaHCO_3$, NaCl and glucose into the bronchial artery. Vertical lines show 20th and 80th percentile values (also in the following Figures)

min after injection. Glucose and NaCl also resulted in a rise of similar time course, however, with a maximum of less than half of the above value. The rise in pulmonary blood flow was significantly higher after $NaHCO_3$ than after NaCl up to the 6th min (2nd min: $p < 0.05$ and after it $p < 0.01$) and significantly higher than after glucose up to the 5 th min (2nd and 3rd min: $p < 0.05$, 4th and 5th min: $p < 0.01$) when the injections were given into the bronchial artery. There was no significant difference between the effects of NaCl and glucose.

The largest increase in *systemic blood pressure* occurred also after hypertonic $NaHCO_3$ solution when injected into the bronchial artery, the effect of the other hyperosmotic solutions was smaller (Fig. 2). The maximum rise in mean arterial blood pressure was 30 mmHg, while NaCl and glucose caused an increase of less than 10 mmHg. The extent of rise in systemic blood pressure after $NaHCO_3$ was significantly higher up to the 3rd min than that observed after the injection of the other two solutions ($p < 0.01$).

The injection of all tested solutions led to increases in *pulmonary arterial pressure* and in *left atrial pressure*, here again $NaHCO_3$ having been the most effective. An injection of $NaHCO_3$ into the pulmonary artery resulted in average

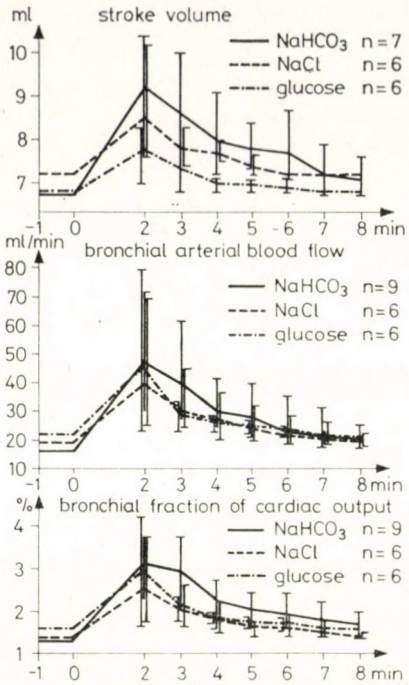

Fig. 3. Changes in stroke volume, bronchial blood flow and bronchial fraction of the cardiac output after injection of $NaHCO_3$, NaCl and glucose into the bronchial artery

rises in pulmonary arterial pressure and left atrial pressure of 1.5 mmHg and 1.1 mmHg, respectively. No significant difference was found between the effects of the three solutions.

Heart rate showed a decrease lasting from the 2nd to the 5th min, with the maximum effect observed at 2 min. The initial heart rate of 175 min^{-1} decreased by 10 and 4 min^{-1} after the injection into the bronchial artery of $NaHCO_3$ and NaCl, respectively, which difference in the response did not prove to be significant statistically.

All solutions given into the bronchial artery led to rises in *stroke volume* (Fig. 3). The most effective of the solutions was again $NaHCO_3$, with significantly higher rises in stroke volume than those evoked by glucose or NaCl between the 3rd and 6th min ($p < 0.01$). The latter two solutions were roughly equipotent.

Injection of all these solutions into the bronchial artery increased *blood flow in the bronchial artery* by more than 100 % in the 2nd min (Fig. 3). Between the 3rd and 8th min, the effect of $NaHCO_3$ was significantly greater than that of glucose ($p < 0.01$), while NaCl caused a rise statistically not different from the effect of the former two solutions.

The *bronchial fraction of cardiac output* showed similar changes (Fig. 3). The effect of $NaHCO_3$ was significantly greater between the 3rd and 6th min ($p < 0.01$) and between the 7th and 8th min ($p < 0.01$) than that of glucose, whereas when compared to the effect of NaCl, the other two solutions were effective to similar extent.

It was again the hypertonic solution of $NaHCO_3$ which was the most effective in reducing *bronchial vascular resistance* (Fig. 4). When compared to the effect of NaCl, $NaHCO_3$ caused greater reductions in the 2nd ($p < 0.05$), 3rd—4th ($p < 0.02$) and in the 8th min ($p < 0.01$). A comparison with the effect of glucose showed significandy greater responses between the 2nd and 8th min ($p < 0.01$).

Pulmonary vascular resistance was reduced by $NaHCO_3$ and glucose but elevated by NaCl after administration into the bronchial artery (Fig. 4). When compared to the effect of NaCl, $NaHCO_3$ was more effective between the 2nd and 5th min and in the 5th min ($p < 0.05$), while when compared to the effect of glucose, a significant difference was found only in the 2nd min ($p < 0.05$).

Total systemic vascular resistance did not change significantly after the injection of any of the applied solutions.

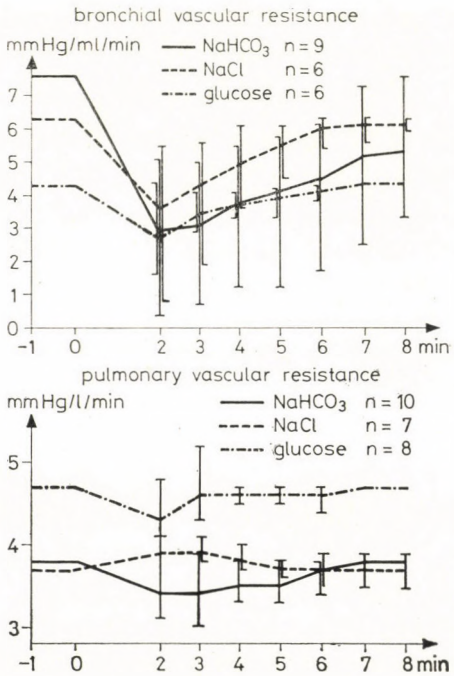

Fig. 4. Changes in bronchial and pulmonary vascular resistances after injections of $NaHCO_3$, NaCl and glucose into the bronchial artery

To assess quantitatively the role of pulmonary circulation in evoking the above effects, the same injections were given also into the pulmonary artery, and the responses were evaluated as differences from those evoked by the injections given into the bronchial artery.

All solutions evoked significantly greater rises in *cardiac output* when injected into the bronchial artery (Fig. 5). The largest increase of cardiac output occurred after $NaHCO_3$ administration. Each of the three solutions caused a higher elevation of *systemic arterial pressure* after bronchial arterial injection (Fig. 5), however, this difference proved to be significant only in the case of $NaHCO_3$.

In the 2nd, 3rd, 7th and 8th min *pulmonary arterial pressure* increased more when $NaHCO_3$ was injected into the bronchial artery than after injection into the pulmonary artery (Fig. 6). Between the 4th and 6th min, however, the injection into the pulmonary artery was more effective. Glucose and NaCl were more effective on administration into the bronchial artery.

The rise of *left atrial pressure* was greater after injection of $NaHCO_3$ and glucose into the bronchial artery than after their administration into the pulmonary circulation. In the case of NaCl no such difference could be observed (Fig. 6).

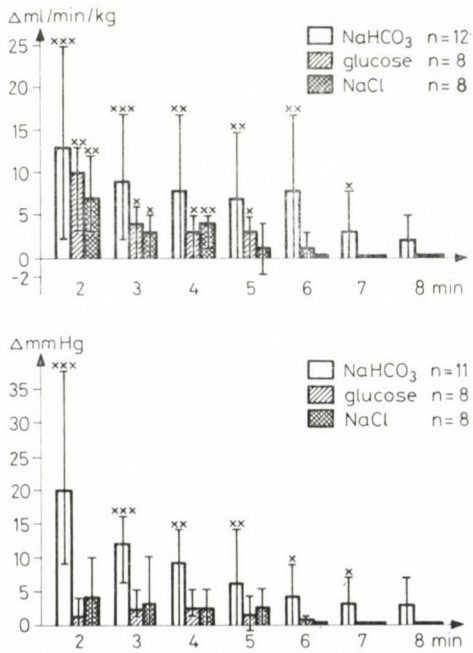

Fig. 5. Differences between the effects of solutions given into the bronchial and pulmonary arteries on cardiac output (upper panel) and arterial blood pressure (lower panel). Levels of significance * $p<0.05$, ** $p<0.02$, *** $p<0.01$ (also in the following Figures)

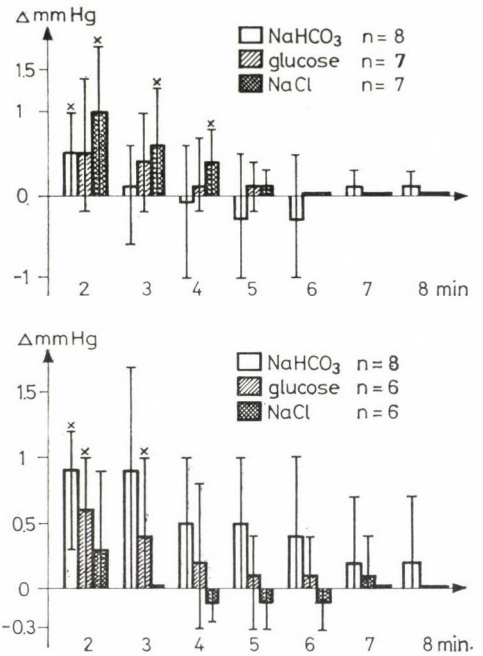

Fig. 6. Differences between the effects of solutions given into the bronchial and pulmonary arteries on pulmonary arterial pressure (upper panel) and left atrial pressure (lower panel)

A greater fall of *heart rate* occurred after $NaHCO_3$ on application into the bronchial artery in the 2nd and 3rd min (Fig. 7). Although NaCl also seemed to be more effective in decreasing heart rate when given into the bronchial artery, owing to the scattering of the data the difference compared to respective values obtained after administration into the pulmonary circulation did not reach statistical significance.

Rises in *stroke volume* (Fig. 7), *bronchial blood flow* (Fig. 8) and *bronchial fraction of cardiac output* (Fig. 8) were all greater after administration into the bronchial artery than after administration into the pulmonary artery irrespective of the solutions used. Here, again $NaHCO_3$ proved to be the most effective solution.

Bronchial vascular resistance decreased to a greater extent in response to injections into the bronchial artery than after administration into the pulmonary circulation (Fig. 9). No consistent difference could be shown in the effects of NaCl and glucose.

Pulmonary vascular resistance dropped to lower values up to the 6th min and up to the 2nd min after $NaHCO_3$ and glucose, respectively, when these solutions were given into the bronchial circulation than after using the other route of administration (Fig. 9). The same parameter increased after NaCl solution on administration into either circulatory system.

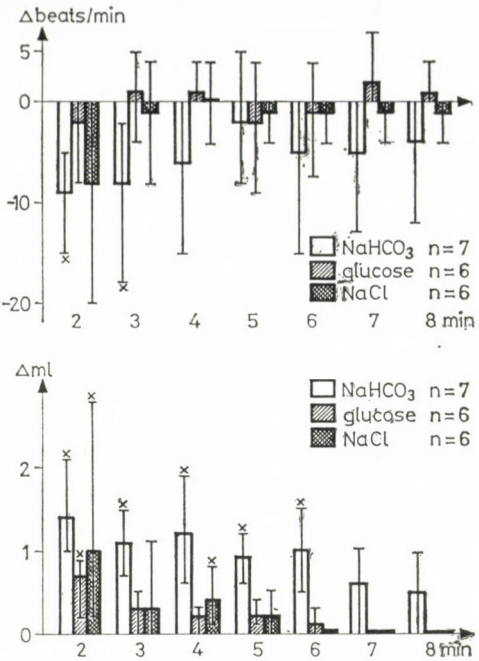

Fig. 7. Differences between the effects of solutions given into the bronchial and pulmonary arteries on heart rate (upper panel) and stroke volume (lower panel)

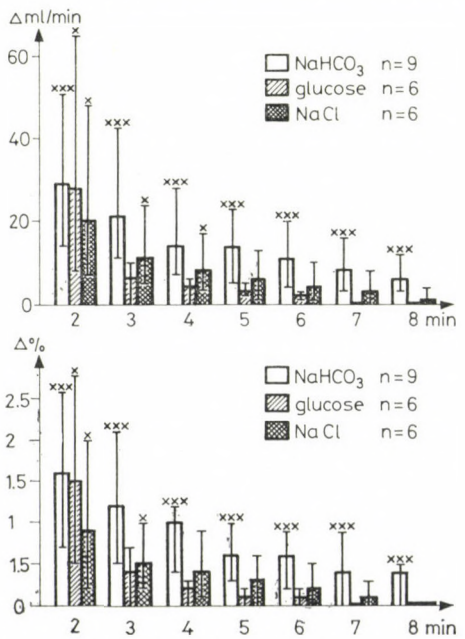

Fig. 8. Differences between the effects of solutions given into the bronchial and pulmonary arteries on bronchial blood flow (upper panel) and the bronchial fraction of cardiac output (lower panel)

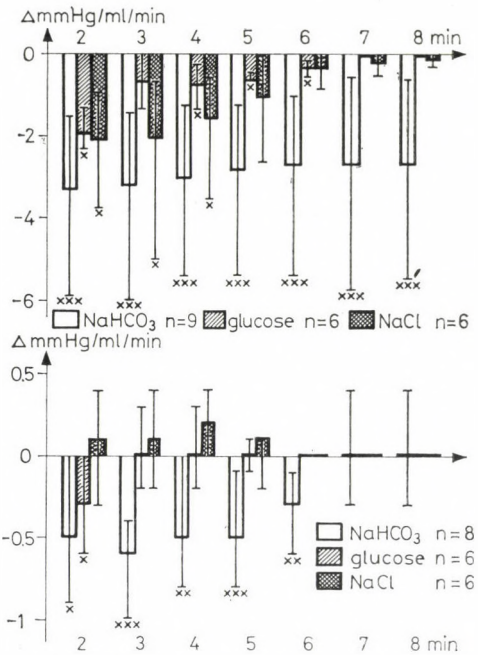

Fig. 9. Differences between the effects of solutions given into the bronchial and pulmonary arteries on bronchial (upper panel) and pulmonary (lower panel) vascular resistance

Osmolarity of the arterial blood was increased by all solutions to the same extent irrespective of the route of their administration (a rise of 10 mOsm L^{-1}). Glucose and NaCl failed to change arterial pH, while $NaHCO_3$ resulted in a rise of pH of 0.03 unit from the initial value of pH 7.43 after injection into either circulation. The initial packed cell volume of 41 % fell to 38 % by the end of the experiments.

Group 2a

In the experiments carried out under normotension, there was no change in systemic blood pressure (initial value, 160/120 mmHg), mean pulmonary arterial pressure (initial value, 10 mmHg) or heart rate (initial value, 140 min^{-1}) in response to injections given either at the level of the bronchial artery or at the level of the diaphragm.

Group 2b

The mean bleeding volume during the one-hour bleeding period amounted to 618 ml. Only one of the dogs had to be reinfused 10 ml blood to maintain a mean systemic blood pressure of 40 mmHg. The mean PaO_2 did not change

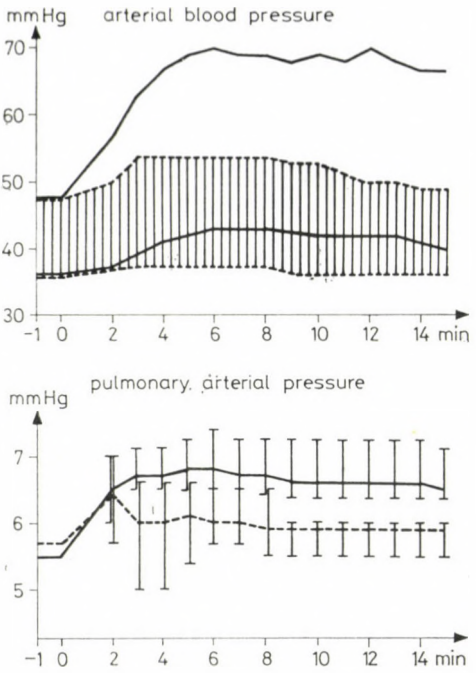

Fig. 10. Changes in systemic (n = 15) and pulmonary (n = 10) arterial blood pressures evoked by the injection of $NaHCO_3$ one hour after bleeding to a level of mean arterial pressure of 40 mmHg. Solid line and broken line show data obtained after injections at the level of the bronchial artery and at the beginning of the abdominal aorta, respectively. The corresponding values of systolic and diastolic blood pressures are connected with a vertical line in the case of injections given below the origin of the bronchial artery

(82 mmHg), while $PaCO_2$ decreased from an initial value of 37 mmHg to 32 mmHg at the time of injections. There was a fall of arterial pH (from 7.26 to 7.24) and a rise in packed cell volume (from 38 % to 44 %).

An injection of $NaHCO_3$, either given above or below the origin of the bronchial artery, resulted in a rise of *systolic blood pressure* (Fig. 10), with a greater rise after injections given above the level of the bronchial artery between the 3rd and 15th min after injection ($p < 0.01$). *Diastolic blood pressure* rose only after injection of $NaHCO_3$ given above the bronchial artery, the extent of this rise having been significantly greater than after administration to the other site from the 4th min onwards (in the 4th and 5th min, $p < 0.05$; after this, up to the 15th min, $p < 0.01$). The rise in *pulmonary arterial pressure* was also greater after injection of $NaHCO_3$ above the bronchial artery (from the 3rd to 8th min, $p < 0.05$; from the 9th to 15th min, $p < 0.01$). Injection of $NaHCO_3$ given into either site did not influence *heart rate*.

When looking for any relationship between maximum changes in systolic blood pressure, on the one hand, and initial values of the blood gases and pH

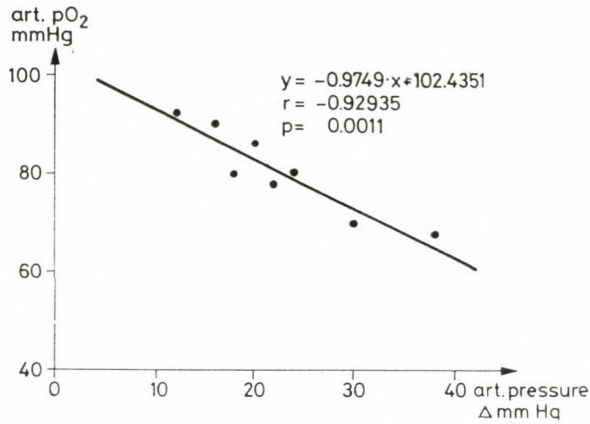

Fig. 11. Relationship between initial values of arterial oxygen tension and its maximum change evoked by $NaHCO_3$ one hour after bleeding

on the other, a strong correlation was found between the extent of rise in blood pressure and the initial value of oxygen tension in arterial blood (Fig. 11). No similar relationship could be observed between the other parameters studied.

Discussion

These extended haemodynamic investigations have supported our earlier observations. Immediately after having been injected into the bronchial artery, $NaHCO_3$ led to significant increases in cardiac output, systemic, pulmonary arterial and left atrial pressures, stroke volume, bronchial blood flow and bronchial fraction of the cardiac output and significant decreases of heart rate and bronchial and pulmonary vascular resistances, all of these changes having been much less marked after injections given into the pulmonary artery. The effects of glucose and NaCl solutions of the same quantity and osmolarity, although qualitatively similar, were in almost all cases much smaller than the corresponding responses obtained after $NaHCO_3$ injection irrespective of the route of their administration. When the haemodynamic effects of the three solutions given into the bronchial artery were compared, it was evident that the rises in cardiac output, systemic blood pressure, stroke volume, bronchial blood flow and bronchial fraction of the cardiac output as well as the decreases in bronchial and pulmonary vascular resistances were significantly greater after $NaHCO_3$ than after the injection of the other two solutions. There was no difference between the effects of glucose and NaCl.

Since the experimental model used in the first series of experiments required major surgical intervention, the haemodynamic effects of the most efficient solution (i. e. $NaHCO_3$) were studied also under more physiological

experimental conditions. Using this latter preparation there were no consistent changes in haemodynamics under normovolaemic conditions. After a one-hour bleeding period resulting in a drop of mean arterial blood pressure to 40mmHg, the administration of $NaHCO_3$ indirectly into the bronchial artery led to significantly greater rises in systemic blood pressure (systolic as well as diastolic) and pulmonary arterial blood pressure than the administration of the same solution given into the lower part of the aorta. In these experiments a strong correlation was found between the extent of changes in systolic blood pressure and the initial level of arterial oxygen tension. The precise mechanism of the observed haemodynamic changes are not known at present. Two characteristics of the applied solutions have to be considered in this respect. An 8 % solution of $NaHCO_3$ influences pH while the other two do not; the common feature of all the solutions applied was their hyperosmolarity.

In previous studies where TRIS buffer was used as a control for recognizing pH-dependent effects, the above haemodynamic changes failed to occur [38] which was consistent with findings of LIANG and HOOD [29] and of ROWE et al. [46]. In addition, at the time of the maximum systemic effect of 8 % $NaHCO_3$ injected in a volume of 0.5 ml kg^{-1}, the pH increased only by 0.03 and no correlation was found between changes of pH and the evoked circulatory responses. This makes it likely that pH changes are not responsible for the observed haemodynamic effects.

There is no agreement on the mechanism of action of hypertonic solutions. The haemodynamic effects of grossly hypertonic solutions injected rapidly should be differentiated from those of mildly hyperosmotic solutions injected more slowly. In the former case a fall in blood pressure, bradycardia, a decreased cardiac output, systemic vasodilatation, pulmonary vasoconstriction and respiratory disturbances have been described to occur [13, 18, 19, 21, 32, 42].

After slow injection of mildly hypertonic solutions cardiac output, systemic blood pressure, pulmonary arterial blood pressure, stroke volume increased and systemic and pulmonary vascular resistances decreased [29]. It is generally agreed upon that the systemic effects are mediated by changes in the pulmonary circulation [10, 12, 17] or, more specifically, in the small pulmonary vessels [1]. It has been suggested that cholinergic [43] or adrenergic mechanisms [26], respiratory chemoreflexes [12], and the release of histamine [47] or catecholamines [29] might be involved in these complex haemodynamic changes.

The observed haemodynamic effects may be classified as follows.

Local effects: increased bronchial blood flow, cardiac output and bronchial fraction of cardiac output and a fall in bronchial vascular resistance after injections of $NaHCO_3$, NaCl and glucose into the bronchial artery. These phenomena can be interpretered as the local vascular resistance decreasing effect of hypertonic solutions [21, 42, 43].

Systemic effects: increased cardiac output, systemic blood pressure, pulmonary blood pressure, left atrial pressure and stroke volume along with decreased heart rate and pulmonary vascular resistance resulting from $NaHCO_3$ glucose and NaCl injected into the bronchial artery. These haemodynamic changes cannot be explained on the basis of relevant data available in the literature.

The site of injection plays a role in influencing the response obtained. The multiple connections existing between the bronchial and pulmonary circulations have amply been described [2—7, 14, 15, 25, 30, 40, 41]. Under physiological conditions, two thirds of the bronchial blood flow is drained by the pulmonary veins *via* precapillary, capillary and postcapillary anastomoses [2, 7, 20, 22, 33, 39]. At a systemic blood pressure of 40 mmHg the flow in the bronchial artery ceases [4]; in this situation bronchopulmonary shunts open up [2—4, 20]. This means that substances injected into the bronchial artery may act not only at this site but also in the small blood vessels of the pulmonary circulation. It should be taken into account that substances reaching this area *via* the bronchial circulation may attain a higher concentration owing to less marked dilution (i. e. a steeper convolutional integral) than substances administered directly into the pulmonary artery. Conversely, substances given into the pulmonary artery may also reach the bronchial circulatory system, particularly if bronchial perfusion is artificially blocked as in the present experiments. It can be inferred, therefore, that although the anatomical site of the effects is likely to be the bronchial circulation or the tissues supplied by it, the role of the pulmonary microcirculation cannot be excluded either. However, the role of receptors localized in the aorta can be excluded on the basis of data obtained during hypotension.

It is probable that a stress situation (such as the major surgical trauma or the haemorrhage in the present study) is a necessary condition for the occurrence of these effects. It should be emphasized that a strong negative correlation could be found between the hypertensive effect and the level of the arterial oxygen tension. The observed slight arterial hypoxia must have been much more severe in the tissues supplied by the bronchial blood vessels, since bronchial blood flow is known to cease at a mean systemic arterial blood pressure of 40 mmHg [4] and tissue oxygen supply severely deteriorates during haemorrhage [9, 36]. The above considerations make it likely that hypoxia-sensitive receptors localized in the bronchial (or pulmonary ?) microcirculatory area play an important role in the development of the systemic haemodynamic effects. The K cells localized in the bronchial mucosa [8, 11, 16, 44] and the neuroepithelial bodies [11, 27, 28, 44] may function as receptors of this kind. These cells are capable of taking up amine precursors, i. e. they belong to the APUD system, and have been demonstrated by fluorescence techniques to possess vacuoles containing serotonin and catecholamines. These vacuoles may be

degranulated during hypoxia. Furthermore, these cells have rich afferent and efferent nerve supply and have in their close vicinity fenestrated bronchial capillaries [8, 11, 17, 28]. According to the present opinion, they may be considered as neuroreceptors involved in the regulation of pulmonary circulation. On the basis of these data, hypertonic solutions may be supposed to exert their effect on these cells osmotically, the response being facilitated by coexistent hypoxia or stress. The observed greater effectiveness of $NaHCO_3$ might be explained by the additional influence of HCO_3^- ions or CO_2, the latter formed during the dissociation process [34].

This hypothesis is sufficient to explain both the changes observed in systemic haemodynamics and the local effects, of the applied hypertonic solutions, considering the rich nerve supply of the bronchial circulatory system [31, 35, 45]. However, further experiments are needed to uncover the exact mechanism of action of these substances.

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REGULATION OF BRAIN PROSTAGLANDINS BY SEXUAL STEROIDS

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Hypothalamic PGF_{2alfa} content was determined by radioimmunoassay in rats. The male animals had significantly higher PGF_{2alfa} level than the females. The hypothalamic PGF_{2alfa} content increased following orchidectomy (GDX), while ovariectomy (OVX) had no such effect. Administration of progesterone (100 μg/kg or 1 mg/kg i.m.) alone or combined with oestrone (100 μg/kg or 1 mg/kg i. m.) resulted in a significant decrease of PGF_{2alfa} level in the hypothalamus, while oestrone (100 μg/kg or 1 mg/kg i.m.) alone was ineffective. Testosterone (1 or 5 mg/kg i.m.) in GDX animals restored the hypothalamic PGF_{2alfa} content to the control level.

The synthesis and breakdown of prostaglandins were also studied in the microsomal and cytosol fraction of brain homogenate. The PGD_2 was the main product of the arachidonate cascade in rat brain microsomes. OVX increased the formation of PGD_2 and diminished the biosynthesis of PGF_{2alfa}. Oestrone administration to OVX rats enhanced the formation of PGF_{2alfa}. Progesterone injection decreased the biosynthesis of PGF_{2alfa} and PGD_2 in OVX animals. Combined administration of progesterone and oestrone to OVX rats failed to restore the PGF_{2alfa} synthesis. In orchidectomized animals the arachidonate cascade was found to be depressed, further the synthesis of prostaglandins was normalized after testosterone substitution. The inactivation of ^3H-PGF_{2alfa} by rat brain cytosol fraction was negligible.

Prostaglandins (PGs) have been identified in the brain of a number of mammalian species [5, 8, 14, 15]. Recent experiments have suggested that PGF_{2alfa} and PGE_2 [12] as well as the thromboxane B_2 [18] are the major products in the catabolism of PG endoperoxides in rat brain homogenates. According to ABDEL-HALIM et al. [1] PGD_2 is the main prostaglandin synthesized by rat brain *in situ*. Prostaglandin release in the brain *in vivo* can be affected by different stimuli as neural, hormonal, pharmacologic and traumatic, as well as by bacterial pyrogens [19]. Recent report indicates [2] that there is no sex difference in the relative amounts of brain PG fractions. BENNETT et al. [3] found larger amounts of PG-like materials in homogenates of female guinea pig brains than that of males. Only limited data are available concerning the changes of PGs in the brain [10], and complete agreement on this point is lacking.

The present experiments were carried out to study the effect of sex steroids on the activity of PG synthetase and PG metabolizing enzymes in the brain, and on the hypothalamic PGF_{2alfa} content.

Methods

Animals. Male and female rats of Sprague-Dawley CFY strain weighing 180—250 g were used. The animals were maintained under a 12 hr light and 12 hr dark regimen, with lights on at 6.00 A.M. The ovaries and testes were removed under pentobarbital anaesthesia (35 mg/kg i.v.). Oestrone and progesterone (1 mg/kg or 100 μg/kg, in 180—250 μl sunflower seed oil) were injected i.m. every other day to ovariectomized (OVX) rats for 14 days. Testosterone (1 or 5 mg/kg in 180—250 μl sunflower seed oil) was administered to orchidectomized (GDX) animals similarly for 14 days. For vehicle control, OVX and GDX rats received 180—250 μl sunflower seed oil. The animals were killed by decapitation 24 hr after the last injection between 9 and 10 A.M. and the brains were quickly removed.

Experimental design. (1) The first series of experiments was designed to determine whether or not ovarian hormones are able to influence PGF_{2alfa} content of the hypothalamus. Therefore PGF_{2alfa} determinations were carried out in ovariectomized as well as oestrone and progesterone treated OVX animals. (2) The second series of experiments was devoted to study the changes of hypothalamic PGF_{2alfa} content in orchidectomized and testosterone treated GDX rats. (3) The PG synthetase activity was assayed in rat whole brain to obtain information about the synthesis of different PGs from exogenous ^{14}C-arachidonic acid in control, OVX, and sex steroid treated rats. (4) The inactivating enzyme system, i.e. 15-OH-PG dehydrogenase and delta 13-reductase, was also measured in the brain.

Materials. 1-^{14}C-arachidonic acid (55 mCi/mM) was purchased from Amersham, England. Charcoal, NADH and beta-NAD were purchased from Sigma Chemical Co., St. Louis, Mo.. Glutathione reduced from (GSH, research grade) was product of Serva Feinbiochemica, Heidelberg, GFR. PGF_{2alfa} radioimmunoassay kit and ^3H-PGF_{2alfa} (120 Ci/mM), ^3H-PGE_2 (200 Ci/mM) were donated by the Institute of Isotopes of the Hungarian Academy of Sciences, Budapest. Norepinephrine bitartarate and pentobarbital-Na (Mebubarbital Sodique) were purchased from Rhône-Poulenc, France. PGE_2, PGF_{2alfa} and their metabolites: 13,14-dihydro-PG (E_2, F_{2alfa}), 15-keto-PG (E_2, F_{2alfa}), 13,14-dihydro-15-keto-PG (E_2, F_{2alfa}) were kindly supplied by Dr. J. E. Pike, Upjohn Co., Kalamazoo, Mich. Oestrone and progesterone were produced by Organon, Oss, Holland. Testosterone propionate (Retandrol) was purchased from G. Richter Ltd., Budapest, Hungary. All other chemicals were of analytical grade, and obtained commercially unless otherwise stated.

Preparations of homogenates of rat brain. The wet weight of brain halves was measured, and homogenized with Ultra-Turrax in Tris/HCl buffer (0.05 M, pH 8.0 containing 0.25 M sucrose) for 20 sec. The suspension was centrifuged at 800 *g* for 10 min. The precipitate was washed with buffer and centrifuged again. The first and second supernatants were collected, then centrifuged at 10,000 *g* for 20 min. The supernatant was further centrifuged at 105,000 *g* for 60 min. This final supernatant was used for the determination of delta-13-PG reductase and 15-OH-PG dehydrogenase (PGDH). The 105,000 *g* microsomal pellet fraction was homogenized with a Potter-Elvehjem homogenizer in 0.05 M Tris/HCl buffer (pH 8.0) and used for PG synthetase assay. The preparation of homogenates was carried out at 0—4 °C.

Assay of PG synthetase enzyme activity. The PG synthesis was measured by the method of Tai et al. [17]. The incubation mixture (1 ml) contained 1-^{14}C-arachidonic acid (0.015 μCi) as substrate, 2 mM GSH and 1 mM noradrenaline as cofactors, Tris/HCl buffer (0.05 M, pH 8.0) and microsomal fraction (1—3 mg protein). Incubation was performed at 37 °C with continuous shaking for 60 min. The enzyme reactions were stopped by the addition of 0.03 ml formic acid (12 M) which reduced the pH to 3.0, and by placing the samples on ice. The enzyme activity was expressed as the amount of PG dpm/mg protein/hr synthesized.

Assay of PG inactivation. The activity of PGDH and delta-13-reductase was determined according to Stone and Hart [16]. The incubation mixture (1 ml) contained ^3H-PGF_{2alfa} (0.01 μCi) as substrate, 0.5 μM beta-NAD as cofactor, phosphate buffer (0.1 M, pH 7.4 containing 4 μM $MgCl_2$) and the 105,000 *g* brain supernatant (3—4 mg protein) as enzyme source. Incubation was carried out at 37 °C for 60 min. The enzyme reaction was stopped by acidification (pH 3.0) and cooling.

Extraction and separation of PGs and PG metabolites. The acidified incubation mixtures were extracted with 2×3 ml of ethyl acetate. The extracts were evaporated under N_2 at 40 °C. Each sample was taken up in ethyl acetate (300 μl) in order to spot on Silica gel G thin layer plates (5×20 cm). When PG synthetase was assayed, the plates were developed with the organic phase of ethyl acetate: acetic acid: iso octane: water (110 : 20 : 50 : 100 by vol.). Chloroform: methanol: acetic acid: water (90 : 8.5 : 1.0 : 0.65 by vol.) was applied as solvent system to separate PGF_{2alfa} and its metabolites. Authentic PGs and PG metabolites were detected with anisaldehyde reagent by the method of Kiefer et al. [9]. Thin layer plates were divided into

5 mm bands, they were scraped off and transferred separately to scintillation vials containing a scintillation cocktail [4]. An LKB 81,000 was used as liquid scintillation counter.

Protein concentrations were determined by a microbiuret method [6].

Radioimmunoassay of hypothalamic PGF_{2alfa} content. The hypothalamus (30—40 mg) was homogenized with Potter-Elvehjem homogenizer in icecold isotonic saline. The neutral lipids were removed by petrolether (boiling point 40—70 °C), then 0.1 N HCl was added to the samples to acidify the homogenates to pH 3.0. PG extraction and determination were carried out according to the Calbiochem guide for PGF radioimmunoassay (RIA) using the PGF_{2alfa} RIA kit of the Institute of Isotopes of Hungarian Academy of Sciences. The results were corrected with the corresponding recovery (76—82 %). The sensitivity of PGF_{2alfa} RIA was around 100 pg. The percentage cross-reactivity with PGF_{1alfa} was 4.3, with PGE_2 2.6, and with PGE_1 1.2. The PGF_{2alfa} content of the hypothalamus was expressed in ng/g wet weight.

Student's *t* test was used for statistical analysis.

Results

PGF_{2alfa} content of the hypothalamus in ovariectomized and oestrone, progresterone treated OVX rats.

The hypothalamic PGF_{2alfa} level of OVX rats did not significantly differ from that of the control. When oestrone was injected in a dose of 100 μg/kg intramuscularly, PGF_{2alfa} content did not change appreciably compared to the OVX group. Progesterone alone (100 μg/kg) decreased PGF_{2alfa} level. The hypothalamic PGF_{2alfa} level could not be restored to control values by the combined injections of oestrone and progesterone (100 μg/kg each) (Table I).

Effect of orchidectomy and testosterone admininstration on hypothalamic PGF_{2alfa} content.

The hypothalamic PGF_{2alfa} concentration was significantly elevated on the 14th day after orchidectomy. Testosterone treatment (5 mg/kg) normalized the PGF_{2alfa} level of GDX rats. Testosterone in a dose of 1 mg/kg was less effective in lowering the PGF_{2alfa} content of the hypothalamus (Table II).

Table I

Effect of ovariectomy (OVX), oestrone and progesterone treatment on hypothalamic PGF_{2alfa} content

Group[a]	PGF_{2alfa} ng/g±S.D.	p	
1. Control	48.1±7.2	—	—
2. OVX	40.8±5.4	NS[b]	
3. OVX+oestrone[d] 100 μg/kg i.m.	49.7±9.2	NS[b, c]	
4. OVX+progesterone[d] 100 μg/kg i.m.	17.9±3.8	<0.0001[b]	<0.0005[c]
5. OVX+oestrone and[d] progesterone 100 μg/kg i.m.	26.4±4.1	<0.001[b]	<0.001[c]

[a] Each experimental group consisted of 10 rats. Each value represents the mean±S.D.
[b] Compared to control rats (1); [c]compared to OVX rats (2); [d]the results were similar when using 1 mg/kg oestrone or progesterone. NS = not significant

The amount of hypothalamic PGF_{2alfa} was found to be higher in male controls than in female ones. The difference was significant ($p < 0.001$) (Tables I and II).

Table II

Effect of orchidectomy (GDX) and testosterone treatment on hypothalamic PGF_{2alfa} content

Groups[a]	PGF_{2alfa} ng/g ± S.D.	p	
1. Control	76.4 ± 5.2	—	
2. GDX	119.3 ± 6.4	<0.001[b]	—
3. GDX + testosterone 1 mg/kg i.m.	97.0 ± 4.1	<0.01[b]	<0.01[c]
4. GDX + testosterone 5 mg/kg i.m.	70.2 ± 3.2	NS[b]	<0.001[c]

[a] Each experimental group consisted of 12 rats. Each value represent the mean ± S.D.
[b] Compared to control rats (1); [c]compared to GDX rats (2); NS = not significant

PG synthetase activity in the brain of male and female rats.

The microsomal fraction incubated with 1-^{14}C-arachidonic acid in the presence of the cofactors norepinephrine and glutathione resulted in the production of PGD_2, PGE_2 and PGF_{2alfa}. The rate of formation of each prostaglandin was in linear relationship with the time of incubation for 60 min.

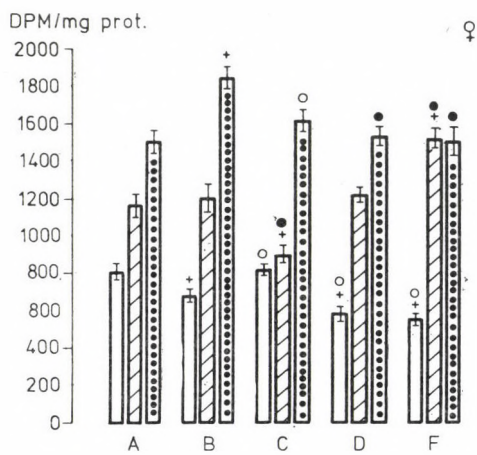

Fig. 1. The activity of PG synthetase in the brain of ovariectomized (OVX) rats. The amount of PG fractions are expressed in DPM/mg protein/h. □ PGF_{2alfa}, ▨ PGE_2, ⁞⁞ PGD_2. A: control, B: OVX 14th day after sugery, C: OVX and oestrone treatment, D: OVX and progesterone treatment, F: OVX and combined administration of oestrone and progesterone. Each block represents the mean ± S. D. of 6 rats. $^+p < 0.01$ compared to control, $^\circ p < 0.01$ compared to OVX, $^\bullet p < 0.001$ compared to OVX

a. Effect of ovariectomy on brain PG synthetase activity.

Ovariectomy decreased the synthesis of PGF_{2alfa} while PGD_2 formation was enhanced. Oestrone administration to OVX rats increased the synthesis of PGF_{2alfa} and diminished the formation of PGE_2 and PGD_2 compared to OVX rats. PGF_{2alfa} production was significantly less both after progesterone and after the combined administration of oestrone and progesterone in ovariectomized animals compared either to the controls or ovariectomized rats. The same experimental conditions produced a decrease in the biosynthesis of PGD_2 compared to that of the OVX rats. The amount of PGE_2 was found to be higher following combined injection of oestrone and progesterone in OVX rats than in the brain of the control or ovariectomized animals (Fig. 1).

b. The effect of orchidectomy and testosterone treatment on PG synthetase activity of the brain.

The amount of each PG fraction synthesized by rat brain microsomal pellet fraction was much less 14 days after removal of the testes. Testosterone administration (1 mg/kg) to orchidectomized rats normalized the biosynthesis of prostaglandins in the brain of GDX animals (Fig. 2).

Prostaglandin metabolism in the brain.

The inactivation of ^3H-PGF_{2alfa} by the 105,000 *g* supernatant fraction of male and female rat brain homogenates was found to be negligible. After gonadectomy or sex hormone replacement, neither 15-OH-PG dehydrogenase nor delta-13-PG reductase activity could be detected.

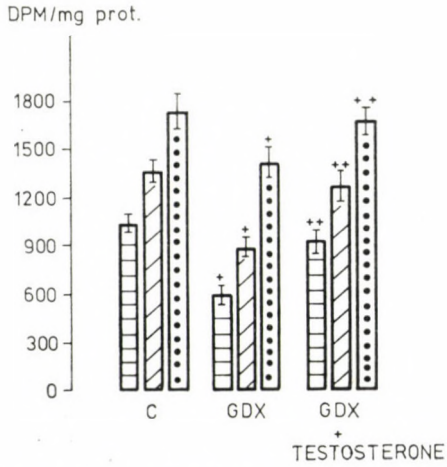

Fig. 2. Brain PG synthetase activity of orchidectomized and testosterone treated rats. The quantity of PG fractions synthesized from 1—^{14}C-arachidonic acid by brain microsomal fraction is presented on the ordinate in DPM/mg protein/h. PGF_{2alfa}, PGE_2, PGD_2. C: control, GDX: 14th day after orchidectomy, GDX + testosterone administration in a dose of 1 mg/kg every other day for 14 days. $^{+}p < 0.001$ as compared to control, $^{++}p < 0.001$ in comparison with the GDX group. Each block represents the mean ± S. D. of 12 rats

Discussion

Prostaglandins may be involved in the feedback regulation of gonadotropin secretion [14]. The primary effect of prostaglandins on gonadotropin secretion was proposed to be at the hypothalamic suprapituitary level [7]. The present experiments suggest that the gonads and sex steroids are capable of influencing PGF_{2alfa} content of the hypothalamus. Orchidectomy of male rats resulted in a considerable increase of hypothalamic PGF_{2alfa} content, and repeated testosterone administration to GDX animals diminished the amount of PGF_{2alfa} to the normal level.

The removal of ovarias, or oestrone injections to OVX rats failed to elicit any change in the hypothalamic PGF_{2alfa} content. In contrast to these data, progesterone treatment significantly decreased PGF_{2alfa} level in the hypothalamus of OVX rats. These observations suggest that testosterone and progesterone may be involved in the regulation of hypothalamic PGF_{2alfa} concentration.

Further experiments were carried out to clarify whether the modulating effect of sex steroids was mediated by affecting the arachidonate cascade or the PG inactivating enzymes in the brain.

The possibility of destruction of prostaglandins in the brain was excluded since we were unable to detect enzymatic catabolism of ^3H-PGF_{2alfa} in the cytosol fraction of rat brain homogenate. Our observations are consistent with the results of ABDEL-HALIM and ÄNGGÅRD [2], NAKANO et al. [11], WOLFE et al. [19].

In agreement with the results of ABDEL-HALIM and ÄNGGÅRD [2], PGD_2 was found to be the major prostaglandin biosynthesized in the brain of male and female rats. According to WOLFE et al. [19] more PGF_{2alfa} than PGE_2 is synthesized by the rat cerebral cortex, however the formation of PGD_2 was not measured in this study.

The consequences of ovariectomy and orchidectomy were different on the prostaglandin synthetase activity of the brain. The quantity of each synthesized prostaglandin (D_2, E_2, F_{2alfa}) was decreased after removal of the testes, whereas ovariectomy resulted only in decreased formation of PGF_{2alfa}, the biosynthesis of PGD_2 being rather enhanced.

Testosterone administration to orchidectomized rats completely restored the altered prostaglandin synthesis. Combined administration of oestrone and progesterone normalized only the formation of PGD_2 while the synthesis of PGF_{2alfa} remained at a low level and quite unexpectedly, PGE_2 formation was increased.

PGD_2, the main fraction of the arachidonate cascade in the central nervous system of the rat, might have an important local role. The results suggest that prostaglandin synthesis in the brain is under the influence of the sex steroids.

Acknowledgement

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DATA ON MUSCLE FIBRE CONVERSION AND FIBRE SPLITTING IN MAN

by

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A high proportion of the "intermediate" muscle fibres, which are semi-dark after both acidic and alcalic preincubation in ATP-ase staining, was found in the thigh muscle of some endurance trained runners and all of the chronically cyanotic children with heart failure. Characteristic patterns of fibre splitting were found mostly in jumpers' muscles. Possibilities of fibre subgroup transformation and fibre hyperplasia due to training are discussed.

One of the most generally accepted method for differentiating muscle fibres is based upon the pH sensitivity of the myosin ATP-ase activity (see Table I).

This sensitivity is attributed to sulphydril groups found in the light chains of the myosin molecule [6, 23]. Types of muscle fibres presented in Table I can be observed in the muscles of individuals performing no particular training.

Fibre type FT_a cannot be detected in endurance trained muscles or they are found only in a small quantity [20, 22].

A high proportion of fibre type FT_c (10—15 %) is considered normal merely in the first years after birth when its percentage decreases below 2 %. In some of the endurance trained muscles 10—12 % FT_c fibres were found [19].

Under the influence of a dynamic strength training in the cat GONYEA et al. [13] found an increase of 19.3 % in the number of fibres in the trained

Table I

pH Sensitivity of myofibrillar ATP-ase activity of different fibre types

Data of BROOKE and KAISER+ (1970) and SALTIN et al. (1977)++

Muscle fiber types	Preincubation
++ST +Typ. I	ATP-ase is labile under pH 3.9 over pH 10.4
FT_a Typ. II_a	ATP-ase is labile under pH 4.9 over pH 10.8
FT_b Typ. II_b	ATP-ase is labile under pH 4.5 over pH 10.8
FT_c Typ. II_c	ATP-ase activity is high after both acidic (under pH 4.5) and alkaline preincubation

extremity. Cross sections of these muscles revealed that fibre splitting had occurred. This is the first publication in which, without any sign of degeneration, hyperplasia is reported in response to training.

Fibre splitting has also been suggested by COSTILL [7] as a possibility for changing the fibre pattern. Recently we have demonstrated fibre splitting and a high occurrence of FT_c fibres in human muscle samples.

Methods

Needle biopsy was carried out by the technique described by BERGSTRÖM [4]; fibre identification was performed using the criteria of BROOKE and KAISER [5] as well as GUTH and SAMAHA [15]. Muscle samples were embedded in AMES TISSUE TEK—II, and samples were further frozen in iso-penthane and liquid nitrogen at K° 134, finally were stored and dissected at a temperature of K° 253. Muscle slices of 6 nm width were stained for ATP-ase by the above method. Muscle samples were obtained from the lateral head of the human quadriceps muscle with the common consent of both the adult sportsmen and the parents of disabled children.

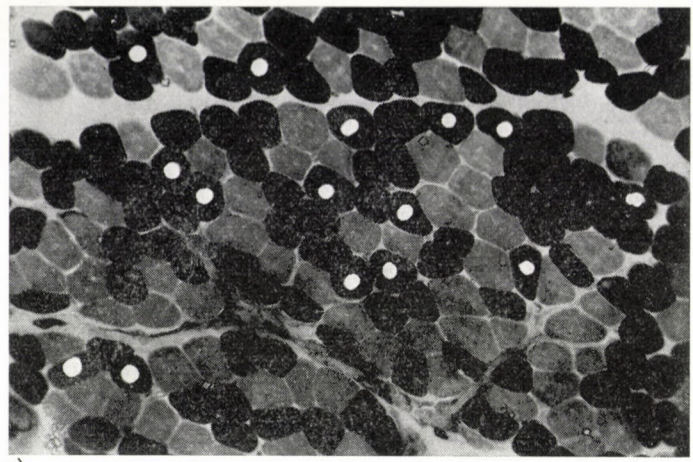
Results

In some of the endurance trained individuals an unusual ATP-ase activity was observed: not only the existence or the lack of this activity could be recognized but fibres with a medium intensity of colour were also found (see Fig. 1/a and b). Fibres being still in active form after either alkaline (pH 10.3) or acidic (pH 4.3) preincubation in the system of BROOKE and KAISERS [5] are considered by SALTIN et al. [22] as FT_c, presumably undifferentiated fibres. In good agreement with recent findings of others such fibres were found in some of our endurance trained athletes in a quantity of 13—16 %. In all the muscle samples obtained from children with Fallot's tetralogy a similar picture was revealed, i. e. 12, 17, 15, 29 and 12 per cent of the muscle fibres proved to be type FT_c.

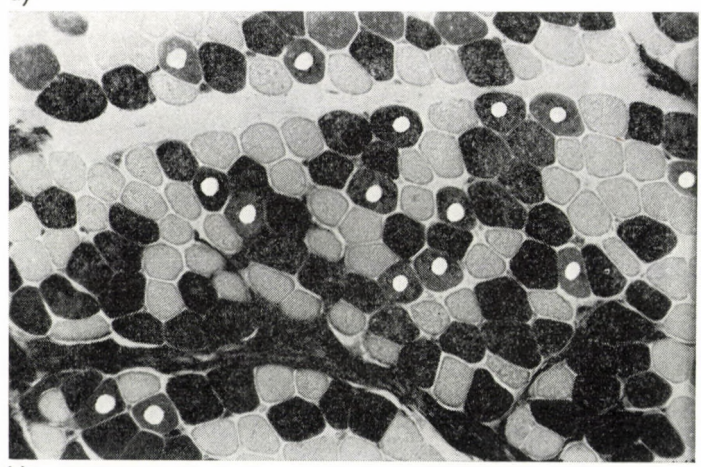
In the muscle samples gained from jumpers characteristic signs of fibre splitting were found (see Figs 2, 3, 4).

Figure 5 shows, instead of the customary mosaic pattern of fibres [3], a pattern of muscle fibres characterized by a high proportion of slow twitch muscles on one hand, and by the presence of well separated groups of fast twitch muscle fibres on the other. ENGEL [10] observed a similar phenomenon ("type grouping") in patients with pathological innervation.

Table II shows the fibre composition of muscle samples of competitors with a remarkable performance in one or other sport event. In accordance with the type of exercise, certain distinctive features are apparent in the proportion of muscle fibres.



a)



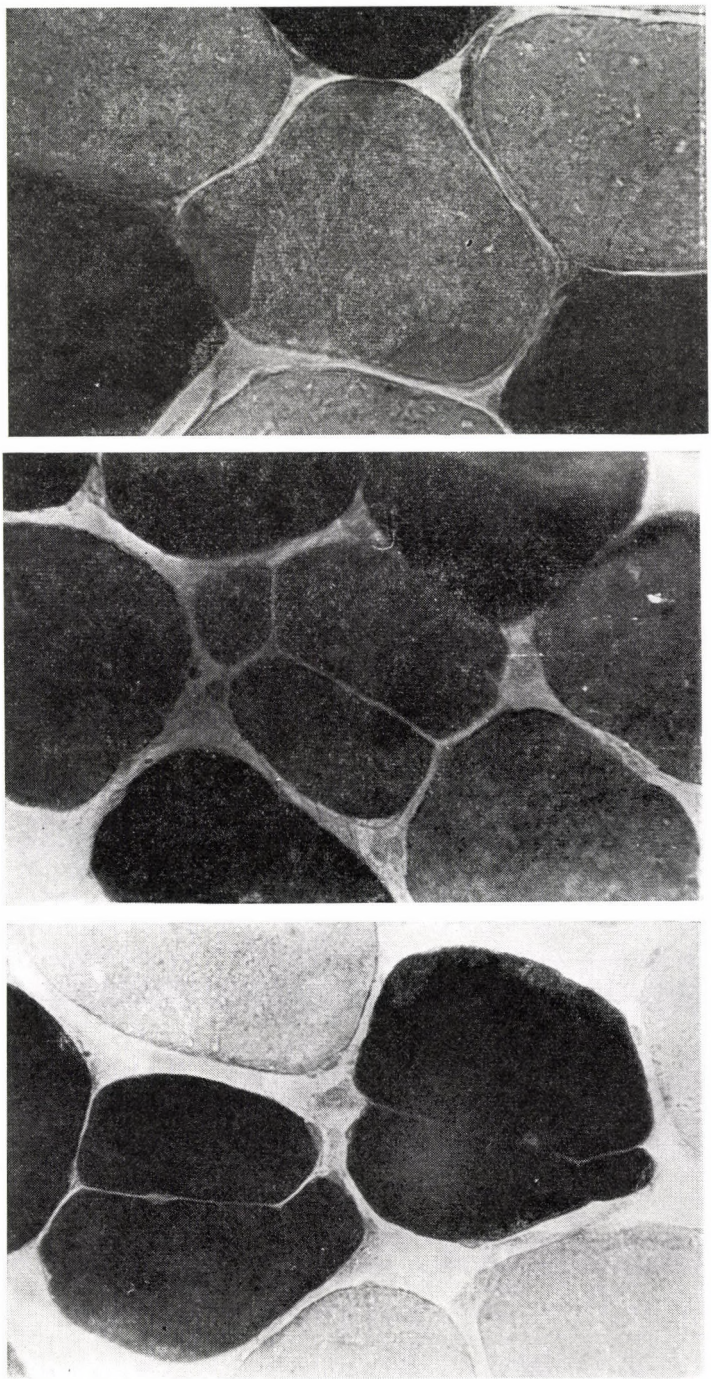
b)

Fig. 1. ATP-ase activity staining of the lateral vastus muscle of cardiac children. a) Preincubated at pH 10.3; b) Preincubated at pH 4.3. Fibres marked with circles are considered as Type II c. (Magnification 100 ×)

Table II

Fibre pattern of m. vastus lateralis of track and field athletes (mean±SD)

	n	ST %	SD
Jumpers (broad and triple jumpers)	6	47.85	5.62
Sprinters	6	50.13	6.18
High jumpers	12	54.18	13.45
Middle distance runners	7	65.84	21.68
Long distance walkers	8	66.23	18.77
Marathon runners	7	83.84	10.87



Figs 2, 3 and 4. Muscle patterns of samples obtained from sportsmen, preincubated at pH 4.3, stained for ATP-ase. Dark fibres are the slow twitch ones. Magnification cca 400 ×

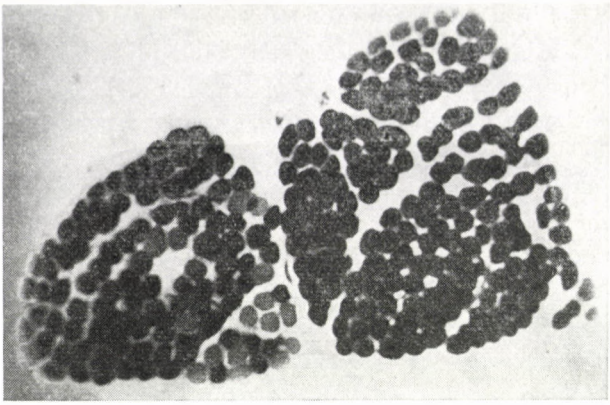


Fig. 5. Lateral vastus muscle of a runner. Dark fibers are the ST ones. Magnification cca 50 ×

Discussion

Dependency on genetic factors of both the muscle fibre type stained for ATP-ase and the contractile property of the muscles has been stressed by several authors (KOMI et al. [17], KOMI and KARLSSON [18], THORSTENSSON et al. [24], GOLLNICK et al. [12]).

While performing endurance training significant changes may ensue in the FT muscle fibres. (Type II). In the muscles of untrained individuals a proportion of 10—20 % of Ft_a (Type II_a) was found while this type of muscle fibres can be observed scarcely or not at all in trained athletes. In the shoulder, deltoid and back muscles of swimmers SALTIN et al. [22] or NYGAARD and NIELSEN [20] could not find FT_b fibres, while in the leg muscles of swimmers FT_b fibres were present. It was found in SALTIN's [22] experiments that immobilization for four weeks caused a decrease in the ratio of FT_a fibres to FT_b fibres, and *vice versa*. The absence of FT_a fibres was regarded by ANDERSEN and HENRIKSSON [19] as a consequence of training. According to them as well as SALTIN et al. [22] disappearance of FT_b fibres may be considered as being a part of adaptation to endurance type of training that involves further an alteration in pH sensitivity, which otherwise also reflects a change caused by overload in the myosin of trained muscle. The myosin structure is altered probably also in its SH groups.

Another observation relevant in this respect is that the FT_c fibres, which has been classified by SALTIN as undifferentiated ones, can be found in a comparatively high proportion in some athletes subjected to endurance training [19]. Our results are in good agreement with these findings. Similarly, a high ratio of FT_c fibres was observed in cardiac children. SALTIN et al. [22] suggested that the reason for this undifferentiation might be the unestablished innerva-

tion of such fibres. Since the fibres can be detected merely in endurance trained muscles, the conclusion may be drawn that multiplication of these fibres be probably a consequence of endurance type overload.

In view of these findings fibre splitting cannot be regarded as the consequence either the conversion from one fibre type to the other or the necrosis of some of the fibres, but it probably involves an alteration of fibre composition. Since the examination of a total cross section of any muscle is practically not possible, direct evidence is not available on the suggested changes in the total number of muscle fibres in the trained muscles of man. When fibre splitting occurs innervation is not altered, only the number of muscle fibres attached to a given nerve is increased.

However, a further possibility for the alteration of the ratio of the various fibre types is provided by collateral reinnervation. Pathologically denervated muscles recover their strength in a remarkably short period of time due to the side branches arising from the surviving intact subterminal nerve fibres. The original end plates are innervated by the most rapidly growing nerve fibre. Its histochemical properties are thus imprinted on the muscle fibres that are reinnervated; at the same time, these fibres regain their original size as well

Table III

Fibre pattern of m. vastus lateralis of non-athletic subjects (mean±SD)

n	ST %	SD or extreme values	Authors
6	52.2	13—96	GOLLNICK et al. 1972
115*	52.0	7.2	HEDBERG and JANSSON, 1976
8	48.0	29—65	EDSTRÖM and EKBLOM, 1972
70	54.0	12.2	DUBOWITZ, 1967
10	44.0	45—60	THORSTENSSON et al. 1976
	43.8		GREEN et al. 1979
	49.1	7.7	KOMI and KARLSSON, 1978
	55.9	11.9	KOMI and KARLSSON
69	55.0		SALTIN et al. 1977
25	50.0		SALTIN et al. 1977
80	46.2	17.6	VIITASALO and KOMI, 1978
20	54.6	12.6	VIITASALO and KOMI, 1978
10	58.8	10.3	VIITASALO and KOMI, 1978
11	39.0	9.2	ÖRLANDER et al. 1978

* Males and females

[3]. When more than 10—15 muscle fibres are in the same pool grouping of muscle fibres of identical histochemical characteristics is considered as a sign of pathological innervation [10].

Table III is review of results reported in the literature on the muscle fibre distribution of nonathletic subjects. It is obvious that the ratio of the ST fibres to the FT ones is almost invariably about 50 %. Table II shows on the other hand, that the muscle fibre distribution in athletes differs from that of normal individuals. This difference might be due either to the selection of subjects or to hyperplasia of certain types of muscle fibres in response to intensive training. It is hardly conceivable that any change would occur in the neuromotor pattern of a conversion from one type of fibres to another would take place in trained subjects. Only ST fibres could be detected in a sample obtained from the vastus lateralis muscle of a marathon runner.

It should be emphasized that muscle fibres are very flexible with respect to certain features e. g. oxidative enzyme activity, intracellular carbohydrate and fat reserves, capillary supply etc., and marked alterations may be caused by training also in man.

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OVERLAPPING EFFECT OF THYROID-STIMULATING HORMONE AND FOLLICLE-STIMULATING HORMONE ON THE THYROID GLAND IN BABY CHICKEN

by

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Follicle-stimulating hormone (FSH) although quantitatively less effective than thyroid-stimulating hormone (TSH) in the thyroid gland, overlapped with the actions of the latter regarding the indices tested. Thus, it increased the follicular diameter and height of epithelial cells. These findings appear to support our earlier observation demonstrating an overlapping effect of tropic hormones in the gonads and suggest that the overlapping action of tropic hormones with related structure is a general phenomenon in the perinatal period.

Members of different hormone groups are known to exert their effect on separate target organs, however being synthetized from a common precursor molecule i. e. because of structural homologies, some overlapping may occur. Thus, due to the identity of alpha subunits and homology of beta subunits of TSH, FSH and LH molecules [10] a minor overlapping among these hormones at the receptors of target organs still occurs in adults [1, 11, 12] but it is so small in magnitude that it does not interfere with the specific action. On the other hand, it appears that at lower stages of phylogenesis [6, 7] and in early periods of ontogenesis the overlapping is more apparent and a member of the hormone group resulting in a differential effect in the mature animal may mimic the action of the original hormone in the newborn. The TSH — gonadotropin overlapping in the gonads of baby chicken is a characteristic example of the latter, when the effect of TSH — i. e. the non-target hormone — is more pronounced on the development of the gonads than that of gonadotropin [2, 3, 4]. Based on these findings it seemed reasonable to examine whether the receptors of the thyroid gland are unable to distinguish between the configuration of the two tropic hormones as it occurred in the gonads.

Methods

Hubbard broyler male baby chicken were used. First day posthatching birds were treated with FSH (United States Biochemical Corporation) or TSH (Ambinon Organon, Oss, Holland) in a dose of 20, 40, 80 and 160 μg given subcutaneously twice daily (every 12 h) for 3 days. The controls received 0.1 ml saline according to the same schedule. Twelve hours after the last injection under ether anaesthesia the left lobe of the thyroid was removed and fixed in

formalin. The paraffin embedded material was sectioned at a thickness of 5 μm. The histological structure of the thyroid was quantitatively evaluated.

Thyroid follicular count per unit area, mean follicular diameter and the mean height of follicular epithelial cells were determined using a calibrated quadratic scale. Follicular count was estimated in 10 randomly chosen nonoverlapping visual fields in each sample. Follicles that were half in and half out of the lower and right-hand lines were included but those half in and half out of the upper and left hand lines were excluded. The mean of 10 determinations was expressed for 1 mm^2 area and taken as follicular count for a given animal. Follicular diameter was determined within the same visual fields in 1—1 follicles chosen randomly. Since the cross sections of follicles are not quite uniform, 3 diameters deviating at an angle of 60° were determined in each follicle and their mean was taken as follicular diameter. The mean of 10 follicular diameters was expressed in μm and taken as mean follicular diameter of a given animal. The height of epithelial cells was determined in the same follicles at 5—5 positions in each cell, where the nucleus was in the place of the section. The follicular diameter and the height of the follicular epithelial cells were expressed in μm. Means $\pm$ S. D. for experimental groups were calculated from individual data. Data were compared by Student's t test. In FSH or TSH groups, correlation analysis was performed between follicular count and follicular diameter, and between follicular diameter and cell height. Correlation analysis, regarding the 3 parameters studied, was also performed between FSH and TSH groups.

Results and discussion

The results (Table I) clearly demonstrate that TSH exerted the actions expected: follicular count per one visual field was diminished with all of the doses tested while follicular diameter and cell height were augmented. These

Table I

Effect of FSH and TSH on certain parameters of the thyroid gland

		Follicular count		Follicular diameter		Cell height	
		No/mm^2	significance	μm	significance	μm	significance
Control		1455.1 $\pm$39.86		25.38$\pm$3.66		2.09$\pm$0.31	
20μ	TSH	657.3$\pm$71.90	$p \ll 0.001$	39.21$\pm$2.77	$0.05 > p > 0.01$	6.22$\pm$0.76	$p < 0.001$
40μ	TSH	570.2$\pm$93.25	$p \ll 0.001$	47.61$\pm$7.76	$0.05 > p > 0.01$	8.21$\pm$0.76	$p \ll 0.001$
80μ	TSH	650.4$\pm$48.26	$p \ll 0.001$	41.90$\pm$4.62	$0.05 > p > 0.01$	8.36$\pm$1.73	$p \ll 0.001$
160μ	TSH	531.1$\pm$110.2	$p \ll 0.001$	47.73$\pm$7.95	$0.05 > p > 0.01$	9.13$\pm$1.10	$p \ll 0.001$
20μ	FSH	1109.5 $\pm$177.34	$0.1 > p > 0.05$	33.05$\pm$1.62	$0.1 > p > 0.05$	2.78$\pm$0.70	n.s.
40μ	FSH	1196.6 $\pm$106.53	$0.05 > p > 0.01$	31.89$\pm$2.34	n.s.	2.66$\pm$0.64	n.s.
80μ	FSH	1090.2 $\pm$118.63	$0.05 > p > 0.01$	32.00$\pm$5.77	n.s.	3.81$\pm$1.03	n.s.
160μ	FSH	815.4 $\pm$ 57.52	$p \ll 0.001$	34.66$\pm$8.27	n.s.	4.92$\pm$0.41	$p \ll 0.001$

changes are essentially dose-dependent a highly significant effect however, was already achieved with the lowest dose.

The effect of FSH was less apparent than that of TSH, but it decreased the follicular count in each instance.

At the same time, it enhanced both follicular diameter and cell height, but these differed significantly from the control only in two instances. This was presumably due to the great variation of data in various groups. Two explanations are offerred for this. Our quantitative method is based on the assumption that the follicles are spherical and the height of the follicular epithelial cells is uniform within one follicle. Actually, this is not the case. The great variation may also be due to the fact that the hormones act differently on various follicle populations (being in different functional state?) and thus depending on the extent of the effect considerable changes might occur in follicular size. Since in the FSH group there was an extreme variation it is suggested that the action of FSH is more selective than that of TSH: it is capable of enhancing the function of a certain population (in functional state?) of follicles only.

Two factors appear to account for the diminution of follicular count per unit area, an increase of large follicles or an enhancement of the parafollicular mass. Although light microscopic observations appeared to indicate that the treatments did not alter the parafollicular mass, the correlation between follicular count and diameter can give a definitive answer. In both FSH and TSH treated groups there was a highly significant correlation ($r = 0.9503$ and $r = = 0.9595$) indicating that the diminution of follicular count was due to a change of the follicular diameter i. e. to an effect on the follicular epithelium and not on the parafollicular mass.

The question arose whether the follicular growth in response to hormonal stimulation was due to an enhancement of cellular dimensions (hypertrophy) and/or increased cellular division (hyperplasia). To solve the problem the correlation between follicular diameter and cell height was studied. The correlation coefficients ($r = 0.9701$ and $r = 0.737$) were significant in both instances but the lower value for the FSH group appear to indicate a significant contribution of cellular division, too. Resemblance between the effects of the two hormones was also compared by correlation analysis of the relevant parameters in the FSH and TSH groups. The high correlation coefficients (follicular count, $r = 0.8782$; follicular diameter, $r = 0.9004$; cell height, $r = 0.7583$) appeared to indicate a similar action by the two hormones. On the other hand, the lower correlation coefficient for cell height rather suggested a hypertrophic response to TSH and a hyperplastic one to FSH treatment.

These findings suggested on overlapping effect by the two hormones under the present experimental conditions i. e. the gonadotropin FSH was capable of acting on the TSH receptors of the thyroid gland in the early stages of ontogenesis. The effect was apparent, thus it could not be ascribed to an

eventual contamination that might occur even in the purest pituitary hormone preparations. The more so, since FSH given in the lowest dose exerted a significant effect on the two parameters, whereas under the effect of higher doses only one of the indices changed significantly. Our hypothesis was further supported by the fact that the same FSH treatment failed to enhance thyroid function in 5-week-old birds. At the same time, a comparison of the present results with those of our earlier study examining the actions of tropic hormones on the gonads showed clearly that overlapping effect of the related hormones was more on the gonads than on the thyroid. This was presumably due to the fact that thyroid function and maturation of its receptors already start in the chick embryo [13] and the onset of endogenous TSH secretion and development of the hypothalamus — pituitary — thyroid axis are complete by day 11 [8], while the onset of gonadal function and receptor maturation are probably terminated later. The examinations by ENGEL and FROWEIN [5] appear to support this view demonstrating that until 2 weeks after birth the Leydig cells are not responsive to hCG in the rat.

PEKOREN and WEINTRAUB [9] have described two types of TSH receptor in the bovine thyroid, one with low affinity responsive to hCG and a TSH-specific one with high affinity. The methods used in the present study did not allow to draw such conclusions in chicken. Postulating however, two types of TSH receptor in the chick, it is possible that their follicular distribution may vary depending on the functional state and the stage of differentiation. In such a case this might be responsible for the selective follicular effect of TSH. A further explanation offered for the quantitative differences between the overlapping effects on the gonadal and thyroid receptors is that the two hormones act on identical receptors in the gonads and on separate ones in the thyroid. This does not of course rule out the fact that thyroid development might be influenced by the presence or an excess of gonadotropins, i. e. an overlapping of hormonal effects at the cellular level may occur in both the thyroid and the gonads.

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EFFECT OF CHOLECYSTOKININ ANTISERUM ON THE BRAIN MONOAMINE CONTENT IN RATS

by

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The effects of intracerebroventricular administration of cholecystokinin (CCK) antiserum (specific for COOH-terminal four amino acids) of CCK were tested in three dilutions on the dopamine (DA), norepinephrine (NE) and serotonin (5-HT) contents of the hypothalamus, mesencephalon, amygdala, septum, striatum and cerebral cortex in rats 24 h following application.

CCK antiserum decreased the DA and NE contents in the hypothalamus, mesencephalon, amygdala and septum, while it increased the DA content and decreased the NE content of the striatum. It had a slight effect on the 5-HT contents of the amygdala, septum and striatum.

During the last years progress in protein biochemistry has made it possible to isolate and structurally define a number of small biologically active peptides of the central nervous system [9]. VANDERHAEGHEN et al. [32] showed with immunohistochemical methods the presence of a gastrin-like peptide of the vertebrate central nervous system, especially in the cortical grey matter. By immunological techniques combined with fractionation procedures, this gastrin-like material has been shown to be related to cholecystokinins, namely to the biological active C-terminal octapeptide of cholecystokinin in its complete sulphated form (CCK-8-SE) [3, 19, 21, 27]. REHFELD [22] reported that this CCK immunoreactivity is present in most of the brain regions except in the epithalamus, cerebellum and pituitary, whereas gastrin-immunoreactivity is present only in the pituitary stalk, posterior pituitary and anterior pituitary. The exact functional role of CCK in the central nervous system is not fully understood. Evidence has, however, been accumulating that these peptides may act as synaptic messenger, possibly neurotransmitter of neuromodulator [10, 26] and/or they may have a role in behavioural, vegetative and endocrine functions [16, 18].

In earlier experiments it was shown that CCK-8-SE caused changes in the brain transmitter content and metabolism following intraventricular administration [6, 7, 30]. It is conceivable that CCK-8-SE exerts its effect on neurotransmitters directly and/or through the cerebrospinal fluid following its release from different brain regions. If this was true, the specific antiserum administered into the lateral ventricle of rats might neutralize the endogenously produced and released CCK, causing in this way a deficit in CCK in the central nervous

system. This approach has successfully been used for vasopressin by DE WIED and his coworkers demonstrating that the action of vasopressin could be reversed by antivasopressin immunosera [4, 5, 15, 31, 33, 34]. Similar effects were observed by us [11].

In the present study an attempt was made to clarify the effect of endogenous CCK on brain monoamine metabolism by a CCK/gastrin antiserum intracerebroventricular administration on the dopamine (DA), norepinephrine (NE) and serotonin (5-HT) content of the hypothalamus, mesencephalon, amygdala, septum, striatum and cerebral cortex.

Methods

In the experiments, CFY adult male rats, weighing 200—250 g were used. For the intraventricular administration of the CCK/gastrin antiserum a stainless steel cannula was implanted by stereotaxic apparatus into the lateral brain ventricle and fixed by dental cement to the skull. The animals were used one week after the operation. The proper position of the cannula was checked at dissection of the brain. The animals were kept on an artificial day-light schedule, 12 h light and 12 h dark, light being started at 6 a. m. Standard commercial food and tap water were given *ad libitum*.

Antiserum was obtained from Dr. J. F. REHFELD (Institute of Medical Biochemistry, University of Aarhus, Aarhus, Denmark); it had been raised in. rabbits against synthetic human gastrin-17 as described by REHFELD et al. [23] and LARSSON and REHFELD [12, 13].

The antiserum (No. 4562) is specific for the COOH-terminal four amino acids (Trp-Met-Asp-Phe-NH_2), which are identical in gastrin and CCK [14].

The antiserum was dissolved in 0.9 % saline and injected *via* the cannula into conscious freely moving rats in 1 : 50, 1 : 10 and 1 : 2 dilutions in 5 μl. The control animals received normal rabbit serum in the same dilutions and volume.

The animals were killed 24 h after the injection. The brain was quickly removed and frozen, and the following brain areas were dissected according to BAUMGARTEN et al. [1]: hypothalamus, mesencephalon, amygdala, septum, striatum and cortex. The DA, NE and 5-HT contents of the different brain areas were measured by the method of SHELLENBERGER and GORDON [28]. Statistical significance was calculated by Student's t test for paired data.

Results

CCK-4 antiserum injected into the lateral ventricle in three dilutions caused the following changes in the DA contents of the different brain areas (Fig. 1). The DA contents were decreased in the hypothalamus after 1 : 10 ($p < 0.05$) and 1 : 2 ($p < 0.001$) dilutions, in the mesencephalon after 1 : 10 ($p < 0.01$) and 1 : 2 ($p < 0.001$), in the amygdala after 1 : 10 ($p < 0.01$) and 1 : 2 ($p < 0.001$) dilutions, in the septum after all dilutions ($p < 0.05$ or $p < < 0.001$). In the striatum it was increased in all dilutions as compared to the control ($p < 0.05$ or $p < 0.001$). There was no change in the DA content of the cortex.

Figure 2 shows the effects of CCK-4 antiserum in three dilutions on the NE contents of the different brain areas. The NE content was decreased in the

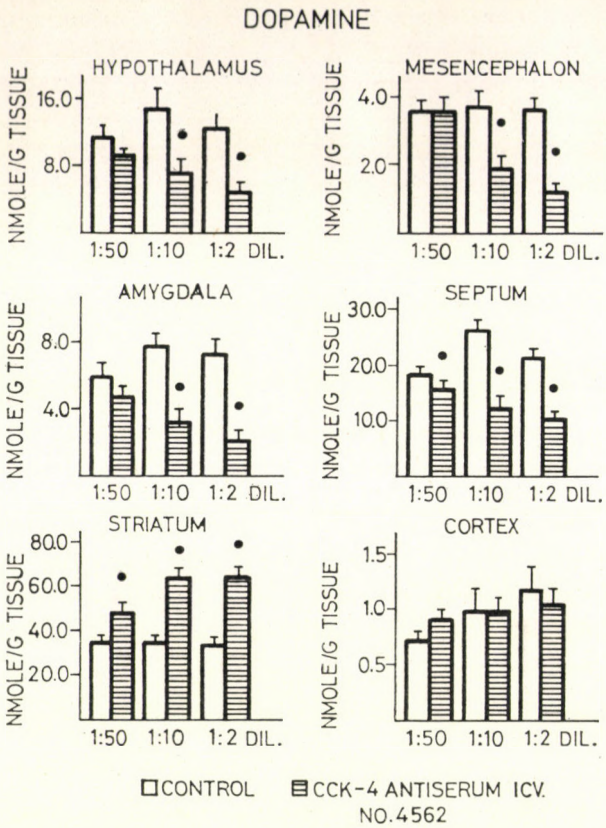

Fig. 1. Effect of CCK-4 antserum (No. 4562) in three dilutions on dopamine content of different brain areas 24 h following intraventricular injection. Each column represents the mean of 8 animals; vertical lines indicate standard error. Abbreviation: — significant changes between CCK-4 antiserum and control

hypothalamus ($p < 0.05$, $p < 0.01$ or $p < 0.001$) and in the mesencephalon ($p < 0.05$ or $p < 0.01$) after all dilutions. In the amygdala it was decreased after 1 : 10 and 1 : 2 dilutions ($p < 0.001$), in the septum it was decreased after 1 : 50 and 1 : 2 dilutions ($p < 0.01$), and in the striatum it was decreased after 1 : 2 dilution ($p < 0.05$). There was no significant change in the NE content of the cortex.

CCK-4 antiserum injected into the lateral brain ventricle in three dilution, caused the following changes (Fig. 3): the 5-HT contents were decreased after 1 : 10 and 1 : 2 dilutions ($p < 0.01$) in the amygdala and after 1 : 10 dilution ($p < 0.05$) in the septum. It was increased after 1 : 10 dilution ($p < 0.01$) in the striatum. There were no significant changes in 5-HT contents of the hypothalamus, mesencephalon and cortex.

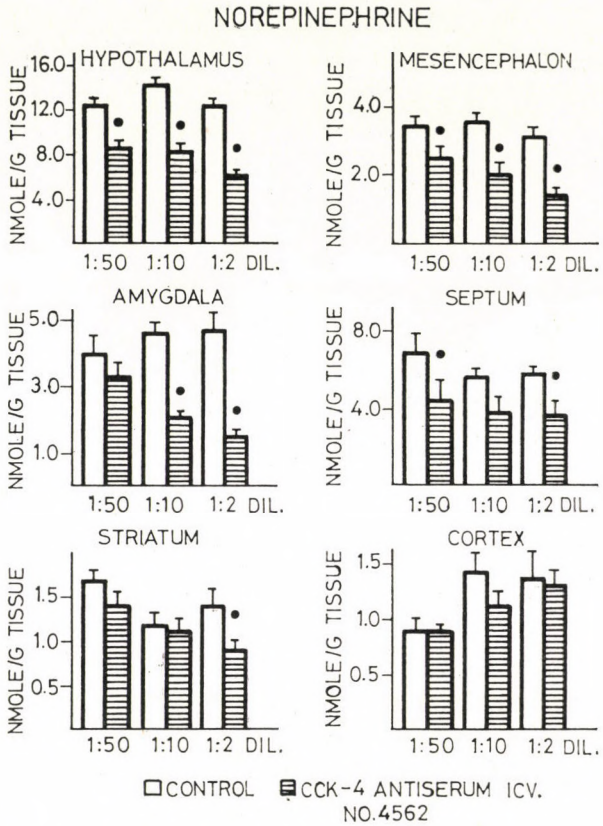

Fig. 2. Effect of CCK-4 antiserum (No. 4562) in three dilutions on norepinephrine content of different brain areas 24 h following intraventricular injection. Symbols as in Fig. 1

Discussion

CCK was first described as a gastrin-like peptide in the brain [32], but it later turned out to be CCK octapeptide [3, 25]. Recent investigations have confirmed this, although whole CCK (CCK triacontapeptide, CCK-33) and other fragments (C-terminal dodecapeptide of CCK-33, CCK-12; C-terminal octapeptide of CCK-33, CCK-8; C-terminal tetrapeptide of CCK-33, CCK-4) have also been identified [14, 25]. Rehfeld and Kruse-Larsen [24] showed that CCK-8, a smaller peptide corresponding to sequence 25—29 of CCK-33, and CCK-4 are present in the human cerebrospinal fluid.

In our earlier studies [6, 7, 30] it was shown that CCK-8-SE injected intraventricularly increased the DA and NE contents of the hypothalamus, mesencephalon, amygdala and septum in a time and dose-dependent manner,

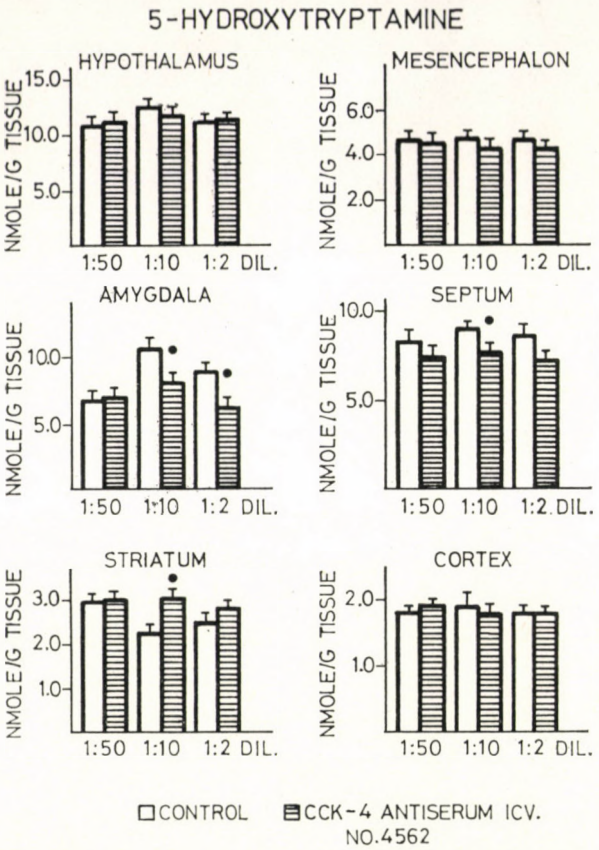

Fig. 3. Effect of CCK-4 antiserum (No. 4562) in three dilutions on serotonin content of different brain areas 24 h following intraventricular injection. Symbols as in Fig. 1

while it decreased it in the striatum. CCK-8-SE was found to decrease the 5-HT contents of the hypothalamus, mesencephalon, amygdala and striatum.

Using specific antiserum for endogenously produced peptide hormones *in vivo* has successfully been used for vasopressin by De Wied and his coworkers demonstrating that vasopressin can elicit a number of behavioural reactions [4, 5]. Neutralizing endogenously produced vasopressin with vasopressin antiserum reversed the action of vasopressin. These data led to the conclusion that endogenously produced vasopressin plays a role in certain behavioural reactions which can be facilitated by exogenous vasopression of diminished or even reversed by antivasopressin serum.

Using cholecystokinin/gastrin antiserum, the present results clearly showed that CCK-4 antiserum, which could neutralize the physiologically active CCK in the brain, elicited an opposite effect in the majority of cases,

lowering the DA and NE contents of the hypothalamus, mesencephalon, amygdala and septum while increasing them in the striatum. There were no opposite effects between CCK-8-SE and CCK-4 antiserum on the 5-HT contents of different brain areas, except in the striatum.

These data supported the assumption that CCK might be a physiological modulator of different monoamines in different brain regions, which could be associated with the documented action of CCK on satiety [2, 8, 17, 20, 29].

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MODIFIED METHOD FOR DIRECT LONG-TERM MEASUREMENT OF AORTIC PRESSURE IN THE RABBIT

by

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A modification of the method of BROOKS and MUIRHEAD [2] for long-term measurement of aortic pressure in rabbits is presented. The original abdominal surgical approach is replaced by a flank extraperitoneal incision and the construction of the catheter is simplified and adapted to the new surgical procedure. The preparation and implantation of the catheter as well as the pressure recording technique are described. Using the modified catheter pulsatile and mean aortic pressure could be measured in conscious rabbits several months.

Owing to the flank approach, surgical trauma and the risk of intra-abdominal infection could be minimized. On the other hand, this technique allowed simultaneous extraperitoneal procedures involving the aorta and kidney, permitting more complex haemodynamic studies.

In conscious rabbits, arterial pressure can be measured either by the ear capsule of Grant and Rothshild or directly by cannulation of the central ear artery. The first method, in addition to its inaccuracy [7], does not provide a possibility for continuous blood pressure recording, whereas the main disadvantage of the second method is that the pressure can be recorded by a single cannulation for a period not longer than 1 week (Korner, 1965). Long-term cannulation of the carotid artery seems to be a more feasible method, however this approach causes a loss of function of the ipsilateral sinocarotid receptors. Moreover, the latter method often leads to local clot formation followed by embolization of the kidneys [2].

In 1972, BROOKS and MUIRHEAD [2] described a technique for the implantation of an aortic catheter in the rabbit that allows the pressure measurements over a period of several months. The method is based on the principle of the cannulation procedure of WEEKS and JONES [6] elaborated for the rat. In an attempt to adapt it to the rabbit, the catheter construction was somewhat modified (Fig. 1-A) by which the aortic wall trauma could be minimized, a better anchoring of the catheter secured, and free neck movements of the animal allowed. On the other hand, the cannulation of the abdominal aorta distal to the origin of the caudal mesenteric artery avoids embolization of the viscera. Considering its functional features, this method seems to be best suited for pressure recordings in the conscious rabbit over a prolonged period of time.

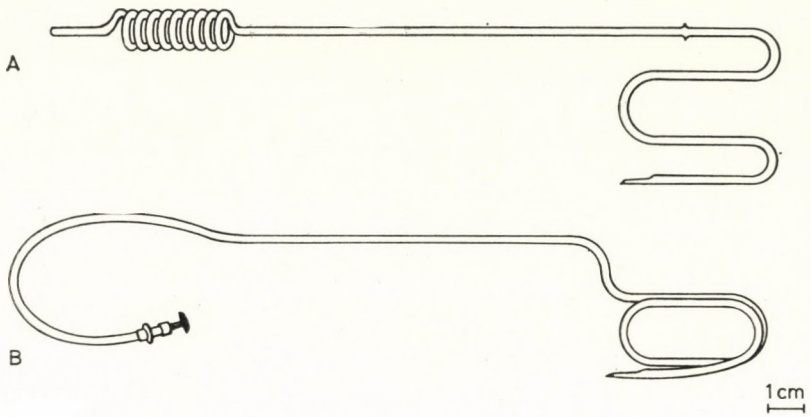

Fig. 1. A—aortic catheter of BROOKS and MUIRHEAD; B—our modification of the catheter construction

The purpose of this work was to test the practical value of our modification of the technique of BROOKS and MUIRHEAD. The original abdominal surgical approach was replaced by a flank incision to allow simultaneous extraperitoneal procedures, to reduce surgical trauma, and to avoid intra-abdominal infections. The catheter construction was simplified and adapted to the new implantation technique.

Methods

Catheter design

Figure 1—B shows the aortic catheter designed by us. With the flank surgical approach, it was more suitable to replace the U-curves in the original construction by parallel coils in form of an ellipse (diameters 4 : 2 cm). Thus this segment became more stable to a sideward pressure and, in the same time, it absorbed quite satisfactory the motion between the aorta and the fixed part of the catheter. On the other hand, this modification allowed one and the same catheter to be used for animals with different body weight. With the new surgical technique the flange was unnecessary. Finally, the curve on the end of the catheter ensures free movements of the animal's neck.

Our catheter was prepared from approximately 40 cm long single pieces of plastic tubing (KIFA—Sweden) with an outer diameter 2 mm and inner diameter 1 mm. The catheter tip was formed by pulling the end segment after heating above a flame. The tapered end was cut at an angle of 45°. The elliptic coils and the other curves were shaped consecutively after the tubing was melted in boiling water for about 1 min. An infusion system needle with a tight plug was inserted into the distal end of the catheter. Before implantation the catheters were treated by ultraviolet rays and kept in absolute ethanol.

Implantation technique

The animals were anaesthetized with pentobarbital sodium (Nembutal, 30—40 mg/kg b. w., intravenously). The lower abdominal aorta was exposed extraperitoneally *via* a left flank incision. The catheter was filled with heparinized saline, the distal part with the tip in a cephalad position was then aligned *in situ* parallel to the aorta below the origin of the caudal mesenteric artery. In this position, the catheter was fixed to the iliocostal muscle by two sutures

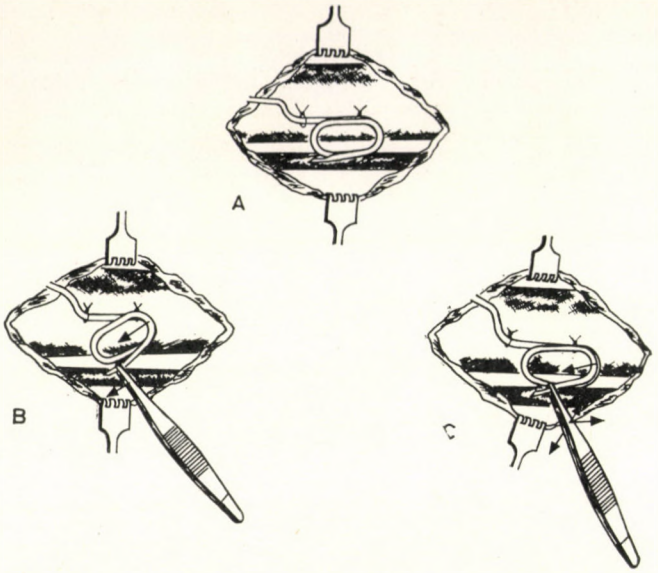

Fig. 2. Stages of the catheter implantation: A—position of the catheter before introduction into the aorta; B—introduction of the catheter into the aortic lumen. C—removal of the trocar

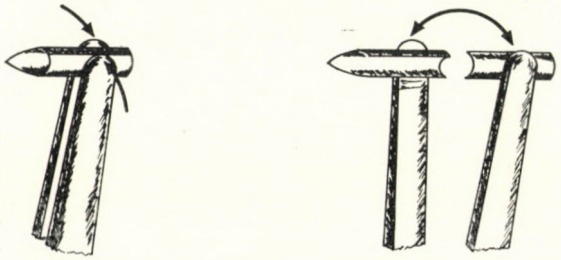

Fig. 3. General view of the trocar

(Fig. 2—A). The catheter tip was introduced into the aorta by a specially designed trocar (Fig. 2—B), consisting of two parts soldered to the tips of a pair of nippers (Fig. 3). This construction allowed removal of the instrument (Fig. 2—C). Using the trocar, bleeding was minimal even though the aorta was not clamped during the puncture. No suture was needed to anchor the catheter tip to the aortic wall. At the next stage of operation the free catheter end was pulled subcutaneously forward to the interscapular region where it was exteriorized and sutured to the skin behind the ear. After the local application of an antibiotic, the incision was closed. Benzyl penicillin, 50,000 units, was injected intramusculary. The catheter was flushed with heparinized saline and plugged. The entire procedure lasted about 30 min.

Recording procedure

The aortic pressure was measured by a pressure transducer (Statham P23Db) connected to a polygraph (Schwarzer, GFR) for continuous recording of the pulsatile and mean pressure. The pulsatile signal was also used for recording of the heart rate measured by a tachometer. To maintain the catheter patency during the procedure, 0.9 % saline (0.05 ml/min) was continuously infused by a constant-flow infusion pump (Braun, GFR) connected to the second outlet of the pressure transducer [4].

Usually the first recording was carried out one day after operation. The animal was placed in a rabbit box where it had at least a 30-minute rest before the measurement. The zero reference line of the Statham transducer was positioned at the right atrial level (approximately midthorax in the anteroposterior plane) [1] after the infusion of saline was started. The catheter end was then cleansed with 70 % ethanol and occluded with shod forceps. The plug was removed and after flushing with saline, the catheter was connected to the transducer by a polyethylene tubing. After recording the catheter was filled with heparinized saline and plugged.

Results and discussion

With practice almost all operations came off successfully. Usually there were no complications in the postoperative period. The animals maintained body weight and had a good disposition. The catheter patency was maintained for more than one month by daily flushing with heparinized saline. In one rabbit we managed to measure the aortic pressure for a period of five months (Fig. 4). After the animal had died of an unrelated disease, the catheter tip lumen was found free from clots.

The mean aortic pressure of the conscious rabbits ranged from 65 to 75 mm Hg which was in agreement with the values reported by BROOKS and MUIRHEAD [2]. The heart rate in resting quiet rabbits was found to be in the range of 180—240 beats/min.

With the original technique of BROOKS and MUIRHEAD the pressure is recorded without infusion into the catheter and the duration of the measurement is not longer than a few minutes. The use of the infusion recording technique allowed us to continue our measurements for practically unlimited time, thus it considerably extended the possibilities of the method.

Good results were obtained with simultaneous extraperitoneal surgical interventions combined with our technique for catheter indwelling. Thus we had a possibility to perform by one and the same surgical approach left kidney denervation and implantation of an aortic catheter, aortic and renal artery occlusion and placement of electromagnetic probe for renal blood flow measurement. The successful performance of complex haemodynamic investigations in chronic experiments was largely due to the present modification of the method for recording of aortic pressure.

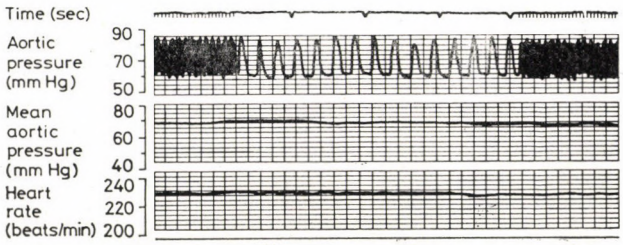

Fig. 4. Aortic pressure and heart rate in a conscious rabbit four months after operation

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EFFECTS OF DRUGS INFLUENCING THE cAMP LEVEL ON HIPPOCAMPAL SEIZURE ACTIVITY

by

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Changes of hippocampal EEG seizure activity elicited by electrical stimulation of the hippocampus or penicillin injection into the hippocampus were investigated under the effect of locally applied drugs influencing the cAMP level in the brain. It was observed that some drugs which elevate the cAMP level such as papaverine, histamine $+K^+$ and dibutyril cAMP elevated the electric seizure threshold while in penicillin-induced epilepsy they reduced the occurrence of ictal activity and the interictal spike frequency. These drugs when applied before penicillin prolonged the time necessary for development of the epileptic focus. The effect of imidazole was the opposite in every respect.

On the basis of these data the possible role of cAMP in the pathomechanism of epilepsy is discussed.

Epilepsy is an excessive and synchronous paroxysmal hyperactivity of a pathologically functioning neuron population. The important role of cAMP in the excitability and metabolic processes of neurons [2, 3, 8, 11] raises the possibility of a relationship between epilepsy and cAMP metabolism. According to present knowledge the disturbances of Na–K–ATP-ase activity, energy metabolism and the transmitter balance are the intraneuronal mechanisms underlying epilepsy [18]. It has been shown that these mechanisms are in connection with cAMP metabolism [2, 16]. On the other hand, some antiepileptic drugs too, influence the metabolism of cAMP [1, 12, 14]. The cAMP level in the cerebrospinal fluid of epileptic patients is lower than in controls [15, 19], and the cAMP level in different brain areas increases during seizures [5]. These data also support the possible connection between cAMP metabolism and epilepsy.

The aim of the present study was to investigate whether locally applied drugs influencing the cAMP level in the brain had any effect on the threshold of seizures elicited by electrical stimulation of the hippocampus, or on penicillin-induced epilepsy in the hippocampus.

Methods

The experiments were carried out on 70 CFY rats. Under ether anaesthesia they were subjected to tracheal cannulation and implantation of electrodes and a cannula. The animals were then immobilized by curarisation (Curarine-Asta, 2 mg/kg) and respirated artificially.

In all cases a cannula and a unipolar recording electrode were introduced stereotaxically into the left dorsal hippocampus. If the electric seizure threshold was investigated, a bipolar

stimulating electrode was also inserted into the hippocampus. The distance between stimulating electrodes was 0.25 mm, while the electrodes and the cannula were placed 0.5—1 mm apart. The stainless steel electrodes were insulated with enamel except 0.5 mm at the tip. Electrical activity of the brain was recorded by a "Hellige Neuroscript" apparatus and the frequency spectrum analyzed with a frequency analyzer.

Histamine $+K^+$ (1 μg + 29 μg) as an adenylcyclase stimulator, papaverine (40 μg) as a phosphodiesterase inhibitor, and dibutyril-cAMP (2 μg) were used to elevate the cAMP level, while to decrease it, imidazole (10 μg) a phosphodiesterase stimulator was employed. The control animals received physiological NaCl solution. The drugs were injected in a volume of 1 μl into the hippocampus with a Holmuth-Wetter microinjector.

When the changes of seizure threshold were examined, physiological saline was injected into the hippocampus after recording the spontaneous EEG activity in order to check the effect of mechanical irritation. 4—5 min later the threshold of EEG seizure activity elicited by electrical stimulation of the hippocampus was determined. Stimulation was performed with a "DISA-Multistim" apparatus through an isolation unit gradually changing the voltage of stimulation (2 sec train of rectangular 0.2 msec pulses at a frequency of 30 c/s). The voltage of stimulation followed by a series of spike discharges with frequency of more than 14.5 c/s and amplitude at least three times higher than of spontaneous activity was considered the seizure threshold. Fifteen minutes after the disappearance of seizure activity, one of the investigated drugs was injected into the hippocampus through the cannula, and 5 min later the seizure threshold was determined again. The seizure threshold was determined 15 min later once more, to see the duration of the drug effect.

In the experiments designed to investigate the influence of drugs on penicillin-induced epilepsy, after recording the spontaneous EEG activity 1000 U penicillin was injected into the hippocampus. The number of paroxysms per 10 min and the frequency of interictal spike discharges of the focal activity developing 3—7 min after penicillin application and changes in these parameters after the local injection of one of the drugs investigated, were determined. In some experiments the drugs affecting the cAMP level were injected 2—3 min before penicillin administration. In these cases the time necessary for development of the epileptic focus was measured.

At the end of the experiments the brains were fixed in 10 % formalin and localization of the electrodes and of the cannula was checked in frozen sections [9].

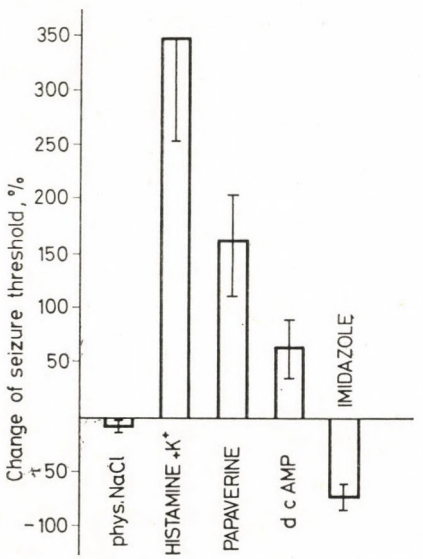

Fig. 1. Changes of the hippocampal electric seizure threshold under the effect of locally applied drugs. Alteration of the seizure threshold is expressed in per cents of the control seizure threshold (in volts) before drug administration. The columns represent the mean ± S. E. M. of 5—6 experiments

Results

The threshold of hippocampal seizure activity elicited by electrical stimulation showed significant changes under the effect of the drugs investigated (Fig. 1). While physiological NaCl solution practically did not influence the electric seizure threshold, histamine + K^+, papaverine and dcAMP markedly elevated it. The effect of imidazole was the opposite. 15—30 min after injection of the drug the seizure threshold returned to the original level. In some cases, however, spontaneous epileptic activity was observed after imidazole application, these animals were not used for seizure threshold determination.

The occurrence of paroxysms of penicillin-induced epilepsy was also influenced by the drugs in question. (Fig. 2). Histamine + K^+, papaverine and

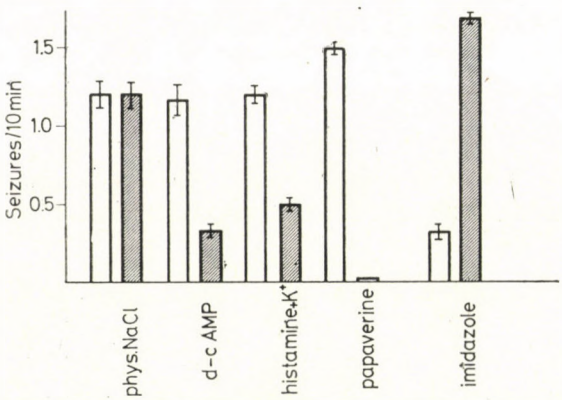

Fig. 2. Number of seizure attacks per 10 min in penicillin-induced epilepsy before (empty bars) and after (striped bars) hippocampal injection of drugs

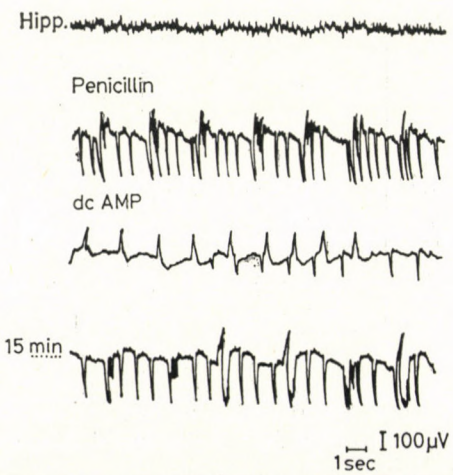

Fig. 3. EEG activity in the hippocampus during penicillin-induced seizure activity and its alteration after dibutyryl cAMP injection

dcAMP reduced the number of seizures, while imidazole increased it. Physiological NaCl solution did not cause any change. Interictal spike frequency was decreased by dcAMP (Fig. 3.). About 15 min after injection of the drug this effect disappeared. A similar depressant effect on EEG activity was displayed by histamine + K^+ and papaverine. The effect of imidazole proved to be opposite: following its injection the epileptic activity increased in intensity (Fig. 4). After physiological saline injection there was no appreciable change in interictal spike activity.

Under our conditions the focal epileptiform activity developed about 5 min after penicillin administration. This latency was not influenced by physiological NaCl injected before the application of penicillin. However, when histamine + K^+, papaverine or dcAMP was injected into the hippocampus 2—3

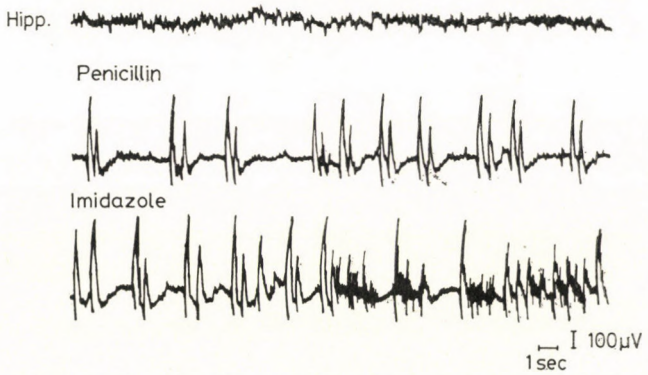

Fig. 4. Effect of imidazole on penicillin-induced hippocampal seizure activity

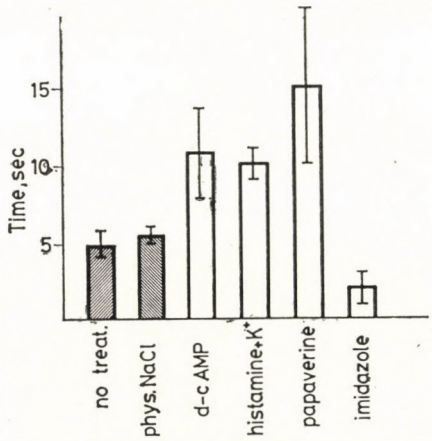

Fig. 5. Effect of drugs influencing the cAMP level on the latency of focal EEG activity after penicillin injection into the hippocampus. The columns represent the mean ± S. E. M. of data received in 3—4 animals

min before penicillin, the development of focal activity was delayed or the focal activity did not appear at all (Fig. 5). On the other hand, imidazole shortened the latency of development of epileptic activity.

Discussion

The drugs applied in our experiments are known to have an increasing or decreasing effect on the cAMP level of the brain [2, 4, 16]. The fact that these drugs influenced the brain seizure activity elicited by electrical stimulation or penicillin injection confirms the connection between tha actual cAMP level of the brain tissue and epileptic events. In the present experiments the agents increasing the cAMP level elevated the electric seizure threshold and elicited a depressant effect on penicillin-induced epilepsy, i. e. they had an antiepileptic-like action. Imidazole, which decreases the cAMP level, had an opposite, epileptogenic effect. This is in agreement with the observations according to which elevation of the cAMP level in different brain regions such as hippocampus, causes a depression in the electrical activity of the same regions [10, 17], and that an increase of the intraneuronal cAMP level elicits hyperpolarization [8, 11]. Some of the authors, however, published contradictory data [6, 20]; after intracerebral cAMP injection they observed epileptiform seizures. We have, however, to take into consideration that electrophysiological manifestations of the cAMP effect may be different depending on whether it exerts its influence on facilitatory or inhibitory structures. Furthermore the actual state of cAMP metabolism in a given brain region can also modify the observable effects.

It has been shown that cAMP accumulation in the cells, especially at the postsynaptic membrane, is increased during enhanced neuronal activity [7, 8] 13]. Taking furthermore into account the hyperpolarizing effect of cAMP and the present results, cAMP seems to participate in a postsynaptic, cybernetic feedback system, which controls the firing rate of the neuron. Since a pathological cAMP metabolism as an aetiological factor has been raised in the case of some diseases like bronchial asthma or gastric ulcer, some anomaly of the cyclic nucleotides might play a role in the pathogenesis of epilepsy.

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PHARMACOLOGICAL BASIS OF DOSAGE FORM OF TWO ANTIMALARIALS: CHLOROQUINE AND MEPACRINE

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(Received April 22, 1980)

Chloroquine and mepacrine appear in the stomach wall and inhibit gastric emptying in the rat after oral or parenteral administration. Measuring their concentration in the stomach wall, it was found that it depended upon the route of application. A smaller proportion of a given dose accumulated in the stomach wall when chloroquine and mepacrine were introduced by avoiding the stomach. The side effect of the two drugs, the inhibition of gastric motility, could be minimized by not only parenteral but also duodenal administration; the latter could be realized by enteric-coated tablets.

A solution of chloroquine or mepacrine given orally to rats, owing to the parasympatholytic and antispasmodic properties of the drugs, diminished gastric emptying and thus their own absorption [5, 7, 8]. This results in a difference between the oral and intraperitoneal LD50 value (Table I). Both chloroquine and mepacrine were shown to be absorbed from the stomach, but also after parenteral administration they were excreted from the blood into the gastric juice, infiltrating the tissues of the gastric wall [6]. It has been shown

Table I

LD50 values of chloroquine and mepacrine in rats by different routes of administration

Route of administration	LD50 mg/kg	
	Chloroquine	Mepacrine
Oral	1080* (885—1.317) n=94	661 (628.7—694.9) n=60
Intraperitoneal	102* (97—107) n=86	142 (133.9—150.5) n=42
Intravenous	60* (56.2—63.8) n=48	41 (39.1—43.0) n=42
Duodenal	210 (203.3±218.0 n=60	260 (251—269) n=60

* VARGA, F.: Arch. int. Pharmacodyn. **163,** 38—46 (1966)
n = number of rats

that chloroquine tablets easily disintegrating caused gastrointestinal complaints and these could be prevented by administrating coated tablets [1].

The present experiments were undertaken to clarify (1) how the concentration of chloroquine and mepacrine in the gastric wall changes depending on the route of administration; (2) the correlation between the concentration of the drugs in the stomach wall and the inhibition of gastric emptying; (3) which route of administration could minimize the quantity of chloroquine and mepacrine appearing in the gastric wall, and lower the incidence of the side effect of these drugs, i. e. the inhibition of gastric motility.

Methods

The experiments were performed on female Sprague-Dawley CFY rats weighing 200—250 g. The animals were deprived of food 24 h before the experiments, water was allowed *ad libitum*. Gastric emptying was measured with phenol-red according to LEVINE [2], chloroquine was estimated by the method of MCCHESNEY et al. [4], mepacrine was determined by a method developed in our laboratory. For details, the reader is refered to a previous paper [6].

Duodenal application for six days was carried out by a flexible polyethylene tube placed into the duodenum after opening the abdomen under pentobarbital anaesthesia. The tube was led under the skin and fixed on the back of the neck, thus the drugs to be tested reached directly the duodenum, imitating the application of an ideal enteric-coated tablet.

LD50 values were determined according to LITCHFIELD and WILCOXON [3].

The data obtained were averaged, standard deviation (S. D.) was calculated, and the results were evaluated statistically by Student's t test.

The gastric emptying half-life as measured in 4 groups each consisting of 10 rats which were given a single dose of chloroquine or mepacrine dissolved in 1 ml volume. The various groups were killed at different times, and the phenol-red in their stomach was determined, then the data were averaged. The averages were plotted against time on a semilogarithmic scale, and a straight line was drawn through the points obtained. Chloroquine and mepacrine were estimated in the stomach wall of the sacrificed animals, too.

In the Tables, maximum chloroquine and mepacrine concentrations in the gastric wall measured in the first 6 h after intravenous administration and 60 min following oral application are indicated.

The drugs used were chloroquine diphosphate (Chinoin); mepacrine (quinacrine) dihydrochloride (Sigma); and phenol-red (Reanal).

Results

The concentration of chloroquine and mepacrine in the stomach wall, the gastric emptying half-life were measured in acute experiments after increasing doses given orally and intravenously. Results are shown in Tables II and III. For the sake of clarity, the rate of phenol-red retention along with the t 1/2 of gastric emptying is given in both Tables.

The data obtained show that the concentration of chloroquine and mepacrine measured in the stomach wall increased in dose dependent manner after oral and intravenous administration. A dose dependent increase of phenol-red retention in the stomach was also detected. When the drugs were introduced intravenously, although a higher portion of the LD50 was injected, a considerably smaller amount of chloroquine and mepacrine was found in the

Table II

Comparative data[1] for chloroquine after oral and intravenous administration

Chloroquine	Intravenous mg/kg			Oral mg/kg		
	10	20	30	20	100	300
Maximum concentration[2] in stomach wall, μg/g	13.4 ±1.1	22.4 ±2.2	38.0 ±10.0	62.0 ±19.0	478.0 ±120.0	887.0 ±95.0
Dose in per cent of LD50	16.6	33.3	50.0	1.8	9.2	27.0
Gastric emptying t 1/2, min	11.0	12.0	13.5	13.0	39.0	114.0
Retention of phenol-red, 30 min after application, per cent	15.8 ±0.1	18.2 ±1.8	21.0 ±7.8	39.9 ±8.9	54.6 ±8.3	72.8 ±4.5

[1] Each value was obtained from groups of 10 rats or more. Mean±S.D.
[2] In the first 6 h following application

Table III

Comparative data[1] for mepacrine after oral and intravenous administration

Mepacrine	Intravenous mg/kg			Oral mg/kg		
	10	20	30	20	100	300
Maximum concentration[2] in stomach wall, μg/g	19.2 ±1.4	51.0 ±6.4	58.0 ±8.0	80.0 ±21.0	270.0 ±57.0	340.0 ±83.0
Dose in per cent of LD50	24.4	48.8	73.2	3.0	15.1	45.4
Gastric emptying t 1/2, min	12.0	13.8	17.0	19.0	54.0	116.0
Retention of phenol-red 30 min after application, per cent	21.0 ±4.0	32.2 ±8.4	35.7 ±16.8	33.6 ±11.4	66.7 ±8.2	81.4 ±13.6

[1] Each value was obtained from groups of 10 rats, or more. Mean±S.D.
[2] In the first 6 h after application

Table IV

Concentrations of chloroquine and mepacrine in the stomach wall after 6-day treatment by gastric or duodenal administration[1]

Drug tested	Route of administration	Initial cumulative dose, mg/kg	Maintenance dose, mg/kg	Concentration in stomach wall, μg/g
Chloroquine	gastric	80.0	40.0	670.7±194.0
	duodenal	40.0	20.0	86.1± 25.9
Mepacrine	gastric	80.0	40.0	198.5± 53.5
	duodenal	40.0	20.0	53.1± 14.4

All data are means±S.D. obtained from groups of 10 rats, or more

stomach wall, and also a proportionally weaker inhibition of gastric emptying, indicated by a shortening of its duration.

Similar findings were obtained in rats after 6-day treatment with chloroquine or mepacrine through duodenal or gastric tube (Table IV). At 24h after

duodenal treatment for 6 days, significantly lower concentrations of chloroquine and mepacrine were found in the stomach wall than after their oral administration in solution.

Discussion

Chloroquine and mepacrine appear in the rat's gastric wall after both enteral or parenteral administration. Their concentration in acute experiments as well as after six days administration, depends upon the dose given and the route of application. The inhibition of gastric emptying correlates with the concentration of chloroquine or mepacrine measured in the stomach wall. The results clearly showed that the quantity of chloroquine and mepacrine in the gastric wall can be minimized by an appropriate choice of the route of administration, i. e. the concomitant inhibition of gastric emptying can be reduced. It seems therefore, to be practical to avoid the stomach; this can be done by parenteral administration. For prolonged therapy, injections are inexpedient, but duodenal application offers an efficacy almost equal with that of the intraperitoneal route (see Table I). On the basis of the present data, the application of chloroquine and mepacrine in entero-coated tablets appearing equipotent with duodenal application, seems to be reasonable, since the delay in gastric emptying can thus be minimized.

Acknowledgement

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Recensiones

J. H. Clark and E. J. Peck, Jr.

Female Sex Steroids. Receptors and Function Monograph on Endocrinology

Springer-Verlag Berlin, Heidelberg, New York, 1979
245 pages, Figs 116, price approx. 43.70 US $

The monograph summarizes the present knowledge concerning female sex steroid receptors and their function, based upon extensive research carried out in the authors' laboratory, complemented by data in the literature.

The monograph is divided into ten chapters, beginning with the action of steroid hormones and the role of receptors in this action, followed by characterization and measurement of receptors. Chapters III and IV deal with the localization of receptors and characteristics of cytoplasmic and nuclear receptors. Chapters V and VI discuss the biologic response and nuclear binding as well as the control of steroid receptor levels and steroid antagonism. Chapter VII gives an insight into the problem of steroid receptors and tumors. Chapter VIII discusses the changes of receptors under dfferent physiological conditions such as development, pregnancy, etc. Chapter IX deals with the action of gonadal steroids on brain function and the role of receptors in the action of steroid hormones in the brain. The final chapter gives a speculation and conclusion which summarizes the authors' opinion on different aspects of the relationship between the action of steroids hormones and receptors. This chapter is somewhat speculative but also stimulative, and will help those working in the field to bild up a model for further experiments. The volume is complemented with over 700 references with full title and subject index.

The book is highly recommended to those interested in biochemical and physiological action of female gonadal steroids, to endocrinologists, gynaecologists as well as scientists working in the field and even to those who would like to enter into this fascinating field.

G. Telegdy

D. A. Kharkevich (Ed.)

Handbook of Experimental Pharmacology

Continuation of Handbuch der experimentellen Pharmakologie

Vol. 53. Pharmacology of Ganglionic Transmission. XIII + 531 pages, with 72 figures and 80 tables. Springer-Verlag, Berlin—Heidelberg—New York 1980. Price DM 245,-; US$ 137. 20.

This volume presents the results of pure and applied research in the field of ganglionic transmission and considers the perspectives of its development. The volume is introduced by the Editor, D. A. Kharkevich in which he presents the history of humoral mediation, emphasizing the leading role of John N. Langely (1852—1926). Langley introduced the terms "autonomic nervous system" and "parasympathetic nervous system".

Chapter 1. Ganglionic Transmission: Morphology and Physiology, by V. I. Skok. Chapter 2, Ganglionic Metabolism, by J. D. Klingman, D. T. Organisciak and G. I. Klingman. Chapter 3. Methods for the Examination of Ganglionic-Blocking Activity, by L. Gyermek. Chapter 4a, Relationship between Chemical Structure and Ganglion-Blocking Activity a/ Quaternary Ammonium Compounds, by V. Trcka. Chapter 4b, Relationship Between Chemical Structure and Ganglion-Blocking Agents, by D. A. Brown. Chapter 6, Action of Ganglion-

blocking Agents on the Cardiovascular System, by D. M. AVIADO. Chapter 7, Action of Ganglion -Blocking Agents on the Gastrointestinal Tract, by D. F. J. MASON. Chapter 8, Absorption, Distribution, Fate and Excretion of Ganglion-Blocking Compounds, by D. F. J. MASON. Chapter 9, Nicotinic Ganglion-Stimulating Agents, by R. L. VOLLE. Chapter 10, Non-Nicotinic Chemical Stimulation of Autonomic Ganglia, by W. E. HOFFELY. Chapter 11, Ganglion Activity of Centrally Acting Neurotropic Agents, by A. NISTRI and J. P. QUILLIAN. Chapter 12, Ganglionic Actions of Anticholinesterase Agents, Catecholamines, Neuromuscular Blocking Agents and Local Anaesthetics, by R. L. VOLLE, Chapter 13, Ganglionic Activity of Cardiovascular Drugs, by M. AVIADO. Chapter 14, Ganglion-Blocking Agents in Internal Medicine, by E. V. ERINA. Chapter 15, Ganglion-Blocking Agents in Anesthesiology, by A. BUNATIAN and A. V. MESCHERJAKOV. Author Index and Subject Index, by V. V. MAISKY.

The volume in an excellent source of informations to pharmacologists, neurophysiologists and biochemists.

K. LISSÁK

K. MAGYAR (Ed.)

Monoamine oxidases and their selective inhibition

Advences in Pharmacological Research and Practice, Proceedings of the 3rd Congress of the Hungarian Pharmacological Society, Budapest 1979. Volume IV

General Editor: J. KNOLL. Pergamon Press, Oxford and Akadémiai Kiadó, Budapest 1980. 160 pages, with 56 figures and 27 tables

The book contains 21 papers of 41 participants from 8 countries. The papers presented at the symposium cover various aspects of MAO inhibitors, their biochemistry (H. BLASCHKO, B. EKSTEDT, J. I. SALACH, A. ANNA-LENA, S. YANEV, H. KALÁSZ, J. NAGY), pharmacology (J. KNOLL, B. KNOLL, L. G. HÁRSING, M. D. MASHKOVSKY), biotransformation (G. P. REYNOLDS) and some clinical studies of MAO inhibition (M. SANDLER, W. BIRKMAYER, M. D. YAHR, E. CSANDA, P. RIEDERER, J. WAJSBORT, U. K. RINNE, GY. MOLNÁR).

The book is an important source of up-to-date information concerning the biochemical mode of actions of deprenyl, the selective inhibitor of MAO-B, and the clinical application of deprenyl in Parkinsonian patients. A number of reports stress the essential role of the dopaminergic mechanisms with respect to the pathological process in Parkinson disease.

The book will be interesting for pharmacologists, biochemists, neurologists and other researchers and clinicians interested in the problem of selective MAO inhibitors.

F. VARGA

AUBREY MANNING

Verhaltensforschung

Eine Einführung. 3rd English edition, translated by G. EHRET and I. EHRET

Springer-Verlag Berlin—Heidelberg—New York 1979. XIII + 320 pages with 97 figures and 5 tables. Price DM 39.80 US$ 21.90.

Today, ethology is one of the most important branches of biology. In spite of ethology being in the foreground of the book, the author emphasizes the importance of its physiological and psychological bases. The book is written for beginning students and for all laymen interested in ethology, in particular in its connections with physiology and psychology. The abundant literature will give a possibility to the interested reader to go deeper into the study of the field.

K. LISSÁK

H. MATTHIES, K. KRUG and N. POPOV (Eds)

Biological aspects of learning, memory formation and ontogeny of the CNS

Akademie-Verlag, Berlin 1979. 514 pages, with 265 figures and 36 tables. Price DM 72,—

The book contains the proceedings of the Fifth International Neurobiological Symposium held in Magdeburg, June 1977, jointly with the Annual Meeting of the Commission "Neurophysiology and Higher Nervous Activity" of the Academies of Sciences of the Socialist Countries (Intermozg). The Symposium was organized by the Society of Pharmacology and Toxicology and the Society of Neurosciences of the GDR.

The volume consists of three separate chapters viz.

(1) basic biological processes of learning and memory (45 papers);
(2) ontogeny and differentiation of the CNS (13 papers);
(3) neurotransmission in the CNS (8 papers).

The first chapter is the most valuable. The papers presented here deal with the molecular neuronal and behavioural correlates of learning and memory processes. The second and third parts represent multidisciplinary approaches to the problem of brain ontogeny and neurotransmission.

The book will contribute to a better understanding of the brain ontogeny and function. The bulk of data presented in this volume will be useful to scientists interested in the research of the different functions of the CNS.

T. KUKORELLI

ROLAND SCHIFFTER (Ed.)

Zentral-vegetative Regulationen und Syndrome

Central autonomic regulations and syndromes

Springer-Verlag, Berlin—Heidelberg—New York 1980. X + 134 pages, with 56 figures, and 6 tables. Price DM 34,—; US$ 20.10.

The book intends to show the decisive significance of the brain in the regulation of vita visceral functions. In clinical medicine and neurology some anatomically and physiologically well-known processes are often regarded as "vegetative dystony". The book surveys the new results of anatomy, physiology, pharmacology and clinical sciences in the central nervous regulation of the heart, circulation, sodium metabolism, neurotransmitters in the limbic and extrapyramidal systems, sleep-and-wake rhythm and sweat secretion. The importance of a close connection between theoretical and clinical sciences is stressed. This would certainly activate neurological and medical research on the vegetative nervous system.

K. LISSÁK

J. SZENTÁGOTHAI, J. HÁMORI and E. S. VIZI (Eds)

Neuron Concept Today

Symposium held in Tihany, Hungary, August 26—28, 1976.
Akadémiai Kiadó, Budapest 1977, pp. 312.

The book is an impressive and daring attempt to confront our present knowledge of neural function with the traditional "neuron doctrine", a theory formulated almost a century ago. As attested by the multidimensional account of the participants of the symposium, the basic tenets of the theory are still valid and it emerges as a monolithic entity. Following a lucid and critical historical introduction of SZENTÁGOTHAI, 73 papers delivered by distinguished

neurobiologists from 12 countries deal with problems of neural functions from almost all possible directions of contemporary research (morphology, comparative aspects, electrophysiology, pharmacology, computer modelling, neuropsychology, etc.). This unusual diversity of approaches is rather tightly linked together by the common goal set by the title of the symposium. At a critical time when a wealth of new and apparently revolutionary information on basic functions, like synaptic morphologies, biochemistry of transmission, microcircuitry, etc., seem to shatter traditional concepts, such an unprecedented attempt at synthesis as was aftered by this symposium, is extremely useful. It helps to consolidate what is really known, it discloses uncertainties and by theoretical reorientation opens up new vistas.

E. GRASTYÁN

J. N. WALTON and F. L. MASTAGLIA (Eds)

The Muscular Dystrophies

British Medical Bulletin Vol. 36 No. 2, 1980.
Published by the Medical Department. The British Council, 65 Davies Street London WIY 2AA.
Price US$ 15.00.

The last 20 years in spite of the immense effort of research on the muscular dystrophies we are still far from understanding the nature of the basic abnormality of the muscle cell which causes it to waste. Within the last 30 years a number of theories concerning the pathogenesis has been developed.

The chapters of the volume are introduction by J. N. WELTON. *Clinical and genetic studies:* Clinical features and classification of the muscular dystrophies, by David GARDENER-MEDWIN; Duchenne muscular dystrophy: genetic aspects, carrier detection and antenatal diagnosis, by Alan E. M. EMERY; clinical biochemistry of muscular dystrophy, by R. J. PENNINGTON; electrodiagnosis of the muscular dystrophies by Michael HAYWARD; the heart in muscular dystrophy, by Stewart HUNTER; the respiratory system in muscular dystrophy, by J. NEWSON-DAVIS; management of muscular dystrophy: pharmacological and physical aspects, by V. DUBOWITZ and J. HECKMATT. *Pathogenesis and current trends in research:* morphological changes in dystrophic muscle, by M. J. CULLEN and F. L. MESTAGLIA; skeletal muscle: regeneration and transplantation studies, by R. H. T. EDWARDS; intermediary metabolism of muscle in Duchenne muscular dystrophy, by David A. ELLIS; muscle cell differentiation and the prospects for genetic engineering, by K. W. JONES; tissue culture of dystrophic muscle cells, by E. J. THOMPSON; is there a membrane defect in muscle and other cells? by J. A. LUCY; animal models: what is their relevance to the pathogenesis of human muscular dystrophy? by J. B. HARRIS and C. R. SLATER; future prospects by A. F. HUXLEY.

Reading the papers, one must agree with the statement of A. F. HUXLEY that it is impossible to say anything that is at the same time specific and reliable about future prospects in research, or improvements of treatment, in muscular dystrophy.

K. LISSÁK

INDEX

PHYSIOLOGIA — PATHOPHYSIOLOGIA

PHARMACOLOGIA

RECENSIONES

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója. Műszaki szerkesztő: Zacsik Annamária

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81.9021 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

ACTA PHYSIOLOGICA

ТОМ 57 — ВЫП. 2

РЕЗЮМЕ

ХАРАКТЕРИСТИКА АНИЗОТРОПНЫХ СВОЙСТВ АРТЕРИЙ С ПРИМЕНЕНИЕМ ЭКСПОНЕНЦИАЛЬНЫХ И ПОЛИНОМИАЛЬНЫХ ФУНКЦИЙ ДЕФОРМАЦИОННОЙ ЭНЕРГИИ СОСУДОВ

ХУДЕЦ А., МОНОШ Э.

С целью определения пассивных, анизотропных, эластических свойств артериальной стенки проводили трёхдимензионные, квази-статистические измерения на изолированных цилиндрических участках art. carotis communis и srt. iliaea. На основании наружного диаметра и на основании соотношения аксиальной расстягивающей силы и интралюминального давления, регистрируемых на различных фиксированных сосудистых участках, в интервалах от 0- до 250 мм ртутного столба высчитали инкрементальные модели аорты Янга и числа Пойсона. В ходе вычислений функцию деформационной энергии артерий, применяли полиномиальные и экспоненциальные модели.

Нами было установлено, что сконструированная нами функция энергии с 4 постоянными, даёт более точные результаты чем полиномная функция с 5 или 12 постоянными. По нашим результатам при низкой тангенциальной растяжении самым большим является аксиальный модуль а при большом тангенциальном растяжении тангенциальный модуль, при обоих типах артерии. Увеличивающееся начальное тангенциальное растяжение сильнее всего увеличивает тангенциальный модуль, потом радиальный и наконец аксиальный модуль. Изменение начального аксиального растяжения одинаково действует на аксиальный и радиальный модуль, но существенно не влияет на величину тангенциального модуля. Соответствующие числа Пойсона таким же образом зависят от величины начальной деформации сосуда.

Различное поведение — по отношению состояния деформации сосуда — двух чисел Пойсона, характеризующих структурную связь между тангенциальными и аксиальными направлениями, указывает на асимметрический характер структурной связи между двумя главными направлениями. По нашему мнению это характерное свойство пассивной сосудистой структуры обьясняется ветвистым расположением коллагеновых волокон.

ИССЛЕДОВАНИЕ РЕНОРЕНАЛЬНЫХ ВАЗОМОТОРНЫХ РЕФЛЕКСОВ

Г. КОТТРА, Б. ТУРЧАНИ и Л. ТАКАЧ

Авторы сообщают об исследовании вызываемого ацетилхолином контралатерального реноренального вазомоторного рефлекса, описанного в 1970 г. Мак-Фарлайном *(MacFarlane)*. В опытах на собаках, наркотизированных нембуталом, ни инфузия ацетилхолина, ни болюсное его введение не оказывали влияния на кровоток в контралатеральной почке, измеряемый с помощью электромагнитного датчика. Активность симпатических нервов, снабжающих почку противоположной стороны, возросла лишь на несколько процентов по отношению к основной активности, изменения показали наличие корреляции с понижением артериального кровяного давления. В конечном результате в экспериментальных условиях не удалось вызвать реноренальный вазомоторный рефлекс.

ВЛИЯНИЕ ДЕНЕРВАЦИИ ПОЧЕК НА ТРАНСТУБУЛЯРНУЮ РАЗНИЦУ ПОТЕНЦИАЛА ПРОКСИМАЛЬНЫХ КАНАЛЬЦЕВ

Г. СЕНАШИ, Г. КОТТРА, П. БЕНЧАТ, Л. ТАКАЧ

Транстубулярная разница потенциала проксимальных почечных канальцев (ПД) была измерена, применяя технику полимикроэлектродов у контрольных крыс (К, н = 10) и после односторонней почечной денервации (Д, н = 10). Острая почечная денервация влекла за собой двукратное увеличение диуреза и четырехкратный натриурез без изменения клубочковой фильтрации. ПД в первом достижимом сегменте проксимального канальца (ЕПТ) в группе К была $+0.27 \pm 0,08$ (S. E.) мВ (н = 16), тогда как в группе Д она была $-0,16 \pm 0,07$ мВ (н = 18) ($P < 0,01$). ПД в средних (МПТ) и последних (ЛПТ) сегментах не изменилась денервацией (МПТ: К = $0,94 \pm 0,05$, н = 21; Д = $0,98 \pm 0,04$, н = 19 NS. ЛПТ: К = $1,04 \pm 0,11$, н = 17; Д = $0,95 \pm 0,06$, н = 18 NS.) Негативный сдвиг ПД в ЕПТ является подкреплением нашего прежнего предложения, что симпатектомия провоцирует понижение активного транспорта ионов и органических веществ в проксимальном канальце. Неизменная ПД в МПТ и ЛПТ может указать на то обстоятельство, что пассивные силы, способствующие реабсорбции в этой части нефрона, сохраняются.

ГЕМОДИНАМИЧЕСКИЕ РЕАКЦИИ СО СТОРОНЫ МАЛОГО И БОЛЬШОГО КРУГА КРОВООБРАЩЕНИЯ НА ГИПЕРОСМОЛЯРНЫЕ РАСТВОРЫ, ВВЕДЕННЫЕ В БРОНХИАЛЬНОЕ КРОВООБРАЩЕНИЕ

ГОНДОШ Т., ПЕНЗЕШ И., ТРОЯН И., КОВАЧ Й., КЕЧКЕШ Л., НАДЬ Ш., КУЛЬКА Ф.

На собаках с открытой грудной полостью, изучали гемодинамические эффекты различных гипертонических растворов (8% р – р $NaHCO_3$, 5,6% NaCl и 34,3% р – р глюкозы) одинаковой осмолярности, введённых в art. bronchialis. При таком введении растворов минутный объём, системное кровяное давление, давление в лёгочной артерии и левом предсердии, пульсовое давление, бронхиальный кровоток и бронхиальная фракция минутного объёма значительно увеличивались а частота пульса, лёгочное и бронхиальное сопротивление уменьшились. При введении вышеуказанных растворов в art. pulmonalis полученные гемодинамические изменения были намного меньше. С целью уменьшения хирургической травмы и устранения открытой грудной полости нам был выработан, новый, более физиологический экспериментальный модель. Применяя этой модель, введение раствора $NaHCO_3$ b art. bronchialis также не получали существенных гемодинамических изменений. Однако введение раствора $NaHCO_3$ в art. pulmonalis через час после снижения кровяного давления до 40 мм ртутного столба путём кровопускания, системное кровообращение и давление в art. pulmonalis опять увеличались. В этих экспериментах мы нашли связь между парциальным давлением O_2 в артериальной крови и величиной изменения кровяного давления. Непосредственный механизм гемодинамических изменений пока не известен. В возникновении их повидимому играют роль гипоксиачувствительные рецепторы, расположенные в области, кровоснабжённой a. bronchialis.

ДЕЙСТВИЕ СЕКСУАЛЬНЫХ СТЕРОИДОВ НА РЕГУЛЯЦИЮ ПРОСТАГЛАНДИНОВ В МОЗГОВОЙ ТКАНИ

ГЕЧЕ А., ОТТЛЕЦ АННА, ТЕЛЕГДИ Г.

Содержание Простагландина Ф2α(ПГФ2α) в гипоталамусе крыс определяли радиоиммунологическим методом. Полученные величины у самцов было статистически значимо выше чем у самок. После орхидектомии содержание ПГФ2α гипоталамуса увеличивалось, в то время как овариэктомия не оказывала нижнего эффекта. Один прогестерон (в дозе 100 мг/кг или 1 мг/кг внутримышечно) или прогестерон в комбинации эстроном (100 мг/кг или 1 мг/кг внутримышечно) в гипоталамусе овариэктомизированных крыс значительно уменьшало содержание ПГФ2α. Введение одного эстрона не оказывало эффекта. Введение

тестостерона (в дозе 1 или 5 мг/кг внутримышечно)орхидектомированным крысам снижало содержание ПГФ2α гипоталамуса до нормального уровня.

Синтез и распад простагландинов изучали в микросомальной и цитосольной фракции гомогената мозговой ткани. Самой большой фракцией превращения архидоновой к-ты оказалось ПГД2. Под действием овариэктомии синтез ПГД2 увеличивалось а синтез ПГФ2α уменьшалось. Введение эстрогена в один раз в дозе 100 мг/кг или 1 мг/кг внутримышечно овариэктомизированным животным повысило синтез ПГФ2α, а введение прогестерона в дозе 100 мг/кг или 1 мг тормозило биосинтез ПГФ2α и ПГД2. Совместное введение эстрона и прогестерона не нормализовало уровень синтеза ПГФ2α. У орхидэктомизированных животных превращение архидоновой кислоты значительно уменьшалось но после введения тестостерона оно нормализовалось.

Инактивацию ПГФ2α меченного H^3 в цитосольной фракции мозговой ткани не могли доказать.

ДАННЫЕ ОБ ИЗМЕНЕНИИ И РАСЩЕПЛЕНИИ МЫШЕЧНЫХ ВОЛОКОН У ЧЕЛОВЕКА

ДЬ. ФЕКЕТЕ и П. АПОР

Исследуя образцы мышц, полученные при биопсии *m. vastus lateralos*, мы нашли в них высокое содержание «промежуточных» мышечных волокон II с типа, как у тренированных на высосливость бегунов, так и у детей-сердечников с цианозом. После инкубирования как в щелочной, так и кислой среде эти волокна сохраняют свою активность и окрашиваются с средней интенсивностью. В мышцах прыгунов в высоту нашли характерную для расщепления волокон картину (гиперплазия). Расщепление, переход одних подгрупп волокон в другие у спортсменов можно объяснить воздействием тренировок.

ПЕРЕКРЫВАЮЩИЙ ЭФФЕКТ ТИРЕОТРОПНОГО И ФОЛЛИКУЛИН-СТИМУЛИРУЮЩЕГО ГОРМОНОВ НА ЩИТОВИДНУЮ ЖЕЛЕЗУ НОВОРОЖДЕННЫХ ЦЫПЛЯТ

ДОБОЗИ О., БАЛКАНИ Л., ЧАБА Г.

У новорождённых цыплят, действие фолликулин-стимулирующего гормона на щитовидную железу — хотя по интенсивности меньше чем действие тиреотропного гормона — по изученным параметрам перекрывает действие последнего. В частности повышает диаметр фолликулов и высоту эпителиальных клеток. Результаты этих исследований подтверждают ранние наши наблюдения, согласно которым перекрывающее действие тропных гормонов наблюдалась и в случаях сексуальных гормонов. Таким образом перекрывающее действие тропных гормонов со сходной структурой является общим явлением в перинатальном периоде.

ВЛИЯНИЕ АНТИХОЛЕЦИСТОКИНИНОВОЙ СЫВОРОТКИ НА СОДЕРЖАНИЕ МОНОАМИНОВ МОЗГА КРЫС

ФЕКЕТЕ М., КАДАР Т., ТЕЛЕГДИ Г.

В боковой желудок мозга крыс, ввели три разных разведений антихолецистокининовой сыворотки, специфичной к Ц-терминальному тетрапептиду молекулы холецистокинина. Через 24 часа после введения сыворотки в гипоталамусе, мезенцефалоне, амигдалярном ядре, прозрачной перегородке, полосатом теле и коре мозга определяли содержание допамина, норадреналина и серотонина.

Под действием сыворотки в гипоталамусе, мезенцефалоне, амигдалярном ядре, прозрачной перегородке содержание допамина и норадреналина уменьшалось, в полосатом теле содержание допамина увеличивалось, содержание норадреналина уменьшалось. Содержание серотонина в амигдалярном ядре и прозрачном теле уменьшалось, а полосатом теле увеличивалось.

МОДИФИКАЦИЯ МЕТОДА ХРОНИЧЕСКОЙ РЕГИСТРАЦИИ КРОВЯНОГО ДАВЛЕНИЯ В АОРТЕ КРЫС

Д. ДИМИТРОВ, Р. ГИРХЕВ

Описывается модификация метода Брука и Мирхеда (1972) для хронической регистрации кровяного давления в аорте крыс. Вместо введения катетера через живот — как это происходит по исходной методике — применяется боковое экстраперитонеальное введение последнего. Описывается оперативный подход, метод введения катетера а также техника регистрации давления. При помощи этой методики мы могли регистрировать среднее артериальное давление в аорте бодрых животных в течение несколько месяцев.

Боковое экстраперитонеальное введение катетера уменьшет хирургическую травму и возможность возникновения интраабдоминальной инфекции до минимума. С другой стороны позволяет при одной операции провести несколько экспериментальных вмещательств на почках и аорте, то есть проведение целого комплекса гемодинамических исследований.

ВЛИЯНИЕ ВЕЩЕСТВ, ДЕЙСТВУЮЩИХ НА cAMP, НА СУДОРОЖНУЮ АКТИВНОСТЬ ГИППОКАМПА

Н. ЛЮДВИГ, А. ВАРТА, Г. ХАРТМАНН и К. ЛИШШАК

Авторы вводили в гиппокамп вещества, изменяющие уровень cAMP в нервной ткани, и исследовали, каким образом, под воздействием этих веществ, изменяется судорожная активность гиппокампа (на основании ЭЭГ). Фокальные эпилептические приступы вызывались электрическим раздражением гиппокампа, а также локальным введением пенициллина. Увеличивающие содержание cAMP папаверин, гистамин + K^+ и дибутирил-cAMP повышали электрический судорожный порог, при эпилепсии же, вызванной пенициллином, уменьшали частоту приступов и интериктальных спайков. Кроме того, все эти вещества удлиняют время, необходимое для развития пенициллиновой эпилепсии. Снижающий уровень cAMP имидазол во всех случаях оказывал противоположное действие.

На основании полученных результатов авторы обсуждают возможную роль cAMP в патомеханизме эпилепсии.

ФАРМАКОЛОГИЧЕСКИЕ ОСНОВЫ ПЛАНИРОВАНИЯ ЛЕКАРСТВЕННЫЙ ФОРМЫ ХЛОРОКВИНА И МЕПАКРИНА

Э. МИНКЕР и Ж. МАТЕЙКА

Хлороквин и мепакрин — как при пероральном, так и парэнтеральном введении, появляются в желудочной стенке и тормозят опорожнение желудка у крыс. Измерения концентрации показали, что количество попадающих в стенку желудка хлороквина и мепакрина зависит от способа их введения. Оказалось, что, при введении хлороквина и мепакрина в обход желудка, в его стенку попадает меньшая часть введенной дозы. Установили, что хлороквин и мепакрин, будучи введены перорально в виде растворов, благодаря своим парасимпатическим и спазмолитическим свойствам, тормозят опорожнение желудка у крыс, а вследствие этого и собственное всасывание. На это указывает разница между значениями ЛД50 при интраперитонеальном и пероральном введении. Как хлороквин, так и мепакрин у крыс всасывается также и из желудка, после же парэнтерального введения они выделяются в желудке, инфильтрируя все его структурные элементы.

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Physiologia—Pathophysiologia

CORTICOSTERONE BINDING IN MYOCARDIAL TISSUE OF RATS AFTER CHRONIC STRESS AND ADRENALECTOMY

By

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Male rats were trained to swim for 9—10 days to assess the effect of chronic stress on the capacity and affinity of specific glucocorticoid binding in the cytosol fraction of myocardial tissue. A significant decrease in the binding capacity for corticosterone (Cpd B) was found 24 hours after the last swimming test, while the affinity constant remained unchanged. Contrary to Cpd B binding, there was no change in the capacity of dexamethasone binding, and there was no decrease in the Cpd B binding capacity in rats which had been adrenalectomized 2 days prior to the training period.

We conclude that daily swimming for 9—10 days alters the number of cytoplasmic Cpd B binding sites in the face of increased adrenocortical activity, while not affecting dexamethasone binding in the cytosol fraction of myocardial tissue.

A large body of evidence indicates that glucocorticoids influence cardiac function through metabolic effects. Previously it has been reported that an increase in adrenocortical activity enhances the pressor effect of catecholamines, followed by an improvement in left ventricular works index and increase in arterial pressure [6, 22]. Glucocorticoids also affect the metabolism of water and electrolytes in myocardial tissue [15, 16], and adrenalectomy diminishes training-induced adaptation in cardiac muscle [17]. BAXTER and FORSHAM [3] suggested that most part of these changes were due to a direct effect of glucocorticoids. Replacement therapy with glucocorticoids proved to be more effective than that with mineralocorticoids [17]. From this point it is interesting that no mineralocorticoid receptors were found in heart muscle [10] while there was a considerable amount of specific glucocorticoid binding sites [2, 10, 12]. The subcellular mechanism of action of adrenal steroids follow a general pattern: initiation of the response by a specific hormone-receptor system in the cytoplasm of the target tissue, followed by interaction of the hormone receptor complex with the genome resulting in activation (or derepression) of transcription, which in turn results in translation of specific

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induced proteins [7]. Such protein receptors were found in the cytosol fraction of glucocorticoid-responsive tissues, and their physicochemical properties as well as their implication in the biological response of various target tissues, e.g. liver, thymus, spleen, kidney, skeletal muscle, brown adipose tissue have been throughly described in several recent publications [1, 4, 9, 14, 19, 20, 23]. However, it is well established that in different target tissues the receptor proteins for the same hormone can be physicochemically distinct ones [1]. On the other hand, as found in spleen and liver, stress-induced changes in metabolism may modify the corticoid receptor activity [1]. Thus, it seems to be important to determine the hormone binding capacity and affinity in the same tissue under different conditions.

The aim of the present study was to establish whether daily swimming for 9—10 days influences the properties of specific glucocorticoid binding in the myocardium. We followed the changes in capacity and affinity of Cpd B and dexamethasone binding sites in the cytosol fraction of cardiac tissue.

Methods

Male inbred Wistar rats aged 8 weeks and weighing 200—280 g were used in the study. For chronic stress a swimming procedure was used. The rats were allowed to swim in a 50 cm deep cylinder-shaped pool (water temperature 32 °C) and were loaded with an extra weight of 5 per cent of their total body weight on the tail. Swimming time, which was 5 min on the first day, was gradually increased till 15 min on the 9—10 day. The animals had to swim every morning at the same time (± 1 hour). Except for the swimming procedure the animals in the control group were treated in the same way as the experimental group. In another series of experiments two groups of animals were adrenalectomized and 2 days later one group was subjected to the same swimming training as the intact animals, the other group serving as control. The trained intact animals were adrenalectomized 1 hour after the last swimming test under ether anaesthesia. The weight of the adrenals was measured on a torsion balance with an accuracy of 0.1 mg. To test the pituitary-adrenocortical activity blood samples were taken under ether anaesthesia from the eye plexus 30 min and 2 hours after the first and the last swimming test. Plasma Cpd B concentration was measured by the technique of MURPHY [21]. The rats were killed by decapitation on the first or third days after the last swimming test and plasma Cpd B level was measured in the trunk blood. The heart was perfused *in situ* with 40 ml ice-cold physiological saline solution *via* the left ventricle, then it was quickly removed, rinsed in ice-cold saline, minced by scissors and homogenized in 1.5 ml of 0.01 M TRIS buffer, (pH = 7.4) containing 1.0 mM EDTA and 1.0 mM dithiothreitol at 0 °C for 60 sec at low speed in an all-glass homogenizer. The homogenate was centrifuged and the supernatant was again spun at 100,000 *g* for 60 min at 0 °C to yield the cytosol fraction. Aliquots of this cytosol were incubated with tritiated corticosterone (1,2,6,7-^{3}H-corticosterone, 117 Ci per mmole) and dexamethasone (1,2-^{3}H-dexamethasone, 38 Ci per mmole, Radiochemical Centre, Amersham). The dilution for both steroids varied from 1.5×10^{-9} M to 5.5×10^{-8} M. The incubation medium was as follows: 50 μl cytosol, 100 μl TRIS buffer, and 50 μl labeled steroid solution. Following incubation at 0—4 °C for 4 hours, the free and bound steroids were separated with dextran-coated charcoal (1 g charcoal, 10 mg dextran, in 200 ml water). One hundred μl dextran-coated charcoal was added to the incubation medium, agitated and 10 min later centrifuged at 2000 *g* for 10 min. Aliquots of 100 μl from the supernatant fraction were assayed for radioactivity in 10 ml counting solution (100 g naphthalene, 7 g 2,3-diphenyl-oxazole, 0.3 g phenyl-oxazolylphenyl-oxazolylphenyl in 1 liter dioxan). The specific binding of steroid was defined as the binding of labeled steroid minus the binding observed in the presence of 300-fold excess of nonlabeled steroid. Radioactivity was counted in a liquid-scintillation spectrometer (Mark II, Nuclear, Chicago). Efficiency rate was determined by sample channel ratio. Protein was determined according to LOWRY et al. [18]. Protein

standard was crystalline bovine serum albumine. Corticosterone and dexamethasone (Fluka AG), bovine serum albumin (Sigma), dextran and charcoal (Reanal, Budapest) were used. All chemicals and solvents were of reagent grade.

Results

Response of adrenal cortex to swimming stress

To determine responsiveness of the adrenal cortex to swimming for 5 min with additional load, plasma Cpd B level was measured 30 min and 24 hours after the first swimming. Blood samples of the control group were taken at the same time. Thirty min after the last swimming plasma Cpd B level was increased from 0.015 ± 0.01 μmol/l to 0.85 ± 0.05 μmol/l ($p < 0.001$). On the next morning plasma Cpd B level was also significantly higher (0.24 ± 0.11 μmol/l). The response to the last stress was significantly higher than that to the first stress (1.21 ± 0.11 μmol/l, $p < 0.02$). The chronic swimming stress increased the weight of the adrenals from 14.7 ± 0.92 mg to 21.2 ± 1.21 mg ($df = 22$, $p < 0.01$).

Changes in the capacity of specific glucocorticoid binding

The individual Scatchard plots, obtained with 5 different steroid concentrations, were used to measure the capacity and affinity of Cpd B and dexamethasone binding in the cytosol fraction of each heart separately (Fig. 1). In trained rats 24 hours after the last swimming test and subsequent adrenalectomy, the number of Cpd B binding sites was significantly lower ($df = 10$, $p < 0.01$) in the trained group than in the untrained control group (Fig. 2). In contrast to these findings there was no difference in the values between chronically stressed and control animals which had been adrenalectomized

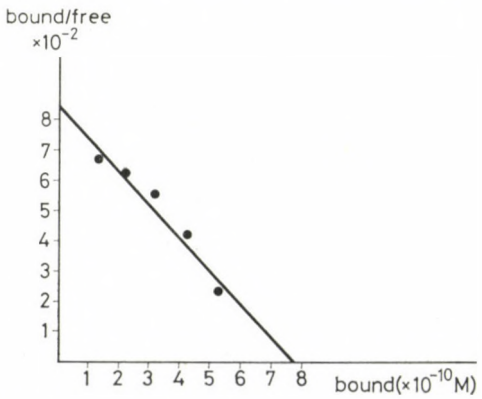

Fig. 1. Corticosterone binding in cytosol fraction of myocardial tissue (example of used Scatchard plot). Nonspecific binding was substracted previously on the each concentration of steroid separately

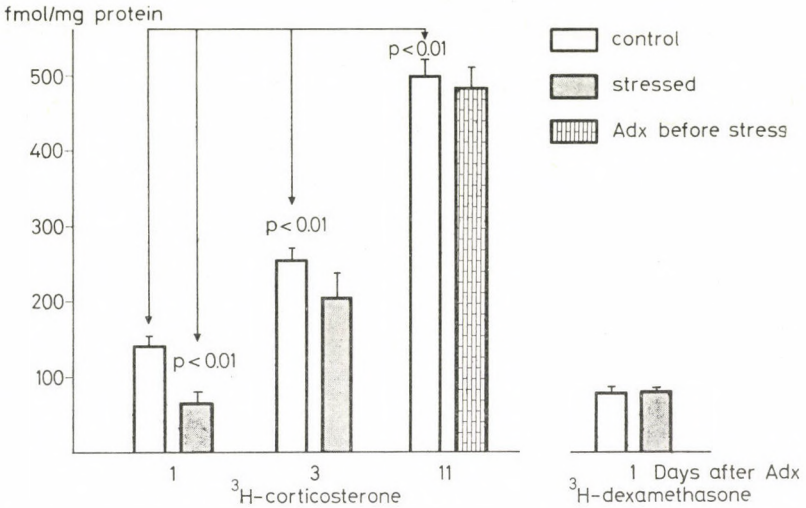

Fig. 2. Capacity of specific binding of ^{3}H-corticosterone (three pairs of columns on the left) and that of ^{3}H-dexamethasone (a pair of columns on the right) in the cytosol fraction of myocardial tissue. Groups are as follows: controls — nontrained and adrenalectomized; stressed — adrenalectomized after training; Adx before stress — adrenalectomized two days before training

Table I

Affinity of specific glucocorticoid binding in cytosol fraction of myocardial tissue after chronic swimming stress in rats ($\times 10^{-8}$ *M*)

Groups	n	Time after the last swim, (days)	^{3}H-corticosterone	^{3}H-dexamethasone
Control	6	1	1.86 ± 0.20	0.75 ± 0.10
Stressed	6	1	1.35 ± 0.19	1.01 ± 0.13
Control	6	3	1.01 ± 0.21	
Stressed	6	3	1.31 ± 0.12	

All figures have been calculated from Scatchard plots

before the training procedure (Fig. 2). The training procedure had no effect on dexamethasone binding (Fig. 2). Corticosterone binding capacity showed a progressive increase after adrenalectomy in both stressed and control groups on the third and eleventh days compared with the first day. The affinity constants were practically unchanged (Table I).

Thermostability of ^{3}H-corticosterone binding

To determine the thermostability of ^{3}H-corticosterone binding in the cytosol fraction prepared 3 days after adrenalectomy, it was incubated at 0—4 °C and at 37 °C for 3 hours with tritiated steroid. Capacity and affinity of ^{3}H-corticosterone binding were not temperature dependent (Table II).

Table II

Thermostability of ^{3}H-corticosterone binding in the cytosol fraction of myocardial tissue

No.	Capacity (fmol/mg protein)		Affinity $K_D/(\times 10^{-8}M)$	
	0—4°	37°	0—4°	37°
1	223	208	1.75	1.70
2	286	278	1.70	1.60
3	190	131	1.85	1.75
Mean ± S.E.	233 ± 28.5	205 ± 31.2		

All figures have been calculated from Scatchard plots

Discussion

The present observations indicate that the capacity of specific Cpd B binding in the cytosol fraction of myocardial tissue is significantly lower as a result of chronic swimming stress in male rats adrenalectomized 24 hours before the measurement. Under the same conditions dexamethasone binding capacity failed to change. The decrement in Cpd B binding capacity was maintained on the first day after the last swimming test and in some animals even on the third and sixth day after the end of the training procedure. Contrary to the capacity, the affinity constant of specific Cpd B binding remained unchanged. There was no decrease in the number of Cpd B binding sites in the cytosol fraction of myocardial tissue in animals which had been adrenalectomized before the training procedure but there was a progressive significant increase of Cpd B binding sites when compared on the first, third and 11th day after adrenalectomy. An increase in the number of glucocorticoid receptors had previously been observed after adrenalectomy in several tissues, such as in rat liver [5, 8] and heart [12]. On the other hand, the training procedure was accompanied with elevated adrenocortical activity which was manifested in higher plasma Cpd B level after single swimming test, hypertrophy of the adrenal glands and in much higher plasma Cpd B level at the end of the training period. Thus the present results provide an

evidence for modulation of the number of cytoplasmic binding sites in heart muscle by adrenal glycocorticoid secretion.

There is, however, some discrepancy in this explanation, since significant increase in the number of specific glucocorticoid binding sites was found for both ^{3}H-Cpd B and ^{3}H-dexamethasone after adrenalectomy [5, 8, 12] and a decrease only in the number of Cpd B sites after conditions associated with elevated Cpd B level in the present experiments. The apparent contradiction between these findings may be resolved by assuming heterogeneity of specific glucocorticoid binding sites in heart muscle. In several tissues several binding sites were found capable of binding glucocorticoids specifically, e.g. in the liver [5, 14], kidney [11], thymocytes [25] and brain [13]. The present data seem to have furnished evidence as to the suggested heterogeneity of specific glucocorticoid binding sites in heart muscle, *viz.* (1) increased adrenocortical activity caused by training affected only Cpd B binding while not having any effect upon the progressive increase of the number of binding sites after adrenalectomy; (2) while specific Cpd B binding proved in this study to be thermostable, specific dexamethasone binding was found thermolabile [12]; (3) we found different sedimentation profiles for specific dexamethasone and Cpd B binding (unpublished data). On the basis of these findings we believe that in the heart, like in some other tissues [5, 8, 13, 25], a trascortin-like binding system is available that binds selectively Cpd B, along with another receptor system binding both Cpd B and dexamethasone.

On the basis of our results it seems that chronic stress does not influence those mechanisms which are responsible for the increase in binding capacity after adrenalectomy. The receptor population which may be affected by chronic stress seems to bind selectively Cpd B and it is probably different from receptors which bind both Cpd B and dexamethasone. The capacity of only this latter type of binding system seems to be increased after adrenalectomy.

Thus the present results provide an evidence for modulation of the number of certain cytoplasmic binding sites by adrenal secretion, however, further studies are required to understand the physiological significance of these events.

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EFFECT OF CAROTID SINUS REFLEX ON THE CIRCULATION OF RATS BEARING GUÉRIN CARCINOMA

By

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The effect of carotid occlusion was studied on cardiac output (Evans blue dilution) and its fractional distribution (Sapirstein's isotope fractionation technique) in rats with and without Guérin carcinoma.

In response to the carotid sinus hypertensive reflex (CSHR), in normal rats blood pressure and TPR were augmented; vascular resistance of the kidneys, intestines, skin and carcass was increased. In tumour bearing rats CSHR resulted in comparable increments of vascular resistance in various organs. Tumour blood flow remained unchanged, whereas vascular resistance of the tumour was considerably increased.

A reduction of blood pressure in the common carotid arteries or their bilateral occlusion is known to result in hypertension (carotid sinus hypertensive reflex = CSHR) and tachycardia. The baroreceptor activity of the carotid sinus has been studied by several investigators [18, 27] in normotensive and spontaneously hypertensive rats (SHR). Data by STRUYKER-BOUDIER et al. [29] suggest that in SHR rats the augmentation of total peripheral resistance (TPR) is the result of baroreflex-induced symphathetic activity. No difference was found in cardiac index between normotensive and SHR rats by PFEFFER and FROHLICH [22]. In contrast, cardiac index was lower in SHR than in normotensive animals [1].

The induction of CSHR — depending in part on the anaesthesia — appears differentially to alter cardiac output, and total and regional resistances in various species [24, 14]. E.g., in the anaesthetized dog, blood pressure elevation is primarily caused by the enhanced vascular resistance. Vasoconstriction ensues especially in skeletal muscles, kidneys, the splanchnic area and the skin vessels [14]. No such detailed study is available for the rat.

In earlier studies [9, 10] it was found that under the effect of alpha or beta adrenergic stimulation or posthaemorrhagic hypotension, the circulation of Guérin carcinoma exhibited uniform response pattern: vasoconstriction in all instances. The present examinations were conducted to find out how the organ blood flow of the host and Guérin carcinoma is influenced by the endogenous reflectory sympathetic activity. To induce the carotid sinus hyper-

tensive reflex, a bilateral occlusion of the common carotid arteries was carried out. Data of carotid occluded rats were compared between groups of normal and tumour-bearing animals.

Methods

Animals: Male rats from the CFY (LATI, Gödöllő) strain were used. They were starved for 24 h prior to the studies, with free access to tap water. They were anaesthetized with 50 mg/kg Na pentobarbital injected intraperitoneally.

CSHR and blood pressure measurement. For the induction of CSHR, the common carotid arteries were ligated below the sinus on both sides while bilateral ligatures were placed also

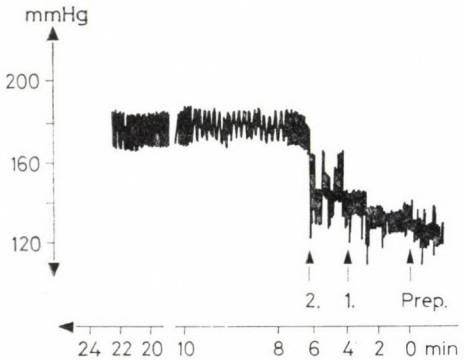

Fig. 1. Effect of bilateral carotid occlusion on blood pressure in the rat

on the internal and external carotid arteries above the sinus. Blood pressure was monitored in the femoral artery, by a mercury manometer. In control animals, the arteries were exposed but no ligation was performed.

In a pilot study (6 rats) blood pressure in the femoral artery was monitored by a Statham P23Db type electromanometer and mean blood pressure was recorded by a Rikadenki Mark II B 36-type compensograph (Japan) at a chart speed of 1 cm/sec. The frequency transmission of the marking pen was 2 Hz per total chart width. Following carotid ligation, blood pressure reached its maximum in 3—5 sec and stabilized at this level throughout the whole observation period (90 min, Fig. 1).

Measurement of cardiac output and its fractional distribution. Organ flow distribution was estimated by the radioactive isotope fractionation technique [25, 26, 32] using 1—2 μCi ^{125}I-antipyrine and 8—10 μCi ^{86}RbCl injected into the femoral vein. Fifty seventy sec after the isotope injection cardiac output was estimated by Evans blue dilution of which 0.3 ml of 0.75% solution was injected. Thereafter, 12—15 blood samples 20 μl in volume were collected at 0.67 sec intervals from the common carotid artery. They were diluted with 3 ml of buffered saline solution and dye concentration was estimated in a Spektromom 360 spectrophotometer (Hungary) at 600 nm. The amount of blood required for the estimation of cardiac output did not alter the ^{86}Rb uptake by organs [32] since by that time more than 95% of the injected ^{86}Rb had already been extracted by the organs from the circulation. Animals were killed by an i.v. injection of saturated KCl, 10—15 sec after cardiac output had been estimated. Organs were weighed and their radioactivity was determined in specimens using a thallium-activated sodium iodide crystal detector and a Nuclear Chicago type 1085 linear scaler (U.S.A.).

Tumour inoculation. Guérin carcinoma cells maintained in rats [19] were injected into the right axillary region under sterile conditions and ether anaesthesia. The cell count was not estimated before inoculation.

Protocol: Tumour-bearing animals were divided randomly into 2 groups. Experiments were performed 2 weeks post-transplantation. Non-tumour-bearing animals are called *normal* in this study.

Variables: The organ fraction of cardiac output is the quotient of radioactivity of an organ and radioactivity of the total body and is given in per cent. In the knowledge of cardiac output, organ fraction and organ weight the nutritive blood flow for 100 g organ was calculated. In the knowledge of blood pressure and organ flows, the circulatory resistance of organs was calculated with the presumption that the central venous pressure is zero. This method is not suitable for the estimation of circulatory resistance of organs with a dual blood supply such as the lungs and the liver [32]. By carcass we mean the parts (skeleton and muscle, spinal cord, peripheral nervous system, etc.) remaining after removal of thoracic and abdominal organs, the skin and the brain.

After the first circulation time the quotient of tumour per total body radioactivity remained unchanged, thus tumour blood flow could be estimated on the basis of ^{86}Rb accumulation [13, 30, 34].

Parameters for brain circulation were estimated by ^{125}I-antipyrine while those for other organs by ^{86}Rb accumulation.

Experimental sequence: Anaesthesia; recording of blood pressure in the femoral artery; bilateral carotid occlusion (CSHR); about 10 min later blood pressure recording; injection of radioactive labels; 50—60 sec later beginning of blood sampling for estimation of cardiac output; 10—15 sec after the blood collection, sacrifice. Sham-operated animals were treated, in the same way except for carotid occlusion.

Statistical evaluation of data was performed by two-tailed *t* test or by F test where appropriate [2, 28].

Results

Results are shown in Table I.

Organ fractions of cardiac output in *normal control* animals where the same as those estimated earlier [9, 10, 11, 30, 33].

In *normal rats with carotid occlusion* arterial blood pressure increased by 26% mm Hg, whereas circulatory resistance of the total body, by 30%. Of the organs tested in response to CSHR, skin blood flow decreased by 30%; circulatory resistance in the kidneys, intestines and carcass increased by 25—30%, while in the skin by 70%; skin fraction of cardiac output was reduced.

In the *tumourous control* animals as compared with the healthy controls, cardiac index was higher by 18%, TPR decreased by 18%; blood flows to skin and carcass were augmented by 25—30%, circulatory resistance in the brain, skin and carcass dropped by 20—25%; the fractions of cardiac output in myocardium, kidney, intestines and carcass were reduced. These changes were similar to those found earlier in this laboratory [9, 10, 30].

In *tumour-bearing carotid occluded rats* as compared to respective control (tumour bearing) values, blood pressure was higher by 27 mm Hg, whereas TPR increased by 30%; blood flow to the brain by 20%, while skin perfusion was reduced by 25%; circulatory resistances were augmented by 15—25% for the kidneys, intestines and carcass, and by 70—80% for the skin and the tumour. Brain, myocardial and kidney fractions of cardiac output were higher whereas its skin fraction was reduced.

In tumour-bearing animals with carotid occlusion as compared with values for *normal carotid ligated rats*, the cardiac index was higher by 22%, TPR by

Table I

Effect of carotid sinus ligation on the circulation of normal and tumour-bearing rats

Number of observations	Normal groups		Tumour groups	
	Control 21	Carotid sinus ligated 15	Control 18	Carotid sinus ligated 18
Total body				
Body weight (BW), g	250.0 ± 6.56	247.0 ± 6.60	264.0 ± 6.42	266.0 ± 5.60□
Blood pressure, mmHg	117.0 ± 2.97	143.0 ± 4.60●	113.0 ± 2.40	140.0 ± 3.76●
Cardiac index, ml/min per 100 g BW	25.9 ± 1.27	24.1 ± 1.15	30.6 ± 1.70○	29.3 ± 0.99■
Resistance, 10^4 cgs/100 g BW	37.8 ± 1.92	48.3 ± 2.11●	31.0 ± 1.80○	39.2 ± 1.83●■
Tumour				
Weight			20.4 ± 1.51	18.4 ± 1.86
Blood flow			24.6 ± 1.77	21.1 ± 2.27
Resistance			40.6 ± 3.42	68.4 ± 10.20●
Fraction			6.06 ± 0.45	4.95 ± 0.61
Brain				
Blood flow	29.7 ± 2.18	31.1 ± 1.54	35.3 ± 2.51	42.6 ± 2.43○■
Resistance	34.3 ± 2.21	37.4 ± 1.50	27.7 ± 2.15	27.9 ± 1.80■
Fraction	0.79 ± 0.046	0.89 ± 0.042	0.78 ± 0.036	0.98 ± 0.074○
Heart				
Blood flow	146.0 ± 9.78	150.0 ± 12.63	144.0 ± 6.19	158.0 ± 9.79
Resistance	7.0 ± 0.54	8.1 ± 0.50	6.5 ± 0.34	7.5 ± 0.49
Fraction	1.87 ± 0.097	1.94 ± 0.13	1.51 ± 0.053●	1.82 ± 0.13○
Kidney				
Blood flow	396.0 ± 17.3	392.0 ± 19.3	394.0 ± 21.1	413.0 ± 15.2
Resistance	2.4 ± 0.11	3.0 ± 0.16●	2.4 ± 0.13	2.8 ± 0.15●
Fraction	16.1 ± 0.45	16.7 ± 0.44	14.1 ± 0.26●	15.4 ± 0.43○□
Intestines				
Blood flow	78.5 ± 4.95	74.4 ± 5.47	86.3 ± 4.83	86.5 ± 5.16
Resistance	12.9 ± 0.89	16.7 ± 1.57○	10.9 ± 0.57	13.8 ± 0.90○
Fraction	18.1 ± 0.76	17.8 ± 0.86	15.1 ± 0.52●	15.8 ± 0.80
Skin				
Blood flow	14.0 ± 0.87	9.78 ± 0.79●	18.2 ± 1.31●	13.1 ± 1.02●□
Resistance	72.1 ± 5.05	124.0 ± 7.68●	54.2 ± 4.31○	97.2 ± 9.02●□
Fraction	10.2 ± 0.47	7.3 ± 0.37●	10.3 ± 0.54	7.7 ± 0.43●

Table I *(continued)*

Number of observation	Normal groups		Tumour groups	
	Control 21	Carotid sinus ligated 15	Control 18	Carotid sinus ligated 18
Carcass				
Blood Flow	16.2 ± 0.90	15.9 ± 0.85	20.3 ± 1.37○	19.3 ± 0.80■
Resistance	61.1 ± 3.33	76.4 ± 3.88●	48.1 ± 3.66○	60.3 ± 3.42○■
Fraction	42.4 ± 1.14	43.6 ± 1.08	40.2 ± 0.97	40.9 ± 1.23

Symbols

Comparisons ○ ● Normal (control) — Normal (carotid sinus ligated)
Normal (control) — Tumour (control)
Tumour (control) — Tumour (carotid sinus ligated)
□ ■ Normal (carotid sinus ligated) — Tumour

with no symbol: not significant

○ □ $p < 0.05$
● ■ $p < 0.01$

Units: Organs: Blood flow ml/min/100 g organ
Resistance: 10^4 CGS/100 g organ
Fraction: per cent

19%; blood flows to brain, skin and carcass were augmented by 20—35% and the vascular resistance of the myocardium (8%), skin (20%) and carcass (20%), and organ the fraction for the kidneys reduced.

Discussion

Induction of CSHR: For the isolation of carotid sinus, vessels were ligated below and above the sinus. In rats with carotid occlusion, blood flow to the brain did not differ from that of sham-operated controls indicating that deterioration of cerebral circulation (cerebral hypoxia) was not essentially involved in the changes observed. In contrast, acute bilateral extirpation of the carotid bifurcation performed under ether anaesthesia in KRIEGER's studies [15, 16] was followed by death of the awake animals. The differences in sensitivity of rat strains and the different responses by awake and anaesthetized animals might serve as explanation. In the dog, cat and rabbit after carotid occlusion the carotid sinus is filled with blood in retrograde way from the vertebral arteries and the circle of Willis and so the normal intrasinusoidal blood pressure is soon restored [8]. Our results appear to suggest a similar response to carotid occlusion by the rat strain used.

Normal animals

Blood pressure, cardiac index, TPR. In the present study, the CSHR-induced blood pressure elevation was unequivocally caused by an augmentation of TPR without changes in cardiac index. OLMSTED et al. [20] suggest that in dogs during the induction of CSHR the change in cardiac output is a function of its basal level: i.e. an initially low cardiac output tends to increase during CSHR whereas a high cardiac output remains unaltered. Our results are in agreement with the majority of observations made in anaesthetized dogs where the CSHR-induced blood pressure elevation could be ascribed to the increased TPR [3, 4, 14, 23, 24].

Organ blood flows and resistances. Blood flow to the skin decreased whereas that to the other organs remained unchanged. The smallest increments in vascular resistance were observed in the coronaries and brain vessels. The increase was higher in the kidneys, the carcass and in the intestines (25—29%), while the highest one was noted in the vascular resistance of the skin (72%). In dogs and cats, CSHR augments the circulatory resistance especially in the skeletal muscles, but renal vasoconstriction is also known to occur under such conditions [5, 6, 7, 14]. In anaesthetized dogs BOND and GREEN [4] found no change in the vascular resistance of the skin during CSHR, other investigators reported on slight vasoconstriction [12, 14]. Blood perfusion of skeletal muscle was not measured in this study, blood flow to the carcass, however, is primarily determined by muscle perfusion. Thus, changes in skeletal blood flow might overlap with those of muscle perfusion, but species differences might also account for the difference. It appears that the skin vessels are the most sensitive to sympathetic stimulation.

Tumour-bearing animals

Blood pressure, cardiac index, TPR, organ blood flows. In earlier studies [10, 30, 31] we have shown that parallel with the growth of Guérin carcinoma an increase occurs in cardiac output and organ blood flows, while TPR and regional resistances fall and there is an overall vasodilation. Similar changes have been observed in the tumourous controls of the present series. In response to CSHR, no changes were seen in cardiac output and organ blood flow with the exception of the skin, whereas TPR and organ vascular resistances were augmented. The differences between the tumourous controls and the tumourous carotid-occluded groups were not different from the respective values between the normal groups. Hence, it appears that the presence of a tumour and the ensuing general vasodilation do not essentially affect the CSHR-induced sympathetic activation as shown by the haemodynamic responses.

Tumour-circulation. The 68% increment in the vascular resistance of Guérin carcinoma is comparable to that of the skin. It therefore seems that in

tumour-bearing animals, in addition to skin blood flow, circulation of the tumour is one of the decisive factors in the CSHR-induced blood pressure elevation. In our earlier examinations [9, 10] a considerable vasoconstriction has been observed in tumour vessels after haemorrhage or phenylephrine treatment. The vasoconstriction during haemorrhage was attributed to the increase in the blood catecholamine level. We suggest a similar mechanism for the constriction of tumour vessels induced likewise by sympathetic activation in the present study.

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EFFECTS OF PROSTAGLANDINS ON CATECHOLAMINE METABOLISM OF THE CENTRAL NERVOUS SYSTEM IN RATS

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The effects of 5 μg prostaglandin (PG) A_1, E_1, E_2 and $F_{2\,alpha}$ injected intracerebroventricularly (i.c.v.) into conscious male rats were studied on the catecholamine contents of certain brain areas at different times after administration. PGE_2 led to increased dopamine (DA) contents in the hypothalamus 10, 20 and 60 min, and in the septal region 20, 60 and 90 min following injection. PGE_1 raised DA levels in the hypothalamus 20 min, and in the septum 20 and 60 min after i.c.v. administration. PGA_1 and $PGF_{2\,alpha}$ proved to be ineffective as regards the DA contents, while none of the PGs studied altered the noradrenaline (NA) contents of different brain areas. Disappearance of DA was significantly enhanced in the hypothalamus and in the septum, while NA disappearance rate was increased only in the hypothalamus after PGE_1 and PGE_2 i.c.v. injection in alpha-methyl-*p*-tyrosine treated rats.

A number of data suggest that there is certain interrelationship between PGs and catecholamines [3] as well as acethylcholine in both the peripheral and central nervous system [2, 9, etc.]. The catecholamines augment PG biosynthesis, and it is possible that these biogenic amines may serve as coenzymes regulating the *in vivo* biosynthesis of PGs (6,8). On the other hand, it has also been suggested that PGs may play the role of a neuromodulator in the catecholaminergic neurotransmission of the CNS [1, 5]. These observations have prompted us to investigate the effects of centrally administered PGs on the catecholamine contents of different brain areas in male rats.

Methods

The experiments were carried out on male CFY albino rats weighing 160—200 g. A polyethylene cannula was implanted into the lateral ventricle and 5—7 days later PGs were injected through the cannula in a dose of 5 μg dissolved in 5 μl physiological saline, while the controls received 5 μl saline. DA and NA contents of the hypothalamus, amygdala, septal region, dorsal hippocampus, striatum and mesencephalon were measured 0, 10, 20, 60, 90 and 120 min after the injection [7]. The catecholamine disappearance rate was studied in alpha-methyl-*p*-tyrosine (α-MPT)-treated animals (250 mg/kg b.w. given i.p. 3 h before decapitation and 1 h prior to i.c.v. PG injection). PGE_1, PGE_2 and α-MPT were purchased from Sigma Chem. Co., St. Louis, Miss. USA, while PGA_1 and $PGF_{2\,alpha}$ were kindly provided by Upjohn Co., Kalamazoo, Mich., USA. Statistical evaluation of the results was made by analysis of variance.

Results

PGE_2 increased the DA content of the hypothalamus 10 ($P < 0.01$), 20 ($P < 0.001$) and 60 min ($P < 0.001$) after the injection (Fig. 1). PGE_2 induced similar changes in the DA levels of the septal region after 20, 60 ($P < 0.001$) and 90 min ($P < 0.01$), while not altering the DA contents of other brain areas (Fig. 1). The i.c.v. injection of PGE_1 produced less marked changes in the DA levels than did PGE_2, however, the tendency was the same, significantly higher DA contents being observed in the hypothalamus 20 min ($P < 0.05$), and in the septum 20 and 60 min ($P < 0.001$) following administration (Fig. 1). 120 min after PGE_1 and PGE_2 treatment the DA levels returned to the starting values in all brain areas.

PGA_1 and $PGF_{2\,alpha}$ had no effect on DA contents, and none of the PGs used were effective in changing the NA contents of different brain areas in the time intervals studied.

An appreciably enhanced DA disappearance in response to PGE_2 was observed in the hypothalamus ($P < 0.001$) and in the septal region ($P <$

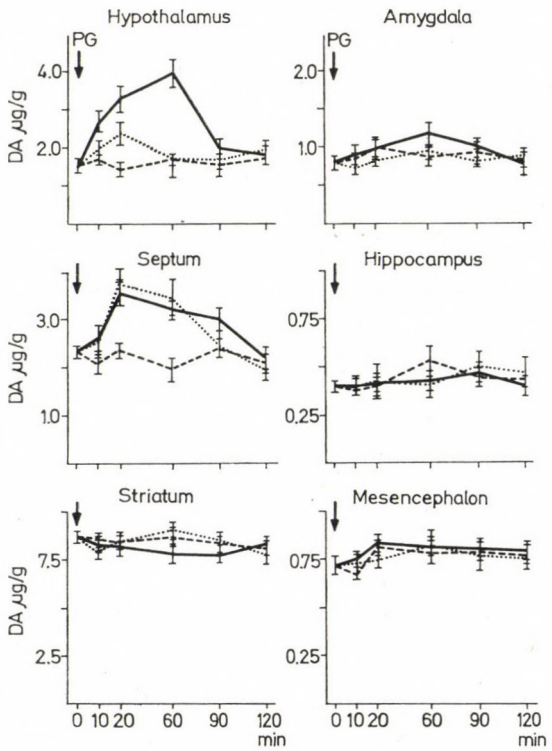

Fig. 1. Effects of injection of 5 µg PGE_1 or PGE_2 into the lateral ventricle of conscious rats on dopamine (DA) contents of different brain areas. Arrows indicate time of i.c.v. administration. Each point and vertical line represent the mean and standard error of 12—24 animals. Broken line: saline-injected control; solid line: PGE_2; dotted line: PGE_1

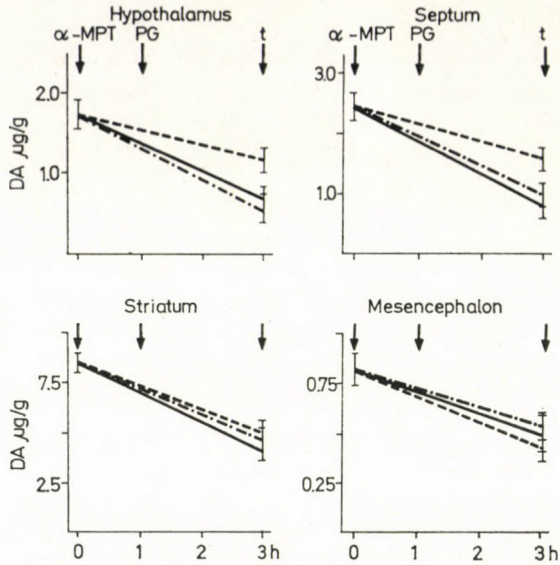

Fig. 2. Effects of 5 µg PGE_1 or PGE_2 administered i.c.v. on dopamine (DA) disappearance induced in different brain areas by alpha-methyl-*p*-tyrosine treatment (α-MPT 250 mg/kg b.w. i.p.). Arrows indicate time of α-MPT and PG administration, and the cross shows the time of decapitation. Broken line: control; solid line: PGE_1; dotted line: PGE_2. Each point represents the mean and standard error of 12—24 animals

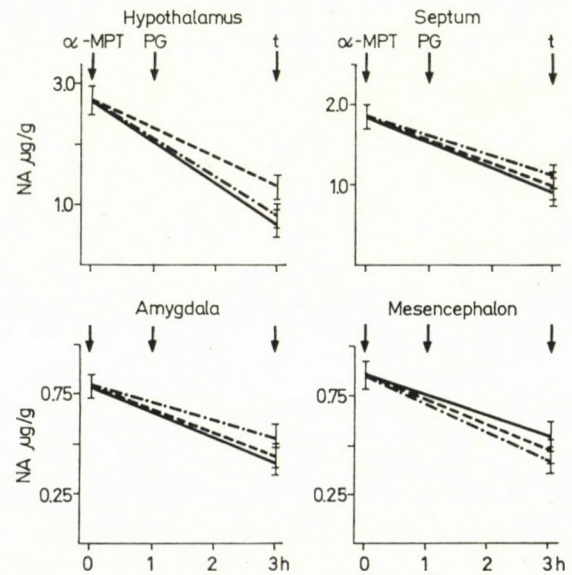

Fig. 3. Effects of PGE_1 and PGE_2 on noradrenaline (NA) disappearance from different brain structures after inhibition of synthesis with alpha-methyl-*p*-tyrosine. (For details see Fig. 2.) Broken line: control; solid line: PGE_1; dotted line: PGE_2. Each point represents the mean and standard error of 12—20 animals

< 0.01), and after PGE_1 injection in the hypothalamus ($P < 0.01$) and in the septum ($P < 0.001$) in α-MPT-treated rats (Fig. 2).

The NA disappearance rate following catecholamine synthesis inhibition with α-MPT was increased in the hypothalamus after i.c.v. injections of PGE_1 and PGE_2 ($P < 0.05$) (Fig. 3).

Discussion

In the present investigation PGE_1 and PGE_2 altered the catecholamine contents of the hypothalamus and septal region. PGE_2 evoked the most pronounced changes in the DA contents of both CNS areas, nevertheless PGE_1 too caused significant increases in DA contents. The fact that PGA_1 and $PGF_{2\,alpha}$ did not alter the DA levels in the same CNS regions suggests that the actions of PGE_1 and PGE_2 might be specific. Investigation of the effects of PGs on α-MPT-induced catecholamine disappearance rate indicated that the rise in DA levels is a result of an increase in the neuronal activity of the catecholaminergic system in the CNS. Our data, demonstrating the actions of PGs from the E series on the central catecholaminergic system, support the concept that a PG–catecholamine interaction may be important in the CNS effects of the two substances [1, 5].

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EFFECT OF PITUITARY INTERMEDIATE LOBE EXTRACT ON STEROID PRODUCTION BY THE ISOLATED ZONA GLOMERULOSA AND FASCICULATA CELLS*

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The effect of intermediate lobe extract (ILE) on aldosterone and corticosterone production of the zona glomerulosa cells and on corticosterone production of the zona fasciculata cells was investigated.

The slope of the dose–response curve of ILE dilution was steeper than that of α_h^{1-39} ACTH measured on zona glomerulosa steroid production. The ED_{50} of both ILE and ACTH was lower when measured on zona glomerulosa than on zona fasciculata steroid production.

It is supposed that a hormone (or some other substance) in ILE alters the sensitivity to ACTH of the zona glomerulosa cells.

In addition to the known factors controlling aldosterone secretion, several authors presume that a so far unidentified hormone exists in the pituitary; the hormone is not identical with ACTH and plays a role in the regulation of aldosterone secretion. Participation of growth hormone in the regulation of aldosterone secretion was repeatedly suggested [9, 10] and rejected [7].

Studies in recent years have called attention to the neurointermediate lobe of the pituitary and its hormones. Our hypothesis was that perhaps in this lobe we could find the "unidentified adrenuloglomerulotropin".

We studied the effect of intermediate lobe extracts on the steroid production by isolated zona glomerulosa and fasciculata cells and compared its effect with that of α_h^{1-39} ACTH, to find out whether besides ACTH a substance existed acting on zona glomerulosa steroid production.

Methods

Chemicals

Synthetic human α_h^{1-39} ACTH was supplied by the National Pituitary Agency, Baltimore, Maryland, USA, anti-aldosterone antiserum (Sheep 088) by the National Institute of Arthritis and Metabolic Diseases, NIH, Bethesda, Maryland, USA The following sub-

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stances were purchased: bovine serum albumin: Phylaxia, Hungary; collagenase (type I): Worthington Chemical Corporation, USA; deoxyribonuclease (DNase I): Sigma Chemical Co., USA; — and (1,2-H^3) aldosterone from the Radiochemical Centre, Amersham, England.

Preparation and incubation of adrenal cells

Male CFY rats of 250—300 g were used. They were fed a stock diet of rat chow (LATI, Gödöllő). In each experiment forty rats were killed by decapitation and the adrenal glands were dissected free of fat and placed till processed in ice cold Krebs–Ringer bicarbonate buffer containing 2 g/l glucose and 40 g/l bovine albumin with the potassium concentration adjusted to 3.7 meq/l (KRBGA). The capsular layer, consisting predominantly of zona glomerulosa cells, was separated from the zona fasciculata, reticularis and medulla under a dissecting microscope, according to the method of TAIT et al. [14]. For dispersion of cells from the capsular and inner zones of the adrenal, the tissue was minced with scissors and incubated at 37 °C under carbogen atmosphere in a shaking incubator (100 cycles/min) for 2×25 minutes with collagenase (2 mg/ml) and DNase (0.1 mg/ml) dissolved in KRBGA, 1 ml for two capsules and 1 ml for the inner layers of one adrenal. During and after incubation, dispersion of the cells was enhanced with mechanical disruption by repeated ($25 \times$) aspiration into a glass pipette. The supernatant containing the dispersed cells was decanted, filtered through nylon gauze (60 μm pore size), washed with KRBGA and centrifuged at 100 *g* three times for 10 minutes. Then the cell pellets were resuspended in KRBGA containing 5 g/l albumin. The volume of the final suspension was adjusted to contain glomerulosa cells from two adrenals or fasciculata cells from one adrenal in one ml KRBGA corresponding usually to $3—3.5 \times 10^5$ cells/ml. Cells were counted in a Buerker chamber.

Forty glomerulosa and 40 fasciculata cell aliquots were incubated in one session in Teflon beakers containing 0.9 ml cell suspension and 0.1 ml intermediate lobe extract or ACTH. Serial two-fold dilutions of the intermediate lobe extract and 7—8 doses α_{1}^{1-39} were added to the tubes in duplicate or triplicate. The doses of ACTH were added in log increments from 10^{-12} to 6.4×10^{-10} M (45.4—2900 pg ACTH/ml). The beakers were shaken at 70 cycles/min at 37 °C in a 95% O_2 + 5% CO_2 atmosphere for two hours. Experiments were carried out in a randomized block format to eliminate bias due to systematic error.

The corticosterone content of the incubation media and fasciculata and glomerulosa cells was determined after chloroform extraction by fluorimetry [3]. Aliquots of the chloroform extract of the glomerulosa incubation media were assayed for aldosterone content by RIA without chromatographic separation, as described below.

Radioimmunoassay of aldosterone in incubation media

The RIA diluent was phosphate-buffered saline pH 7.4 + bovine serum albumin 5 g/l. Aliquots from the chloroform extracts were evaporated and taken up in 1 ml absolute ethanol. Three aliquots from each incubation vial were pipetted into siliconized glass tubes. Aldosterone standards of 10—640 pg were also prepared in 10—640 μl of assay diluent. Equal volumes (0.5 ml) of diluent containing (1,2-^{3}H) aldosterone (6000 cpm) and anti-aldosterone serum (1.4×10^5) were added and shaken vigorously for two minutes, then incubated at 4 °C for 18 h. Separation of bound and free hormones was achieved by the rapid addition of 0.5 ml cold dextran-coated charcoal suspension, followed by centrifugation at 1600 rpm at 4 °C for 5 minutes; 0.5 ml of supernatant was transferred into scintillation vials, and 1 ml ethanol and 10 ml counting solution were added. Radioactivity was determined in a Packard TRI-CARB Model 3390 liquid scintillation spectrometer.

In order to evaluate the validity of direct RIA the aldosterone content of 24 samples of incubation medium was determined by direct assay and also by extraction and paper chromatographic separation [13]. The simultaneous assay of multiple samples of incubation medium by both assay methods gave the following result: $y = 1.011x + 3.162$, $r = 0.977$, $P < 0.01$, whereas x corresponds to pmol aldosterone measured after paper chromatographic separation (range: 10—100 pmol).

Preparation of intermediate lobe extract (ILE)

After decapitation the pituitaries were quickly removed and under a stereomicroscope first the anterior and neurointermediate lobes, then the intermediate lobe islands were separated with the aid of fine forceps and Graefe knife. The intermediate lobe tissue from 10 rats was homogenized in 200 μl 0.1 N HCl (20 μl was put aside for protein determination), centrifuged at 2000 rpm for 5 minutes, the supernatant decanted, the precipitate rehomogenized

with 2000 μl 0.1 N HCl, centrifuged as above and the supernatants were combined. Extracts were frozen at —20 °C till assayed. After several preliminary experiments eight different extracts were assayed, of which, only five are tabulated as some of the assays could not be evaluated since the sensitivity of the cells in these assays was either too low or too high. Forty μl of ILE corresponding to one intermediate lobe was diluted by 50—3200 and added to the cell suspensions at a two-fold dilution curve corresponding to 100—6400 ng protein of ILE. Protein was determined according to LOWRY et al. [6].

Statistical analysis

The slopes of the dose–response curves of ILE and α_h^{1-39} ACTH and the ED_{50} values were calculated after logit-log transformation. Aldosterone concentrations were calculated by a computer program based upon the logit-log transformation of the standard curve [11].

Results

The slopes of the dose–response curves of the intermediate lobe extracts were steeper than that of α_h^{1-39} ACTH measured on zona glomerulosa corticosterone ($P < 0.01$), aldosterone ($P < 0.01$) — and zona fasciculata corticosterone (N.S.) production (Table I). The dose–response curves of α_h^{1-39} ACTH and that of ILE measured on aldosterone production of zona glomerulosa cells are illustrated in Fig. 1.

Table I

Slope of the dose–response curves of intermediate lobe extracts after logit-log transformation

(mean ± S.E.M., n = 5)

Zona glomerulosa				Zona fasciculata corticosterone	
aldosterone		corticosterone			
ILE	ACTH	ILE	ACTH	ILE	ACTH
—1.85	—1.26	—1.82	—1.30	—1.69	—1.26
±0.300	±0.297	±0.074	±0.136	±0.290	±0.012
$P < 0.05$		$P < 0.01$		N.S.	

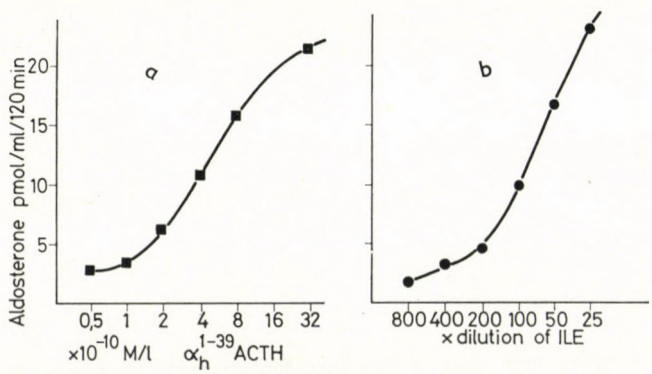

Fig. 1. Dose–response curve of α_h^{1-39} ACTH (a) and that of ILE (intermediate lobe extract, b) measured on zona glomerulosa aldosterone production

Table II

ED_{50} of ILE (dilution) and α_{1-39}^{1} ACTH (fmol/ml) measured on zona glomerulosa aldosterone, corticosterone and zona fasciculata corticosterone production (n = 5)

Zona glomerulosa				Zona fasciculata corticosterone	
aldosterone		corticosterone			
1 ILE	2 ACTH	3 ILE	4 ACTH	5 ILE	6 ACTH
1/148.1[a]	677.42	1/211.4	560.60	1/323.3	353.18
−4.998[b]	6.518	−5.354	6.329	−5.779	5.867
0.513[c]	0.198	0.548	0.165	0.469	0.100

a: Geometric mean
b, c: the mean and S.E. of the natural logarithm of the data
3 — 5: $P < 0.05$, 4 — 6: $P < 0.01$

Table III

The proportion of ED_{50} of ILE and ACTH between zona fasciculata and glomerulosa corticosterone production
(mean ± S.E.M., n = 5)

Fasciculata/Glomerulosa ILE ED_{50}	Fasciculata/Glomerulosa ACTH ED_{50}
0.663 ± 0.059	0.637 ± 0.049

Since the slopes of the ACTH curve and of ILE were not parallel, we could not calculate the ACTH content of the extracts by usual methods. In order to compare the effect of ILE on the two cell systems, we calculated the ED_{50} of ILE dilution, and calculated the ED_{50} of ACTH on both cell systems as well (Table II). A higher dilution of ILE was more effective on fasciculata cell corticosterone production than on glomerulosa cell corticosterone and aldosterone productions ($P < 0.05$). The ED_{50} of ACTH was measured on zona glomerulosa was nearly twice that measured on the zona fasciculata corticosterone production ($P < 0.01$).

The proportion of ED_{50} of ILE measured on glomerulosa and fasciculata corticosterone productions was the same as the proportion of the ED_{50} of ACTH measured on the two cell systems (Table III).

Discussion

The widely used method of zona glomerulosa and fasciculata cell separation described by HANING et al. [4] allowed to differentiate between specific glomerulotropic substances and those affecting both glomerulosa and fasciculata steroid production. It was supposed that the origin of the "unidentified aldosteronotropic factor" was the intermediate lobe of the pituitary. In order to test this hypothesis we studied the steroidogenic effect of ILE on zona glomerulosa corticosterone and aldosterone and on zona fasciculata corticosterone production. First we compared the slopes of the dose–response curves of ILE and of α_h^{1-39} ACTH on both cell systems. If they were parallel, we could have measured the ACTH content of the extract on steroid production by the glomerulosa and fasciculata zones. It was, however, found that the slopes of ILE and of α_h^{1-39} ACTH were different when measured on steroid production by the zona glomerulosa and there was a trend of non-parallelism measured on zona fasciculata corticosterone production, too.

What does the difference of the slopes mean?

According to ZIMMERMAN and KRAICER [15] pars intermedia ACTH activity is composed of four separate entities, the dose–response curve for all four is parallel to that of the Corticotrophin Reference Standard. Thus, it is not an ACTH-like material which causes the difference between the slopes. Besides ACTH, there are α-MSH [12], β-lipotropin [5], endorphins [1] and calcitonin [2] in the intermediate lobe, all of which may alter the sensitivity of adrenal cells against the ACTH of ILE.

Because of the non-parallelism of the slopes we could not measure the ACTH content of ILE by the usual four or six-point assays commonly used in ACTH bioassay. In order to compare the effect of ILE on the two cell systems, we compared the ED_{50} of ILE on zona glomerulosa and fasciculata steroid production. There is a significant difference between ED_{50} of ILE measured on the zona glomerulosa, and on corticosterone production by the zona fasciculata. The cause of the difference between the two cell systems towards ILE could be explained by the difference of the ACTH sensitivity of glomerulosa and fasciculata cells. The ED_{50} of ACTH was nearly 1.5 fold measured on glomerulosa corticosterone, and two-fold when measured on aldosterone then on fasciculata corticosterone productions. As the proportion of ILE ED_{50} measured on fasciculata and glomerulosa corticosterone productions is the same as that of $\alpha_h^{1\ 39}$ ACTH ED_{50} (Table III), we may conclude that the glomerulotropic effect can be attributed to the ACTH content of ILE.

According to MORIARTY and MORIARTY [8] the concentration of ACTH is 15 ng/mg neurointermediate lobe, in their experiment only two doses of the extract were applied. In our experiments the ED_{50} of ILE measured on zona fasciculata cell corticosterone production was $1/323 \times$ dilution corresponding

to 0.98 μg protein of the extract. The ED_{50} of ACTH on zona fasciculata cell suspension was 353.2 fmol = 127.3 pg. If the steroidogenic effect of ILE is supposed to be attributable mainly to ACTH, and some other hormones only alter the sensitivity of the cells to it, one may say that the ED_{50} of ILE corresponds to the ED_{50} of ACTH. Taking this in account, the ACTH content of ILE is 129.6 pg/μg protein corresponding to 21.1 ng/mg intermediate lobe; this agrees fairly well with the earlier data of MORIARTY and MORIARTY [8].

What is the answer to the hypothesis, that we have detected a new glomerulotropic substance? The answer is not equivocal. In the pars intermedia, besides ACTH there was a substance causing a steeper dose–response curve than that of ACTH, and this effect is more pronounced when measured on the corticosterone and aldosterone production of the zona glomerulosa than on the corticosterone production of zona fasciculata cells. To clarify what substance in ILE is responsible for this effect is a question for future experiments.

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THE EFFECT OF HAEMORRHAGIC SHOCK ON BLOOD PRESSURE AND HEART RATE RESPONSES TO ADRENALINE IN THE CONSCIOUS DOG*

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The effects of haemorrhagic shock on blood pressure and heart rate responses to exogenous adrenaline (2 μg/kg i.v.) were studied in conscious dogs with chronically implanted vascular cannulae. The animals were bled to a mean arterial pressure of 40 mmHg, the duration of hypovolaemia being two hours. Adrenaline was injected before bleeding, at the beginning and at the end of controlled hypotension, before and 15 minutes after reinfusion. Plasma adrenaline and noradrenaline levels were determined by a radioenzymatic method.

In shock, blood pressure responses and their duration decreased. Heart rate responses were changed from a bradycardia type before bleeding to a tachycardia during shock. After reinfusion biphasic responses were dominating. A significant negative correlation was found between plasma noradrenaline levels and blood pressure responses to adrenaline.

In haemorrhagic shock the activity of the sympathetic nervous system is increased [7]. Plasma levels of catecholamines are elevated [10, 20, 27, 28] and contribute to the development of selective vasoconstriction in certain vascular areas.

Adrenaline (A) and noradrenaline (NA) are secreted by the adrenal medulla but NA is released mainly by the postganglionic nerve endings. As NA in blood is derived primarily from the sympathetic nerves, especially from those supplying the cardiovascular system [21], its concentration is a sensitive index of the sympathetic activity [23].

Several investigators have examined the catecholamine sensitivity of blood vessels in shock during anaesthesia [5, 15, 17, 18, 19, 22],

The present study was devoted to the blood pressure and heart rate responses to exogenous A and also to the changes in the level of sympathetic activity estimated by measuring the plasma levels of NA in haemorrhagic shock. As anaesthetics tend to modify the function of the cardiovascular system and its reactivity to haemorrhagic shock [8, 9, 30, 31] our experiments were performed in conscious dogs.

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Our aim was also to study the relationship between the plasma concentration of endogenous NA and the blood pressure response to a test dose of A and to establish whether the latter simple method is suitable to indicate the level of sympathetic activity in shock.

Methods

Twenty-three mongrel dogs of either sex (average weight 12.4 kg) were used. Several days before the experiment heparin-filled Silastic cannulae were implanted into the left carotid artery and jugular vein under aseptic conditions under sodium pentobarbital anaesthesia (30 mg/kg). The cannulae were led through subcutaneous tunnels and exteriorized at the dorsal side of the neck. They were protected with a bandage encircled by adhesive tape.

The conscious animals were placed into a large size plexiglass box and handled very gently throughout the experiment. Before bleeding 750 U/kg b.wt. of heparin was injected for anticoagulation. The arterial cannula was used for blood pressure measurements, for bleeding and for taking blood samples. The venous cannula was used for injection of A and for the reinfusion.

The dogs were bled to a mean arterial pressure of 40 mmHg (Fig. 1). In the period of controlled hypotension this pressure was maintained for one hour by further bleeding or reinfusion. A graduated glass reservoir and interconnecting Silastic and Tygon tubings were used for the bleeding. In the compensation period bleeding was stopped and the compensation mechanisms were allowed to operate for one hour. Then all shed blood was reinfused. The bleeding volumes were recorded at the beginning and at the end of the controlled hypotension period.

Before haemorrhage, at the beginning and at the end of controlled hypotension, before and 15 minutes after reinfusion, arterial blood samples were taken for plasma catecholamine, blood pH and haematocrit measurements. Plasma NA and A were measured in 3 ml blood samples by the radioenzymatic method of PASSON and PEULER [26]. Radioactivity was measured in a Beckman LS-100 C liquid scintillation spectrometer. Blood pH was determined by a Radelkis pH-meter. Haematocrit values were measured by the microcapillary method.

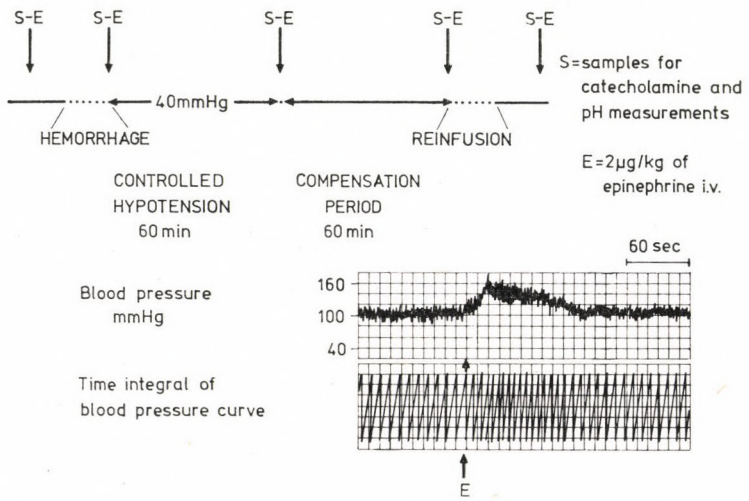

Fig. 1. Experimental protocol for haemorrhagic shock, blood sampling and injection of adrenaline. An original record shows blood pressure response evoked by a single dose of adrenaline and its integral

During the experiments mean arterial pressure and heart rate were recorded continuously on a Beckman Dynograph recorder. Mean arterial pressure was measured using a Beckman transducer. Heart rate was measured with a cardiotachometer coupler (Beckman 9857).

Immediately after taking blood samples a bolus of 2 μg/kg A was injected intravenously. Blood pressure responses to adrenaline were analyzed in terms of time integral of blood pressure curve, the maximum change of blood pressure in mmHg and duration of responses in seconds. A Beckman resetting type integrator coupler (9873 B) was used for time integration of blood pressure curves. The time integral of the blood pressure curve is given as the increase of recorded peaks over the preinjection level (Fig. 1). Heart rate responses were determined by the minimum and maximum rates and duration of responses. Duration of blood pressure and heart rate responses were measured as the time in seconds from the injection of A until the blood pressure and heart rate crossed the preinjection level.

Two control experiments were performed to determine spontaneous changes in plasma catecholamine levels over a period of 3 hours without haemorrhagic shock and injection of A. In addition, we also measured the effect of exogenous A on the plasma level of catecholamines in haemorrhagic shock.

The results were evaluated by nonparametric statistical methods. Variation is given as median $\pm$ semi-interquartile range. Semi-interquartile range is defined as one half of the difference between the 75th and 25th percentiles [14, 16]. Stochastic probability was estimated using the Wilcoxon's rank sum test and signed rank test for intergroup and within group comparisons, respectively [34]. A P value of 0.05 was taken as the critical level of stochastic significance [13]. Spearman's rank correlation coefficient was used for testing association.

Results

In haemorrhagic shock the blood pressure responses to exogenous A (2 μg/kg i.v.) were gradually decreased. The rise in blood pressure given as the time integral of blood pressure curve, the duration of pressure responses and the maximum change of blood pressure following the A bolus are presented in Fig. 2. At the beginning of controlled hypotension the rise in blood pressure given as the integral, the duration of response and the maximum change of blood pressure were the same as before haemorrhage. At the end of the controlled hypotension, however, all three parameters were significantly ($P < 0.01$) decreased. During the compensation period pressure response was further decreased and in some dogs it completely disappeared before reinfusion. After reinfusion only the duration of response returned to the control. The rise in blood pressure and the maximum change were also increased, however, both were significantly ($P < 0.02$ and < 0.01, respectively) smaller than the control values.

Heart rate and heart rate responses to A are shown in Fig. 3. After the initial haemorrhage heart rate increased to a significantly ($P < 0.01$) higher value than the control. Then it was further increased till reinfusion. After reinfusion it was decreased but remained significantly ($P < 0.01$) higher than the control. Before haemorrhage A elicited usually bradycardia but in some dogs a biphasic response was observed. During shock most of the biphasic responses were changed into tachycardia. In four dogs no response was produced to a bolus of A given immediately before reinfusion. In three dogs a heart rate response of bradycardia type was observed. After reinfusion the

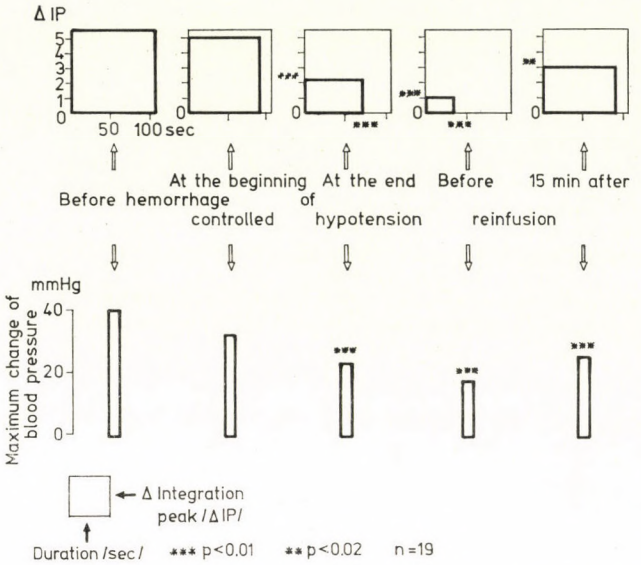

Fig. 2. The effect of haemorrhagic shock on blood pressure responses to adrenaline (2 μg/kg i.v.) in conscious dogs. Blood pressure response is illustrated by a rectangle: on the ordinate the integral in Δ integration peak units is presented and on the abscissa the duration of pressure response is shown in seconds. The maximum change of blood pressure is presented by the bars

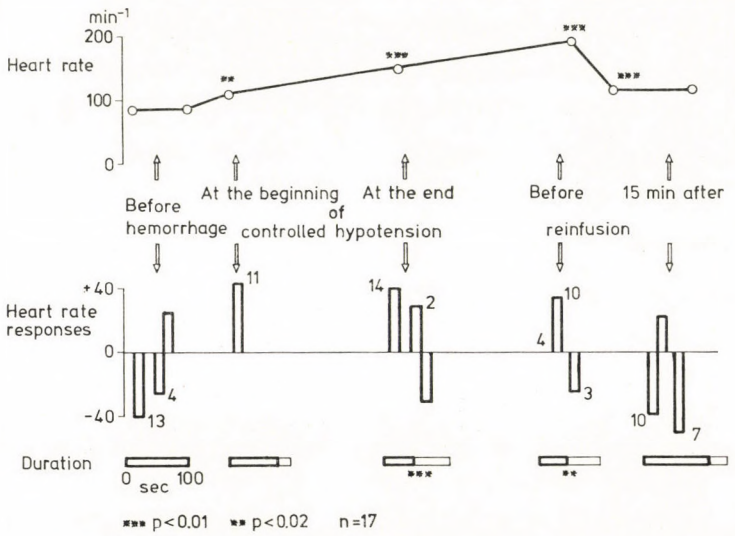

Fig. 3. The effect of haemorrhagic shock on heart rate responses to adrenaline (2 μg/kg i.v.) in conscious dogs. The upper curve shows the heart rate changes. The heart rate responses to adrenaline are given as the minimum and maximum rates and duration of responses. The bars represent the heart rate responses to adrenaline. The figures at the bars show the number of dogs

Table I

Plasma catecholamines, arterial blood pH, bleeding volume and haematocrit values of conscious surviving dogs in haemorrhagic shock

n = 19	Before haemorrhage (control)	At the beginning of controlled hypotension	At the end of controlled hypotension	Before reinfusion	15 min after reinfusion
Adrenaline (pg/ml)	205.5 (188 ± 54)	3154.9[a] (3090 ± 1703)	6543.8[a] (6515 ± 1737)	4440.0[a] (3915 ± 2081)	997.4[a] (669 ± 304)
Noradrenaline (pg/ml)	313.8 (329 ± 116)	2270.7[a] (1032 ± 1034)	2674.8[a] (2125 ± 781)	2207.1[a] (2135 ± 843)	682.4[a] (539 ± 231)
Arterial blood pH	7.42 (7.41 ± 0.03)	7.46 (7.47 ± 0.03)	7.31[a] (7.33 ± 0.08)	7.31[b] (7.32 ± 0.05)	7.28[a] (7.31 ± 0.04)
Bleeding volume (ml/kg)		39.4 (39 ± 7)	52.9 (52 ± 6)		
Haematocrit (%)	39.0 (39 ± 3)	37.9 (39 ± 3)	36.4 (36 ± 4)	36.4 (36 ± 3)	40.4 (40 ± 3)

Values represent mean and median ± semi-interquartile range (in parentheses)
Significant difference from control values: a = $P < 0.01$; b = $P < 0.02$

biphasic responses were dominating. Duration of the response was significantly ($P < 0.02$) decreased till reinfusion, but after reinfusion it increased slightly above the control.

Table I shows plasma levels of catecholamines, arterial blood pH, bleeding volumes and haematocrit values of the surviving dogs in haemorrhagic shock. Both catecholamines were markedly increased but the increase of NA was less than that of A. After reinfusion plasma A and NA decreased, however, both remained above the control levels. Thirty minutes after haemorrhage arterial blood pH (7.34) was significantly ($P < 0.02$) lower than the control and then decreased further. Immediately after reinfusion the pH was 7.24, significantly ($P < 0.01$) lower than before reinfusion, although the pH of the reservoir blood was the same as that of blood in dogs before reinfusion. After 15 minutes the pH increased to the value measured before reinfusion, however, still remaining significantly ($P < 0.01$) lower than the control. At the end of controlled hypotension the bleeding volume was significantly ($P < 0.01$) greater than at the beginning of the period. Haematocrit values were slightly but not significantly decreased.

Table II

Plasma levels of catecholamines in the control experiments without haemorrhagic shock

n = 2	Control	20	80	140	175
		minutes after the control sample			
Adrenaline (pg/ml)	233.0	256.0	315.5	268.0	174.0
Noradrenaline (pg/ml)	295.0	434.5	362.5	466.0	401.5

Table III

The effect of exogenous adrenaline (2 μg/kg i. v.) on plasma levels of catecholamines in haemorrhagic shock

		Control	30	60	120	180	300
		second after the injection of adrenaline					
Adrenaline (pg/ml)	Before shock	205.5	6593.5	2456.5	924.5	424.0	368.0 NS
	In	6543.8	12 921.0	11 436.5	5775.0	3854.5	5164.3 NS
Noradrenaline (pg/ml)	Before shock	309.7	842.5	431.5	370.0	320.0	382.4 NS
	In	2913.3	4447.5	2 466.0	1713.5	1749.0	2453.1 NS
n		13	2	2	2	2	13

NS = not significant compared to the control value

The results of the control experiments without haemorrhagic shock show that neither A nor NA levels in plasma were changed during the experiment (Table II).

Table III demonstrates the effect of exogenous A on the plasma level of catecholamines in haemorrhagic shock. Before haemorrhage, 30 seconds after the injection of A a thirtyfold increase was observed in the plasma level of A. Five minutes after the injection, however, plasma A did not differ significantly from the level measured before the injection. In shock the plasma level of A was doubled after the injection while two minutes later it decreased to the preinjection level. The plasma level of NA was not significantly altered after the injection of exogenous A.

Figure 4 demonstrates that there is no relationship between the blood pressure responses to A and the plasma A level.

On the other hand, as shown in Fig. 5, a nonlinear relationship was found between the blood pressure responses to A and the plasma NA level. Prior to haemorrhage the control blood pressure responses showed considerable variation and plasma NA levels fell in a narrow range. At the end of con-

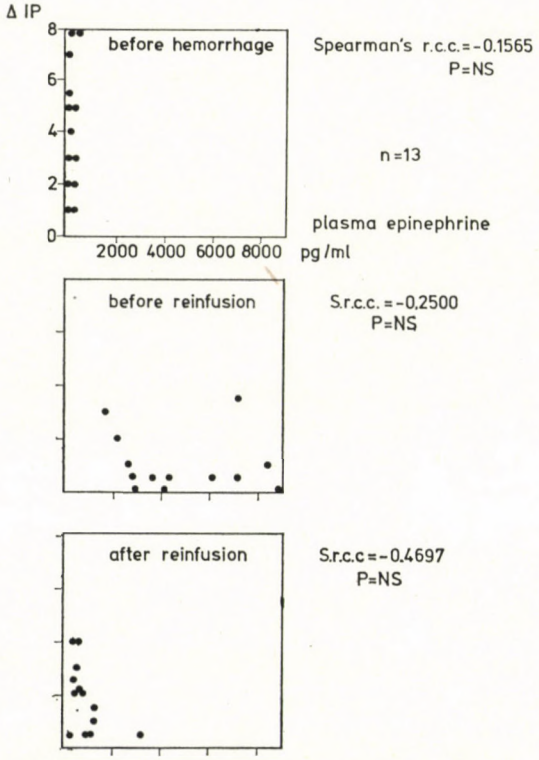

Fig. 4. Relationship of blood pressure responses to adrenaline (2 μg/kg i.v.) and plasma adrenaline levels in conscious dogs. NS = not significant

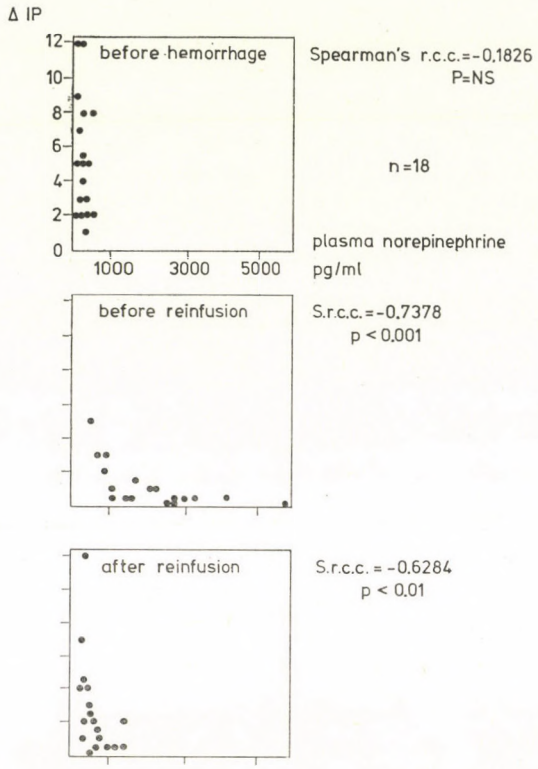

Fig. 5. Relationship of blood pressure responses to adrenaline (2 μg/kg i.v.) and plasma noradrenaline levels in conscious dogs. NS = not significant

trolled hypotension the Spearman's rank correlation coefficient ($r = -0.4623$) was not significant (not shown in the figure); on the other hand there was a significant negative relationship ($r = -0.7378$; $P < 0.001$) between the pressure responses and the plasma NA levels before reinfusion which remained so even after reinfusion ($r = -0.6284$; $P < 0.01$). The responses were larger at the lower levels of plasma NA than at the higher levels.

A log-log type relationship was found between blood pressure responses to A and plasma NA levels (Fig. 6). When the logarithms of the integral of blood pressure curves are plotted against the logarithms of the plasma NA levels a linear relationship is obtained.

The relationship of blood pressure responses to A and arterial blood pH is demonstrated in Fig. 7. Before haemorrhage various responses were obtained at normal pH values. In shock, A produced only blunted responses, especially at low pH ($r = 0.6652$; $P < 0.01$). After reinfusion neither blood pH nor blood pressure responses returned to control levels ($r = 0.4964$; $P < 0.05$). As revealed by the Spearman rank correlation coefficients, the relationship

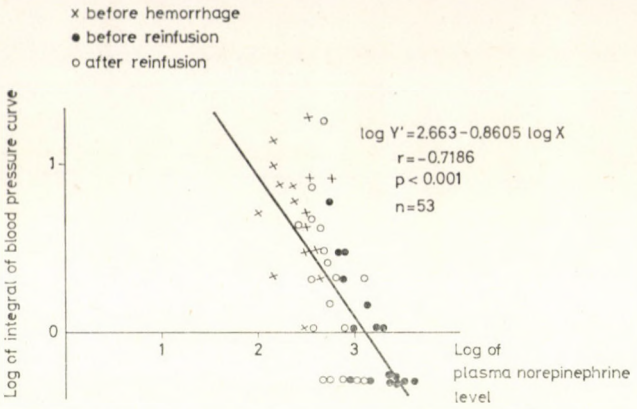

Fig. 6. Correlation between blood pressure responses to adrenaline (2 μg/kg i.v.) and plasma noradrenaline levels in conscious dogs. Ordinate: log scale of blood pressure responses given as the integral. Abscissa: log scale of plasma noradrenaline levels

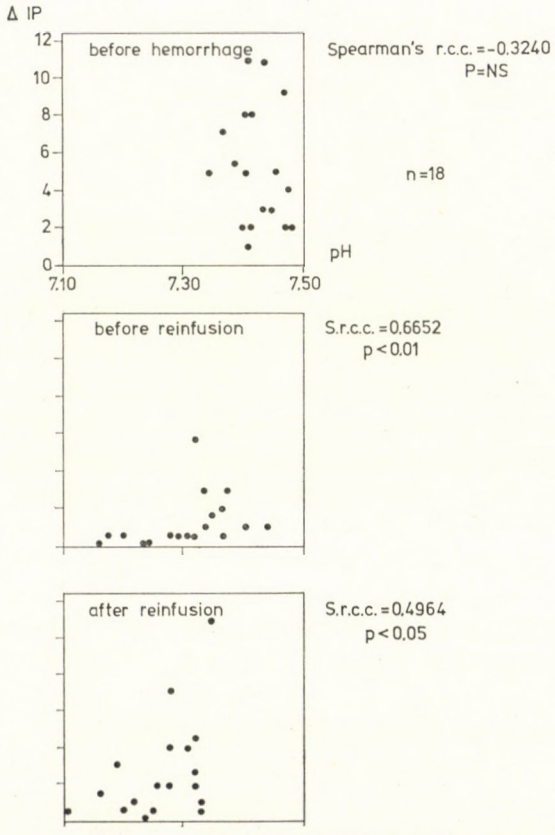

Fig. 7. Relationship of blood pressure responses to adrenaline (2 μg/kg i.v.) and arterial blood pH in conscious dogs. NS = not significant

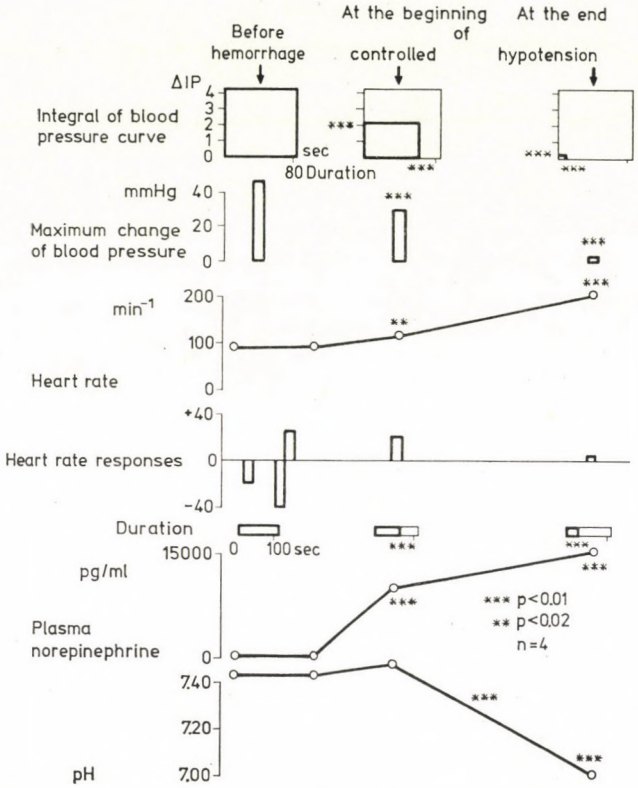

Fig. 8. The effect of haemorrhagic shock on blood pressure and heart rate responses to adrenaline (2 μg/kg i.v.) in conscious nonsurviving dogs

was closer between the blood pressure responses and plasma NA levels than between the blood pressure responses and blood pH.

The blood pressure and heart rate responses to A in nonsurviving dogs are demonstrated in Fig. 8. These dogs died about 20 minutes (average 19.7 minutes) after the end of the controlled hypotension period. They responded to A quite differently from the survivors. At the beginning and at the end of controlled hypotension, blood pressure responses were significantly ($P < 0.01$) smaller than in the control period and also significantly ($P < 0.01$) smaller than in the survivors. In shock, heart rate increased and at the end of controlled hypotension it was significantly ($P < 0.01$) higher than that of survivors. Heart rate responses to A were similar to those of the other group. However, before death A elicited no response in two dogs.

Table IV shows plasma NA levels, arterial blood pH, bleeding volumes and haematocrit values of the nonsurviving dogs. In shock, plasma NA reached a significantly higher level than in the control group ($P < 0.01$) and than in the survivors ($P < 0.01$). At the end of controlled hypotension arterial

Table IV

Plasma noradrenaline, arterial blood pH, bleeding volume and haematocrit values of conscious nonsurviving dogs in haemorrhagic shock

n = 4	Before haemorrhage (control)	At the beginning of controlled hypotension	At the end of controlled hypotension
Noradrenaline (pg/ml)	255.5 (313 ± 316)	10,048.5$^{a}_{c}$ (8128 ± 2182)	15,886.0$^{a}_{c}$ (13,872 ± 12,054)
Arterial blood pH	7.45 (7.43 ± 0.05)	7.51 (7.52 ± 0.09)	7.051 (7.06 ± 0.20)
Bleeding volume (ml/kg)		50.3^{b} (49 ± 6)	58.5 (61 ± 10)
Haematocrit (%)	42.2 (42 ± 4)	45.0 (45 ± 6)	45.2 (45 ± 6)

Values represent mean and median ± semi-interquartile range (in parentheses)
Significant difference from control values: a = $P < 0.01$
Significant difference from values of the surviving dogs: b = $P < 0.05$; c = $P < 0.01$

blood pH was significantly lower than in the control period ($P < 0.01$) or in the survivor group ($P < 0.01$). Bleeding volume necessary to lower blood pressure to 40 mmHg was significantly ($P < 0.05$) larger than that of the survivors. Haematocrit values did not change.

Discussion

Several investigators demonstrated changes of catecholamine sensitivity in haemorrhagic shock, however, all these studies were carried out on anaesthetized animals. HILTON [17] found no significant differences in the rise of blood pressure before and after bleeding with a similar dose of A. However, these dogs were bled to only 10% of the measured blood volume. According to the results of KOVÁCH and TAKÁCS [22] the A sensitivity was reduced after a blood loss of 35 ml/kg but after reinfusion it rose to normal. In contrast to this CALVERT and LUM [5] found reduced pressor responses to NA and A immediately after reinfusion, however, the responses gradually returned toward the control levels over a period of several hours.

These conflicting findings may probably be explained by the different degree of anaesthesia and by the different severity of haemorrhagic shock.

In our experiments the effects of haemorrhagic shock for two hours on blood pressure and heart rate responses to exogenous A were studied in conscious dogs. Our results are in consistent with the above-mentioned data. Blood pressure responses to 2 μg/kg A were gradually decreased with the

duration of shock. After reinfusion, however, responses were further diminished despite the restoration of circulating blood volume.

In four dogs A elicited greater responses at the beginning of hypovolaemia than in the control period. We supposed that these responses might be due not only to the effect of A but also to the operation of blood pressure compensation mechanisms. If this were true, the bleeding volume in the first 10 minutes should be less in those dogs which showed diminished responses to A. Bleeding volumes were, however, the same, thus the pressure responses to A seem not to be affected by the compensation mechanisms.

As shown by the relationships between the blood pressure responses and the plasma catecholamine levels the elevated plasma NA levels are responsible for the change in the responses. Although the mean of the responses is the same at the beginning of controlled hypotension and before haemorrhage, only high levels of plasma NA were found in dogs which responded slightly to A (mean level, 1865.2 pg/ml). In dogs showing greater responses, plasma NA levels were lower (mean level, 774.5 pg/ml). This was particularly apparent at the end of shock when most of the responses became smaller than during the control period. The relationship between the blood pressure responses and plasma NA levels is verified by the value of the Spearman's rank correlation coefficient which is highly significant. This correlation is maintained after reinfusion when neither NA levels nor the blood pressure responses returned to control values. There is a nonlinear relationship between the two parameters, however, their logarithms present a linear relationship.

It is known that in irreversible shock plasma catecholamine concentration tend to rise to extremely high levels [32]. In the present study dogs which died after an hour had higher plasma NA level than the surviving ones. At the same time the nonsurvivors responded to A only slightly or not at all already at the beginning of shock. In neither of the dogs could a response be elicited immediately before death. Thus, the reactivity of the blood vessels to A may completely disappear in the irreversible stage of shock. These results are contrary to those of ZAPATA-ORTIZ and STASTNY [37], or CALVERT and LUM [5] who found that the blood vessels maintained their reactivity to catecholamines even in the stage of irreversible shock. Our results suggest that the higher the level of sympathetic activity the lower is the sensitivity of blood vessels to catecholamines.

In addition to sympathetic overactivity arterial blood pH is another factor which may be important in the development of the refractoriness to A. In shock inadequate tissue perfusion caused by the decreased blood flow results in an anaerobic shift of metabolism. Many studies suggest that acidosis reduces the responsiveness of blood vessels to catecholamines and affects the function of the myocardium [2, 3, 4, 6, 11, 25, 29, 35]. Moreover, in addition to hypotension, acidosis *per se* may also stimulate the release of catechol-

amines from the adrenal medulla [12, 24, 36]. It is known that responses to catecholamines may be restored to normal by the correction of a low arterial blood pH [6, 11]. In our experiments blood pH began to fall already in the early stage of shock. Thirty minutes after the initial haemorrhage the pH was significantly lower than the control and remained at a low level till reinfusion. After reinfusion it further decreased indicating the so-called wash-out phenomenon. Lactic acid and other acid metabolites which accumulated in the inadequately perfused tissues were released into the circulation during reinfusion. The acidosis was significantly more severe in the nonsurviving dogs. In these dogs the extremely high level of plasma catecholamines may account for diminished tissue perfusion and also for the metabolic acidosis. We assume that the low arterial blood pH may also contribute to the diminished blood pressure responses and the failure of responses to recover after reinfusion. This is suggested by the significant positive correlation between pressure responses and blood pH before and after reinfusion. The relationship between the blood pressure responses and blood pH, however, is not as close as between the blood pressure responses and the plasma NA levels. In this context it seems relevant that ANDERSEN, BORDER and MOURITZEN [1] found a maintained responsiveness to A in pure metabolic acidosis produced by lactic acid infusion down to a pH of 6.8.

According to our results, the plasma levels of catecholamines were not affected by exogenous A. The high levels of plasma A caused by the injection of A quickly disappeared. Five minutes after the injection of A, plasma levels of catecholamines were similar to the preinjection level.

In these experiments the effect of haemorrhagic shock on the heart rate responses to A was also studied. It is known that in the conscious state low doses of A cause a bradycardia of vagal origin, however, large doses may override the vagus effect [33]. Before haemorrhage we also observed a bradycardia evoked by the injected A. In shock at low arterial pressure the bradycardia type of response reversed to tachycardia and after reinfusion it was changed in part to bradycardia and to biphasic responses. In shock the sensitivity of the baroreceptors is decreased and the direct effect of A on the heart rate seems to prevail. After shock the heart rate responses to A were slightly modified.

Plasma NA levels were determined in small samples of plasma using the radioenzymatic method of PASSON and PEULER [26]. This method, although very sensitive and specific, is rather laborious and expensive. Since we found a relationship between the blood pressure responses and plasma NA levels and, as the latter is known to reflect the degree of the sympathetic activity [21, 23], we suggest that the blood pressure responses to exogenous A may serve as a measure of the level of the sympathetic activity in haemorrhagic shock.

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STRIATAL DOPAMINE LEVELS AFTER UNILATERAL LESIONS OF THE SUBSTANTIA NIGRA: EVIDENCE FOR A CONTRALATERAL DECREASE

By

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Unilateral electrolytical and chemical (6-hydroxydopamine) lesions in the substantia nigra (SN) of rats were followed 7 days later by considerable bilateral decreases of neostriatal dopamine (DA) levels. Similarly, the DA content of the substantia nigra decreased not only ipsilaterally but contralaterally as well. Positive correlations were found between ipsi- and contralateral nigral DA levels, ipsi- and contralateral striatal DA and between the DA level of the SN and the striatum of the corresponding side both ipsi- and contralaterally to the lesion.

A contralateral decrease of neostriatal dopamine (DA) level was found previously [4] one week after unilateral lesion in the pallidum (GPL). Others have demonstrated that evoked potentials can be elicited by caudate stimulation in the contralateral caudate nucleus in the rat and cat [9], unilateral pallidal lesion influences contralateral striatal firing rate [5] and a decrease of DA concentration of the contralateral caudate and substantia nigra (SN) follows unilateral caudate lesion [1] in the cat. These data suggest that there is a functional connection between the two nigrostriatal systems even though the anatomical substrate of such a connection is not known. These phenomena, however, may also be attributed to the destruction/stimulation of non-nigrostriatal elements in the sites in question and may not occur after manipulations interfering with the activity of *other* sites containing nigrostriatal elements. This possibility is the more probable because SAAVEDRA et al. failed to show any decrease of contralateral striatal DA in the rat two months after unilateral nigral lesions [8], a manipulation which is certain to affect the nigrostriatal elements of the caudate. Furthermore, WALTERS et al. [10] when measuring the contralateral striatal DA within 2 days and 12 days after the interruption of nigrostriatal fibres but not in the period between the 2nd and 12th postoperative days did not find any decrease either. POIRIER and SOURKES [7], on the other hand, found decreased contralateral striatal DA levels in the *Rhesus macacus* several months after unilateral nigral lesion. The discrepancy between findings in the rat and monkey may be explained by species differences in the organization of nigrostriatal function while the contradiction between our findings after unilateral pallidal lesion [4] and data

of SAAVEDRA et al. [8] and WALTERS et al. [10] can be reconciled in two ways: first, it may be suggested that, in the rat, the contralateral decrease is transient and it is confined to a short period between the 2nd and 12th postoperative days. The 7th day, on which our previous measurements were made, is exactly in the middle of this period. The other possibility is that the contralateral effect is not connected with nigrostriatal function.

The present study was made to establish whether unilateral nigral lesions have any effect on the contralateral striatal DA levels in the rat. Unilateral SN lesions were made and DA contents of the ipsilateral and contralateral striatum and SN were measured one week later. The 7th day was not covered in the study of WALTERS et al. [10] and it was at this time that we obtained the contralateral decrease after pallidal lesion.

Methods

Fifty male rats of the CFY strain weighing 280—350 g (LATI, Gödöllő, Hungary) were used. The animals were transferred to the laboratory 10 days before the experiment and kept on standard laboratory diet. The animals were caged individually.

Operations were carried out under pentobarbital (Nembutal) anaesthesia. Stereotaxic coordinates (A: 2.2, L: 2.0, V: —1.7) of DEGROOT [2] were used. Both electrolytical and chemical lesions were made; the former with a glass-insulated stainless steel electrode (2 mA anodal DC for 10 sec), the latter with 8 μg of 6-hydroxydopamine (6-OHDA) in 0.8 μl physiological saline containing 0.1% ascorbic acid, injected at a rate of 0.5 μl/min. Further details have been described elsewhere [6]. Sham-operation consisted in locating the cannula tip above the SN.

The animals were killed by decapitation 7 days after the operation without anaesthesia; the brains were removed from the skulls rapidly, frozen and stored at —25 °C. Samples of the caudate-putamen complex were taken as described previously [4]. SN samples were taken with similar technique after a crosswise section at the posterior end of the SN.

Accurate lesion localization was difficult in these experiments since the requirements of DA determination and histology were contradictory, DA content being measured in the site of the lesion, too. The correctness of the lesion was ascertainted of with visual inspection of the cross-sected brains in every case and in randomly chosen animals the SN was not used for DA determination but histologically examined in frozen sections. The latter procedure gave, naturally, a picture about the accuracy of the lesions only in a general way. The striatal DA depletion also gave an important information on the correctness of the lesion: only those animals were considered in which the *ipsilateral* DA depletion was higher than 40% after electrolytical lesion or higher than 75% after chemical lesion.

DA determination was made with a fluorimetric micromethod [3].

Analysis of variance and analysis of covariance were used for the statistical evaluation of the data.

Results

Ipsilateral striatal DA levels in animals subjected to either electrolytically or chemically induced lesions were significantly lower than corresponding concentrations in sham-operated animals. There was no difference between the two experimental groups in this respect (Fig. 1/A).

Contralateral striatal DA levels also decreased in both groups subjected to lesion in comparison to sham-operated animals. The DA concentrations

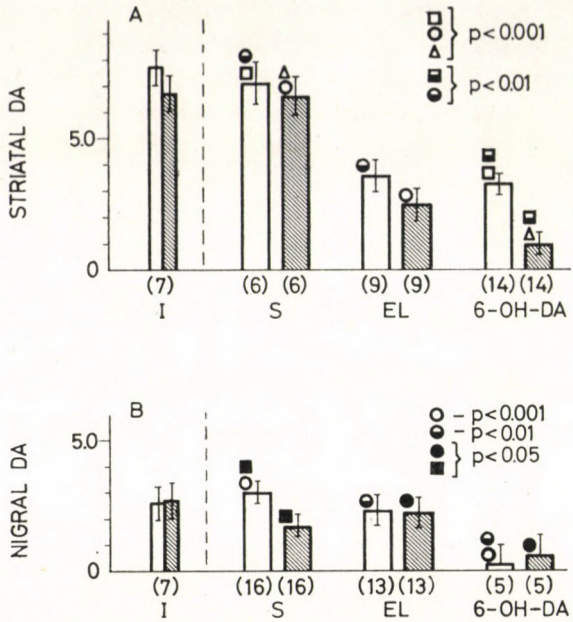

Fig. 1. Striatal (Section A) and nigral (Section B) dopamine concentrations 1 week after unilateral electrolytical (EL) and chemical (6-OHDA) lesions of the substantia nigra and in intact (I) and sham-operated (S) animals (μg/g fresh tissue). Hatched bars represent ipsilateral levels, open bars show contralateral values (means $\pm$ S.E.M.). The numbers of measurements in each group are given in parentheses below the bars. Differences between groups labelled with the same symbol are significant at the level assigned to the symbol in the figure

were higher contralaterally than ipsilaterally in 6-OHDA lesioned animals. Contralateral concentrations in the rats subjected to electrolytical or chemical lesions were similar (Fig. 1/A).

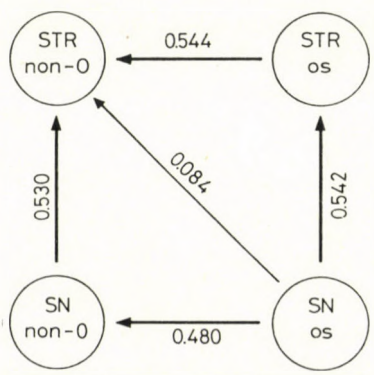

Fig. 2. Summary of the correlations between DA levels of the sites studied. SN = substantia nigra, STR = striatum, OS = operated side, non-O = non-operated side. Thick arrows indicate significant correlations

Positive correlation was found between the levels on the lesioned side and their contralateral counterparts (Fig. 2). There was no definite difference in ipsilateral nigral concentrations between sham-operated and lesioned animals. A significant difference was observed, however, between the electrolytically and chemically lesioned groups and between the latter and intact animals ($p < 0.01$, not indicated in the Figure) in this respect (Fig. 1/B).

The contralateral DA level in the SN of chemically lesioned animals was significantly lower than that of sham-operated or electrolytically lesioned animals while there was no difference between sham-operated and electrolytically lesioned animals (Fig. 1/B).

Ipsilateral DA levels in the SNs of sham-operated animals were markedly higher than the contralateral levels (Fig. 1/B).

A positive correlation was found between the ipsilateral and contralateral SN as well as between nigral and striatal concentrations on both sides. There was no significant correlation between the concentrations in the ipsilateral SN and the contralateral striatum (Fig. 2).

Discussion

The situation after unilateral nigral lesion is very similar in terms of striatal DA levels to what has been found after GPL [4]. Quite like after GPL, a contralateral decrease was found. The contralateral effect of 6-OHDA was not an artifact caused by some of the drug passing into the cerebrospinal fluid or exerting a generalized effect in any other way since electrolytical lesion had a similar effect. Dopaminergic specificity and the coincidence of the sites the destruction of which causes contralateral decrease of striatal DA with the course of the nigrostriatal bundle, point to this structure as the one responsible for the contralateral changes.

A decrease of contralateral nigral DA concentration followed unilateral chemical lesion of the SN while electrolytical lesions were not effective enough to produce a significant difference between sham-operated and lesioned animals. The same is true about ipsilateral levels. The mechanism underlying the latter finding is more complicated, however; it seems that sham-operation itself causes a localized decrease of ipsilateral nigral DA. Still, since sham-operation had *no contralateral* effect while 6-OHDA definitely *reduced* the contralateral nigral concentration, it may be assumed that the substantia nigra is involved in the mechanism of contralateral DA decrease after unilateral nigrostriatal lesions.

It is clear according to the present data that, though the contralateral changes after unilateral lesions are transient in the rat, there is an interdependence of DA levels of the two sides in this species, too.

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A RAPID CENTRIFUGATION METHOD FOR THE ISOLATION OF POLYMORPHONUCLEAR LEUCOCYTES FROM HUMAN BLOOD

By

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A rapid one-step gradient centrifugation method to prepare PMN leucocytes has been worked out by which a 98—99% pure, washed granulocyte suspension of 98% viability can be obtained in 30—40 minutes. The cells prepared by this method displayed higher NBT reduction upon ingesting the same soluble DNA — anti DNA complexes than those prepared by the dextran sedimentation method.

The cellular immune response has gradually gained attention in recent years. In addition to lymphocytes, the granulocytes have gained wide interest. In 1978, we reported on the alteration of immune complex phagocytosis by PMN leucocytes in SLE [7]. For the purpose of these studies, a two-step centrifugation method had been worked out to separate PMN-s; the method was a modification of BØYUM's [1] original technique. This method, however, was time consuming and did not yield a sufficient number of viable cells. Kinetic studies were, therefore, difficult to perform.

Beside many techniques by which small amounts of granulocytes would be obtained in excellent functional condition [7] methods are widely used which combine gradient separation with subsequent dextran sedimentation [2, 8]. Their cell output is favourable but for testing phagocytic function, intact cells are needed. Earlier we have observed that between dextran sedimented cells and those prepared by centrifugation over Ficoll-Uromiro gradient there existed striking differences which could not be revealed by trypan blue viability testing.

Viability was 98% for both. Still, dextran prepared cells seemed to have less stability and functional capacity than those prepared by our two-step centrifugation technique [7]. Cellular yields and the time factor were, however, more favourable with the latter procedure. The aim, therefore, was to find a suitable centrifugation technique that took less time and yielded more cells of sufficient purity and functional activity. This way best expressed by the

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immune complex phagocytosis measured spectrophotometrically by quantitative NBT reduction [8]. The final goal was to produce from the venous blood sample within 30—40 min. a granulocyte suspension that fulfils these requirements.

Methods

Venous blood was taken from seven volunteers whose systemic illness was excluded by routine laboratory tests. Eight ml blood was mixed with 10 mg heparin substance (137 IU./mg), diluted twofold by Parker's TC 199 medium followed immediately by overlayering on Ficoll-Uromiro gradients.

Separation of PMN leukocytes

a) *Centrifugation + dextran sedimentation*

Three ml sample of venous blood diluted twofold was overlayered on 3 ml 1.077 g/m Ficoll-Uromiro solution in a centrifuge tube.

To prepare the 1.077 g/ml solution, 21.6 g Ficoll was dissolved in 250 ml distilled water and 56.6 ml 60% Uromiro and the mixture was diluted to 340 ml with deionized water.

Centrifugation was carried out at 500 *g* at 5 °C for 30 min. One ml diluted serum was pipetted off from the top of the tubes followed by removal of the visible interphase band containing lymphocytes. Then the Ficoll-Uromiro solution was removed and the erythrocyte + granulocyte pellet at the bottom of the tubes collected and mixed with 1 ml of patient serum plus 0.4 ml of 6% dextran [2] dissolved in 0.15 mol/l phosphate buffer pH 7.4. This mixture was allowed to sediment in 15 mm tubes at room temperature for one hour. The upper phase was decanted, washed twice with medium TC 199 and the cells were counted by an electronic cell counter. Viability was assayed by the trypan blue exclusion method. The specific gravity of Ficoll-Uromiro solutions was repeatedly determined by a picnometer.

b) *New centrifugation method*

A 3 ml sample of venous blood diluted twofold was overlayered on 1.0794 g/ml (25 °C) Ficoll-Uromiro solution placed into centrifuge tubes in equal amounts. Centrifugation was carried out at 500 *g* at 5 °C for 5, 10, 15, 20, 25 and 45 min.

To prepare the 1.0794 g/ml solution, 21.6 g Ficoll was dissolved in 250 ml distilled water and 58.24 ml 60% Uromiro was added, and the mixture diluted to 340 ml with deionized water.

After centrifugation the following phases were collected separately: solution above the interphase band, interphase band, that below the interphase band, and the erythrocyte mass at the bottom of the tube.

The collected leucocytes from each phase were washed twice. The cells were counted and subjected to viability tests. Finally, the cellular suspension was sedimented on microscopic slides in sedimentation chambers built according to the description of KOLAR and ZEMAN [6]. All studies were performed in siliconized glass tubes.

Method for measuring phagocytosis of immune complexes

The method described by MOLNÁR et al. [7] was used. Briefly, a cellular suspension, 0.2% NBT, autologous inactivated serum and a mixture containing immune complexes were mixed in equal amounts of 100 µl of each. After incubation at 37 °C for 30 min, the reaction was stopped by 0.5 N hydrochloric acid and centrifuged at 1500 *g* for 15 min. The formazan formed was dissolved in pyridine by placing the tubes in boiling water for 15 min and the water clear supernatant was used for measuring colour intensity at 515 nm against a pyridine blank, in a Spekol 20 type spectrophotometer of Zeiss, Jena, GDR.

Preparation of immune complexes

Hundred µl high titre anti-DNA serum obtained from a severe case of SLE was added to 55 µg of high molecular weight DNA (Calf thymus, A grade, Lot: 300032, Calbio-

chem, San Diego, California, USA), dissolved in 1.0 ml 140 mmol NaCl and 10 mmol K_2HPO_4, pH 8.0. After mechanical stirring, the mixture was incubated at 37 °C for 60 min and then allowed to precipitate at 4 °C for 24 h, according to the method of PINCUS [9]. The actual amount of the stabilized, soluble immune complex was determined as follows. 1.1 ml ammonium sulphate solution saturated at 4 °C and adjusted to pH 8.0 was mixed with the above incubation mixture according to FARR [3]. After incubation at 4 °C for 60 min centrifugation at 3000 *g* was performed for 15 min. The DNA content of the supernatant and sediment fractions was determined by the method of GILES and MYERS [5] and the amount of bound DNA was expressed in per cent. The serum used in these experiments revealed a 38 $\pm$ 2% binding activity.

Results

The new centrifugation technique

The effectiveness of separation measured by the output cell counts and the spectrum of cells was determined every 5 min from the 5th to the 45th min. of centrifugation. Four phases had separated in each tube. The phases could clearly be distinguished (Fig. 1). The phases above the interphase bands (A), the interphase bands (B), the phases between interphase and erythrocyte sediment (C), and the sedimented erythrocytes (D) were collected separately. Results of cell counting and of differential counting are shown in Fig. 2, as a function of the centrifugation time. It is evident that granulocytes tended to sediment gradually downwards from the phase above the interphase band through the interphase towards the sedimented erythrocyte mass. An optimum

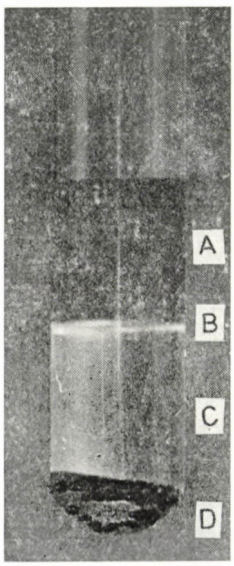

Fig. 1. Isolation of human leucocytes from twofold diluted blood on Ficoll-Uromiro solution of 1.0794 g/ml specific gravity (25 °C). A = upper phase; B = interphase; C = lower phase; D = pellet phase

Localization of fraction	Cell type	Centrifugation time: 5 min	10 min	15 min	20 min	25 min	45 min
		number of cells x μl^{-1}					
Upper phase	Ly.	960	810	890	495	300	50
	Segm.	240	90	10	5	–	–
Interphase	Ly.	5104	6400	6175	3895	4214	3800
	Segm.	696	900	325	205	86	200
Lower phase	Ly.	300	100	50	100	50	200
	Segm.	5700	3700	3100	1900	1450	600
Pellet phase		Erythrocytes	Erythrocytes	Erythrocytes	Erythrocytes	Erythrocytes	Erythrocytes

Fig. 2. Differential counts of lymphocytes and granulocytes in the function of centrifugation times determined from different fractions over Ficoll-Uromiro gradients

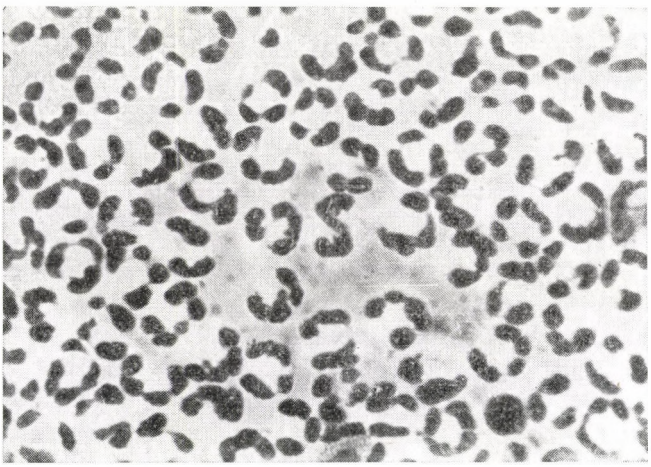

Fig. 3. Photomicrograph of the cellular suspensions taken after 7 min of centrifugation at 500 *g* from the lower phase (C) of Ficoll-Uromiro gradients

time could be found at which they left the interphase band but have not yet reached the already sedimented mass of erythrocytes. Centrifugation interrupted at this point resulted in a pure granulocyte suspension when phase (C) was collected. This could be achieved in the 7th min. The photomicrograph in Fig. 3 shows the sedimented granulocytes collected in this manner.

The viability determined by trypan blue exclusion was 98% and the fraction contained 1—2% lymphocytes and less than 10% erythrocytes. The yield was $2.0—2.5 \times 10^7$ granulocytes from 8 ml whole blood and the cells were ready for use including two successive washes 30—40 min after the blood had been taken.

In contrast, centrifugation + dextran sedimentation yielded after 30 min centrifugation at 1400 r.p.m. followed by 60 min sedimentation with

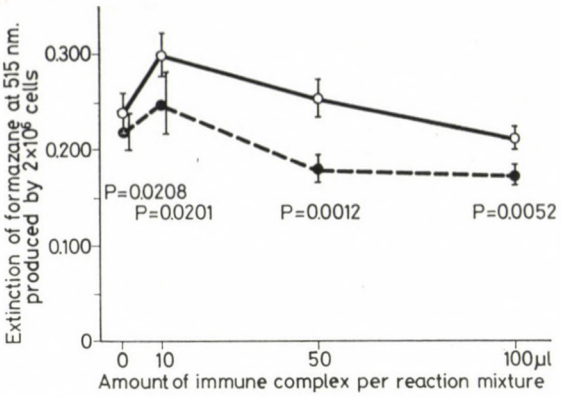

Fig. 4. Quantitative NBT reduction in isolated human granulocytes as the function of immune complex containing reaction mixtures. ○—○: Granulocytes obtained by the new centrifugation technique; ●—●: Granulocytes obtained by the dextran sedimentation method

dextran and successive washes $2.5-3\times10^7$ granulocytes from 8 ml of whole blood, and the fraction contained 5—8% lymphocytes and up to 40% erythrocytes. The trypan blue method revealed a 97% viability of the granulocytes.

Immune complex phagocytosis of granulocytes obtained by different methods

The immune complex induced NBT reduction of different amounts of DNA anti-DNA containing reaction mixtures was measured in the granulocyte suspensions obtained from 7 blood specimens prepared by the two methods. Results are shown in Fig. 4. The values were higher with granulocytes obtained by the new centrifugation technique than with those obtained by the dextran sedimentation method. The greatest increase in NBT reduction was found at 10 μl of the immune complex containing reaction mixture. The differences were significant statistically.

Discussion

Separation of granulocytes in sufficient number and condition is a prerequisite of functional immunological methods. Recently, a few attempts have been made to modify BÖYUM's classic method [1]. FERRANTE and THONG [4] published a simultaneous separation procedure of mononuclear cells and granulocytes from horse blood. Our earlier method [7] was time consuming. It provided, however, a pure granulocyte fraction that displayed higher values in NBT reduction than these cells prepared by the dextran sedimentation method. This fact together with other published data [4] have made it neces-

sary to analyse critically the results obtained in different experimental systems using dextran-prepared granulocytes.

Our studies showed equivocally that the Ficoll-Uromiro gradients can be modified in a way to obtain a practically pure granulocyte mass in a short time. The exact specific gravity of such solutions was 1.0794 g/ml.

It has to be pointed out that trypan blue exclusion cannot reveal differences in the functional activity of othervise intact cells.

Application of our rapid centrifugation method can provide an opportunity for many laboratories engaged in clinical immunology to carry out functional tests in multiple blood samples under conditions reflecting a higher level of standardization than before.

This in turn can improve the further investigation of functional and biological properties of PMN granulocytes, and to obtain a similar precision regarding their function as that of mononuclear cells.

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METABOLIC AND VASCULAR VOLUME OSCILLATIONS IN THE CAT BRAIN CORTEX*

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Earlier studies from our laboratory suggested that the cerebrocortical NADH fluorescence oscillations evoked by severe arterial hypoxia are not due to the oscillations of cerebral perfusion pressure and cerebral blood flow [10]. In the present work further data will be presented on the possible mechanism of other kinds of cerebral blood flow and metabolic oscillations. The experiments were performed on 20 cats anaesthetized by alpha-D-glucochloralose, immobilized by gallamine triethyliodide and ventillated artificially. The changes in cortical vascular volume and NADH fluorescence were measured with a fluororeflectometer through a glass window implanted into the right parietal bone.

In 5 experiments the oscillations in cortical vascular volume and NAD–NADH redox state were promoted by the so called Traube–Hering waves in arterial blood pressure. The vascular volume waves had a delay of 1—2 sec compared to the MABP waves and their frequency varied between 2—3 cycles/min. The NADH fluorescence waves lagged 2—10 sec behind the vascular volume waves. This type of oscillation was depressed transiently by i.v. administration of glucose and was stopped after MABP stabilization.

In 5 experiments the mean arterial blood pressure was decreased to 30—40 mm Hg by bleeding. When nitrogen anoxia was induced or glucose was administered, periodic alterations in NADH fluorescence appeared that were independent from the changes in vascular volume. The frequency of these waves was 1 cycle/2—5 min. These NAD–NADH redox state oscillations have been suggested to be pure metabolic oscillations that were described earlierly in yeast cells [2, 3].

In 10 experiments the cortical vascular volume and NADH fluorescence oscillations occurred already in the control period or were evoked by additional administration of alpha-D-glucochloralose. The frequency of this oscillation varied between 5—10 cycles/ min. During oscillation MABP was constant and ECoG was characterized by a low spike activity. The NADH fluorescence oscillations lagged 2—4 sec behind the vascular volume waves. The oscillation was insensitive to glucose but it could be abolished transiently by arterial hypercapnia and hypoxia and direct electrical stimulation of the brain cortex.

Oscillatory phenomena are observed at all levels of biological organization from the social to the molecular level, with periods ranging from seconds to years. Oscillations in the order of one min and of a few sec closely resemble those involving metabolites which control enzymatic reactions [5, 7, 11, 17]. The latter periodicities have evoked considerable interest in recent years owing to their putative link with biological clocks. With more sophisticated tech-

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niques it was possible to show NAD–NADH redox state oscillations even in intact organs [6, 7, 10]. Results from our laboratory indicated that arterial blood pressure waves, governed possibly by CNS ischaemic reflex oscillation [13], are based on the glycolytic oscillation of the medullary cardiovascular center [10].

In the present study an attempt was made to explore the conditions necessary to cause oscillations in the cerebrocortical NAD–NADH redox state and vascular volume. On the other hand we tried to characterize the various types of NAD–NADH oscillations that occurred in the living brain cortex.

Methods

The experiments were performed on 20 cats anaesthetized by alpha-D-glucochloralose (60 mg/kg b.w.), immobilized by gallamine triethyliodide (Flaxedil, 2—4 mg/kg b.w.) and respirated artificially (Harvard respirator, No. 667). A glass window, described earlier, [18] was implanted into the right parietal bone for optical monitoring of NADH fluorescence and UV reflectance of the cat brain cortex. The NADH fluorescence was measured at 450 nm, the UV reflectance at 366 nm with appropriate filters and photomultipliers [18]. The changes in fluorescence and reflectance have been expressed in per cent of the initial values of the normoxic brain cortex. To avoid apparent changes in NADH fluorescence caused by the variations of cortical blood volume the correction method of HARBIG et al. [16] was employed. The so-called corrected NADH fluorescence shows the real changes in tissue NADH concentration. The reflectance measured at 366 nm was used to indicate the alterations of cortical blood content and vascular volume. When the blood content of the brain cortex is increased the reflectance decreases and *vice versa* when the blood content is diminished. Theoretically the changes in haemoglobin oxygenation, when the absorption of the haemoglobin is measured at 366 nm, also may cause alterations in the intensity of the reflected light [20], however, since the red blood cells rather diffract than absorb the exaltation light this wave length seemed to be a satisfactory indicator of blood content alterations.

The trachea, femoral arteries, one of the lingual arteries and femoral veins were prepared and cannulated. The arterial blood gases and haemoglobin concentration were determined by an ABL-1 analyzer (Radiometer). The artificial respiration was adjusted to maintain the arterial pO_2 and pCO_2 close to 100 and 35 mm Hg, respectively. In the experiments where bleeding was applied the femoral artery was connected to a buffer-reservoir system.

Electrocorticogram was recorded *via* copper screws fixed into the frontal and occipital bones. Arterial blood pressure and intracranial pressure were measured by Statham P23/d electromanometers. The rectal temperature of the animals was maintained at 37 °C using a YSI 73/A temperature controller and an infralamp. The following parameters were recorded routinely on a 8-channel Grass polygraph: arterial blood pressure, intracranial pressure, cerebrocortical reflectance, NADH fluorescence, corrected NADH fluorescence and ECoG.

To characterize the different types of cortical NAD–NADH redox state and vascular volume oscillations, the following tests were applied: i.v. or i.a. infusion of glucose solution, direct electrical stimulation of the brain cortex (parameters of stimulation: 8—10 V, 0.5 msec, 15 c.p.s. electrical square wave impulses), nitrogen gas inhalation for 1—1.5 min, 5% CO_2 inhalation, arterial bleeding. To assess the connection between the different events the phase relationship of the various waves was also analyzed.

The experiments were divided into 3 groups depending on the characteristics of the oscillations (frequency, their dependence on arterial blood pressure variations, the conditions under which the NADH fluorescence oscillations appeared or were evoked, etc.). In the first group (5 experiments) Traube–Hering arterial blood pressure waves were seen. In the second group (5 experiments) the cortical NADH oscillations were evoked by nitrogen breathing and by glucose administration following a long duration of arterial hypotension. In the third group (10 experiments) the arterial blood pressure was constant and the frequency of the NADH fluorescence oscillations varied between 5—10 waves/min.

Results

In the present report 3 types of cerebrocortical oscillation will be described.

The first type of NADH oscillation is associated to the so-called arterial blood pressure waves of Traube and Hering (Fig. 1). In this experiment the cycle length of one wave was around 25 sec. The arterial blood pressure and cortical reflectance waves were almost in phase, the corrected NADH fluorescence waves were lagging 1—2 sec behind them. The close phase relationship between the arterial blood pressure and reflectance waves indicates the loss of cerebral blood flow (CBF) autoregulation when the Traube—Hering waves are present. The cerebrocortical vascular volume passively followed the fluctuations of arterial blood pressure. The spontaneous rise in arterial blood pressure led to the dilatation of cerebral vasculature and to the increase of CBF and cerebral oxygen supply. The better oxygen supply resulted in NADH oxidation (decrease in NADH fluorescence). The NAD reduction was promoted by opposite changes in cerebrocortical vascular volume and CBF.

Figure 2 demonstrates that the amplitude of cerebrocortical vascular volume and NAD–NADH redox state oscillations can be decreased by i.v. administration of glucose, however, the effect of glucose is not specific since the amplitude of arterial blood pressure waves is also decreased.

Direct electrical stimulation of the cerebral cortex transiently abolished the oscillations in the vascular volume and decreased the amplitude of NADH fluorescence oscillation. When the phase relationship between NADH fluorescence and arterial blood pressure oscillations is analysed during electrical stimulation it is obvious that the arterial blood pressure waves constitute the primary oscillation (Fig. 3). It is worth to note that electrical activation of

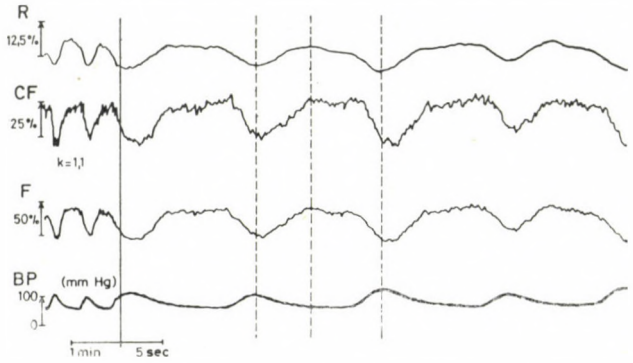

Fig. 1. Vascular volume (R), corrected NADH fluorescence (CF) and uncorrected NADH fluorescence (F) oscillations in the cat brain cortex that were associated with the so-called Traube and Hering arterial blood pressure waves. Abbreviations: k = correction factor determined by artificial haemodilution, BP = arterial blood pressure. Note that the vascular volume changes are inversely related to the reflectance (R) alterations

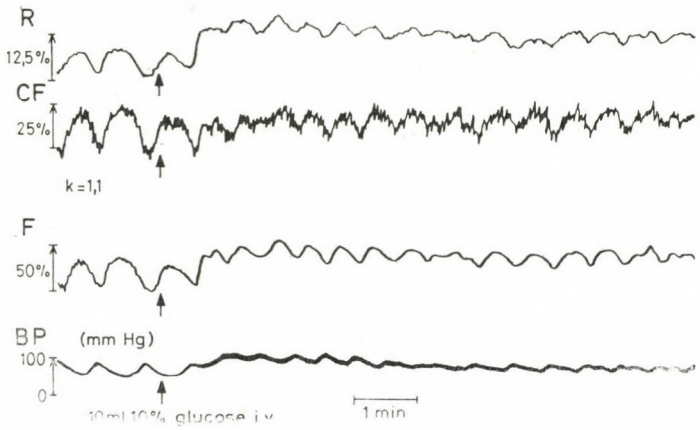

Fig. 2. Effect of the injection of 10 ml 10% glucose solution on the oscillations in the same experiment as in Fig. 1

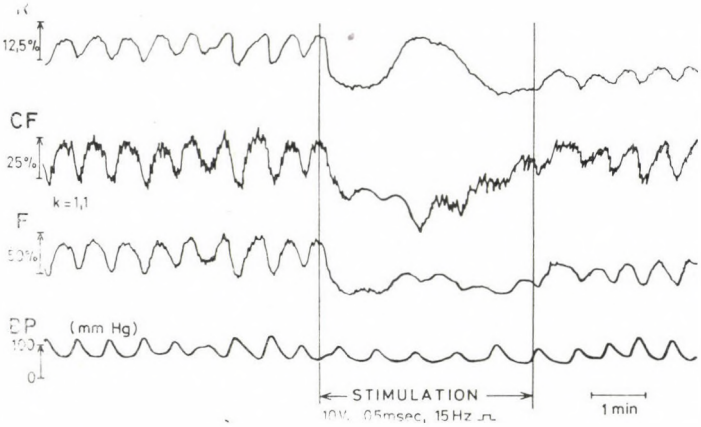

Fig. 3. Effect of direct electrical stimulation of the cerebral cortex on the oscillations demonstrated in Fig. 1. The duration of stimulation is marked by 2 vertical lines

the cerebral cortex in this case resulted in NADH oxidation in agreement with the impairment of CBF autoregulation [9].

When the arterial blood pressure is stabilized the oscillations in cortical vascular volume and NAD–NADH redox state vanished (not shown in the diagram).

The second type of cerebrocortical NADH oscillations appeared when the oxygen supply of the brain was restricted to a certain critical level and this condition was maintained for a long period. In our experiments this was achieved by decreasing the arterial blood pressure to 30—40 mm Hg for 2—3 hours. After this procedure oscillations in cerebrocortical vascular volume and NADH fluorescence could be provoked by nitrogen gas breathing and by

systemic glucose administration. Figures 4, 5 and 6 show such an experiment where the oscillations appeared after a nitrogen cycle. During nitrogen anoxia the cerebrocortical vascular volume did not change, since the vessels were already maximally dilated owing to the low arterial blood pressure (Fig. 4). This result rules out the possibility that the oxy-deoxy-haemoglobin changes would have caused significant alterations in the cerebrocortical reflectance measured at 366 nm as it had been suggested by KRAMER and PEARLSTEIN [20].

Following the nitrogen cycle NAD–NADH redox state transiently recovered, and subsequently it was shifted towards a more reduced state. There were also some fluctuation in the cerebrocortical vascular volume, however, these were less consistent than the fluctuations in the NAD–NADH

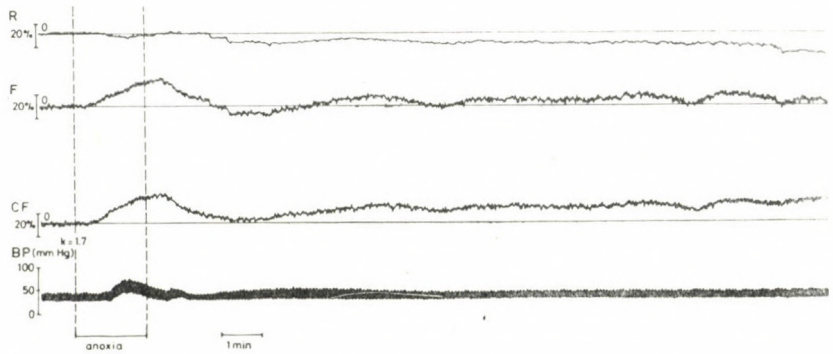

Fig. 4. Effect of nitrogen anoxia on the cerebrocortical vascular volume, uncorrected NADH fluorescence, corrected NADH fluorescence and arterial blood pressure during severe arterial hypotension in a typical experiment. Abbreviations as in Fig. 1. Note irregular oscillations in R, F and CF

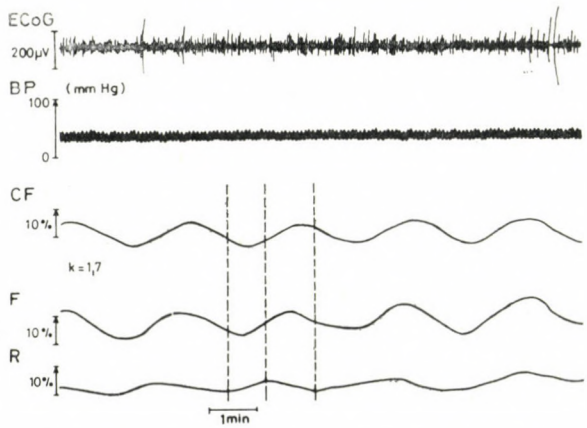

Fig. 5. Rhythmic alterations in cerebrocortical corrected NADH fluorescence, uncorrected NADH fluorescence and in vascular volume in the recovery phase following nitrogen anoxia in the same experiment as in Fig. 4. Note that the experimental traces were rearranged as compared to Fig. 4

redox state. Subsequently the rhythmic variations of cerebrocortical vascular volume and NAD–NADH redox state became more regular (Fig. 5) and the vascular volume waves preceded the NADH fluorescence waves. However, the oscillations of the NAD–NADH redox state could not have been governed by the periodic alterations of the vascular volume and CBF, since during the last phase of the oscillation the vascular volume waves vanished while the NADH fluorescence oscillations still persisted (Fig. 6).

Figure 7 shows an experiment where the cortical NADH fluorescence oscillations were evoked by the intra-arterial injection of 10% glucose solution.

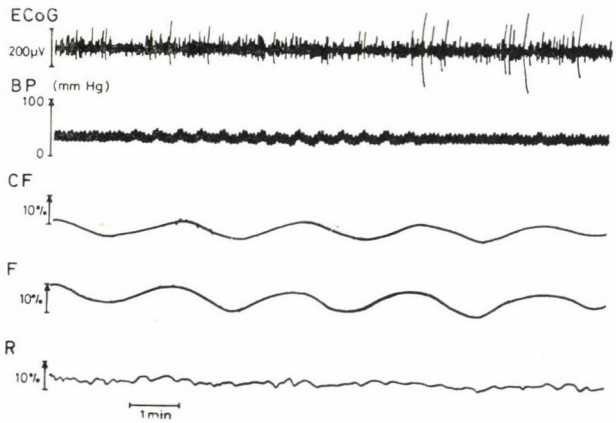

Fig. 6. Rhythmic alterations in cerebrocortical corrected NADH fluorescence and uncorrected NADH fluorescence before the oscillations ceased spontaneously in the same experiment as in Fig. 4

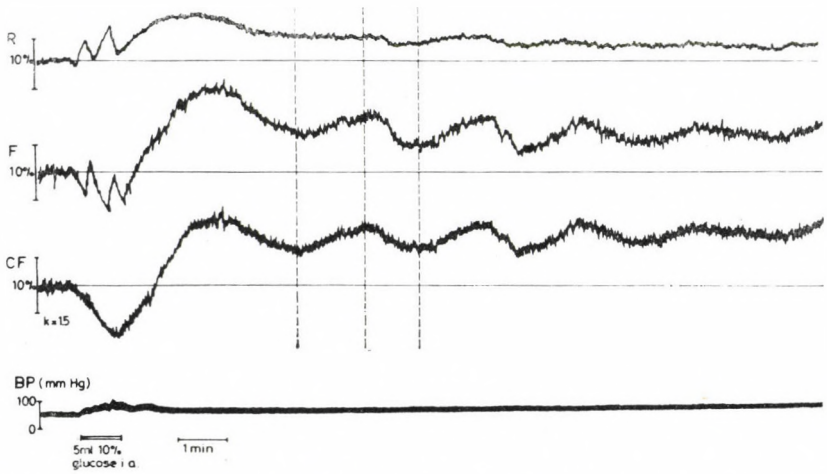

Fig. 7. Vascular volume and NAD–NADH redox state oscillations in the cat brain cortex evoked by glucose administration into the lingual artery in a single experiment

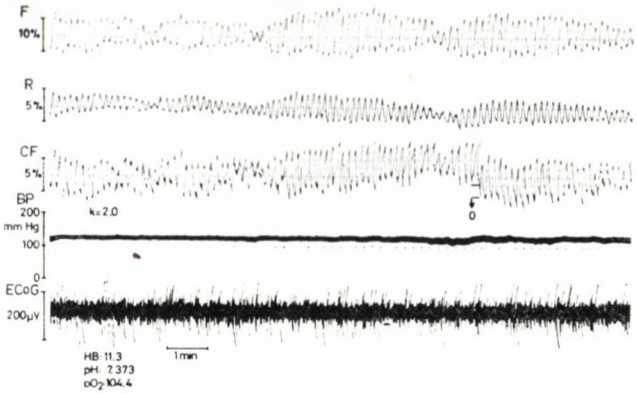

Fig. 8. Spontaneously appearing oscillations in the cerebrocortical uncorrected NADH fluorescence, vascular volume and corrected NADH fluorescence in a typical experiment. Arterial pH, pO_2 and Hb values are also shown

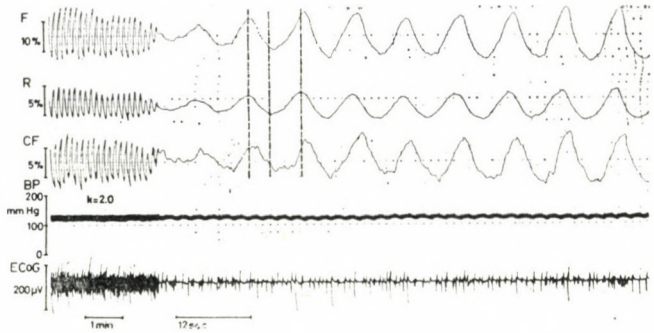

Fig. 9. Cerebrocortical vascular volume and NAD–NADH redox state oscillations in the same experiment as in Fig. 8. For more accurate evaluation of the phase relationships between the various waves the paper speed was increased

The administration of glucose transiently increased the arterial blood pressure which later returned nearly to the initial level. The glucose injections resulted in transient increase of reflectance and uncorrected NADH fluorescence, obviously as a consequence of haemodilution. Corrected NADH fluorescence decreased this time, probably as a result of the improved oxygen supply of the brain. Following this initial period vascular volume decreased, NADH fluorescence increased spontaneously and, subsequently NADH oscillations became regular. During the regular cycle of NAD–NADH redox state oscillations the vascular volume changes were inconsistent pointing again to the independence of this type of NAD–NADH redox state oscillations from changes in vascular volume and blood flow.

The third type of cerebrocortical NAD–NADH redox state oscillation is demonstrated in Figs 8 and 9. The cycle length of these waves varied between 6 and 12 sec. The arterial blood pressure was constant and there was no sign

of oedema upon inspection of the brains. Analysing phase relationships (Fig. 9) it is obvious that the NAD–NADH redox state oscillations were lagging by 1—2 sec behind the vascular volume oscillations. The amplitude of the oscillations was modulated within periods of 10—20 waves which was less pronounced in the corrected NADH fluorescence trace.

This type of NADH oscillation was not sensitive to glucose administration (Fig. 10). The transient shift in the reflectance and uncorrected NADH fluorescence traces during glucose infusion was caused by the haemodilution.

Since the vascular volume waves preceded the redox state waves it seemed reasonable to investigate the effect of 5% CO_2 inhalation on the oscillations. In agreement with earlier findings of GYULAI et al. [14], CO_2 inhalation basically resulted in an increase in vascular volume of the brain cortex and shifted the NAD–NADH redox state towards oxidation (Fig. 11). CO_2 inhalation abolished the oscillations in vascular volume and corrected NADH fluorescence. Since the corrected NADH fluorescence oscillations vanished before the vascular volume oscillations during hypercapnia it might be suggested that this type of redox state oscillation is a threshold phenomenon which occurs when the CBF oscillation reaches a critical value.

Direct electrical stimulation of the brain cortex dilates the vessels and leads to an increase in NADH fluorescence when strong stimuli are applied [9]. This type of NADH fluorescence response is very sensitive to trauma and it is absent when autoregulation of CBF is disrupted. In agreement with these previous results we were able to show that the brain is not affected adversely when these frequent NADH oscillations occur. In Fig. 12 the direct electrical stimulation of the brain cortex led to a marked increase in vascular volume

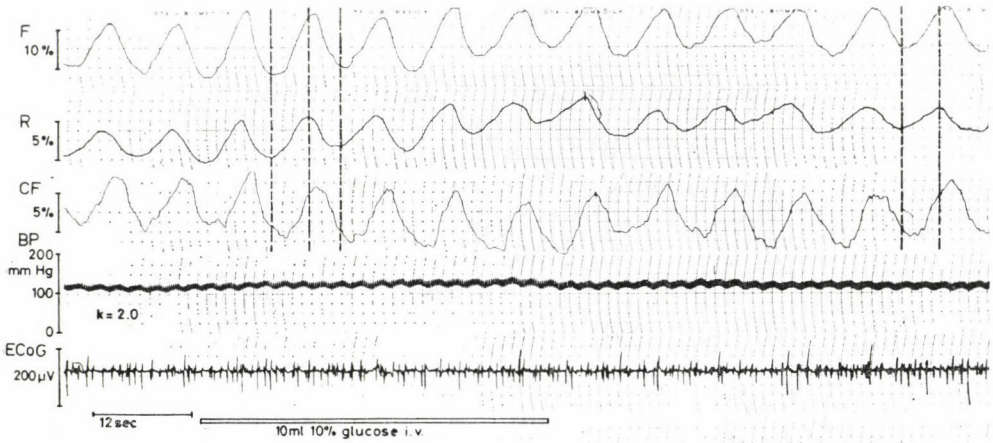

Fig. 10. Effect of glucose infusion on the frequent cerebrocortical vascular volume and NAD NADH redox state oscillations in a single experiment. The duration of glucose infusion is marked by a horizontal bar

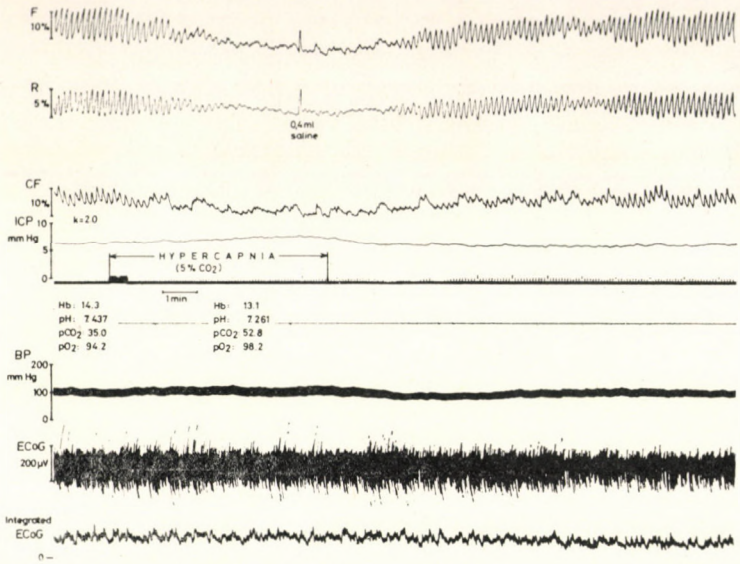

Fig. 11. Effect of the inhalation of 5% CO_2 on the frequent oscillations of cerebrocortical vascular volume and NAD–NADH redox state in a single experiment. The values of arterial Hb, pH, pCO_2 and pO_2 before and during hypercapnia are also shown. Abbreviations: ICP = intracranial pressure measured *via* metal tubes fixed into the wall of the glass window

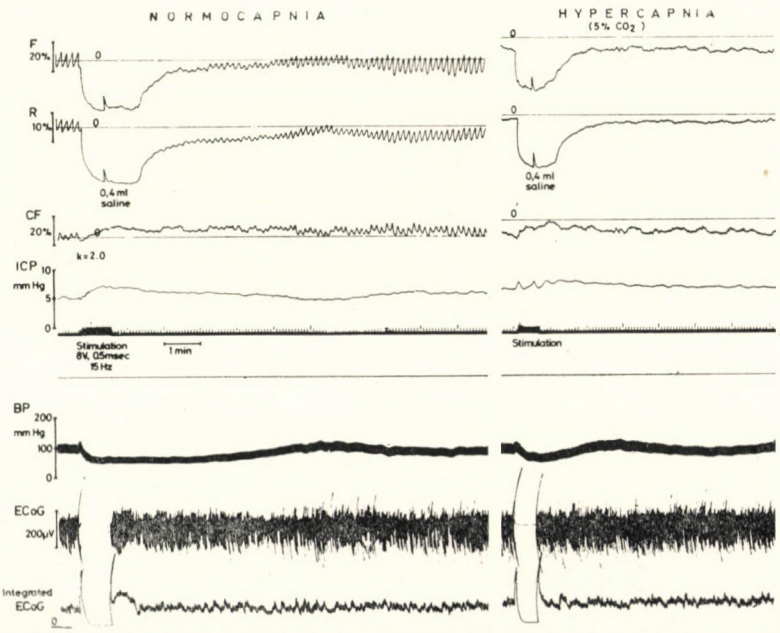

Fig. 12. Effect of direct electrical stimulation of the brain cortex in an oscillatory experiment during normo- and hypercapnia. The duration of the stimulation is marked on the time scale

and to a slight NAD reduction. The NADH oscillations vanished during stimulation, while reappearing following the onset of the recovery of the vascular volume oscillations. Owing to the strong electrical stimuli arterial blood pressure decreased during stimulation and epileptic electrical phenomena persisted for nearly 40 sec after the cessation of electrical stimulation.

The second stimulation was applied during hypercapnia. In agreement with the results of GYULAI and KOVÁCH [15], the inhalation of 5% CO_2 failed to influence the stimulation-induced increase in vascular volume indicating that CO_2 and increased electrical activity of the brain probably do not affect the same segment of the cerebral vasculature.

The effect of nitrogen breathing on the cerebrocortical vascular volume and NADH fluorescence oscillations is shown in Fig. 13. As shown in this diagram anoxia abolished the oscillations, however, they reappeared about 10 min after the cessation of nitrogen inhalation.

When analysing more carefully the experiments where frequent NADH fluorescence oscillations appeared we found that ECoG was invariably depressed. It was therefore suggested that the deepness of chloralose anaesthesia might play a role in the appearance of these waves. To test this hypothesis we administered additional doses of anaesthetics to evoke oscillations. Such an experiment is demonstrated in Fig. 14. In this experiment the oscillations appeared spontaneously already in the control period though the brain looked normal and the arterial blood pressure and the arterial blood gases were in the physiological range. Following a 2-hour pause the vascular volume oscillations appeared only sporadically and there was no oscillation in the corrected NADH fluorescence trace. During these 2 hours the arterial blood pressure increased considerably and the ECoG contained much more high

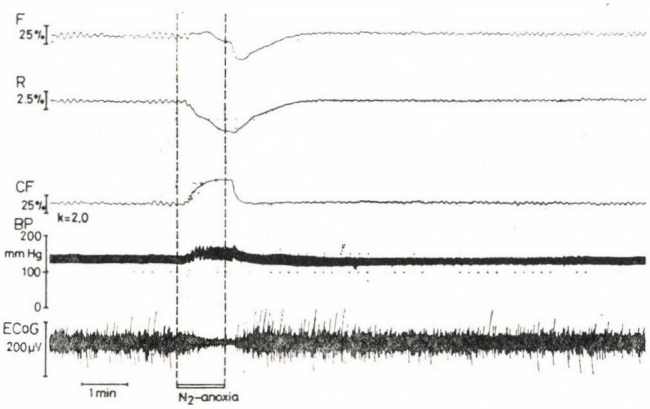

Fig. 13. Effect of nitrogen anoxia on the cerebrocortical vascular volume and NAD–NADH redox state oscillations in a single experiment at normal arterial blood pressure. The duration of nitrogen breathing is marked by two vertical dashed lines

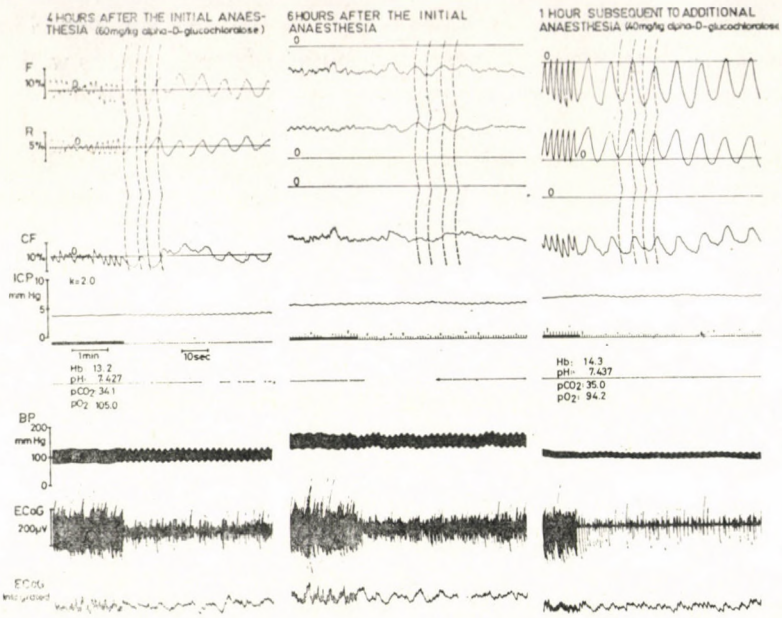

Fig. 14. Effect of the deepness of alpha-D-glucochloralose anaesthesia on the frequent vascular volume and NAD–NADH redox state oscillations in a typical experiment. The vertical dashed lines mark the phase relationships between the various waves

frequency components than before. As a result of increased arterial blood pressure the autoregulatory mechanism of CBF was activated [19], i.e. the vascular volume decreased and the NAD–NADH redox state was shifted towards oxidation.

After this stage 40 mg/kg alpha-D-glucochloralose was infused slowly into the femoral vein. As a result of this, arterial blood pressure decreased to a mean of 100 mm Hg, the high frequency components in the ECoG disappeared and the ECoG was characterized by low spike activity. The deep anaesthesia led to remarkably regular oscillations in the cerebrocortical vascular volume and NAD–NADH redox state.

Discussion

The physiological significance of the biochemical and other kinds of biological oscillations is not yet cleared. It is possible that these periodical events provide reliable timing mechanisms for the rhythmicity of certain biological phenomena at the cellular level. The measurement of tissue NADH fluorescence in this respect could be very useful since it can be monitored continuously in a non-invasive way; on the other hand the NAD–NADH

redox state is a complex parameter of cellular metabolism. The NAD/NADH redox ratio is influenced by the oxygen supply of the tissue, as well as by the adenylate charge and substrate fluxes.

In our experiments 366 nm exaltation light was used to excite the NADH molecules and to monitor the alterations in cortical blood content, i.e. vascular volume. KRAMER and PEARLSTEIN [20] claimed that the 366 nm light can not be used to monitor the changes in tissue blood content since this wave length is not isobestic for oxy- and deoxy haemoglobin. If the red blood cells rather absorbed the incident light than scatter it, the changes in haemoglobin due to oxygen uptake would cause additional alterations in the intensity of 366 nm reflected light, though the tissue blood content might not have been changed. In addition, these authors stated that from the same reason the reflected light measured at 366 nm can not be used for the correction of NADH fluorescence changes caused by the alterations in blood content. To avoid all these "artifacts" KRAMER and PEARLSTEIN suggested the use of isobestic fluore-reflectometry where the changes in blood content are measured at 549 nm (isobestic wave length for oxy- and deoxy haemoglobin) and the changes in NADH fluorescence are monitored at 448 nm (isobestic wavelength for oxy- and deoxy haemoglobin).

Though theoretically these arguments are valid, it should be considered that the red blood cells scatter rather than absorb the incident light. If the absorption effect and the oxy-deoxy conversion of haemoglobin had a considerable influence on the intensity of the reflected light one would expect a marked decrease in reflectance during a nitrogen cycle when the cerebrocortical vessels were previously dilatated maximally. However, this was not the case. While nitrogen anoxia applied during physiologically normal conditions resulted in a marked decrease of reflectance (Fig. 13), nitrogen anoxia employed at 40 mm Hg mean arterial blood pressure (i.e. where the cerebrocortical vasculature is maximally dilatated) failed to cause any measurable change in cerebrocortical reflectance (Fig. 4). On the other hand, CHANCE et al. [4] showed that the use of short cut interference filters (at 448 nm) and of broad band filters (450 nm $\pm$ 30 nm) during a nitrogen cycle yields identical results in the case of brain, while recordings made with 448 nm interference filters are less reliable owing to the inferior signal-to-noise ratio. CHANCE et al. [4] attributed the negligeble interference of haemoglobin oxygenation changes in NADH fluorescence readings to the fact that the decrease in the absorption of excitation was approximately corrected for by an increase of transmission at the emission wave length.

In the present study we were able to show that the so-called presso-receptor autonomic arterial blood pressure waves [12] may promote oscillations in the cerebrocortical vascular volume and blood flow which lead to oscillations in the NAD–NADH redox state. These periodical changes in tissue

energy metabolism must be small since, periodical alterations in ECoG were not observed. When the changes of arterial blood pressure were buffered the oscillations in cortical blood flow and NAD–NADH redox state ceased.

The second type of NADH oscillation demonstrated here was elicited by the long lasting restriction of cerebral oxygen supply and by additional nitrogen breathing or glucose administration. The oscillations of the cerebrocortical vascular volume were irregular and mostly out of phase compared to the NADH fluorescence oscillations. These oscillations were regarded as pure biochemical oscillations described in detail in yeast cells [2, 3]. In this case the cytoplasmic NAD–NADH redox state oscillates as a result of the multiple feed-back between substrate metabolism and mitochondrial oxidative phosphorylation [3, 11, 17]. The key organizator is probably the phosphofructokinase enzyme [3, 4, 17]. In the cat brain cortex the glucose-induced NADH fluorescence changes are also certainly of cytoplasmic origin since, except for the initial period, the oxygen supply of the brain is not altered. CUMMINS and BULL [7] were also able to elicit, by the addition of glucose, biochemical oscillations in isolated brain slices preincubated in substrate-free medium. Our results are, however, the first to show that biochemical oscillations can be generated in intact living brain. It is presumed that the underlying mechanism of the biochemical oscillations in the mammalian brain and yeast cells and other cells are the same, this being a general phenomenon of the living material.

The third type of cerebrocortical NAD–NADH redox state oscillation was characterized by a cycle length of 6—12 sec. Since the oscillations in cerebrocortical vascular volume and blood flow preceded the NAD–NADH redox state oscillations by 1—2 sec it is presumed that the metabolic oscillations were the consequences of blood flow oscillations. This indicates that blood flow, oxygen supply and the NAD–NADH redox state are closely coupled in the brain. The strict coupling is also suggested by findings which show that the cerebral blood flow and NAD–NADH redox state oscillations can be transiently abolished by hypercapnia, nitrogen anoxia and functional hyperaemia. Since redox state oscillations appeared already in response to small variations of the cerebrocortical vascular volume and of blood flow, it appears that the normoxic brain is not overperfused with oxygen.

This kind of blood flow and redox state oscillation was observed only when the electrical activity of the brain was depressed by chloralose anaesthesia.

The oscillations, as their frequency is concerned, resemble very much to the cerebral oxygen tension oscillations described by DAVIES and BRONK [8]. These authors attributed the rhythmic oxygen cycles to periodic variations of the vasomotor tone governed by some central mechanism. The participation of the sympathoadrenal system seems to be well established, since the amplitude of oxygen tension oscillations can be influenced by alpha receptor blockade [1]

and by stimulating the sympatho-adrenal system [21]. However, further experimental work is needed to clarify the underlying mechanism of this type of cerebral blood flow and metabolic oscillation. According to our preliminary data (DÓRA, unpublished data) atropine has no influence on these oscillations.

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THE INCREASE OF CEREBELLAR cAMP LEVEL AFTER DECAPITATION: THE EFFECT OF PROPRANOLOL

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The increase of cAMP level of the rat cerebellum induced by decapitation was studied. Administration of 5 mg/100 g propranolol 1 hour before decapitation completely prevented this increase. Neither the depletion of catecholamine pools, inhibition of their synthesis, nor barbiturate treatment influenced the increase of cAMP level evoked by decapitation.

It has been concluded that noradrenergic neurotransmission is involved in the cerebellar cAMP level increase after decapitation.

Extraordinary sensitivity of the brain to hypoxia is a well-known fact. In mild hypoxia brain cGMP and glucose levels as well acetylcholine synthesis are decreased, while there is no change in cAMP level [8]. Complete cessation of oxygen supply has an essentially different effect: ATP level begins to decrease within seconds and the energy reserves are depleted within about one minute and the animal dies [6, 7]. Complete abolition of the blood supply to the brain (i.e. decapitation) leads to fast and temporary increase of cAMP level, while cGMP level is unchanged. After the exhaustion of energy reserves the level of both cyclic nucleotides is decreased below a basic level [21].

After decapitation, cAMP content of different brain areas changes rapidly in rabbits [12, 13], in mice [21], and in rats [4, 11, 16, 17, 18].

Barbiturate anaesthesia alters the brain energy reserves and their consumption [7], however, it does not alter the cAMP level of different brain areas [14].

The present paper deals with measurements of cerebellar cAMP level. Enzymatic processes were stopped by liquid nitrogen at different intervals after decapitation. The postmortem change of cAMP level and its causes were investigated. Inhibition of the observed increase by propranolol suggests a role of postsynaptic beta adrenergic mechanisms in this phenomenon.

Methods

Adult female R-Amsterdam rats were used. Propranolol (Sigma), alpha-methyl-tyrosine methyl ester HCl (Sigma), and pentobarbital sodium (Nembutal, Serva) were dissolved in physiological saline. These and reserpine (Rausedyl), pindolol (Visken), and phentolamine

(Regitin) were administered intraperitoneally. The control rats were treated with physiological saline. The animals were killed by decapitation, their skulls were opened, and the cerebellums were excised and thrown into liquid nitrogen. A strong reduction in the intensity of boiling of liquid nitrogen marked the freezing of the tissues. Fifteen to 120 seconds passed between the handling of the animal and the chilling of the cerebellum.

The frozen tissues were weighed and homogenized in 4 ml 0.5 M trichloroacetic acid solution using a glass homogenizer with a glass pastle. The mechanical destruction and the denaturation by trichloroacetic acid preceded the warming up of the tissue.

After centrifugation of the homogenate, the trichloracetic acid was extracted by diethyl ether, and cAMP content was determined by a protein binding method [5].

Means of four parallel samples and standard error were calculated; Student' *t* test was used for statistical evaluation of the results.

Results

The cAMP level of the cerebellum 120 seconds after decapitation was unchanged by treatment of the rats with 5 mg/100 g phentolamine or 2 mg/100 g pindolol, while it was significantly lowered by 4 mg/100 g propranolol (Fig. 1). Rats were decapitated 60 min after the administration of the drugs.

From 15 to 120 seconds the cAMP level of the cerebellum increased to a value three times higher (Fig. 2). The increase of cAMP level was totally prevented by the administration of 5 mg/100 g propranolol. cAMP level was significantly lower 15 seconds after decapitation in the propranolol treated rats than in the controls. This finding indicates that the increase of the cAMP content started before 15 seconds. In the propranolol treated animals there

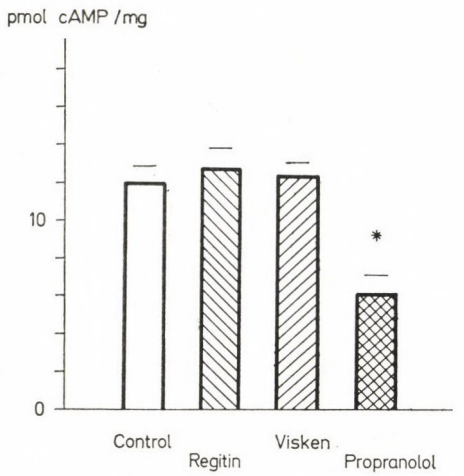

Fig. 1. Effect of 5 mg/100 g phentolamine (Regitin), 2 mg/100 g pindolol (Visken), and 5 mg/100 g propranolol on the cerebellar cAMP content. Rats were decapitated 60 min after treatment. The cerebellums were chilled 120 seconds after decapitation. * $p < 0.05$ compared to control

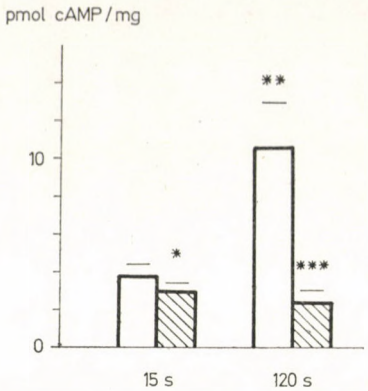

Fig. 2. Effect of 5 mg/100 g propranolol on cerebellar cAMP content. Rats were decapitated 60 min after treatment. The cerebellums were chilled 15 or 120 seconds after decapitation. Open bars: controls; hatched bars: treated rats. * $p < 0.05$ compared to the respective control; ** $p < 0.01$ compared to control at 15 seconds; *** $p < 0.01$ compared to control at 120 seconds

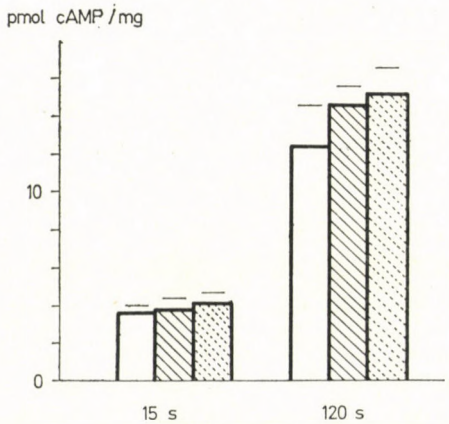

Fig. 3. Effect of the depletion of catecholamine pools and the inhibition of catecholamine synthesis on cAMP content of the cerebellum. The cerebellums were chilled 15 or 120 second after decapitation. Open bars: controls; hatched bars: 0.5 mg/100 g reserpine 24 hours earlier; dotted bars: 0.5 mg/100 g reserpine 24 hours and 300 mg/100 g alpha-methyl-tyrosine methyl ester HCl 3 hours prior to the experiment

was no significant difference between the values obtained at chilling the cerebellums after 15 or 120 seconds (Fig. 2).

Neither depletion of catecholamine pools by 0.5 mg/100 g reserpine given 24 hours earlier, nor the combined depletion of pools and inhibition of the synthesis of catecholamines (0.5 mg/100 g reserpine 24 hours, and 300 mg/100 g alpha-methyl-tyrosine methyl ester HCl 3 hours prior to the experiment) changed the cAMP levels 15 or 120 seconds after decapitation (Fig. 3).

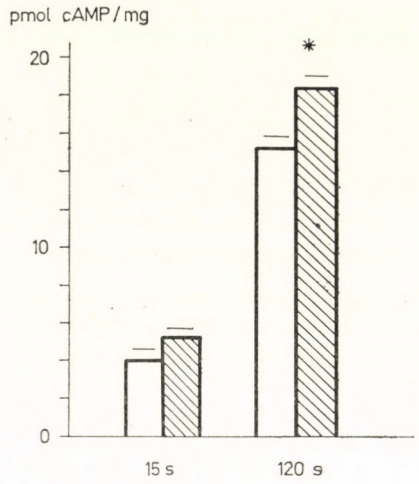

Fig. 4. Effect of anaesthesia on cAMP content of the cerebellum. Rats were decapitated; 30 min after treatment. The cerebellums were chilled 15 or 120 seconds after decapitation. Open bars: controls; hatched bars: treated with 5 mg/100 g pentobarbital sodium. * $p < 0.01$ compared to the respective control

The cAMP level of the cerebellum of pentobarbital anaesthetized rats was unchanged 15 seconds after decapitation (Fig. 4). The increase could also be observed, and even the level after 120 seconds was significantly higher than that found in the untreated rats.

Discussion

An explanation for the fast brain cAMP level increase that followed decapitation was sought. This phenomenon was recognized just after understanding the importance of cAMP in the central nervous system [4]. There are significant species differences; thus cAMP level of the cerebral cortex increases about eight times 90 s after decapitation in rabbits [12, 13]. A similar change was observed in the cerebral cortex and the cerebellum of mice [21], while there was only a slight change in the cAMP level of the cerebral cortex of rats [18].

To measure the undisturbed cAMP levels, new methods like microwave killing [20] and freeze-blowing method [23] have been developed recently. The phenomena, which include the rise in cAMP level, complicate the measurement of adenylate cyclase activity and the assessment of activation by transmitters [9].

The activity of adenylate cyclase can be measured only in a cell-free system [9], however, destruction of the cells can apparently activate the enzyme [22]. Measurement of adenylate cyclase activity in tissue slices yields

only indirect results [19], and changes of cAMP levels must also be taken into consideration [9, 12, 13].

The cause of increased cAMP level in the brain after decapitation is unknown [17]. The phenomenon can be explained by hypoxia and the cessation of glucose supply or by the release and unspecific activation effect of neurotransmitters [12, 13]. On the other hand, it is open to debate whether the increase of cAMP content can be attributed to the activation of adenylate cyclase or to inhibition of phosphodiesterase activity [11].

In rats the most marked increase of cAMP level is observed in the cerebellum [18], while adenylate cyclase activity is the lowest here [11]. Probably the ratio of adenylate cyclase to phosphodiesterase activity is of paramount importance, and this ratio is the highest in the cerebellum in the rat [24].

An alpha adrenergic blocking agent (phentolamine), and a beta blocker were found to be ineffective in inhibiting the cAMP level increase after decapitation. Propranolol, the other beta blocker prevented completely the observed phenomenon. Propranolol used in the same dosage as in this study inhibited the increase of cAMP level in the cerebral cortex induced by electric stimulation of the locus coeruleus [15]. Considering that the noradrenergic innervation of the cerebellum arises from the locus coeruleus [1], we can not exclude the possibility that propranolol exerts its effect, at least partly, through the locus coeruleus.

The inhibitory effect of propranolol suggests that noradrenaline is involved in the observed phenomenon. The depletion of catecholamine pools and even the inhibition of their synthesis failed to modify the increase of cAMP level after decapitation, however, similar depletion and inhibition stopped the cAMP level increase in the cerebral cortex induced by electric stimulation of the locus coeruleus [15]. The proposed senzibilization of adenylate cyclase after reserpine treatment [2] and the high turnover of noradrenaline in the cerebellum [10] might explain this apparent discrepancy.

The inhibitory effect of propranolol on the increase of cAMP level points to extracellular processes. Noradrenaline is released from the synapses of catecholaminergic neurones, and propranolol inhibits noradrenaline binding to postsynaptic receptors in the synaptic cleft; thus the extracellular effect of propranolol prevents the activation of adenylate cyclase in the cell [3]. Hypoxia as a result of disturbed energy supply develops intracellularly. Changes in cell metabolism occurring within seconds caused by a drastic interruption of oxygen supply [6, 7] may be responsible for the increased sensitivity of the cell to activators, and a weak stimulus may result in a major response. Thus the failure of reserpine and alpha-methyl-tyrosine methyl ester to affect this phenomenon may be explained by the altered sensitivity. It may be concluded that noradrenergic neurotransmission is involved in the increase of cerebellar cAMP level after decapitation.

Anaesthesia alters the energy reserves of the brain [7], while not effecting cAMP level [14]. The finding that cAMP content 2 min after decapitation is higher in pentobarbital treated rats than in controls might be explained by a higher energy supply of anaesthetized rats [7], cAMP production from ATP apparently decreasing only somewhat later.

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THE FATE OF *p*-BROMO-METHYLAMPHETAMINE (V-111) IN THE BODY

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The fate of *p*-bromo-methylamphetamine (V-111) in the body was studied by means of its radioactive labelled forms in mouse and rat experiments. It was found with the whole body autoradiographic method and liquid-scintillation measurements that the compound is rapidly absorbed by whatever routes of administration and it is rapidly taken up by the tissues from the blood stream. In the central nervous system, it reaches higher concentration than methyl-amphetamine and it leaves the central nervous system more slowly. We have shown with differential centrifugation that V-111 is bound much more avidly to the mitochondrial and microsomal fractions of rat brain than methyl-amphetamine and *o*-bromo-methyl-amphetamine (V-104). The intensity of binding is proportional to the lipid solubility of the compounds. V-111 and its metabolites are excreted mainly in the urine, and they can be found in small amounts also in the stool. In the case of V-111-3-^{14}C a small amount of $^{14}CO_2$ appeared in the expired air, too, which is a consequence of the disintegration of the molecule. It has been shown by the radiochromatographic and gas chromatographic, mass-spectrometric analysis of the metabolites that V-111 is excreted partly in unchanged form, nevertheless, the N-demethylated and subsequent products, viz. p-bromo-phenyl-acetone, *p*-bromo-phenylpropanol, *p*-bromo-benzoic acid and *p*-bromo-hyppuric acid are also excreted in the urine. The main metabolic pathway of amphetamine and of its methyl-derivative in rat is *p*-hydroxylation, which does not take place in the case of *p*-halogenated V-111. Thus the secondary metabolic pathway (demethylation, oxidative desamination) becomes the main metabolic route of V-111 in this species. The vigorous demethylation of V-111 was proved both *in vivo* and *in vitro*. In the rat, demethylating activity increases during prolonged treatment. The latter fact has to be taken into consideration when interpreting the pharmacological tolerance that develops during chronic administration of the compound.

A decade ago extensive research started in our department aiming at the development of amphetamine derivatives which selectively influence the central nervous system. Two of the compounds thus elaborated is phenyl-isopropyl-methyl-propinylamine or deprenyl, a selective inhibitor of the "B" form of monoamine oxidase [4, 5, 6, 8, 9, 25], and *p*-bromo-methyl-amphetamine (V-111) which exerts its main effect on the serotonin (5-TH) regulation of the central nervous system. The pharmacological and biochemical effects of V-111 have been published previously [3, 7, 11, 14, 16, 17, 18, 19, 20, 22, 24, 26, 27, 28, 29, 30, 31, 32]. In these papers we have described that the compound inhibits selectively and lastingly the uptake of ^{3}H-5-HT into rat brain synaptosomal preparations. A release of 5-HT is also induced by V-111 from the synaptosomal fraction.

The duration of action of V-111 concerning inhibition of the uptake of 5-HT is much longer than that of amphetamine or methylamphetamine, therefore it seemed worth to study how this long-lasting effect is related to the fate of the compound in the body.

In the present paper the results of our experiments on the fate of V-111 are summarized. The radioactive labelled analogues of the compounds were used in these studies.

Methods

Labelled compounds. 1-(4-bromophenyl)-2-methylamino-propane-(1-^{14}C) hydrochloride (V-111-1-^{14}C); 1-(4-bromophenyl)-2-methylamino-propane-(3-^{14}C) hydrochloride (V-111-3-^{14}C) specific activity, 3.67 mCi/mmol; (+)-1-(4-bromophenyl)-2-methylamino-propane-(methyl-^{14}C) hydrochloride (+)-V-111-methyl-^{14}C), specific activity, 0.704 mCi/mmol; (−)-1-(4-bromophenyl)-2-methylamino-propane-(methyl-^{3}H) hydrochloride (−)-V-111-methyl-^{3}H), specific activity, 401.0 mCi/mmol; (±)-1-(4-bromophenyl)-2-methylamino-propane-(methyl-^{3}H) hydrochloride (±)-V-111-methyl-^{3}H), specific activity, 282.0 mCi/mmol; 1-phenyl-2-methylamino-propane(3-^{14}C) hydrochloride (MA-^{14}C), specific activity, 1.56 mCi/mmol; 1-(2-bromophenyl)-2-methylamino-propane-(2-^{3}H) hydrochloride (V-104-^{3}H), specific activity, 636.0 mCi/mmol; 1-phenyl-2-methylamino-propane-(2-^{3}H) hydrochloride (MA-^{3}H), specific activity, 690.0 mCi/mmol; 1-(4-bromophenyl)-2-amino-propane-(1-^{14}C) hydrochloride (nor-V-111-1-^{14}C), specific activity, 3.82 mCi/mmol; 4-bromobenzoic acid-(7-^{14}C), specific activity, 9.84 mCi/mmol; 4-bromohippuric acid-(7-^{14}C), specific activity, 9.84 mCi/mmol; 4-bromophenyl-acetic acid-(1-^{14}C), specific activity, 3.62 mCi/mmol.

The labelled compounds were synthetized in the Radiochemical Laboratory of the Drug Research Institute.

Animals. Male CFLP mice weighing 20 ± 1 g and male CFY rats of 100 ± 5 g were used in our experiments.

Whole body autoradiography. The method was carried out according to ULLBERG [39]. Male (20 g) and pregnant (35—40 g) mice were used. Pregnant animals were 4 days before delivery. V-111-3-^{14}C was given intravenously: 5 μCi/20 g, 18.1 mg/kg of a 25 μCi/ml solution. At various time following the administration of the compound (30 sec—24 hours) the mice were superficially anaesthetized with diethyl ether and were embedded in water saturated carboxy-methylcellulose (CMC). Then the embedded animals were immersed into hexane, cooled to −75 °C by the use of solid carbon dioxide. The freezed block containing the animal was stored at −15 °C for several hours and the sectioning was carried out at the same temperature. 20 μm thick sagittal sections were prepared with Leitz 1300 type microtome and fixed on a special adhesive tape (Minnesota Mining and Manufacturing Co., No. 810).

The sections were freeze-dried at −15 °C, stored at room temperature, then pressed on X-ray films (Structurix, Gaevert) and after a suitable exposure time the films were separated from the sections and developed.

In one case a pregnant mouse was injected intravenously with 500 μCi of V-104-^{3}H in a volume of 0.2 ml (10 mg/kg). V-104-^{3}H was diluted with inactive material. In this case Illford Nuclear 5 plates were used for the exposure.

Estimation of the relative partition coefficients. Test substances (MA-^{14}C, V-111-3-^{14}C; V-104-^{3}H) were transferred to 20 ml glass stoppered tubes, containing 5.0 ml petroleum ether (BHD b.p.: 60—80 °C) and 5.0 ml 0.1 M phosphate buffer (pH 7.2). The concentration of the tested compounds was 0.5 mg/ml and each tube contained 5 μCi radioactivity. The specific activity was adjusted by the addition of unlabelled material. After shaking for 20 min at 22 °C samples were taken both from the buffer and the organic phases and the radioactivity was measured in Bray solution with the liquid scintillation method (Intertechnique SL 40). The absolute activity measured in petroleum ether was compared to that measured in the buffer.

The relative partition coefficients of the compounds compared to that of methylamphetamine were also calculated.

Quantitative estimation of radioactivity in tissues. Male mice of 20 g were given radioactive compounds intravenously, subcutaneously or orally. At various times following the treatment the head of the animals was immersed for 10—20 sec into hexane saturated with solid carbon dioxide. Blood samples were obtained by heart puncture, then samples were taken from selected tissues. Precisely measured blood (20—25 mg) and tissue samples (below 100 mg) were placed in liquid scintillation vials and treated with 0.5 ml Soluene TM 100 (Packard) for 12 hours at room temperature. Radioactivity was measured with the liquid scintillation method. Toluene containing 0.5% PPO and 0.01% POPOP was used for the measurement. Relative activity (cpm) was corrected to absolute activity (dpm) by means of the external standard device. The radioactivity of the organs related to 100 mg tissue in dpm was expressed as the mean ± SEM measured in five experiments. In one set of our experiments the animals were treated with the mixture of ^{3}H- and ^{14}C-labelled compounds and the double labelled technique was used. The Intertechnique SL 40, when adequately programmed, properly counts the ^{3}H and ^{14}C radioactivity. Otherwise the experimental conditions were the same as in the case of single isotope labelling.

Subcellular distribution of amphetamine derivatives. Male rats weighing 100 g were injected intravenously with 20 μCi V-111-1-^{14}C (19.8 mg/kg), or 20 μCi MA-^{14}C (23.7 mg/kg) or 30 μCi V-104-^{3}H (20 mg/kg). V-104-^{3}H was diluted with inactive compound. The rats were decapitated 30 min after the treatment and the brains were removed. Brain homogenate of 10% was prepared in 0.25 M sucrose with a glass homogenizer (Potter). Subcellular fractions from the homogenate were prepared by differential centrifugation. The radioactivity of 0.1 ml samples of the subfractions was determined with the liquid scintillation method after Soluene TM 100 treatment. Radioactivity of the sediment at different speeds of centrifugation was expressed as a percentage of the activity of the initial homogenate ± S.E.M.

Elimination studies. Rats weighing 100 g were treated intravenously with 10 μCi of V-111-1-^{14}C, V-111-3-^{14}C or MA-^{14}C (9.9; 7.23; 11.8 mg/kg, respectively). The animals were placed in a Simax metabolic cage for 72 hours and their urine and stool were collected separately. After petroleum ether extraction the excreted radioactivity was determined with liquid scintillation method. The cumulative urinary excretion was calculated as the percentage of the whole administered dose.

In one part of the experiments rats were treated daily with unlabelled V-111 (15.0 mg/kg; s.c.) and at the end of the treatment they were given 10 μCi (9.9 mg/kg) V-111-1-^{14}C intravenously, then urine was collected for 48 hours. The quantity of the excreted radioactivity was determined as described above.

Chromatographic study of the excreted V-111 metabolites. One half of the 24-hour urine of male rats treated with labelled V-111 (V-111-1-^{14}C or V-111-3-^{14}C) was extracted with ether at pH 10, then at pH 1. Metabolites extracted at basic pH were of *basic* or *neutral* character (extract A), while in the case of acidic pH *acidic* metabolites were extracted (extract B). The etheric phase was distilled and the residue was dissolved in methanol. The other half of the urine was hydrolyzed for an hour at 100 °C with 5 N hydrochloric acid before the extraction.

Chromatograms were prepared on Kieselgel $PF_{253+366}$ layer except when quantitative measurements were performed by scraping off the layer. In this case Kieselgel-G was used. After thorough drying contact autoradiogram was made from the chromatograms with FORTE X-ray film at an exposure time of 60—120 hours. For the chromatography the following solvent systems were used:

I. Butanol—water—acetic acid (4 : 1 : 1)
II. Butyl acetate—butanol—cc. NH_4OH (20 : 10 : 1)
III. Cyclohexane–dioxane–acetic acid (35 : 35 : 1)

Chromatograms of one and two dimensions were prepared. In one part of our experiments spots corresponding to V-111-1-^{14}C and to the demethylated metabolites (nor-V-111-1-^{14}C) were scraped off from the thin-layer chromatogram of rat urine and their activity was measured by liquid scintillation.

Gas chromatographic—mass spectrometric (GC/MS) examination of the metabolites. Urine collected for 0—24 hours from rats treated with 15 mg/kg V-111 was filtered on Amicon UM-2 membrane. The membrane protects the filtration of compounds having a molecular weight of more than 1000 daltons. The ultrafiltrate was extracted first with ether at pH 9 then at pH 1 and the two organic phases were evaporated. The rest was dissolved in methanol and 1-μl quantities were put in the GC/MS equipment (Jeol, JMS-D 100 + JGC 20K). The gas chromatographic peaks were detected by means of a total ion monitor (TIM) and mass spectrum was recorded by means of the peaks representing the separated substances. On the basis of mass spectrum peaks containing bromine were analysed with computer technique.

The examination of N-demethylation in in vitro *experiments.* The N-demethylation of V-111 was studied by means of the N-methyl labelled alteration of the compound. Liver microsomal fraction was prepared according to the method of SUTHERLAND et al. [37]. The liver was homogenized in three volumes of 0.15 M KCl solution which contained 0.02 M Tris buffer (pH 7.4). The homogenate was centrifuged for 15 min at 9000 *g*, the supernatant for 60 min at 100,000 g. The microsomal residue prepared from 10 g tissue was rehomogenated in 5.0 ml buffer solution. The incubation mixture (0.15 M KCl and 0.02 M Tris buffer, pH 7.4) in a final volume of 3.0 ml contained 5 mM glucose-6-phosphate, 5 mM $MgCl_2$, 0.33 mM NADP and 0.7 Kornberg Unit of glucose-6-phosphate-dehydrogenase (BDH) prepared from yeast. The substrate concentration was $1-8\times10^{-4}$ M. An aliquot of the microsomal fraction to produce 1 mg/ml final concentration of protein was added to the incubation mixture. The reaction was started after 5 min preincubation (37.5 °C) by the addition of the substrate. It was stopped after 10 min incubation by adding 1.0 ml 5% $ZnSO_3$. The denaturated protein was sedimented for 10 min at 1500 *g* and the supernatant was used for measurement.

The active formaldehyde formed during N-demethylation was separated from the unchanged V-111 by Amberlite XAD-2 resin [38]. The resin had been pretreated two times for 2 hours with the bed volume of methanol. It was washed three times with distilled water, the quantity of which was equal to that of the methanol, then a 1×8 cm column was prepared. The column was washed with 50 ml distilled water before adding the sample. One ml of protein-free supernatant was placed on the column and eluated with 50 ml distilled water. The first 5—8 ml fraction of the eluate contained the radioactive formaldehyde. Its quantity was measured with the liquid scintillation method (1.0 ml sample in 10 ml Bray solution). The rate of demethylation was expressed by the quantity of formaldehyde formed during the reaction in nmol/g protein. The column is suitable for the separation of further samples after washing with 50 ml distilled water and the measurement can be repeated 10—15 times without the increase of the background activity. Then the total regeneration was carried out with 50 ml methanol and 100 ml distilled water. The recovery of the column was checked with inactive formaldehyde which was measured according to NASH [34]. The recovery of formaldehyde was 99.5 $\pm$ 1% in the first 10.0 ml.

In another series of experiments the N-demethylating capacity of the microsomal fraction prepared from human liver was examined by using (+)-V-111-methyl-^{14}C substrate.

Results and discussion

The time dependence of distribution of V-111-3-^{14}C is presented in whole body autoradiograms obtained in mouse experiments (Figs 1 through 6). The strongly lipid-soluble compound (Table I) is quickly distributed in the tissues and already 30 sec after its administration peak level is reached in the central nervous system (Fig. 1). Its concentration keeps increasing until the 5th min (Fig. 2), remaining on this high level. Even on the 4-hour autoradiogram its quantity in the brain is similar to that of the liver and the spleen (Fig. 4). Twenty-four hours later the major part of the compound is eliminated from the body but even then considerable activity is present in the central nervous system of the mouse (Fig. 6).

The concentration of V-111 quickly increases in the brown-fat tissue, similarly as in the central nervous system. This parallelism between the two tissues can be often observed during the distribution of lipid-soluble compounds. However, while the brain concentration keeps increasing and remains constant, that of the brown-fat decreases significantly after 5 min (Fig. 2) and at the 20—60th min only background activity of the latter can be recorded (Figs 3, 4). The difference in the concentration of radioactivity in the two tissues

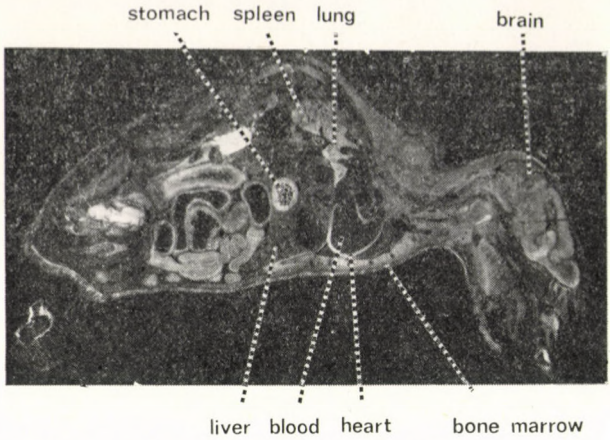


Fig. 1. Whole body autoradiogram of male mouse 0.5 min after intravenous injection of V-111-3-^{14}C (5 μCi/animal; 18.1 mg/kg; in a volume of 0.2 ml)

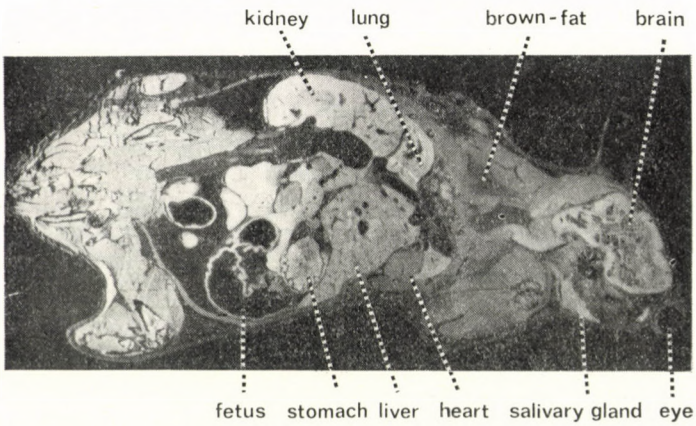


Fig. 2. Whole body autoradiogram of pregnant mouse 5 min after the intravenous injection of V-111-3-^{14}C (10 μCi/40 g mouse, 18.1 mg/kg, in a volume of 0.4 ml)

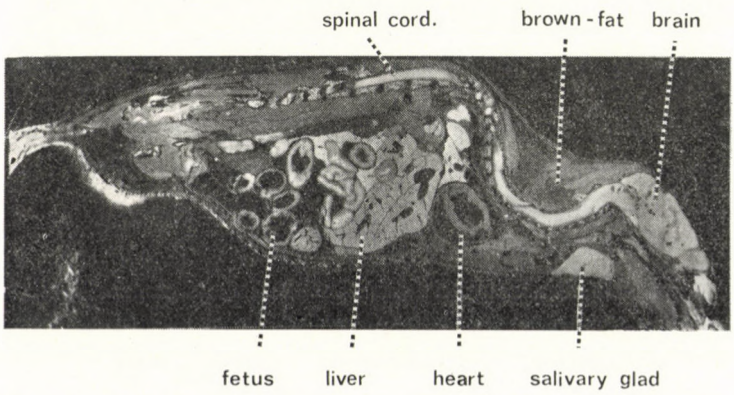


Fig. 3. Whole body autoradiogram of pregnant mouse 20 min after the intravenous injection of V-111-3-^{14}C. Experimental circumstances same as in Fig. 2

refers to the strong binding of V-111 or of its metabolite to the central nervous system. The lung also shows a quick increase of radioactivity, the 24-hour level being practically the highest compared to the other organs. The tissue concentration of the liver increases more slowly and it reaches its peak only in the 5th minute (Fig. 2). Later on the activity in the liver decreases as shown in the autoradiograms. The rapid decrease of radioactivity in the blood goes parallel with the fast increase of V-111 in the organ levels proving the intensive uptake of the compound by the tissues. Thirty sec after treatment the heart tissue contains more V-111 than the blood, and during the experimental period, marked activity can be recorded in the myocardium.

Similarly to other lipid-soluble basic compounds [35], V-111 is intensively excreted into the stomach (Figs 1, 2, 5).

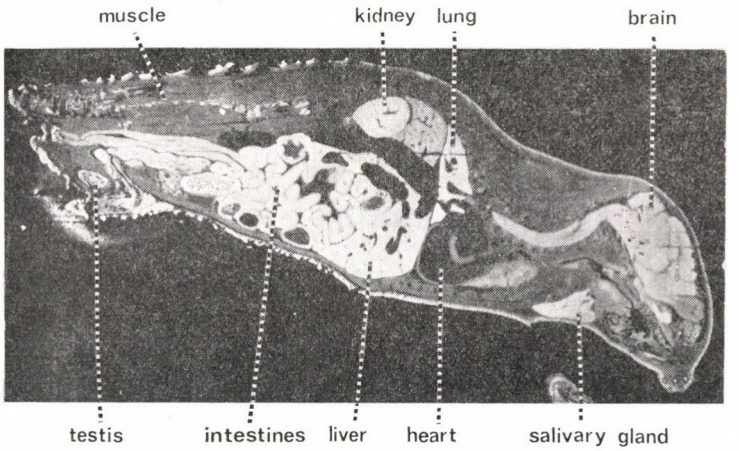


Fig. 4. Whole body autoradiogram of male mouse 1 hour after the intravenous injection of V-111-3-^{14}C. Experimental circumstances same as in Fig. 1

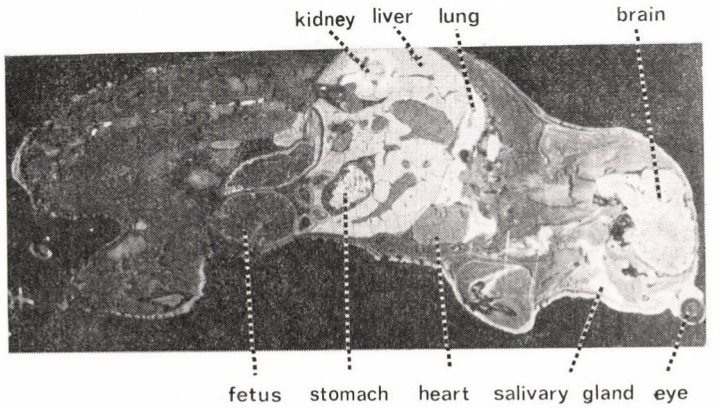


Fig. 5. Whole body autoradiogram of pregnant mice 4 hours after the intravenous injection of V-111-3-^{14}C. Experimental circumstances same as in Fig. 2

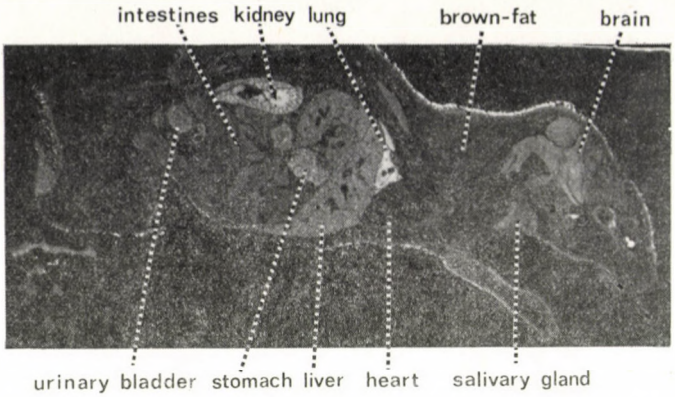


Fig. 6. Whole body autoradiogram of male mouse 24 hours after the intravenous injection of V-111-3-^{14}C. Experimental circumstances same as in Fig. 1

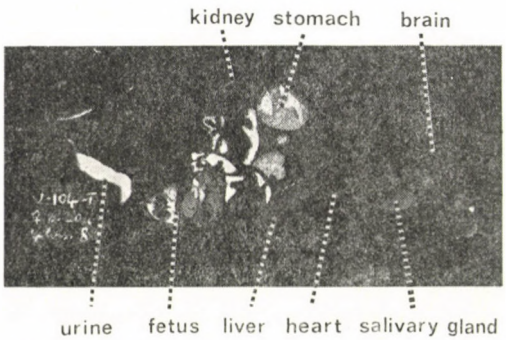


Fig. 7. Whole body autoradiogram of pregnant mouse 4 hours after the intravenous injection of V-104-^{3}H (500 μCi/animal; 10 mg/kg; in a volume of 0.2 ml)

Figures 2, 3, and 5 are autoradiograms of pregnant mice made at different times after injection. Apparently, foetal tissues hardly contain any radioactivity, however considerable activity is present in the wall of the placenta. The compound poorly penetrates into the eye though some radioactivity can be recorded in the lens (Fig. 2).

In the skeletal muscles low concentration of V-111 could be detected during the whole experimental period. The high activity in the kidney (Figs 2, 4, 5, 6) and the urinary bladder points to the urinary secretion of the compound. It is also excreted through the salivary glands (Figs 2, 6).

The intestinal wall contains a significant quantity of the isotope until the 20—60th min, while low activity was recorded in the lumen of the intestine. This indicates that the compound is only negligibly excreted with the stool.

Figure 7 shows the distribution of V-104-^{3}H 4 hours after the administration of the compound. The autoradiograms show two essential differences as

Table I

Relative partition coefficients of amphetamine derivatives

Compounds	Partition coefficients Concentration in petroleum ether / concentration in buffer	Relative solubility in petroleum ether
MA-^{14}C	0.0703 ± 0.002	1.0
V-104-^{3}H	0.3558 ± 0.027	5.09
V-111-1^{14}C	0.5628 ± 0.003	8.03

Petroleum ether BDH, boiling point: 60—80 °C
Phosphate buffer: 0.1 M, pH 7.2
Temperature: 22 °C, average of 5 experiments ± S.E.M.

compared to the distribution of V-111. Low radioactivity was observed in the central nervous system, while a considerable amount was found in the foetal tissues. The two compounds differ from each other only in the position of the bromine, V-111 being para- while V-104 orto-halogenated. This structural difference caused also a significant change in the lipid solubility of the compounds (Table I).

The absorption of V-111 was studied by means of V-111-1-^{14}C. Mice were given 5 μCi radioactive material (24.7 mg/kg) i.v., s.c. or p.o. Radioactivity in the blood obtained by heart puncture was determined with the liquid scintillation method. The results indicate that the compound is rapidly absorbed from the subcutaneous tissues and from the gastrointestinal tract (Fig. 8). The

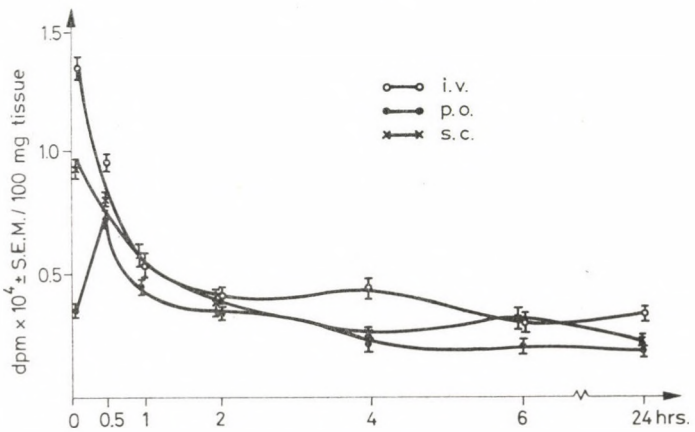


Fig. 8. Changes in the radioactivity of the blood after the intravenous, subcutaneous and oral administrations of V-111-1-^{14}C in mice. Dose: 24.7 mg/kg; 5 μCi/20 g mouse. Results represent means ± S.E.M.

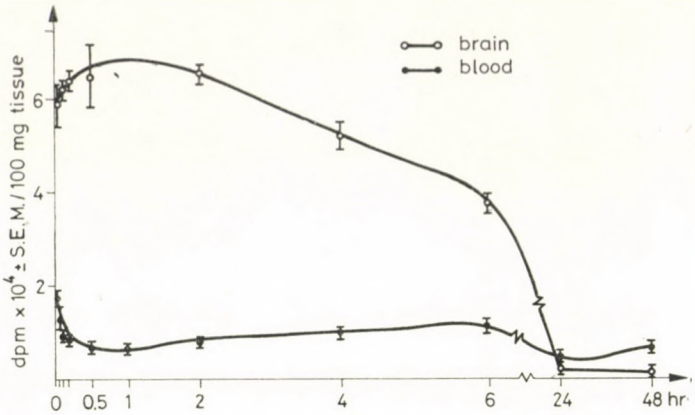


Fig. 9. Changes of the radioactivity in brain tissue and blood after the intravenous administration of V-111-1-^{14}C in mouse. Experimental circumstances same as in Fig. 8

highest blood level of the compound was measured 10 min after s.c. and 30 min after p.o. administration. A fast decrease in the blood concentration was observed after i.v. administration of V-111, also shown by the autoradiograms, indicating intensive tissue uptake of the compound. When the areas under the 24-hr curves (AUC) were compared, the following results were obtained: the AUC recorded after s.c. and p.o. administration were 81.3 and 64.9% in relation to the 100% AUC of i.v. administration which indicates a good absorption of the compound.

In quantitative studies the changes in brain concentration of V-111 were followed in mice treated with V-111-1-^{14}C i.v. The brain levels of V-111 were compared to that of blood and the results are presented in Fig. 9. Quantitative determinations have also proved, in accordance with the whole-body autoradiographic examinations, that the concentration of V-111 rapidly increases and accumulation is long-lasting in the mouse brain tissue.

The changes of brain concentrations were compared also to that of brown-fat. As our numerous whole body autoradiographic examinations proved the lipid-soluble, slightly basic compounds, at the pH of the body, rapidly reach high concentrations in both organs, the time course of these changes being similar in the brain and in brown-fat [10, 13, 21, 23, 25, 33]. The changes in radioactivity in the two organs are shown in Fig. 10. The highest level of radioactivity was reached rapidly in both organs, while the quick subsequent decrease was observed only in brown-fat tissue. The comparison of the AUC-s shows that in a period of 24 hours 4.54 times more V-111 was present in the brain than in brown-fat.

The high and long-lasting brain concentration of V-111 is in good agreement with the prolonged pharmacological effects of the compound which exceed that of amphetamine [3, 6, 11, 20, 24, 27].

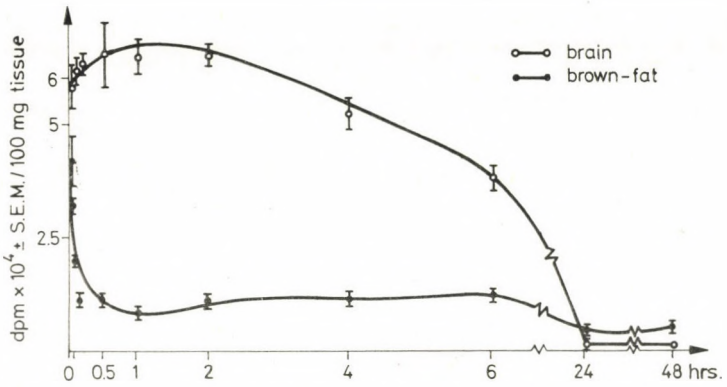


Fig. 10. Changes in the radioactivity of brain and brown fat tissue after the intravenous administration of V-111-1-^{14}C in mouse. Experimental circumstances same as in Fig. 8

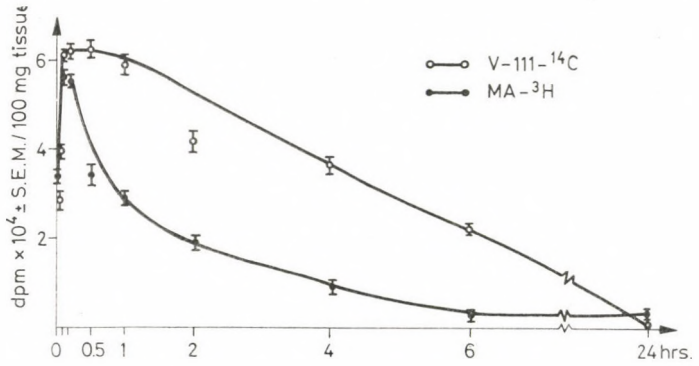


Fig. 11. Changes of ^{3}H and ^{14}C activity in the brain tissue of mice after the intravenous administration of V-111-1-^{14}C and MA-^{3}H. The mixed solution of the two labelled compounds was administered in a volume of 0.2 ml. Dose: 9.9 mg/kg; 2 μCi/20 g animal in the case of both compounds. The concentration of MA-^{3}H was adjusted by the inactive substance. Results are given as the means $\pm$ S.E.M.

In another series of experiments the changes in the brain level of V-111-1-^{14}C and MA-^{3}H were also determined. The two labelled compounds were mixed in a proper ratio before injection. The mixture of the compounds was administered i.v. to mice and the changes in the activity of ^{14}C and ^{3}H were followed parallelly in the central nervous system (doses, including radioactivity, are indicated in the legend to Fig. 11).

When using this method the possible competition for the binding site of the two compounds must be taken into consideration. This disadvantage is more than offset by the advantage of measuring brain concentration of both compounds in the same animal at the same time. On the basis of our findings published elsewhere we have concluded that the tissue binding sites are almost unsaturable [36] thus at the concentrations used in the present study the

competition of the two compounds for binding sites is probably not an important factor. The situation is different in the case of studies devoted to specific receptor binding, when very low concentrations of high specific activity compounds are used [2]. In distribution studies, when relatively high doses are injected, it would be misleading to conclude from the appearance of the compound in an organ to specific receptors.

As shown in Fig. 11 the concentration of V-111 in the central nervous system exceeds that of MA, and its level decreases more slowly. To each curve indicating the brain concentrations of the two compounds two exponential lines can be adjusted. This analysis was performed by a computer. The calculated half-time in the case of methylamphetamine in the phase of the rapid decrease was 27.8 $\pm$ 1.6 min while in the slower phase it was 280 $\pm$ 4.8 min. The same parameters for V-111 were 41.9 $\pm$ 6.04 and 361 $\pm$ 44 min. When comparing the AUC values we observed that in a 24-hour period the quantity of V-111 in the brain tissue was 2.51 times more than that of MA. The numerical ratio of V-111 and MA in the blood and in the brain tissue is shown in Table II. In the first few seconds the concentration of MA was higher than that of V-111, while subsequently the concentration of V-111 exceeded that of MA.

The data of Table III show the subcellular distribution of MA-^{3}H, V-104-^{3}H and V-111-1-^{14}C, in the fractions of rat brain homogenates obtained by differential centrifugation. There was no substantial difference in the binding of the three compounds to the nuclear fraction and to the fraction which

Table II

Ratio of concentration of V-111-1-^{14}C and MA-^{3}H in mouse blood and brain tissue after the simultaneous intravenous administration of the two compounds

Time (min)	Blood	Brain
	V-111/MA	V-111/MA
0.5	0.24	0.83
5	0.11	1.08
30	0.32	1.78
60	0.25	2.03
120	0.20	2.56
360	0.35	3.84

Brain concentrations were calculated from the data of Fig. 11. Blood levels were obtained from the same experiment. For details of experimental data see the legend to Fig. 11

Table III

Subcellular distribution of methylamphetamine derivatives in rat brain homogenate

g×min fraction	%* + S.E.M.		
	MA-^{14}C	V-104-^{3}H	V-111-1-^{14}C
1500×10	R** = 1	R = 1.3	R = 0.9
Unbroken cells, nuclei, myelin	10.45 ± 0.7	13.73 ± 1.7	9.71 ± 1.1
12,000×15	R = 1	2.3	4.3
Mitochondria, myelin, nerve endings	13.42 ± 0.7	31.49 ± 0.5	58.11 ± 1.8
100,000×60	R = 1	-1.3	1.89
Microsomal fraction	9.80 ± 0.9	13.30 ± 0.8	18.61 ± 1.6
230,000×300	R = 1	0.86	0.69
Molecular weight over 80,000	1.71 ± 0.6	1.48 ± 0.3	1.19 ± 0.11
230,000×300	R = 1	0.64	0.29
Supernatant molecular weight under 80,000	62.53 ± 3.2	40.48 ± 2.6	18.44 ± 1.7
Recovery	97.30 ± 1.9	96.99 ± 1.2	103.23 ± 1.6

%* = radioactivity of the fractions is expressed as per cent of the initial radioactivity of the homogenate
R** = ratio compared to MA

sedimented together with haemoglobin, while V-111 was bound strongly, V-104 less firmly and MA weakly to the mitochondrial and microsomal fraction. The mitochondrial binding of the compounds was particularly marked. The order of binding corresponds to the lipid-solubility of the compounds (Table I). As expected, in the supernatant of the 230,000 $g \times 300$ min pellet the order of the concentration of the compounds was reversed.

The cumulative urinary excretion of V-111-1-^{14}C, of V-111-3-^{14}C and MA-^{14}C is shown in Fig. 12. MA-^{14}C was more intensively excreted than V-111. The diagram also shows the long-lasting urinary excretion of V-111. The differences observed in the elimination of V-111-1-^{14}C and V-111-3-^{14}C and/or of their metabolites are not considered as being consequent to methodical faults. Presumably the V-111 molecule is slightly disintegrated in the body and its metabolite is oxidized to $^{14}CO_2$. This hypothesis is verified by the fact that, when the expired air of the animals treated with V-111-3-^{14}C was led through soluene, it bounded $^{14}CO_2$. Its quantity was 5—7% of the administered radioactivity during 48 hours.

The urinary excretion of V-111-1-^{14}C after V-111 pretreatment is shown in Table IV. The pretreatment for 7 days significantly increased the excretion

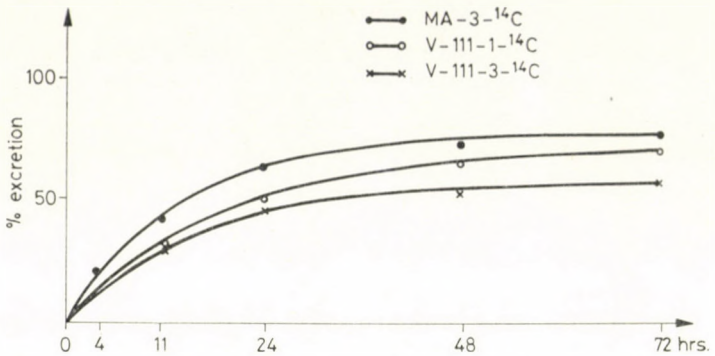


Fig. 12. Cumulative urinary excretion of radioactivity after the intravenous administration of V-111-1-^{14}C, V-111-3-^{14}C and MA-^{14}C in the rat. Dose: 9.9 mg/kg V-111-1-^{14}C; 7.23 mg/kg V-111-3-^{14}C; 11.8 mg/kg MA-^{14}C. The radioactive dose was 100 μCi/kg in the case of all the three compounds. Average of 3 experiments

of V-111 or its metabolites. The majority of the administered compound was eliminated in the first 12 or 24 hours. Treatment for longer than 7 days did not increase further the rate of elimination.

In our experiments radioactivity excreted in the stool was also determined. The stool was extracted with ether at acidic pH (pH 1), then at basic pH (pH 10). In the pooled extract 10.15% of the administered radioactivity could be found over 48 hours.

In conclusion V-111 is excreted slowly in the rat. After a single dose, within 48 hours 50.3% of the injected compound was excreted in the urine, 5—7% through the expired air, 10.15% with the stool (altogether: 65—70%).

Table IV

Effect of V-111 pretreatment on the excretion of V-111-1-^{14}C and of its metabolites in rat experiments

V-111 treatment 15 mg/kg s.c.	Activity found in the urine in per cent of the administered dose (+S.E.M.)		
	0—12 h	0—24 h	0—48 h
Single dose	15.9 ± 2.20	23.75 ± 4.05	50.3 ± 3.04
4 days	14.53 ± 1.19	36.91 ± 6.55	66.33 ± 3.61
7 days	35.99 ± 2.53*	54.58 ± 1.98*	69.74 ± 2.25
12 days	30.92 ± 3.43*	51.42 ± 2.51*	65.78 ± 2.48

Dose of V-111-1-^{14}C: 9.9 mg/kg i.v., 10 μCi/animal
Number of experiments: 5
* = $p < 0.05$ level of significance as computed using Student's t test

The identification of V-111 metabolites extractable from the urine with ether at acidic and basic pH was attempted with thin-layer chromatography. Metabolites remaining in the watery phase after etheric extractions were not examined.

As shown in Fig. 13, after acidic hydrolysis a new spot appears in extract A (extracted at pH 10) while the intensity of one of the spots of extract B and that of the spot of the watery phase markedly decreased. Extracts A were also run in the 2nd solvent system (Fig. 14).

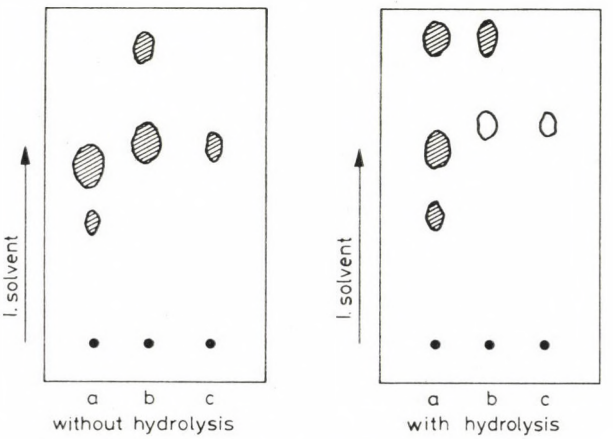


Fig. 13. Thin-layer chromatogram of V-111 metabolites extracted from the urine with ether. a = Metabolites extracted at pH 10, extract A; b = metabolites extracted at pH 1, extract B; c = watery phase

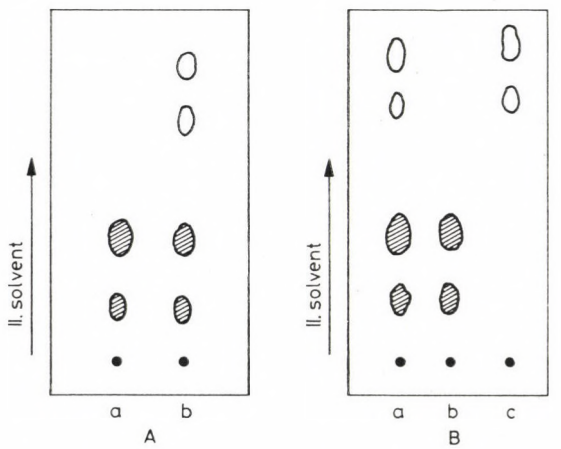


Fig. 14. Thin-layer chromatogram of extract A. A: a = extract A before hydrolysis, b = extract A after hydrolysis. B: a = extract A after hydrolysis, b = extract A extracted at acidic pH (pH 3), watery phase; c = extract A extracted at acidic pH, etheric phase

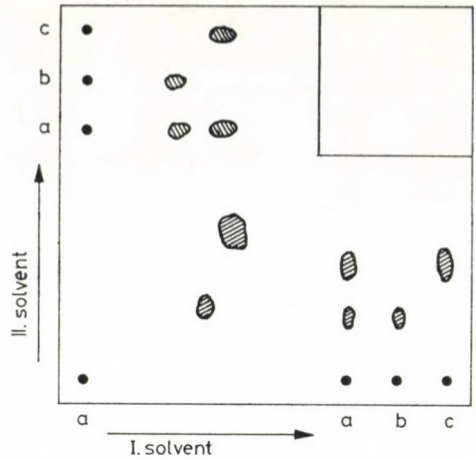


Fig. 15. Two-dimensional thin-layer chromatogram of the substances dissolving in hydrochloric acid (pH 3) of extract A. a = Extract A dissolved in hydrochloric acid (pH 3); b = V-111-1-^{14}C; c = nor-V-111-1-^{14}C

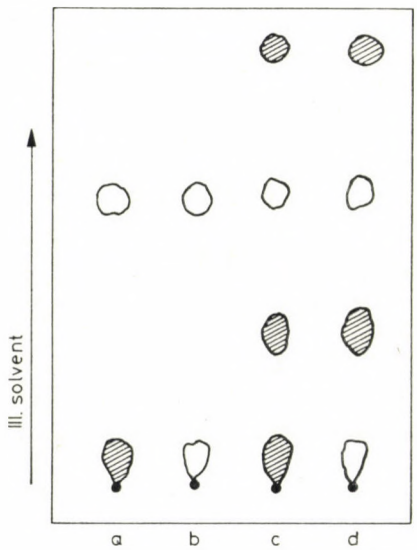


Fig. 16. Thin-layer chromatogram of extract B. a = Extract B of V-111-3-^{14}C; b = extract B after acidic hydrolysis of V-111-3-^{14}C; c = extract B of V-111-1^{14}C; d = extract B after acidic hydrolysis of V-111-1-^{14}C

We have found that two further spots appeared in the hydrolyzed urine (Fig. 14, Part A). The probable reason for this is that, after hydrolysis, the conjugates of V-111 are decomposed and can be extracted from the urine with ether. When extract A was shaken with diluted hydrochloric acid the two lower spots remained in the watery phase, which suggests that they have a

basic character, while the two upper spots originating from hydrolysis were found in the etheric solvent, i.e. they are of neutral character (Fig. 14, Part B).

It seemed probable that the substances dissolved at acidic pH contained basic amino group, so they could be the unchanged or demethylated V-111 (*p*-bromoamphetamine, nor-V-111). The identification of these metabolites was carried out by two-dimensional chromatography. As shown in Fig. 15, the spots move similarly to the reference substances in both solvent systems.

It should be emphasized that the qualitative chromatographic picture of extract A was the same in the case of V-111-1-^{14}C and V-111-3-^{14}C.

Extract B was examined in the 3rd solvent system. Figure 16 shows that the intensity of the spot at the point of application decreases considerably, due to the effect of acidic hydrolysis indicating that the supposed conjugates are decomposed but the emerging substances are not of acidic character. In the urine of animals treated with V-111-1-^{14}C, two spots can be seen which do not appear in the case of V-111-3-^{14}C. This can be due to the metabolic changes leading to disintegration of the molecule. The difference observed in the urinary excretion of the V-111-1-^{14}C and V-111-3-^{14}C refers also to the process mentioned above (Fig. 12). The metabolic conversion of the molecule is probably due to microsomal oxidation, resulting in substances of acidic character. These may be *p*-bromo-phenylacetic acid. The substances of acidic character in the urine of animals treated with V-111-1-^{14}C were compared with the above mentioned reference substances on two-dimensional chromatogram.

Figure 17 shows that in extract B only the two spots which were identical with *p*-bromo-benzoic acid and *p*-bromo-hyppuric acid could be identified in the case of V-111-1-^{14}C.

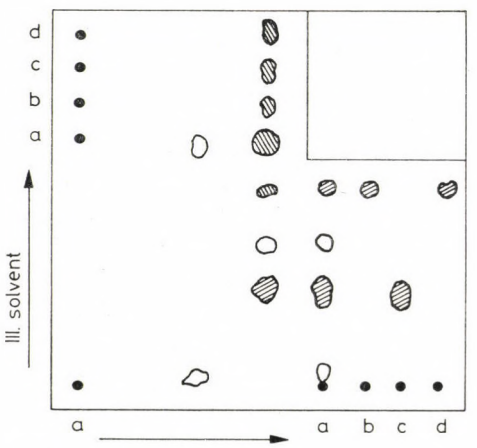


Fig. 17. Two-dimensional thin-layer chromatogram of extract B. a = Extract B of V-111-1-^{14}C after acidic hydrolysis; b = *p*-bromo-benzoic acid-1-^{14}C; c = *p*-bromo-hyppuric acid-1-^{14}C; d = *p*-bromo-phenylacetic acid-1-^{14}C

It should be noted that we could not differentiate between *p*-bromo-benzoic acid and *p*-bromo-phenylacetic acid in the employed chromatographic systems.

From the thin-layer chromatograms (Kieselgel-G) of the urine samples collected during 0—12, 12—24, 24—48 hrs in rats treated with V-111-1-^{14}C, spots identical with nor-V-111 and V-111 were scraped off and their activity was determined with the liquid scintillation method. We found that the unchanged V-111 and its demethylated derivative were excreted in the urine in considerable quantity. 6.85% of V-111 and 15.05% of nor-V-111 was found in the urine collected during the first 24 hours, as computed for the administered dose of the original compound. The quantity of nor-V-111 found in the urine significantly increased with time as compared to V-111. The ratio of the two compounds (nor-V-111/V-111) was 1.19 in the urine during the first 12 hrs, 5.94 during the second 12 hrs, reaching 11.5 in the urine in the period of 24—48 hrs. *p*-Bromo-benzoic acid and *p*-bromo-hyppuric acid represented 7.9% of the total administered doses in the urine collected during the first 24 hrs (Fig. 19).

The gas chromatographic analysis of metabolites excreted in the urine was performed with total ion monitor. The results are shown in Fig. 18. As shown 41 relatively well-separable peaks could be detected in the etheric

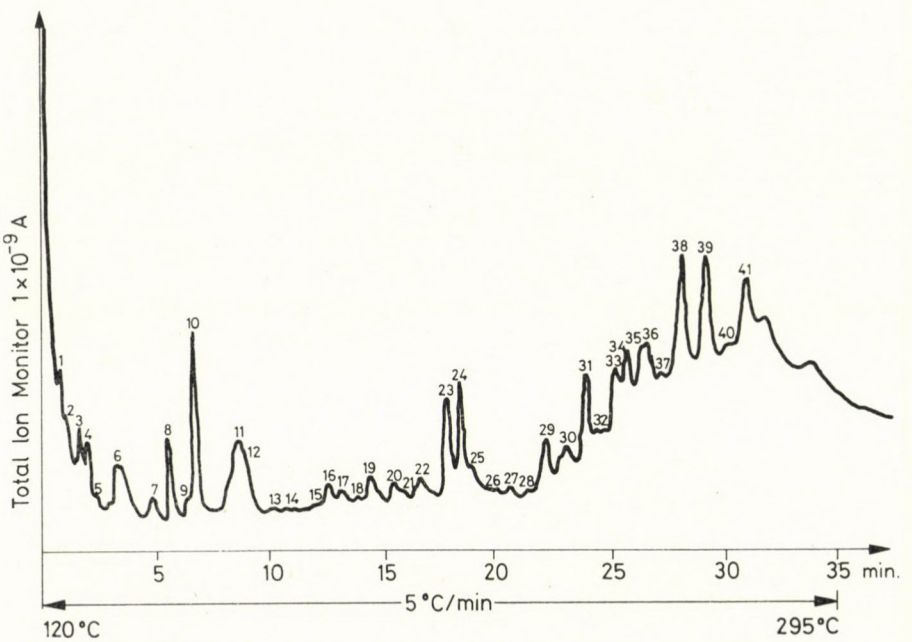


Fig. 18. Gas chromatographic separation in GC/MS combination of metabolites extracted with ether at acidic and basic pH from the urine of rats treated with V-111. Rats were treated with 15 mg/kg of V-111 intravenously. Urine was collected for 24 hours

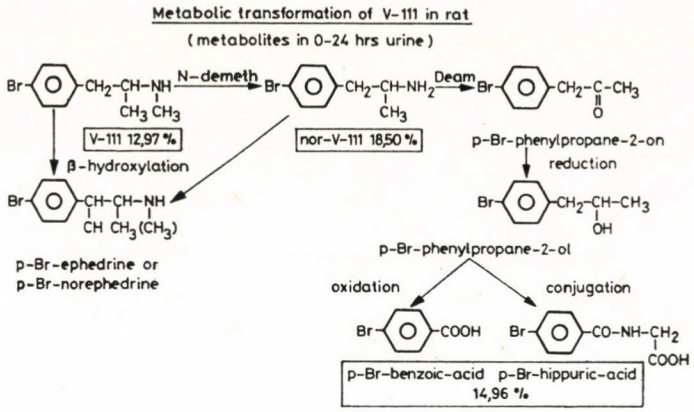


Fig. 19. Metabolic conversions of V-111 in the rat. Metabolites were identified in 24-hour collected urine. Values in the frame indicate the quantity of metabolites in per cent of the administered dose. Dose of V-111-1-^{14}C was 9.9 mg/kg, 10 μCi/rat, administered intravenously

extract of the urine. From the mass-spectra of components separated with gas chromatographic analysis, only those metabolites were analysed with computer which contained bromine. Our investigations with CG/MS technique supported the presence of V-111, nor-V-111, *p*-bromo-benzoic acid and *p*-bromo-hyppuric acid, identified also on thin-layer in the urine. By using this method we could detect the presence of metabolites which could not be identified on thin-layer in the absence of reference substances. So we found a molecular ion with mass number of 212 which might have been *p*-bromo-phenylacetone and a fragment ion with mass number of 169 which might have originated from the former by the decomposition of $-COCH_3$ (-43).

As we have identified the *p*-bromo-benzoic acid and *p*-bromo-hyppuric acid on thin-layer which are also the end-products of the metabolism of amphetamine — naturally without *p*-bromo-substitution — *p*-bromo-phenyl-acetone can be expected to be present in the urine as an intermediate product (Fig. 19). As it can be seen in Fig. 19 *p*-bromo-phenylpropanol may also be formed as an intermediate product through the reduction of *p*-bromo-phenyl-acetone. This compound was not found in the urine but we identified a fragment ion with mass-number of 196 (C_9H_9Br) originating from the compound through the withdrawal of water which might have been identical with *p*-bromo-phenyl-1-2-propene. We also found a fragment ion with mass-number of 170 (C_7H_7Br) which could be *p*-bromo-toluol. It can be regarded as the decarboxylated end-product of *p*-bromo-phenyl-acetic acid.

According to our investigations the main metabolic pathway of V-111 is N-demethylation which leads to *p*-bromo-benzoic acid and by further enzymatic transformation to *p*-bromo-hyppuric acid. The results are summarized in Fig. 19. We proved the presence of V-111, nor-V-111, *p*-bromo-phenylacetic

Table V

Parameters of gas chromatographic, mass spectrometric examinations

Gas chromatograph, mass spectrometer	JG (20K + JMS) — 100
Column	2 cm×3 mm
Resin	10%; PEG 20 M; gas chrom Q 100—120 mesh
Flux rate of helium	2 kg/cm²
Temperatures	
Injector	270 °C
Column	120—250 °C, 5 °C/min
GC-MS	250 °C
Sensitivity	1×10^{-9}
Vacuum	1×10^{-5} Torr
Ionizing power	27 eV

acid, *p*-bromo-phenylacetone in the urine and *p*-bromo-phenylpropanol can also be regarded as an intermediate product. We have found and identified also on thin-layer *p*-bromo-benzoic acid and *p*-bromo-hyppuric acid in the urine. Beta-hydroxilation of the compound as a theoretical possibility is also shown in the Table. Its significance has been proved by other authors in the case of amphetamines [1].

The metabolism of amphetamines in rat also differs qualitatively from the above pattern, as its main metabolic pathway is *p*-hydroxylation. The *p*-hydroxylated product represents 60% of the amphetamine metabolites excreted in the urine. The *p*-hydroxylation of V-111 can not take place because of the *p*-bromo-substitution. That is why the metabolism of V-111 differs from that of amphetamine as it has been proved in rat. N-demethylation and the consecutive oxidative desamination of methylamphetamine take place in

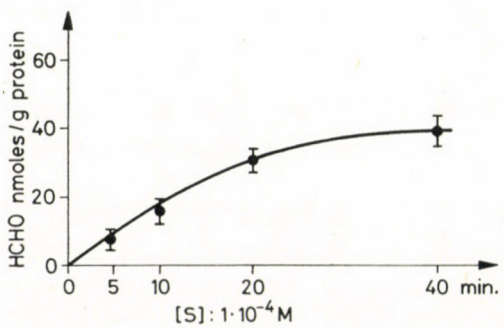


Fig. 20. Time dependence of N-demethylation of (+)-V-111-methyl-^{14}C

only insignificant quantities in the rat, while in the case of the *p*-halogenated V-111 it becomes the main metabolic pathway.

In our experiments the N-demethylation of V-111 was studied *in vitro* in rat liver microsomal preparation by the aid of the methyl labelled variants of the compound. Our experiments showed that the N-demethylation of V-111 *in vitro* progresses linearily for 10 minutes (Fig. 20). The substrate dependence of the reaction is shown in Fig. 21.

As we possessed the N-methyl labelled variants of the stereoisomers of V-111, their N-demethylation was also studied separately. The results of

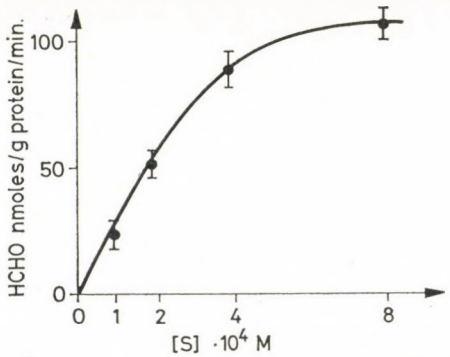
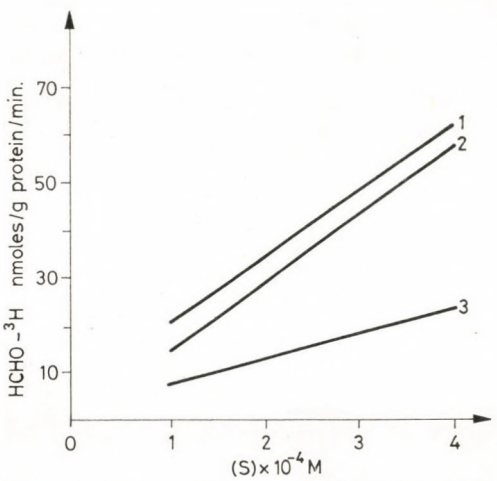


Fig. 21. Substrate dependence of N-demethylation of (+)-V-111-methyl-^{14}C



1./ a_0=6.33±1.29 a_1=13.83±0.48 r=0.996 (+)-V-111-methyl-^{14}C
2./ a_0=0.87±0.46 a_1=14.71±0.48 r=0.995 (±)-V-111-methyl-^{3}H
3./ a_0=2.84±0.46 a_1=5.13±0.42 r=0.979 (−)-V-111-methyl-^{3}H

Fig. 22. N-demethylation of the N-methyl-labelled stereoisomers of V-111. The deviation between the 1st and 3rd line is significant at $p < 0.05$. Statistical evaluation was carried out with Student's *t* test

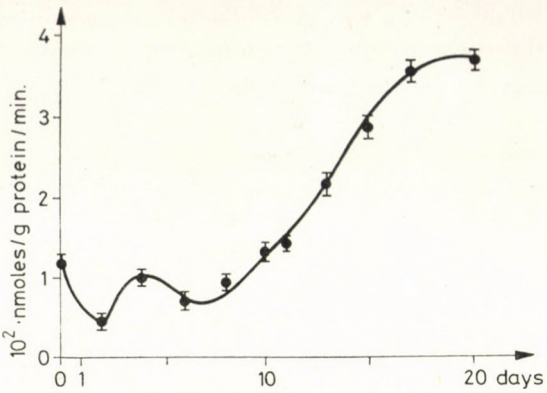


Fig. 23. N-demethylation of (+)-V-111-methyl-^{14}C in the liver microsomal preparation of rats pretreated with the compound. During pretreatment the animals were given daily 15 mg/kg inactive V-111 subcutaneously. The concentration of (+)-V-111-^{14}C was $8 \cdot 10^{-4}$ M in the incubation medium

experiments using 3 different substrate concentrations are shown in Fig. 22. It is obvious from the equations that the N-demethylation of (+) V-111 is significantly more intensive than that of the (−) isomer. The demethylation of the racemic compound does not reach that of the (+) isomer, but the difference between them is not significant.

As pretreatment with V-111 increases the urinary excretion of the compound or its metabolites in the rat, we studied how pretreatment influences the N-demethylating activity of the liver microsomal fraction. Figure 23 shows that N-demethylation increases with time and three weeks later it increases to the threefold of the initial activity. Thus pretreatment with V-111 increases its own metabolism. The significance of this phenomenon in the development of tolerance to the compound requires further experiments.

Table VI

N-demethylating activity of human liver and rat liver microsomal preparation in vitro *at different substrate concentrations*

Species	Activity nmol/g protein/min		
	1×10^{-4} M	2×10^{-4} M	4×10^{-4} M
Human liver	28.88 ± 0.7*	48.72 ± 1.4	96.07 ± 1.3
Rat liver	18.30 ± 2.7	40.23 ± 3.1	86.40 ± 9.65

Substrate: (+)-V-111-methyl-^{14}C
Statistical evaluation was done using Student's *t* test

* $p < 0.05$

The metabolism of amphetamine and methyl- amphetamine in man differs from that observed in the rat, since in man the main metabolic pathway is the N-demethylation [1]. We examined the relationship between the N-demethylating activity of human liver microsomal preparation and that of rat liver on the basis of the protein content of the same fractions.

According to these experiments (Table VI) *in vitro* activity of human and rat liver microsomal preparation differs significantly with low substrate concentrations. In this case the N-demethylating activity of human liver microsomal fraction exceeds that of the rat liver.

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EFFECT OF PROSTAGLANDIN F_2 ALPHA ON THE ISOLATED COMMON BILE DUCT OF THE DOG AND THE RABBIT

By

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In an isolated spiral muscle preparation from the common bile duct of the dog and rabbit contraction was elicited by PGF_2 alpha, in a concentration dependent manner. The contraction was long-lasting and reversible. As opposed to PGF_2 alpha, morphine, noradrenaline and barium chloride did not induce a contractile response.

The effect of prostaglandins (PG) on the smooth muscle of the human gastrointestinal tract has widely been investigated, but little is known on the effect of PG exerted on the different parts of the biliary system [1, 3, 4, 5]. Experimental results concerning the physiology and pharmacology of the bile duct were obtained mostly with manometric methods [9, 10]. The disadvantage of these methods is that they cannot distinguish to what degree the obtained effect originates from the smooth muscle of the sphincter of Oddi or of that of the bile ducts. It is not possible to excise the common bile duct of a human being during surgery, and isolated preparations from animals [8] have so far been made only from the Oddi sphincter or the lower part of the common duct connected with the sphincter [6, 7]. Therefore, there are no data available on the sensitivity to PG of the smooth muscle of the common bile duct *in vitro*. We have, therefore, attempted to study under controlled conditions the response to PG of a circular muscle preparation. It was assumed both from our own experimental results and from those obtained by other authors under conditions *in vitro* that PGF_2 alpha would have an agonistic effect.

We have therefore studied on the *in vitro* of the dog and rabbit common bile duct circular muscle, whether PGF_2 alpha had any effect on the preparation, and if it did what was the direction of the response it depended on the PGF_2 alpha concentration of the organ bath.

This work was partly supported by the Hungarian Ministry of Health. Grant No. 4-18-0101-01-0/M.

Methods

The common bile duct of anaesthesized (30 mg/kg i.v. pentobarbital) bastard dogs of both sexes and rabbits of 2.5 to 3.0 kg weight, was exposed from upper median laparotomy. The duct was transected above the sphincter of Oddi removed, cleaned under the stereomicroscope from the serosa in Tyrode solution of 38 °C then a spiral strip was cut from it. A 20 mm portion of the strip was suspended in Tyrode organ bath of 38 °C bubbled with a mixture of 95% oxygen and 5% carbon dioxide, and loaded with 0.5 g, and its movements were recorded isometrically. Before the experiment, the preparations were equilibrated for 60 minutes.

Contractions were obtained at different concentrations of PGF_2 alpha. The responses to the single concentrations were plotted. Every point of the curve was derived from at least five experiments. The mean values and the standard error of the mean ($\bar{x} \pm S_{\bar{x}}$) are shown.

The pD_2 value PGF_2 alpha was calculated in the conventional manner. The drugs used were PGF_2 alpha (Chinoin), phentolamine (Ciba), atropine sulphate (EGYT), morphine hydrochloride (EGYT), acetylcholine bromide (Berlin-Chemie), barium chloride (Reanal), noradrenaline bitartarate (Merck).

Results

PGF_2 alpha elicited contraction of the circular muscle layer of the common duct of the dog and rabbit in a concentration dependent manner. The minimum effective PGF_2 alpha concentration was 5×10^{-6} mol/l, for both species. The concentration response curves are shown in Fig. 1. The mean pD_2 value, characteristic of the affinity of agonists, was 3.50 ± 0.08 in the dogs and 4.34 ± 0.26 in the rabbits. The circular muscle layer of the common duct of either the dog or the rabbit, failed to contract under the effect of

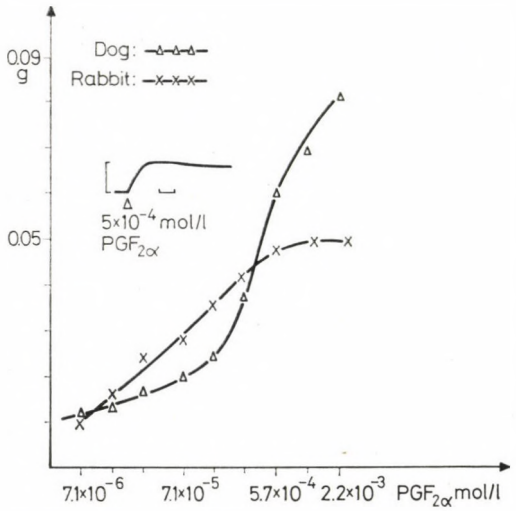

Fig. 1. Isolated circular smooth muscle of the common bile duct of dog and rabbit. Concentration effect curves. Abscissa: concentration of PGF_2 alpha, mol/l; ordinate: contractile force, g. Inset: Isolated circular smooth muscle of dog common bile duct, contraction elicited by PGF_2 alpha. Calibrations: 0.1 g and 1 min

morphine (1.3×10^{-3} mol/l), noradrenaline (2.3×10^{-4} mol/l), acethylcholine (2.7×10^{-3} mol/l), and barium chloride (5.1×10^{-2} mol/l). Since only PGF_2 alpha elicited contractions, it was concluded that only PGF_2 alpha could be regarded as an intrinsic stimulant of the muscle of the common duct.

In a particular female rabbit (No. 3) a high activity was recorded; the contractile force of this preparation was about four times higher than the average.

Contraction of the dog and rabbit muscle reached slowly a plateau. The contractile force of the preparation exposed to submaximum PGF_2 alpha concentration, did not diminish during exposition. After its termination by washing in fresh Tyrode solution, the tonicity of the muscle returned to the baseline value. The effect of PGF_2 alpha is therefore reversible. The contraction elicited by PGF_2 alpha was not affected by phentolamine and atropine in the concentration range of 1×10^{-8} to 1×10^{-5} mol/l.

In course of the experiments, tachyphylaxis was not observed on any preparation. Desensitization, if any, presented itself only after exposition to very high concentrations, thus 5×10^{-3} in the dog and 2×10^{-3} mol/l in the rabbit.

Discussion

It has been shown that in the isolated dog and rabbit common bile duct preparation gives positive response to PGF_2 alpha. The response seems to be specific because it was not affected by atropine or phentolamine. The PGF_2 alpha affinity of the rabbit muscle as calculated from the pD_2 value was 3.6 times higher than that of the dog.

The high minimum effective concentrations as well as the low pD_2 value refer to a low PGF_2 alpha affinity of the tissues. Nevertheless, three conclusions can be drawn. (1) If contraction is induced by PGF_2 alpha, it will be long-lasting. (2) The sensitivity to PGF_2 alpha varies in individual animals. (3) The muscle preparation is sensitive only to PGF_2 alpha. The experiments clearly demonstrated that the smooth muscle of the common duct was not sensitive to morphine. The stepwise increase of intraluminal pressure, generally observed by the manometric method after morphine may be attributed to a concentration of the sphincter of Oddi [2]. This is in good agreement with the well-known fact that sphincters contract after morphine administration.

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РЕЗЮМЕ

СВЯЗЫВАНИЕ КОРТИКОСТЕРОНА В ТКАНЯХ СЕРДЦА У КРЫС В УСЛОВИЯХ ХРОНИЧЕСКОГО СТРЕССА И ПОСЛЕ АДРЕНАЛЭКТОМИИ

А. ЭЛЛЕР, Ч. НЯКАШ, Г. САБО и Э. ЭНДРЕЦИ

Авторы изучали влияние хронического стресса (крыс заставляли ежедневно плавать, в течение 9—10 дней) на емкость и аффинитацию специфических рецепторов, связывающих глюкокортикоиды клеточной плазмы сердечной мышцы. Кортикостерон-связывающая емкость достоверно уменьшается во время ее измерения спустя 24 часа после последнего плавательного теста, в то же время в дексаметазон-связывающей емкости изменений не наблюдали. У крыс с удаленными надпочечниками, однако, плавательная тренировка не оказывала влияния на сязывания кортикостерона. Сделан вывод, что плавательные упражнения, в результате повышенной деятельности надпочечников, вызвали изменение числа рецепторов, связывающих кортикостерон клеточной плазмы.

ВЛИЯНИЕ РЕФЛЕКСА КАРОТИДНОГО СИНУСА НА КРОВООБРАЩЕНИЕ КРЫС С КАРЦИНОМОЙ ГУЕРИНА

ДЕБРЕЦЕНИ Л. А., ЭРЖЕБЕТ БЕККЕР, ЛИДИЯ МОХАИ и ТАКАЧ Л.

Авторы изучали влияние перевязки каротидной артерии на минутный объём (методом разведения синьки Эванса) и на распределение минутного объёма по органам (методом фракционирования изотопного индикатора по Шапирштеину) у здоровых крыс и крыс с карциномой Гуерина.

У контрольных животных под действием гипертензивного рефлекса со стороны каротидного синуса, давление крови и минутный объём увеличились, сосудистое сопротивление почек, кишечника кожи и каркаса также увеличивалось. У животных с карциномой Гуерина рефлекс каротидного синуса также привело к увеличению сосудистого сопротивления в различных органах. Кровообращение опухоли не изменялось, но сосудистое сопротивление значительно увеличивалось.

ВЛИЯНИЕ ПРОСТАГЛАНДИНОВ НА ОБМЕН КАТЕХОЛАМИНОВ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ У КРЫС

Д. ТЭЛЕГДИ

Мы изучали действие простагландинов A_1, E_1, E_2 и $F_{2\text{альфа}}$ на содержание катехоламинов в различных отделах головного мозга у бодрствующих крыс-самцов, в разное время после введения простагландинов в боковые желудочки мозга. PGE_2 увеличивал содержание дофамина в гипоталамусе спустя 10,20 и 60, а в перегородке спустя 20, 60 и 90 мин после его введения. PGE_1 увеличивал содержание дофамина в гипоталамусе на 20-й минуте, а в перегородке на 20-й и 60-й мин после его введения. PGA_1 и $PGF_{2\text{альфа}}$ не оказывали влияния на содержание дофамина. На содержание норадреналина не влиял ни один из проста-

гландинов. После введения PGE_1 и PGE_2 животным, предварительно получившим альфа метил-р-тирозин, скорость исчезновения дофамина увеличивалась в гипоталамусе и перегородке, тогда как содержание норадреналина уменьшалось только в гипоталамусе.

ВЛИЯНИЕ ВЫТЯЖКИ СРЕДНЕЙ ДОЛИ ГИПОФИЗА НА ВЫРАБОТКУ СТЕРОИДОВ ИЗОЛИРОВАННЫМИ КЛЕТКАМИ *z. glomerulosa* И *z. fasciculata*

К. С. САЛАИ

Мы изучали в экспериментах действие вытяжки средней доли гипофиза на продукцию альдостерона и кортикостерона клетками *z. glomerulosa* и кортикостерона клетками *z. fasciculata.*

Кривая зависимости доза–эффект вытяжки средней доли гипофиза (измеряя по выработке альдостерона клетками *z. glomerulosa*), чем кривая доза–эффект альфа$_h{}^{1-39}$ АКТГ. ED_{50} как вытяжки средней доли гипофиза, так и АКТГ ниже при измерении продукции стероидов в *z. glomerulosa*, чем в *z. fasciculata.*

Мы предполагаем, что какой-то гормон (или любое другое вещество) в средней доле изменяет чувствительность клеток к АКТГ.

ВЛИЯНИЕ ГЕМОРРАГИЧЕСКОГО ШОКА НА РЕАКЦИИ СО СТОРОНЫ КРОВЯНОГО ДАВЛЕНИЯ И ЧАСТОТЫ СЕРДЦЕБИЕНИЙ, ВЫЗВАННЫЕ ВВЕДЕНИЕМ АДРЕНАЛИНА У БОДРСТВУЮЩИХ СОБАК

А. АДАМИЦА, К. ТАРНОКИ и Ш. НАДЬ

В экспериментах на собаках мы изучали влияние геморрагического шока на реакции со стороны кровяного давления и пульса, которые вызывались внутривенным введением 2 мкг/кг адреналина. За несколько дней до опытов в левую сонную артерию и яремную вену имплантировали канюли *(Silastic)*. Кровопусканием понижали кровяное давление у собак до 40 мм рт. ст. и затем поддерживали на этом уровне в течение двух часов. Тестированные дозы адреналина вводились перед кровопусканием, в начале и конце контролируемой гипотензии, перед реинфузией и спустя 15 мин после реинфузии. Радиоэнзиматическим методом определяли уровень адреналина и норадреналина в плазме.

Результаты настоящих экспериментов показали, что в шоке уменьшаются величина и продолжительность реакции кровяного давления на адреналин. Введенные перед геморрагией адреналин вызывал реакцию типа брадикардии, которая сменялась тахикардией во время шока. После обратной инфузии адреналин вызывал двухфазные реакции. Показали существование статистически значимой отрицательной корреляции между уровнем норадреналина в плазме и величиной реакции со стороны кровяного давления в ответ на введение адреналина.

СОДЕРЖАНИЕ ДОФАМИНА В ПОЛОСАТОМ ТЕЛЕ ПОСЛЕ ОДНОСТОРОННЕГО ПОВРЕЖДЕНИЯ ЧЕРНОГО ВЕЩЕСТВА: ДОКАЗАТЕЛЬСТВА УМЕНЬШЕНИЯ УРОВНЯ ДОФАМИНА НА ПРОТИВОПОЛОЖНОЙ СТОРОНЕ

З. ХАН, З. КАРАДИ и Л. ЛЕНАРД

Через 7 дней после одностороннего электролитического или химического (6-гидроксидофамин) разрушения черного вещества *(substantia nigra)* наблюдалось значительное *двустороннее* уменьшение концентрации дофамина (DA) в *neostriatum*. Подобным же образом, уровень дофамина снизился в черном веществе не только на стороне поражения, но и на противоположной. Выявлена положительная корреляция между содержанием дофамина ипси- и контралатеральных полосатых тел, а также между содержанием дофамина в черной субстанции и полосатом теле той же стороны, как на стороне повреждения, так и на противоположной стороне.

МЕТОД БЫСТРОГО ЦЕНТРИФУГИРОВАНИЯ ДЛЯ ВЫДЕЛЕНИЯ ПОЛИМОРФОНУКЛЕАРНЫХ ЛЕЙКОЦИТОВ ИЗ ЧЕЛОВЕЧЕСКОЙ КРОВИ

Л. МОЛНАР, Я. ЛЕЙБИНГЕР и М. БАЛО БАНГА

Нами разработан быстрый, одноступенчатый метод центрифугирования для препаровки полиморфонуклеарных лейкоцитов. С помощью этого метода можно за 30—40 мин приготовить суспензию мытых гранулоцитов (чистота 98—99%), 98% которых сохраняют жизнеспособность. Полученные таким образом клетки обнаруживают более высокую редукцию NBT по ходу поглощения растворимых комплексов ДНК-анти-ДНК, чем лейкоциты, полученные методом осаждения декстраном.

ОКИСЛИТЕЛЬНОЕ—ВОССТАНОВИТЕЛЬНОЕ СОСТОЯНИЕ НАД-НАД·Н И ОСЦИЛЛЯЦИЯ ОБЪЕМА СОСУДОВ В КОРЕ ГОЛОВНОГО МОЗГА КОШКИ

Е. ДОРА и А. ДЬ. Б. КОВАЧ

Как показали наши предыдущие исследования, осцилляции флуоресценции НАД·Н мозговой коры при тяжелой артериальной гипоксии не являются следствием осцилляции мозгового перфузионного давления и мозгового кровотока (Дора и др., 1979). В настоящей работе мы сообщаем дальнейшие данные о вероятном механизме различного происхождения осцилляций мозгового кровотока и обмена веществ мозговой ткани.

Мы производили эксперименты на кошках (20) с управляемым дыханием, наркотизированных альфа-Д-глюкохлоралозой и иммобилизованных триэтилиодидом галламина. Изменения объема сосудов коры головного мозга и флуоресценции НАД·Н измеряли флуорорефлектометрическим методом, через стеклянное окошко, которое укреплялось в отверстии правосторонней париетальной кости черепа.

В 5 опытах осцилляции объема сосудов мозговой коры и окислительно-восстановительного состояния НАД-НАД·Н были вызваны изменениями артериального кровяного давления (т. н. волнами Траубе—Геринга). Волны сосудистого объема на 1—2 сек отставали от волн артериального кровяного давления, и частота их колебалась в пределах 2—3 волн в мин. Волны флуоресценции НАД опаздывали по сравнению с волнами сосудистого объема на 2—10 сек. Осцилляции такого типа временно уменьшались под воздействием введенной внутривенно глюкозы, стабилизирование же кровяного давления приводило к исчезновению этих осцилляций.

В 5 экспериментах кровопусканием снижали кровяное давление до 30—40 мм рт. ст. Если после этого вызывали состояние аноксии (вдыхание азота) или вводили глюкозу, то появлялись осцилляции флуоресценции НАД·Н, которые не зависели от изменений сосудистого объема. Волны появлялись каждые 2—5 мин. Мы полагаем, что эти осцилляции окислительного-восстановительного состояния НАД-НАД·Н имеют чисто метаболическое происхождение, подобные же осцилляции были описаны ранее другими авторами у дрожжевых клеток (*Chance* и др., 1964).

В 10 опытах возникновение осцилляций сосудистого объема в коре головного мозга и флуоресценции НАД·Н наблюдалось уже в контрольном периоде или после повторного введения альфа-Д-глюкохлоралозы. В таких случаях частота волн осцилляций была 6—12 в мин. Во время осцилляций кровяное давление было постоянным, для электрокортикограммы была характерна низкая спайковая активность. Осцилляции НАД·Н запаздывали на 2—4 сек относительно осцилляций сосудистого объема. Осцилляции не были чувствительны к введению глюкозы. В то же время они временно исчезали под влиянием артериальной гиперкапнии, гипоксии и при прямом электрическом раздражении коры головного мозга.

ПОВЫШЕНИЕ УРОВНЯ cAMP В МОЗЖЕЧКЕ ПОСЛЕ ДЕКАПИТАЦИИ: ВЛИЯНИЕ ПРОПРАНОЛОЛА

Г. САБО и Э. ЭНДРЕЦИ

Мы занимались исследованием явления увеличения содержания cAMP в мозжечке у крыс после декапитации. Введение 5 мг/100 г пропанолола за час до обезглавливания предотвращало быстрое посмертное повышение уровня cAMP. На повышение уровня с AMP не оказывали влияния ни опустошение катехоламиновых депо с помощью резерпина, ни торможение синтеза катехоламинов, на введение барбитурата.

Результаты настоящих экспериментов позволяют нам сделать вывод, что в увеличении содержания cAMP в мозжечке после декапитации играет роль норадренергическая нейротрансмиссия.

СУДЬБА p-БРОМ-МЕТИЛАМФЕТАМИНА (V—III) В ОРГАНИЗМЕ

К. МАДЬЯР, К. ТЭКЕШ, Г. ЗОЙОМИ, Т. СЮЧ и Й. КНОЛЛ

Судьбу p-бром-метиламфетамина (V—III) в организме мы изучали в экспериментах на мышах и крысах с помощью радиоактивных модификаций этого соединения.

Авторадиографические исследования и жидкостно-сцинтилляционные измерения показали, что это соединение быстро всасывается и распределяется в тканях. В центральной нервной системе оно достигает более высоких концентраций, чем метиламфетамин, и медленнее оттуда выводится. Метод дифференцированного центрифугирования показал, что V—III лучше, чем метиламфетамин и его орто-бромное производное (V—104), связывается с митохондриальной и микросомальной фракциями.

Интенсивность связывания с фракциями прямо пропорциональна липоидной растворимости соединений. Соединение V—III и его метаболиты выделяются в основном с мочой, малая часть их, однако, находится также и в кале. Если животным предварительно давали соединение V—III—3—^{14}C, то в выдыхаемом ими воздухе появляется также небольшое количество $^{14}CO_2$, что является результатом распада молекулы. Радиохроматографические исследования, а также газовая хроматография и масс-спектрометрия (GC/MS) показали, что V—III частично выделяется с мочой в неизмененном виде, однако с мочой выделяются также его деметилированное производное и следующие за ним p-бром-фенилацетон, p-бром-фенилпропанол, p-бром-бензойная кислота и p-бром-гиппуровая кислота. Главный путь метаболизма амфетамина и метиламфетамина у крыс — p-гидроксилирование, этот путь бывает ингибирован при p-галогенировании V—III. Таким образом, у крысы вторичное метаболическое превращение (деметилирование, окислительное дезаминирование) становится главным путем метаболизма V—III. Протекание интенсивного деметилирования V—III удалось показать в экспериментах на крысах, как в условиях ***in vivo***, так и ***in vitro***.

N-деметилирование V—III усиливается у крыс в ходе продолжительного введения этого соединения. Это последнее явление (индукция) должно приниматься во внимание также и при объяснении фармакологической толерантности, которая развивается в это время.

ДЕЙСТВИЕ ПРОСТАГЛАНДИНА $F_{2-альфа}$ НА ИЗОЛИРОВАННЫЙ ОБЩИЙ ЖЕЛЧНЫЙ ПРОТОК СОБАКИ И КРОЛИКА

М. ПОЦИК, Ч. БАРТА и Э. МИНКЕР

Авторы разработали метод приготовления препарата круговых спиральных мышц ***in vitro*** из общего желчного протока кролика и собаки. В экспериментах на этом препарате они показали, что простагландин $F_{2-альфа}$ дозозависимо сокращает круговую мышцу общего желчного протока у собаки и кролика. Вызываемое простагландином $F_{2-альфа}$ сокращение является продолжительным и обратимым. Морфин, адреналин и хлористый барий не вызывали сокращения этого препарата в дозах, применявшихся при исследовании.

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BÉLA ISSEKUTZ
1886—1979

Béla ISSEKUTZ was born in Kőhalom, a small village in Transylvania, and studied medicine at Kolozsvár University. When he graduated he had already committed himself to pharmacological research, a field he never abandoned throughout his long life. As a young man he saw the birth of the receptor theory, the discovery of neurochemical transmission, of some important hormones, the beginning of chemotherapy and the hypothesis on the relationship between chemical structure and pharmacological effect. He always attempted to follow the basic new lines in his laboratory at the Institute of Pharmacology.

In 1908, heroin was introduced as an antitussive superior to morphine. This released a wide debate. In 1911 ISSEKUTZ showed that there was no difference between morphine and heroin in their effect on respiration. He also demonstrated the synergism between the natural opium alkaloids, morphine, noscapine and papaverine.

Issekutz's first success in the field of structure–activity relationships was the synthesis of quaternary derivatives of atropine and hematropine. In 1917 he showed that such drugs, with an increased peripheral and a weakened central effect, were advantageous parasympatholytics; of these compounds hematropine methylbromide is still in use.

The year 1922 was a significant date in clinical medicine. It was in this year that insulin was first used. The discovery was marked by the Nobel prize of Banting and Macleod in 1923, but the very effect of the hormone had not been clarified. ISSEKUTZ spent much time on the problem between 1924—

34 and provided experimental evidence of the increase of glycogen in the liver under the effect of insulin.

In 1928 he published a study of great practical importance showing that the recently introduced mercurial diuretics were increased in effect and decreased in local irritability by their combination with theophylline. For many decades, such type of diuretic combinations was applied all over the world.

In the 30-ies he published a couple of papers dealing with the relationship between spasmolytic effect and capillar activity. Structure-activity relationship studies resulted in collaboration with Chinoin to the development of new spasmolytics, like perparine and ethaverin, and later in 1960, to the introduction of No-Spa.

The year 1935 was one of the turning points in clinical medicine: in that year did Domagk publish his discovery of the activity of prontosil against coccal infections. Though Domagk used the trial and error method, his result was a clear proof of the validity of Ehrlich's theory of the chemotherapy of bacterial infections. As Tréfouel, Nitti and Bovet had clarified the mechanism of action of sulphonamides, the way was opened for the successful new line of the research of antivitamins and antimetabolites. The papers of ISSEKUTZ and his pupil Jancsó written in these years show well his sense to touch upon important problems in due time. In 1933 they showed that suramine acts as a specific enzyme poison, inhibiting the oxygen and sugar consumption of trypanosomes so that the degenerating parasite falls victim to the RES. Their subsequent papers, too, dealt with mechanisms selectively damaging the metabolism of parasites; this line closely coincided with the conclusions drawn from the effect of sulphonamides.

In the 40-ies ISSEKUTZ was concerned with two closely related problems, the mechanism of action of thyroxine and amphetamine. He devoted several papers to show that thyroxine acts on the central nervous system. He also showed that amphetamine increased the metabolic rate and body temperature by stimulating the brain stem. For measuring the oxygen consumption in the rat, ISSEKUTZ, who was always fond of using simple methods, constructed a special device, adapting Warburg's technique for a rat-sized animal.

After World War II ISSEKUTZ was mainly concerned with new problems of structure–activity relationships and by re-evaluating his older experiences he succeeded to develop several new drugs.

His work was always appreciated and supported. In 1919 he was appointed to the chair of Pharmacology at Kolozsvár University, then half a year later at the new Szeged University. In 1931 he took over the chair of Pharmacology in Budapest and soon was elected a corresponding, then in 1945 a full, member of the Hungarian Academy of Sciences.

The wisdom and charm of Issekutz always attracted many young people to his institute, where they found a wide range of possibilities for

research and the promise of success. In our days his pupils and their pupils are leading all the Hungarian and a number of foreign departments of pharmacology, and many pharmacological laboratories in drug research throughout the world.

ISSEKUTZ had been studying and teaching in all his life. His monograph on the chemotherapy of cancer was published in 1969 in English, and his excellent book on the history of drug research came out in 1971 in German. His most popular work was his "Pharmacology", a concise handbook for students and practitioners which still serves as a gospel for every practising doctor in Hungary.

One could hardly characterize ISSEKUTZ's life and work better than he did in an interview on his 90th birthday: ". . . I have been a scientist whose task was to organize pharmacological research in Hungary. My achievement is that drug research flourishes in our country; my pupils or the pupils of my pupils are engaged in work in almost every laboratory where such research is carried out. The achievement of my life is that the Hungarian pharmaceutical industry is able to produce what it produces. I did my best and I believe I did it in the best way I could."

Béla ISSEKUTZ will always be remembered as an excellent scholar of 20th century Hungarian science, a prominent representative of experimental medicine and as the founder of modern drug research in Hungary.

J. KNOLL

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Physiologia—Pathophysiologia

THE INHIBITORY ACTIONS OF ESERINE AND OUABAIN ON THE K, Rb AND Cs UPTAKE IN SLOW AND FAST TWITCH MUSCLES OF THE RAT

By

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Comparative, *in vitro* studies were carried out on the ^{42}K, ^{86}Rb, and ^{131}Cs uptake in fast twitch extensor digitorum longus (EDL), slow twitch soleus (SOL) muscles of the rat, and in fast muscle (sartorius) of the frog. The inhibitory action of ouabain ($10^{-4}M$) and eserine ($10^{-3}M$) on the influxes of alkali cations was investigated. The rate of potassium influx in isolated EDL muscles was higher than that of SOL, while no difference could be found in ^{86}Rb or ^{131}Cs influx in the two types of mammalian muscles under *in vitro* condition. Ouabain inhibited to about the same extent the influx of K (25%) in both types of mammalian muscles and also in fast amphibian muscle. On the other hand, the eserine sensitive component of ^{42}K influx in fast twitch mammalian muscle (EDL) was about one-fourth of the total influx and even less in slow twitch mammalian muscle (SOL), while in frog muscle it amounted to about two-third of the total. The "residual" potassium influx, which represents the influx remaining after simultaneous treatment of the muscles with cardiac glycoside and eserine was about half of the total in EDL and SOL, but it was only a fraction of it in the frog sartorius muscle. The results may be explained on the basis of the morphological differences of the transverse tubular and sarcoplasmic reticulum systems of fast and slow mammalian muscles.

The sarcoplasmic reticulum and transverse tubular system of the slow mammalian muscle are less developed than that of the fast one [11, 12, 14]. Furthermore, fast and slow mammalian muscles differ also in their intracellular ionic composition. Slow muscles have a higher cellular Na^+ and a lower K^+ concentration than fast muscles [7, 13, 15]. Comparative studies on the potassium, rubidium and caesium uptake *in vivo* also revealed marked differences between the two distinct types of mammalian muscles [3, 4, 5, 6].

It has already been established that the effect of cardiac glycosides on potassium fluxes in frog muscle is remarkably dependent on the intracellular sodium ion concentration [9, 16]. Application of strophantidine reduces the potassium influx to about 75—80 per cent of the normal value in freshly

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dissected striated muscle cells. Sjodin [16] concluded that under normal conditions about one fifth of the potassium influx is coupled to the sodium efflux and the remaining part of the potassium movement is an electrically coupled K: K exchange.

We have reported on a marked inhibitory effect of eserine on the inward potassium movement in frog muscle which involved the ouabain-insensitive K: K exchange process located in the membrane of transverse tubules [10, 15].

The aim of this study was to compare the effect of ouabain and eserine on K-, Rb-, and Cs-uptake in rat SOL and EDL muscles and to test the possibility that the different morphological features of slow and fast mammalian muscles are associated with different pharmacological sensitivities in the cation transport.

Materials and Methods

Experiments were performed in a paired muscle design, using extensor digitorum longus (EDL) and soleus (SOL) muscles from female albino rats of CFY strain weighing 100—120 g, and sartorius muscles of the frog *Rana esculenta*. Muscles were dissected and cleaned with special care under stereomicroscope, retaining only sufficient tendon for their attachment by non-capillary thread to glass frames. The average weight of EDL was 113 ± 8 mg and soleus 93 ± 9 mg. Temperature of incubation was 33 °C for mammalian muscles, and Tyrode fluids were bubbled through gently with oxygen. For comparative studies, Ringer fluid was used at 20 °C to bath the frog muscle.

Muscles tied to glass frames and stretched to their resting length were preincubated for one hour in these solutions then transferred to the same fluid containing ^{42}K, ^{86}Rb or ^{131}Cs for different uptake periods. Eserine (10^{-3}M) and ouabain (10^{-4}M) were added 30 min before transferring the muscles from the inactive solution to the radioactive one; the drugs were present during the whole uptake period. At the end of incubation the muscle and its frame were passed successively through fresh changes of 8 ml of non-radioactive K-free choline solution. For wash-out of the extracellular tracer, the duration (min) and number of washes in non-radioactive fluid were 0.5×5, 1×3, and 2×2, successively. At the end of wash-out, muscles were cut down, trimmed, blotted on moistened filter paper and weighed for wet weight. The muscles were then dried in an oven for 4 hr at 95 °C and their dry weight determined. The dry samples were digested in 1 ml 30% H_2O_2 (Merck) after digestion and evaporation the residue was appropriately diluted in distilled water and analysed for radioactivity in a well-type scintillation counter and scaler. The concentration of Na^+ and K^+ was measured by a flame photometer equipped with a photomultiplier.

In order to compute intrafibre concentration from analytical values for whole muscle, the extracellular space was estimated using ^{58}CoEDTA as a marker [1]. At a carrier concentration of 0.8 mM · l^{-1} CoEDTA, a mean CoEDTA space of $17.2 \pm 1.2\%$ (mean $\pm$ S.E.; n = 7) was found for EDL muscle soaked for 60 min in Tyrode solution, and $21.2 \pm 1.7\%$ for SOL.

Tyrode solution had the following composition (mM · l^{-1}): NaCl, 144; KCl, 5.5; $CaCl_2$, 2.5; $MgCl_2$, 1.2; glucose, 5.5 Composition of Ringer solution was (mM · l^{-1}): NaCl, 120; KCl, 2.5; $CaCl_2$, 1.8; $MgCl_2$, 2.0. The solutions were adjusted to pH 7.35 (Tyrode) or 7.20 (Ringer) with 5 mM · l^{-1} Tris (hydroxymethyl) aminomethane-HCl buffer.

Radioactive isotopes were obtained from the Isotope Institute of the Hungarian Academy of Sciences. Eserine (physostigmine sulphate, Calbiochem) was dissolved directly in the incubation solution, ouabain (Serva) was diluted from freshly prepared alcoholic solution. The ethanol concentration in Tyrode (Ringer) solution was less than 0.5‰.

Results

Electrolyte balance

Water content, sodium, and potassium concentrations in the two different types of muscle are summarized in Table I. When incubated in oxygenated Tyrode solution at 33 °C, muscles did not show statistically significant changes

Table I

Muscle water and calculated intrafibre concentration of Na, K in isolated extensor digitorum longus (EDL) and soleus (SOL) muscles of the rat

		n	H_2O	mmol · kg^{-1} fibre water	
			ml · kg^{-1} wet weight	Na	K
Freshly dissected	EDL	8	775.9±6.8	17.1±2.8	155.6±7.2
	SOL	8	779.0±5.4	23.4±2.1	126.5±6.6
Incubated (120 min in Tyrode)	EDL	12	777.5±5.2	22.5±4.9	149.3±9.7
	SOL	12	778.7±6.1	27.2±6.6	121.4±7.8

Values expressed are the mean ± S.E.

in the intrafibre concentration of electrolytes, as compared to freshly dissected muscles, except for the increase of Na^+ concentration in EDL.

Kinetics of K exchange in slow and fast twitch muscles

The K uptake in isolated EDL and SOL muscles was followed for periods of incubation of 30, 60, and 90 min. At the end of incubation the standard wash-out procedure for extracellular ^{42}K was used for all muscles. Figure 1 shows that the uptake of radioactive potassium was significantly higher in EDL than in SOL muscle for each duration of incubation. The reduced rate of K influx in SOL muscle was maintained relatively constant for 90 min, although uptake tended to decrease between the 60th and 90th min in both muscles.

The slower rate of K influx in SOL muscle could be demonstrated even if the standard wash-out procedure was omitted (Table II). One muscle from each pair was incubated for a shorter period of time (15, 30 min) and the other muscles of each pair for a longer period (60, 120 min) in a radioactive solution. At the end of each incubation period the surface of the muscles was gently blotted on moist filter paper and radioactivity was determined. The K^+ uptake was calculated from the difference of the amount of K^+ exchanges during the incubation and K influx was corrected for simultaneous backflux (Table II).

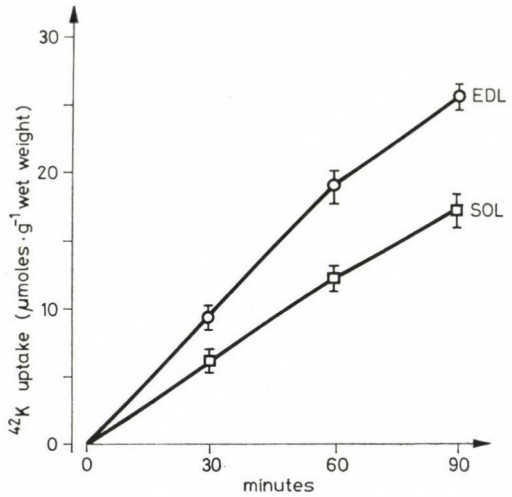

Fig. 1. Time course of the ^{42}K uptake in isolated EDL and SOL muscles of the rat, in oxygenated Tyrode solution at 33 °C. Points and vertical bars represent the mean ± S.E. of 18 determinations

In order to determine whether some restriction of K^+ movement through the interfibre space in the slow muscle might be responsible, the slower rate of potassium uptake, the fractional loss of ^{42}K with time was studied in SOL and EDL muscles of the same wet weight (Fig. 2). About 53% of the total ^{42}K in the EDL muscle and about 38% in the SOL muscle exhibited a relatively slow efflux and followed a single exponential ($t_{1/2}$ = 80 and 75 min, resp.; Fig. 2a). This would represent an intracellular potassium content. To further analyse the fast exchanging component of the flux curve, the exponentials characterizing the slow exchange process was subtracted from the per-

Table II

K exchange and influx in rat SOL and EDL muscles in normal Tyrode solution

	Exchanged ^{42}K (μmol · g^{-1} w.wt.)*		K influx** Δ pair nmol · g^{-1} · min	Exchanged ^{42}K(μmol · g^{-1} · w.w.t)*		K influx** Δ pair nmol · g^{-1} · min
	t_1 = 15 min	t_2 = 60 min		t_1 = 30 min	t_2 = 120 min	
EDL	5.8±0.5 (4)	18.5±1.2 (4)	311±21	9.6±0.8 (5)	34.1±2.8 (5)	301±29
SOL	6.2±0.6 (4)	17.3±0.4 (4)	258±17	9.8±0.9 (5)	29.9±1.4 (5)	245±31

All values are mean ± S.E.

* Exchanged $^{42}K = {}^{42}[K]$ in muscle · $[K]_0/{}^{42}[K]_0$ · wet weight

** Corrected influx = $M_i = M_i' \cdot k_0[\exp(-k_0 t_1) - \exp(-k_0 t_2)]$

M_i' = difference of exchanged K between pairs; k_0 = for EDL $1.35 \cdot 10^{-3}$ min^{-1}; for SOL $1.26 \cdot 10^{-3}$ min^{-1}

t = time of exposition in radioactive Tyrode

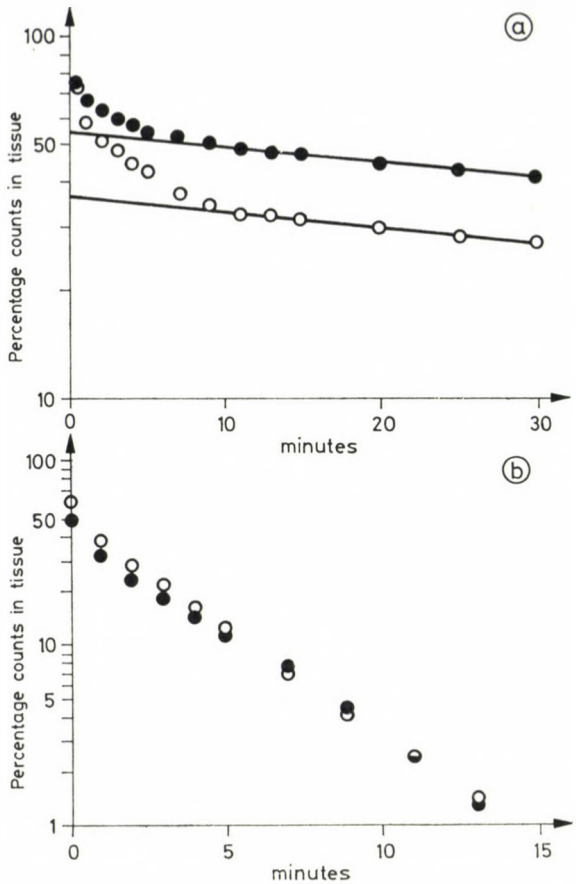

Fig. 2. Efflux of ^{42}K from the EDL (•) and SOL (o) muscles of the rat at 33 °C. (a) Total counts in tissues. The curves approach single exponentials which are indicated by the straight lines. (b) Counts in tissues after subtraction of the slow exponential component. Ordinates: counts expressed as a percentage of the tissue content at the beginning of wash-out (log. scale). Abscissa: time in min after start of the wash-out

centage of initial counts remaining in the tissue. The differences are plotted in Fig. 2b. In both muscles the wash-out of ^{42}K from the tissue was complex in that more than one exponential would be required to describe the entire process between zero and 15 min. The data suggest that the K^+ exchange in extracellular space exhibits a similar kinetics in slow and fast muscles.

The influx of ^{42}K, ^{86}Rb and ^{131}Cs in slow and fast muscles.

When RbCl or CsCl at 5.5 mmol · l^{-1} was substituted for KCl in Tyrode, the rate of influx of Rb and Cs (measured over a 30 min incubation) was about half of the K^+ influx (Fig. 3). However, no difference was found between the

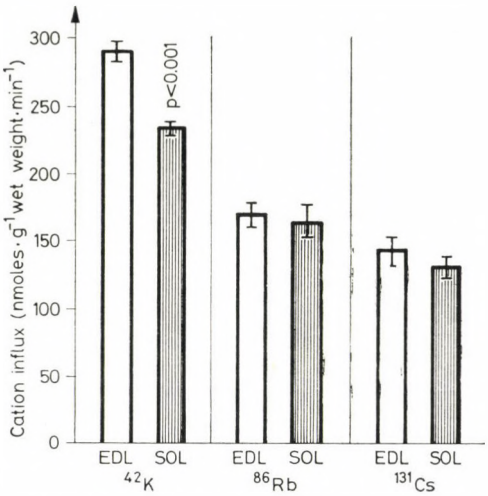

Fig. 3. Comparison of the resting ^{42}K, ^{86}Rb, and ^{131}Cs influx measured over the initial 30 min in fast twitch EDL and slow twitch SOL muscles of the rat. The bars in each set indicate the mean ± S.E. of 8 determinations

rate of Rb^+ or Cs^+ influx in the fast and slow muscle in contrast to the influx of ^{42}K, which was significantly higher in EDL than in SOL muscles.

Our *in vitro* results are inconsistent with the *in vivo* findings of KERNAN [4]. KERNAN and MCDERMOTT [6, 7], KERNAN and MCCARTHY [5]. They reported that the relative specific activity of soleus muscles was at least twice higher than that of EDL muscle in rat after i.p. injection of ^{42}K or ^{86}Rb. Although unilateral section of the sciatic nerve 48 hr prior to radioisotope injection resulted in a marked reduction of ^{86}Rb uptake in the soleus and in the denervated EDL muscles, ^{86}Rb uptake increased by about 75% [6, 8].

In order to determine whether the higher rate of ^{86}Rb influx of the rat soleus *in vivo* might have been due to a higher electrical activity, the effect of nerve section of ^{86}Rb influx into soleus and EDL muscles was studied by KERNAN's method [6]. Table III shows count ratios of intracellular to plasma

Table III

Effects of unilateral sciatic nerve section in SOL and EDL muscles on ^{86}Rb uptake over 4 hr in vivo

	Ratio of activity		
	n	(counts in fibre water/counts in plasma)	
		EDL	SOL
Control	(6)	29.8±2.2	56.3±2.0
Denervated	(6)	35.1±3.7	30.2±6.4

Mean ± S.E.

activity in muscles after a 4 hr exposure to the isotope. The section of sciatic nerve was carried out immediately before i.p. injection of ^{86}Rb. The Rb uptake was reduced by about 46% in soleus while in denervated EDL it was increased by about 16%, however, the later change was not significant statistically. Data indicate that the difference between the rate of Rb influx into soleus and EDL muscles disappears after nerve section and that denervated slow and fast muscles take up the same amount of tracer Rb both *in vivo* and *in vitro*.

Inhibition of K and Rb influx by ouabain and eserine in fast and slow mammalian muscle. In further experiments the inhibitory effects of eserine at a concentration of 10^{-3} M and ouabain (10^{-4} M) were compared in the two different types of muscles. Preincubation with eserine, followed by the presence of the inhibitor in the radioactive medium, resulted in a lower uptake of ^{42}K for each duration of incubation in fast and slow muscle (Table IV). Eserine blocked about 26% of the total K^+ influx in EDL muscle, while in SOL, the eserine-sensitive influx was only 18% of the total influx. There was no significant difference in the degree of hydratation or net ion content between the control and inhibited companion muscles. Since initial influx was linearly related to time in control and eserine treated muscles, the 30 min K^+ influx was selected for subsequent experiments on K^+ uptake.

Previously we have shown that eserine reduces the ouabain insensitive components of resting potassium influx of frog sartorius muscle, thus it seemed interesting to examine the additive effect of ouabain and eserine also on mammalian fast and slow muscles. In Tyrode of normal ionic composition, ouabain, at a concentration of 10^{-4} M, produced a significant reduction in resting K^+ influx measured under the same condition as described above for

Table IV

Effect of eserine on resting K influx in rat SOL and EDL muscles incubated for various periods in normal Tyrode solution; $[K]_0 = 5.5$ *mM*

	Uptake duration (min)	n	K influx (nmol · g^{-1} · min^{-1})		p*
			control	eserine	
EDL	30	(7)	316±16	252±38	<0.02
	60	(7)	305±48	215±29	<0.01
	90	(4)	299± 8	211±29	<0.01
SOL	30	(7)	254±27	211±23	<0.005
	60	(7)	257±34	204±29	<0.01
	90	(4)	245±18	204± 6	<0.02

p* by paired sample *t* test

Table V

Additive effects of eserine and ouabain on K influx into the rat slow and fast muscles

	K influx (nmol · g^{-1} · min^{-1})	
	EDL	SOL
Control	299±14	252±10
Ouabain (0.1 mM)	227±12	189±13
(n = 12) p^+	<0.001	<0.001
Eserine (1 mM) (Control)	246± 6	204±10
Eserine + ouabain	165±11	144±10
(n = 12) p^+	<0.001	<0.001

p^+ by paired sample *t* test

eserine. Following preincubation with ouabain, K^+ influx was reduced by about 25% of control both in EDL and SOL (Table V).

In a separate experiment, the effects of eserine and ouabain were compared to eserine alone (Table V). Eserine produced a further inhibition of K^+ influx in the presence of ouabain both in SOL and EDL muscles.

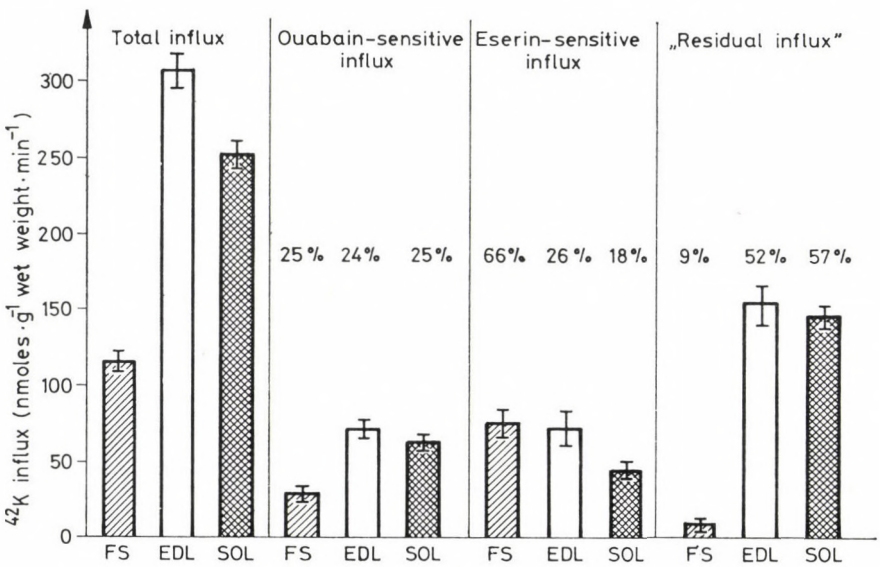

Fig. 4. Comparison of the total, ouabain, eserine sensitive and "residual" influx of ^{42}K in frog sartorius (FS), extensor digitorum longus (EDL) and soleus (SOL) muscle of the rat. Each bar represents the mean of 10 determinations ±S.E. The numbers represent the influx values expressed as per cent of the total

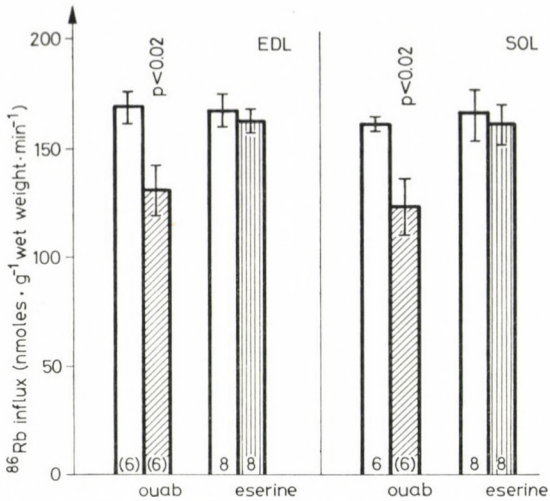

Fig. 5. Effects of ouabain (10^{-4}M) and eserine (10^{-3}M) on the influx of ^{86}Rb in fast twitch EDL and slow twitch SOL muscles. Open bars represent influx in the absence of inhibitors, pointed and hatched bars indicate influx with ouabain or eserine (mean ± S.E.)

The relationship between total, ouabain and eserine sensitive and "residual" influx, *i.e.* influx remaining after the admission of the two drugs, in frog sartorius and in mammalian fast and slow muscles is illustrated in Fig. 4. Total K^+ influx in frog sartorius muscle is only about one-third of that in rat EDL muscle.

The ouabain sensitive influx, though it has different absolute values in rat and frog muscles, amounted to about the same fraction of total influx in mammalian and amphibian muscles. Furthermore, there was no difference between the ouabain sensitive component of the resting K^+ influx in fast and slow muscles of the rat. On the other hand, there was a significant difference in the eserine sensitive influx. Eserine inhibited about 66% of the total K influx in frog sartorius, while in rat EDL the eserine sensitive K influx was about 26% and in SOL muscles it was only 18% of the total. The most remarkable difference between frog and rat muscles was found in the "residual" influx. In frog sartorius it was only about 10% of the total K influx, while in rat muscles it amounted to more than half of the total resting K influx.

Finally, the inhibitory effect of ouabain and eserine on ^{86}Rb influx was compared in fast and slow muscles of the rat. Figure 5 demonstrates that ouabain inhibited one-fourth of the total ^{86}Rb influx and the difference between the two types of muscles was not statistically significant. Eserine failed to have any effect on the ^{86}Rb influx both in EDL and SOL (Fig. 5).

Discussion

The results obtained in these experiments demonstrate conclusively that the ^{42}K uptake in isolated fast twitch EDL muscles of the rat is significantly higher than in slow twitch SOL muscles, but there is no difference in Rb or Cs uptake. These results seem to be inconsistent with the finding of KERNAN [3], KERNAN and MCCARTHY [5], KERNAN and MACDERMOTT [6, 8], showing a twice higher specific activity in soleus muscles as compared to EDL muscles after i.p. injection of ^{86}Rb and ^{42}K. VRBOVÁ [19] reported that when electromyographic activity was recorded in slow and fast muscles of conscious rabbit, the soleus muscle was almost continuously activated no matter whether the animal was moving, standing or sitting whereas the fast muscle was activated only when the animal changed its posture or responded to a noxious stimulus. The electromyographic activity ceased immediately after tenotomy [19]. KERNAN and MCDERMOTT [6] found that the influx of ^{86}Rb into tenotomized soleus muscles was about 40% lower than that observed in the contralateral pairs. Data on Table III demonstrate that the higher rate of Rb influx into soleus muscle significantly decreases immediately after the section of the sciatic nerve, indicating that the higher cation uptake of slow muscles *in vivo* is partly due to the continuous activation by motoneurons. Furthermore, the different rates of K influx in slow and fast muscles result from the dissimilar properties of the membrane system and are not the outcome of a delayed diffusion of potassium ions through the interfibre space of slow muscles (Fig. 2).

A consistent finding in these experiments is the inhibition of resting potassium influx by eserine. We have established that the reduction of K influx in frog muscle by eserine is confined to a K: K exchange mechanism, the inhibition being observed in the presence of ouabain [10]. The present results demonstrate that the influx of potassium in mammalian fast and slow muscles can also be blocked partially by eserine and the inhibitory actions of eserine and ouabain are additive (Table V).

It is noteworthy that eserine inhibits about two-third of the total K influx in frog sartorius muscle, whereas in rat EDL muscle the eserine-sensitive influx is about one-fourth of the total, and even less in soleus muscle (Fig. 4). Although the total potassium uptake of frog sartorii is about one-third of the K uptake of mammalian muscles, ouabain blocks about one-fourth of the total uptake both in mammalian and amphibian muscles (Fig. 4 and Table V).

We reported that after disruption of the transverse tubules in frog sartorius by hypertonic glycerol treatment the eserine sensitive component of the K influx markedly decreased but neither Rb influx nor the ouabain sensitive K influx changed [15]. Our earlier data provide a confirmation for the assumption that ouabain sensitive K influx as well as Rb influx can be

located on the sarcolemma, whereas the eserine sensitive K: K exchange on the membrane of the T tubules.

Some of the data in Fig. 4 may bear a relationship with this. The smaller proportion of eserine sensitive K influx in soleus muscles can be accounted for the less developed transverse tubular system in slow muscles of the rat [16]. The lack of the difference in Rb influx (Fig. 5) and the ouabain sensitive K influx of similar magnitude suggest a similar rate of K or Rb transport across the sarcolemma of fast and slow mammalian muscles.

From the above results it may be concluded that inward potassium movement can be separated by ouabain and eserine in at least three fractions. From this it follows that in normal frog muscle about 65 per cent of potassium influx is involved in an eserine sensitive K: K exchange, about 25 per cent is involved in an ouabain sensitive Na: K exchange, and 10 per cent is a "residual" one representing the influx remaining after treatment with cardiac glycoside and eserine. Present studies show that about one-half of the total influx of potassium ions in mammalian muscles appears to occur *via* the "residual" influx channels (Fig. 4).

Whether this represents an electrogenic passive flux or a metabolically driven ion movement with an uncleared coupling process are the questions to be solved. Since the "residual" influx amounts to a significant proportion of the total potassium influx, it stresses the importance of further studies on the possible role and characteristics of this transport system in mammalian muscles.

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INVOLVEMENT OF PEDAL NEURONS IN CARDIO-RENAL REGULATION AND THEIR CONNECTIONS WITH IDENTIFIED VISCERAL CELLS IN *HELIX POMATIA* L.

By

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Involvement of the cells of pedal ganglia in the regulation of heart activity and their connections with visceral neurons was studied in the central nervous system of *Helix pomatia* L. It was found that

(1) numerous cells of pedal ganglia receive inputs from the heart. The reaction of the pedal cells to heart afferents was characteristic: initial high frequency increase of firing followed by inhibition, then a secondary tonic increase occurred in activity. Some of the pedal cells were activated after a brief delay;

(2) many of the cells of the visceral and right parietal ganglia were activated simultaneously with pedal neurons during tactile stimulation of the heart. In different ganglia the answer to heart afferents can be observed to the same or opposite directions but its duration was identical in every cell;

(3) one cell of the left pedal ganglion (LP3) formed monosynaptic connections with several cells of the visceral ganglion. The axon of the visceral cells, connected monosynaptically to the neuron LP3, runs into the intestinal nerve. LP3 caused EPSPs on the visceral cells;

(4) the heart excitatory motoneuron V41, simultaneously with a number of pedal cells, receives excitatory inputs from the heart. The heart excitatory motoneuron is involved in the afferent and efferent pathways of heart regulatory network;

(5) a motoneuron (V43) was identified to cause relaxation of the heart, giving branches into the intestinal nerve and similarly to other motoneurons activated by heart afferents.

The distribution of certain neurons in the CNS involved in the regulation of cardio-renal system has been demonstrated by retrograde degeneration [4] as well as by retrograde transport of cobalt chloride [15, 11].

The role of these neurons, located to the visceral and parietal ganglia, in heart regulation has been studied and described in detail [11, 12, 13]. In the *Aplysia* heart, regulatory neurons were found in the abdominal ganglion, and in pedal and pleural ganglia [9, 6, 7, 17].

The aim of the present investigations was to examine the physiological role of pedal cells in the innervation of the heart and to study the interrelation between pedal and visceral neurons taking part in heart regulation.

Materials and Methods

The experiments were carried out on active snails *Helix pomatia* L., at room temperature (20—24 °C). For the investigations semi-intact preparations developed earlier [14, 11] were used, comprising the central nervous system, the intestinal nerve and cardio-renal system (*e.g.* heart, pericardium, blood vessels, kidney and liver). Identification of the neurons was made by the morphological and physiological criteria determined earlier [11]. Nomenclature of the ganglia and numbering of the neurons was described earlier [14]. For the right and left pedal ganglia the abbreviations RP and LP, respectively, were applied (Fig. 1).

Intracellular activity of two selected central neurons, and extracellular activity of the intestinal nerve as well as the heart beats were recorded simultaneously. Intracellular activity of the neurons was registered with conventional glass microelectrodes filled with 2.5 M KCl, having a resistance of 5—10 MOhm. Extracellular activity of the intestinal nerve was registered by platinum electrodes. Contractile activity of the heart was registered using the photo-optic method [19]. For recording the cell activity a high input impedance amplifier [18] was used with a bridge circuit, assuring polarization of the soma membrane. A four channel Tektronix oscilloscope and a Gould-Brush recorder were employed. For tactile stimulation of the heart, a point stimulator with dosing device was applied.

Results

1. *Reaction to heart stimulation of the nerve cells located at pedal ganglia*

Figure 1 shows distribution of the neurons studied. The majority of the cells of pedal ganglia show a double reaction to tactile stimulation of the heart: an initial increase in firing is followed by inhibition (Figs 2B and 3A). Another increase in activity appears 15—20 sec after tactile stimulation of the heart.

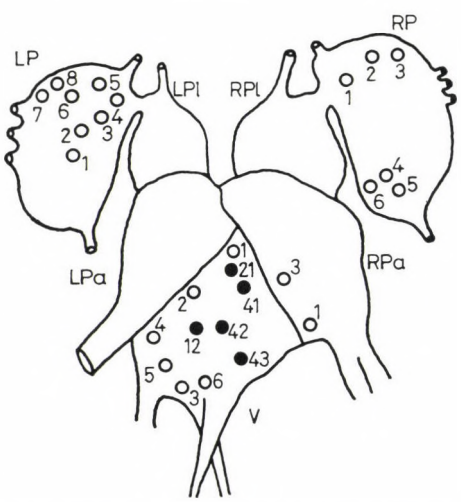

Fig. 1. Distribution of the investigated neurons in the central nervous system of *Helix pomatia* L. LPl — left pleural, LPa — left parietal, V — visceral, RPa — right parietal, RPl — right pleural, RP — right pedal and LP — left pedal ganglia. Numbering of cells according to earlier scheme (S.-Rózsa, 1976)

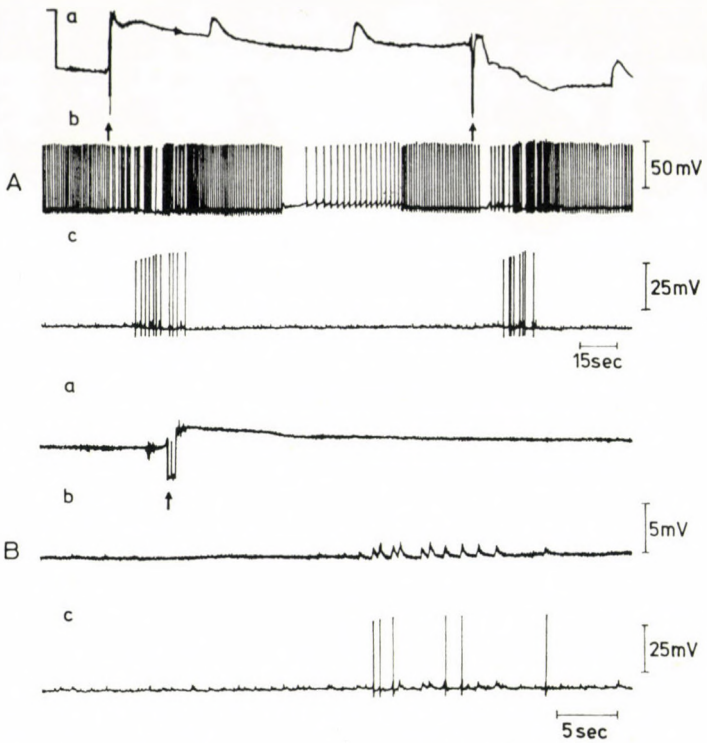

Fig. 2. Reaction of central neurons to tactile stimulation of the heart. A — simultaneous recording of heart contractions (a) activity of neurons RPa3 (b) and LP2 (c). Here and in the following Figures the moment of applying tactile stimuli to the heart is shown by an arrow. B — Reaction of the heart (a) and activity of the neurons LP8 (b) and RPa3 (c)

Some of the pedal neurons reacted to tactile stimulation of the heart only by delayed synaptic activation. They reacted similarly as did the LP8 neuron demonstrated in Fig. 2B. Delayed activation took place synchronously in numerous cells of the CNS after tactile stimulation of the heart. The activation of a cell located in the right parietal ganglion (RPa3) appeared after a delay as in the pedal cell demonstrated in Fig. 2B. Delayed activation of cell RPa3 after stimulation of the heart afferents coincided with the reaction in the LP2 neuron, but here the response proved to be an opposed one (Fig. 2A).

Around cell V2, as well as around LP3, numerous paired neurons were discovered reacting synchronously to heart stimulation. One couple responded to heart stimulation with excitation (Fig. 4A), then the neuron located at the vicinity of the cell LP3 underwent a long-lasting inhibition. During this period EPSPs orginating from unknown sources appeared (Fig. 4A) causing AP generation, coinciding with the inhbitiion of the visceral partner cell. During tactile stimulation of the heart, a silent cell located at the vicinity of cell V2 showed a long-lansting hyperpolarization, while in another one, near cell

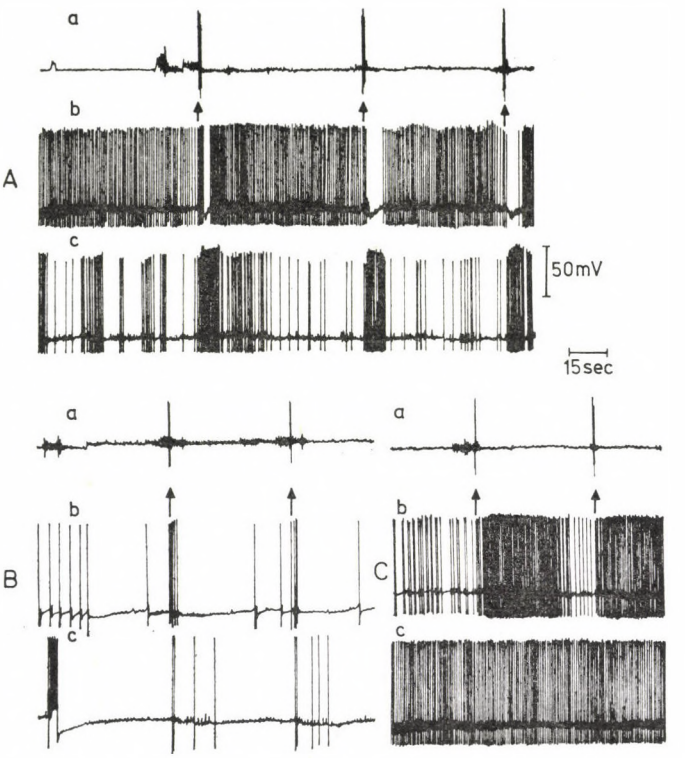

Fig. 3. Reaction of the neurons to heart stimulation. A — activity of the heart (a), a visceral neuron in the vicinity of V21 (b) and a cell in the left pedal ganglion near to LP3 cell (c). B — activity of the heart (a), and two cells in the left pedal ganglion (LP4 — b, LP8 — c). C — activity of the heart (a), neurons LP5 (b) and RPl (c)

RP3, the firing was eliminated while EPSPs were observed (Fig. 4B). The answer to the activation of heart afferents was prolonged for 12—15 sec, and it stopped simultaneously in both cells.

The neurons LP3 and LP4 reacted to heart stimulation with fast burst-firing, partly overlapping each other in time, but the activity of cell LP4 was somewhat longer (Fig. 3B). One cell in the vicinity of LP3 also reacted to heart stimulation with typical high frequency burst-firing and this activity correlated with the pattern of a visceral cell characterized by biphasic action (Fig. 4A). With repeated activation of heart afferents, the response of the two cells appeared again and again, although the inhibition was slightly facilitated (Fig. 3A) and excitation declined, which can be seen when comparing the answers after the first and third stimulation (Fig. 3A). After an initial excitatory-inhibitory effect, the LP5 neuron underwent a long-lasting activation in response to heart stimulation (Fig. 3C). At the right pedal ganglion the neuron RP1 showed a slight increase in frequency during the first excita-

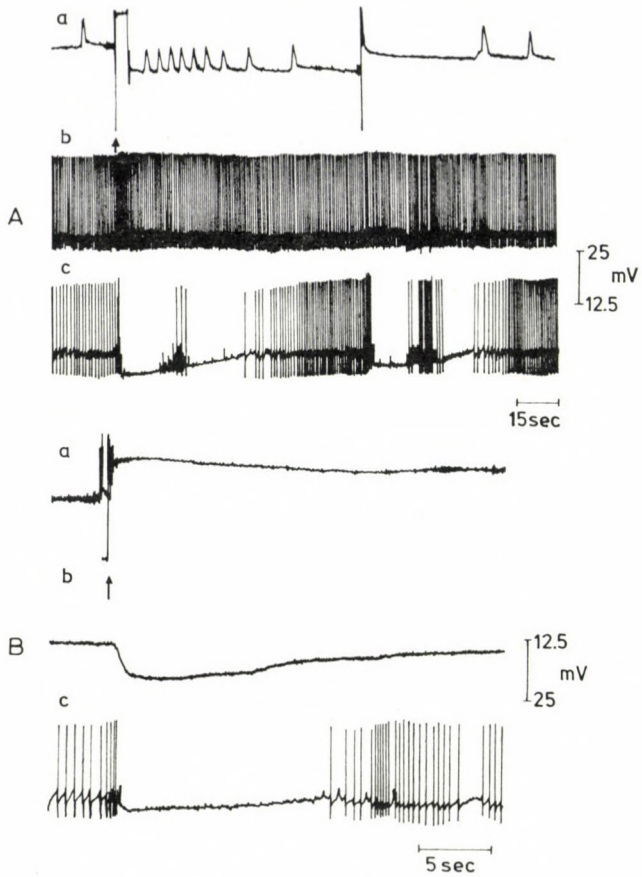

Fig. 4. Reaction of the neurons to tactile stimulation of the heart. A — heart activity (a), activity of neurons V2 (b) and LP3 (c). B — heart activity (a), activity of a visceral neuron (b) and a neuron in the left pedal ganglion (c)

tion of LP5 (Fig. 3C). Cell RP1, however, failed to respond to repeated stimulation of the heart (Fig. 3C).

The soma firings of pedal neurons could not be identified in the intestinal nerve. Depolarization of the soma membrane of cells LP2 and LP8 failed to influence the heart activity. The firing pattern of the investigated pedal cells showed no correlation with the heart beat.

2. *Interrelations between visceral and pedal neurons*

In the visceral and pedal ganglia, numerous cells were connected by common inputs or efferent pathways. Neuron LP3 proved to be connected monosynaptically to several cells of the visceral ganglion. Among these cells

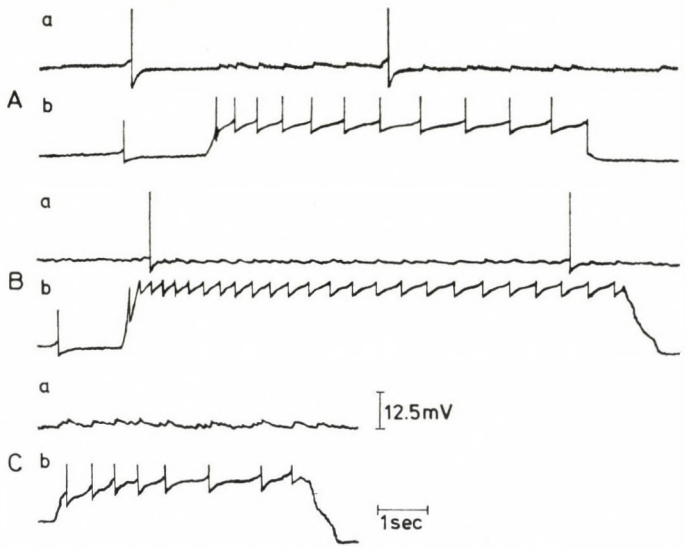

Fig. 5. Connection between the neurons of visceral and pedal ganglia. A, B and C "a" correspond to the activity of various cells from the identified cell cluster of the visceral ganglion; b — firing of cell LP3 spontaneously or caused by depolarization of the soma membrane. Each spike of cell LP3 correlates with the synchronous EPSPs of the visceral neurons

a group of neurons and two single cells were identified; all were stained on retrograde injection of $CoCl_2$ through the intestinal nerve [11]. The group was found at the border of the visceral and right parietal ganglia, located close to the bottom of the dorsal surface. One synchronously firing visceral cell was found between the above group of neurons and the motoneuron V12, near to the motoneuron V41, while the other visceral cell was located at the border of the visceral and left parietal ganglia (Fig. 1).

The connection of the LP3 cell with three neurons of the identified visceral group is demonstrated in Fig. 5. The spontaneous or evoked firing of cell LP3 caused EPSPs in the coupled visceral cells (Figs 5A, B, C). The connection of cell LP3 with three different visceral neurons is shown in Fig. 5. The third cell also received EPSPs originating from other sources (Fig. 5C).

Depolarization of LP3 also caused EPSPs in the visceral cell located at the vicinity of motoneuron V41 (Fig. 6A). The same EPSPs appeared in the above cell during spontaneous firing of LP3 (Fig. 6B). The second single cell connected to the LP3 showed the same response as the first one, namely produced EPSPs (Fig. 7). Near to this second cell, the third single cell also showed EPSPs during depolarization of LP3, but here the AP of LP3 and EPSP of the visceral neuron had no close correlation and EPSPs appeared without LP3 depolarization at lower frequency (Fig. 8).

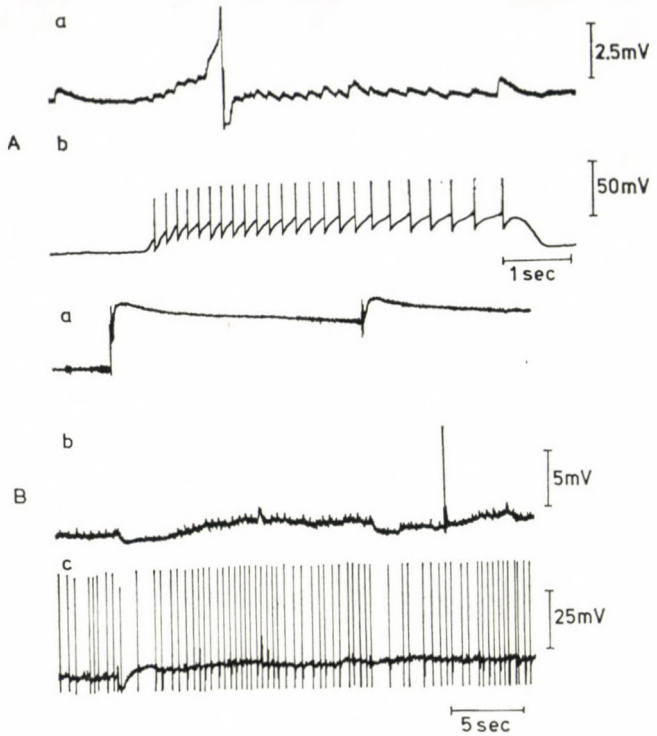

Fig. 6. Connection between neurons LP3 and V6. A — firing of cell LP3 caused by depolarization (b) and the synchronous reaction of neuron V6 in the visceral ganglion (a). B — reaction of neurons LP3 (b) and V6 (c) to tactile stimulation of the heart (a)

The connection between LP3 and the monosynaptically coupled visceral cell did not prove to be electrotonic, as depolarization of the visceral cells caused no changes in the activity of LP3. The presence of chemical synapsis between these cells is also supported by the increase in EPSP amplitude during hyperpolarization of the visceral neurons. The mean latency of EPSP was 8 msec following the peak of AP in cell LP3. The high frequency and long-lasting firing of cell LP3 failed to decrease the amplitude of EPSPs in the follower cells. The connection between cell LP3 and its follower seemed to be monosynaptic, with chemical synapses.

The visceral follower cells of LP3 send an axon into the intestinal nerve and each receives inputs from the cardio-renal system. The cell in the vicinity of motoneuron V41 belongs to the inhibitory system regulating heart activity [11, 13]. The group of visceral cells coupled to LP3 was connected only with the afferents of the heart.

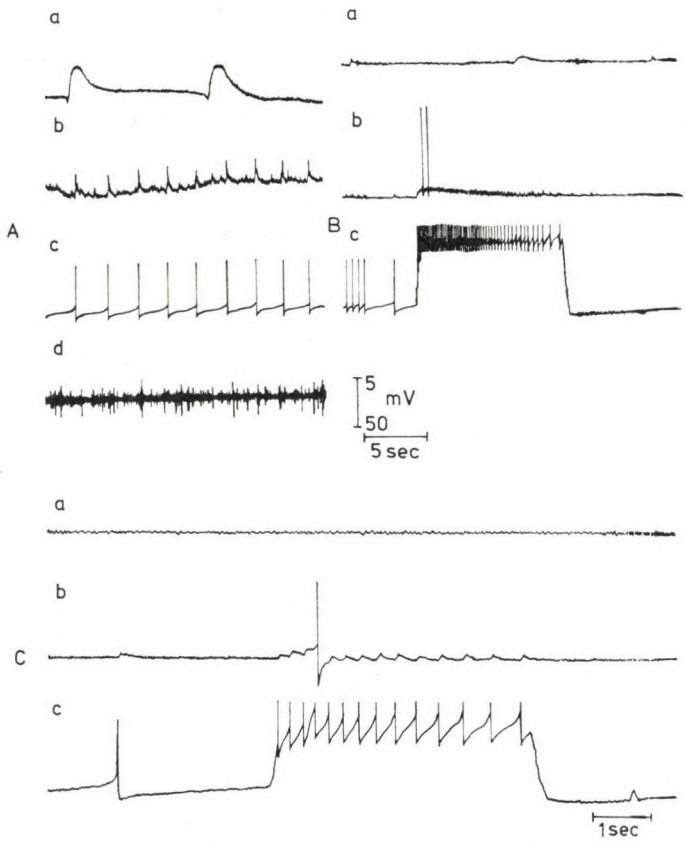

Fig. 7. Connection between neurons LP3 and V4. A — spontaneous firing of LP3 (c) and a synchronously generated EPSP of neuron V4 (b); a — heart activity, d — activity of intestinal nerve; B — high frequency firing caused by depolarization of cell LP3 (c) and the reaction in neuron V4 (b); a — heart activity. C — reaction of neuron V4 (b) to firing of LP3 (c) caused by depolarization (recording made at higher speed). a — heart activity

3. *Connection of pedal neurons with the cells modulating heart activity*

The effect and connection of a heart excitatory motoneuron (V41) identified earlier [11] and a newly found heart-relaxing motoneuron (V43) was investigated in relationship to the heart and pedal cells. Depolarization of the excitatory motoneuron V41 caused a contraction on the stopped heart and an extrasystole on the beating heart (Fig. 9). The reaction of the heart could be elicited repeatedly without adaptation, but only above the critical firing frequency of the motoneuron. The cell reacted to tactile stimulation of the heart with an increase in spike frequency. The nature of this excitation was similar to the activation of several pedal cells located in the vicinity of neuron LP3 (Fig. 10C). The increase in frequency of cell V41 above a critical level

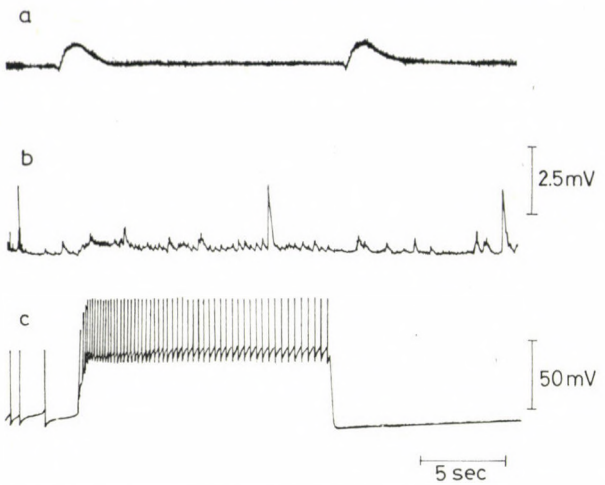

Fig. 8. Connection between neurons LP3 and V5. Firing of LP3 caused by depolarization (c) and a synchronous reaction of neuron V5 (b); a — heart activity

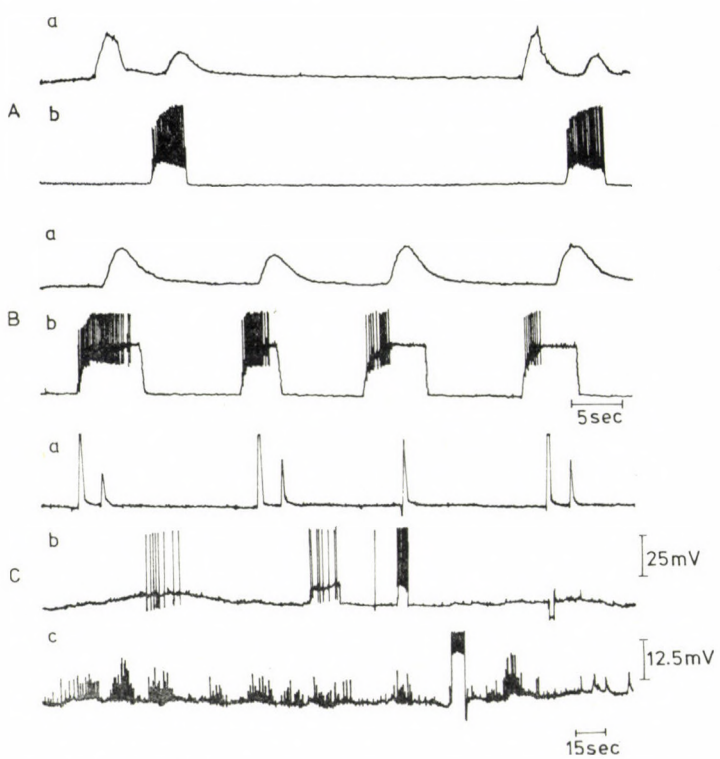

Fig. 9. Reaction of the heart to the activation of cell V41. A — depolarization of neuron V41 (b) caused an extrasystole (a). B — reaction of the heart (a) to repeated depolarization of cell V41 (b). C — reaction of the heart (a) to activation of neurons V41 (b) and a pedal cell (c) in the vicinity of LP3. Heart contractions are evoked by several APs of V41. Activation of the pedal neuron failed to influence heart activity

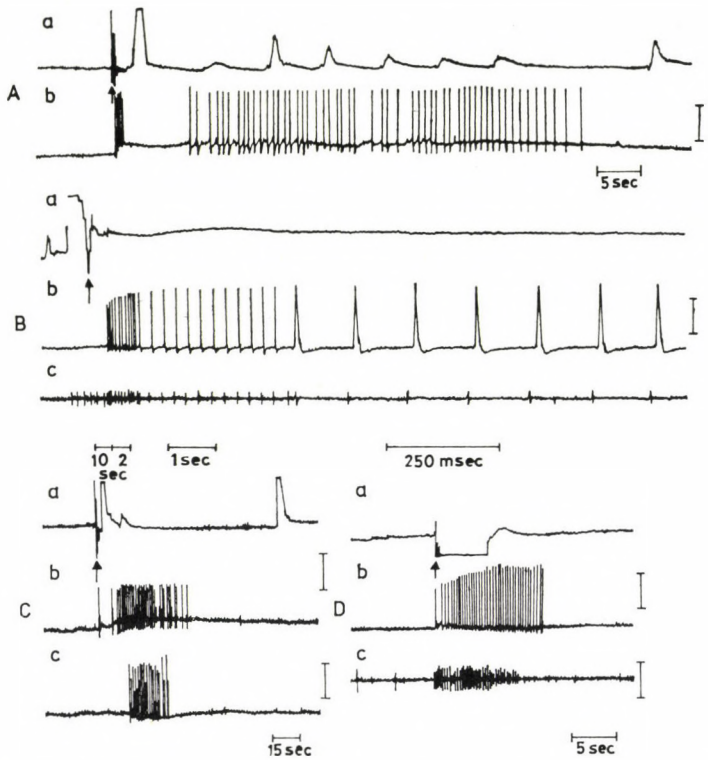

Fig. 10. Reaction of cell V41 to tactile stimulation of the heart. A — activity of the heart (a) and of neuron V41 (b). B — activity of heart (a) neuron V41 (b) and intestinal nerve, C — answer of neuron V41 (b) and a pedal neuron (a) in the vicinity of LP3 to tactile stimulation of the heart

appeared as an answer to heart stimulation and caused an extrasystole (Fig. 10B, C). The action potentials of neuron V41 after heart stimulation were preceded by synchronous potentials at the intestinal nerve (Fig. 10B). This means that neuron V41 receives afferent signs from the heart and causes an increase in heart beat with efferent pulses; in this aspect it is similar to the neuron V21, identified as an interneuron of double function [11]. In contrast with neuron V21, the spontaneous firing pattern of neuron V41 showed no correlation with heart beats.

The V43 neuron was studied also after $CoCl_2$ staining. Depolarization of the cell V43 caused a relaxation on the heart (Fig. 11A). The time from the soma depolarization to heart relaxation was 1.5—2 sec. Long lasting depolarization of V43 caused tonic relaxation of the heart (Fig. 11B, C). The neuron V43 also received inputs from the heart, which caused an increase in firing frequency (Fig. 12), resulting again in relaxation of the heart. In the firing of cell V43 sometimes an increase in frequency occurred originating from an

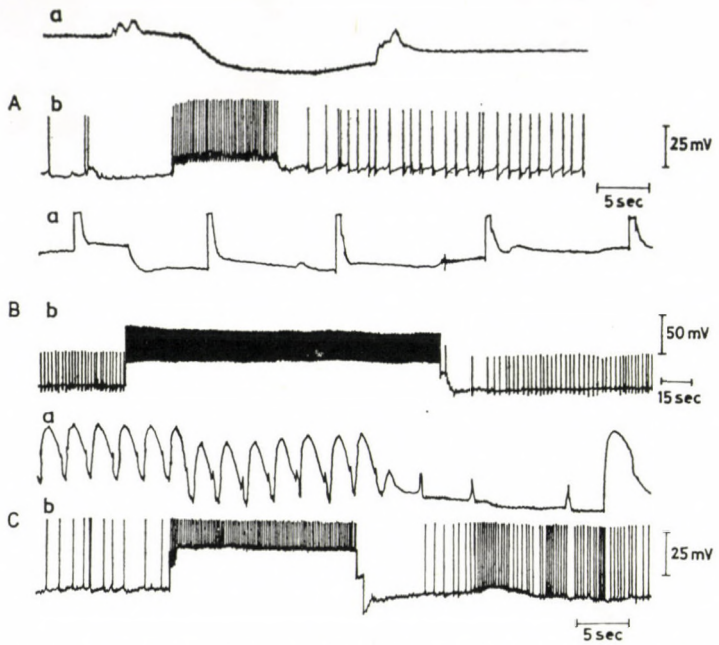

Fig. 11. Response of the heart to depolarization of neuron V43. A — depolarization of neuron V43 (b) causes relaxation of the heart (a). B — Relaxation of the heart (a) persists during long-lasting depolarization of cell V43 (b). C — Increase in firing of neuron V43 as an answer to depolarization (b) failed to alter the frequency of spontaneous heart beats (a) but caused relaxation (b)

unknown source; this caused relaxations, too (Fig. 12B). A neuron in the vicinity of cell LP3 was activated simultaneously with the V43 neuron during tactile stimulation of the heart (Fig. 12A, B). The heart-relaxing neuron V43 sends an axon into the intestinal nerve and its soma spike could be identified in the activity pattern of the nerve.

Discussion

The results demonstrate that numerous cells of the pedal ganglia receive inputs from the heart. The reaction of pedal neurons to tactile stimulation of the heart differed from that described for visceral and parietal cells [11, 12, 13]. The response of pedal cells to heart afferents has as a rule three phases. The initial step is a high frequency primary firing, which is followed by an inhibitory phase, then a tonic increase in firing is observed. The results support the earlier suggestion that the interoceptive information can be diverged to a large number of neurons throughout the central nervous system [11, 12, 13, 14]. Morphologically, 9 pedal neurons have been found to send direct branches

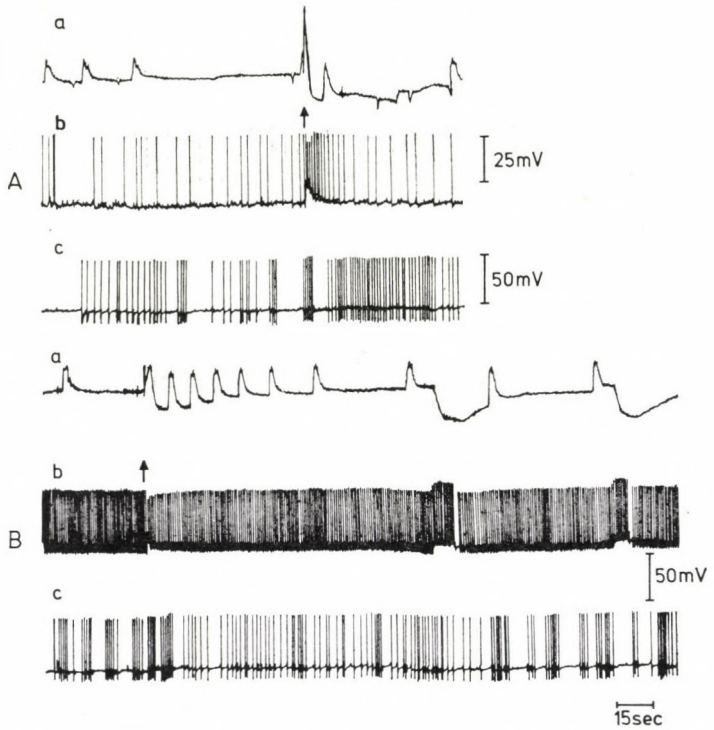

Fig. 12. Reaction of neuron V43 (b) and of a pedal neuron in the vicinity of LP3 (c) to tactile stimulation of the heart (a)

to the intestinal nerve and to the heart in *Gastropods* [4]. By physiological methods, more neurons could be identified which are connected with the heart, since heart stimulatory neurons are activated by mono-, and polysynaptic pathways. A large number of coupled cells in the visceral and pedal ganglia received synchronous inputs from the heart. These common inputs can be excitatory in both cells, but they may vary and be excitatory on pedal, and inhibitory on visceral neurons (Fig. 3).

In the experiments, monosynaptic connection of the cell LP3 was observed with many follower cells of the visceral ganglion. Supposedly, the cell LP3 of *Helix* is a homologue of the giant dopaminergic neuron (GDN) described at the left pedal ganglion of *Planorbis corneus* [8, 1, 2, 3]. The suboesophageal ganglionic ring is composed of the same ganglia in both species and the LP3 neuron of *Helix* proved to be the largest cell of the left pedal ganglion similarly to the GDN of *Planorbis* [1]. Also, the localization of LP3 and GDN proved to be similar in both species. In *Planorbis,* numerous follower cells of GDN were found at the visceral and right parietal ganglia generating EPSPs, IPSPs or biphasic synaptic potentials according to the soma spikes of

GDN [3]. The role of GDN in the regulation of functions has not, however, been investigated. Our data showed that this cell of the left pedal ganglion receives inputs from the heart, evokes EPSPs in visceral cells, sending direct branches to the heart and in this way can take part in the afferent and efferent pathways of the heart reflexes. The connection of LP3 with visceral neurons was found at the same area of the visceral ganglion of *Helix* where the endings of GDN were demonstrated in *Planorbis* by the use of various marker techniques [10].

In *Aplysia depilans* the central representation of sensory inputs from the heart is distributed in the abdominal, pleural and pedal ganglia [17]. All the neurons but one receiving inputs from the heart in *Aplysia* were able to modify the heart activity. In *Helix pomatia* only one pedal cell was connected to the inhibitory system of the heart. It cannot, however, be excluded that pedal ganglia contain more motor and interneurons involved into heart regulation.

In the visceral and right parietal ganglia of *Helix pomatia*, two inhibitory and four excitatory motoneurons, and one interneuron have been identified in the heart regulatory network [11, 12, 13, 16]. Among them the motoneuron V41 received similar inputs from the heart as a pedal neuron located in the vicinity of LP3.

A new element in the network regulating cardiorenal system was also observed. Cell V43 influenced only the tone of the heart causing relaxation and had no effect on the strength or frequency of the heart beats. The degree of relaxation was in close correlation with the firing frequency of neuron V43. Supposedly, cell V43 may be a motoneuron responsible for the relaxation of the heart. The axon of motoneuron V43 was identified in the intestinal nerve [11]. The activity of the motoneuron relaxing the heart was influenced also by heart afferents; this motoneuron has not been described in other *Gastropoda*.

According to our data it seems rather common that in *Gastropoda* the motor and interneurons receive sensory inputs from the innervated organ. This type of network organization can be characteristics of the majority of motoneurons [5]. This means that the formation of outputs is in close correlation with sensory information and can be considered to represent the effect of the network.

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LH—RH INDUCED CHANGES IN cAMP CONTENT OF THE ANTERIOR PITUITARY GLAND IN MALE AND FEMALE RATS *IN VIVO* AND *IN VITRO*

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Incubation of male anterior pituitary tissue with 10^{-7} M LH—RH resulted in a marked accumulation of cAMP in the 3rd hour of the incubation period. The presence of LH—RH for 20 min was enough to initiate a delayed accumulation of cAMP. Cycloheximide in the incubation medium inhibited the LH—RH induced increase of cAMP content. In contrast with males, the female pituitary showed accumulation of cAMP under the effect of LH—RH only after 6 hours incubation time and the increase was less than in the males. Ovariectomy or sex steroid treatment did not influence reactivity of the female pituitary.

Four LH—RH analogues with either agonistic or antagonoistic action on LH release were tested on cAMP accumulation of pituitary tissue *in vivo* and *in vitro*. One of the antagonists inhibited the LH—RH induced increase of cAMP, while the other antagonist elicited an increase of the cAMP content *in vitro*. Intravenous administration of LH—RH and its analogues led to an elevation of the cAMP content within 15 minutes, with the exception of one of the antagonists. LH—RH itself did not induce long-term accumulation of cAMP, while its analogues with either agonistic or antagonistic properties caused a significant rise in cAMP content in the 3rd hour after injection.

LH—RH did not influence the adenylate cyclase and phosphodiesterase activity of anterior pituitary tissue, and failed to modify the sensitivity of adenylate cyclase to NaF.

The present observations led to assume that the LH—RH induced accumulation of cAMP requires a factor synthetized during the incubation period. Moreover, it was found that the sex difference in LH—RH action on the adenylate cyclase–cAMP system may be attributed to the different time patterns and kinetics in the response rather than the refractoriness of the female pituitary. In the development of LH—RH response on cAMP accumulation of male rats, a significant increase occurred at about 40 days of age. These findings support the assumption that a complex biochemical reaction is involved in the LH—RH induced cAMP accumulation.

There are some observations which suggested the mediating role of the adenylate cyclase–cAMP system in the LH—RH action on pituitary gonadotropin release [2, 9, 13, 17]. LH—RH administration provokes a rapid rise in the plasma LH level, but the response *in vitro* to LH—RH requires a time to develop [7, 15, 18]. LH—RH enhanced the LH release during the first hour of incubation, but pituitary cAMP and cGMP levels were not altered during this period [18].

An interesting feature of LH—RH action on the cAMP content of the rat pituitary is that it occurs exclusively in the male rats [14, 15]. There was no effort to elucidate the sex dependence of this action and experiments deal-

ing with LH—RH induced changes of the cAMP level were carried out in male rats.

Several antagonists of LH—RH which inhibit FSH and LH release *in vitro* were also effective in inhibiting the LH—RH induced accumulation of cAMP in rat hemipituitaries [1]. The authors reported parallel changes in the cyclic AMP level and FSH and LH release indicative of a close correlation between activation of the adenylate cyclase system and gonadotropin release. Concerning the mechanism of the LH—RH action, the findings showed that Ca^{2+} is absolutely required for the stimulation of cAMP accumulation and FSH/LH release in rat's anterior pituitary gland *in vitro* [4].

Taking into consideration the *in vivo* data concerning the LH—RH action on the adenylate cyclase–cAMP system, the involvement of cAMP in LH release cannot be accepted. Administration of either dibutyryl–cAMP or aminophylline increased the pituitary cAMP level, but these changes were not associated with any increase in LH release [18].

In the present investigation we have studied the time pattern and some characteristics of the accumulation of cAMP in rat hemipituitaries after intravenous injection of LH—RH and its analogues. Special attention was paid to age and sex difference in the LH—RH response and in the effect of LH—RH analogues with either agonistic and antagonistic properties on LH release. Moreover, we have studied the possible action of LH—RH on the adenylate cyclase and phosphodiesterase enzymes as well as its effect upon the sensitivity to NaF of adenylate cyclase.

Materials and Methods

R-Amsterdam albino rats were used in the study. For the experiments *in vitro* the animals were killed by decapitation, the brain was quickly removed, the anterior pituitary was dissected, halved and placed into incubation vials which contained 2 ml Krebs—Ringer bicarbonate buffer (11 mM glucose, pH 7.4). Incubation was performed under shaking in O_2/CO_2 (95: 5 v/v) atmosphere at 37 °C. Preincubation usually lasted for 60 min, except for several experiments which are indicated in the Results. At the end of preincubation, the buffer was exchanged and the peptide was given to the medium. After incubation the tissue was homogenized in glass homogenizer tubes with 0.5 ml 0.5 M trichloroacetic acid (TCA). The precipitate was centrifuged and the TCA was removed by three consecutive extractions with 2 ml water-saturated ether. The competitive protein binding technique was used for determination of the cAMP content; the receptor was extracted from bovine adrenal cortex [5].

In experiments *in vivo* the peptides were dissolved in physiological saline and given into the tail vein. The rats were killed by decapitation at 15 and 180 min after the injection. The time between decapitation and pituitary dissection was not more than one minute.

Adenylate cyclase activity was measured by the method of KRISHNA et al. [10]. In the experiments I, the total volume of the samples was 80 μl (0.5 mM ^{3}H—ATP, diluted with unlabelled ATP; 2 mM $MgSO_4$, 10 mM theophylline, 64 mM Tris-HCl pH 7.6) and contained 30—40 μg protein. An incubation time of 4 min was selected because the reaction was linear under this experimental condition. In the experiments II the anterior pituitary was homogenized with the buffer containing all ingredients necessary for the enzymatic reaction and sampling was performed in the 1st, 3rd and 5th minutes. The reaction was stopped by placing the tubes into boiling water for 3 min and then aliquots were taken for determination of the labelled cAMP content. Enzyme activity was measured at 30 °C.

Phosphodiesterase activity was determined after 3 hours incubation; the pituitary was incubated without and with 5×10^{-7} M LH—RH. The tissue was homogenized in 0.6 ml buffer (10 mM Tris-HCl, 0.5 mM EGTA, pH 7.6), and aliquots of homogenate were diluted twice with the buffer containing different concentrations of cAMP (80 mM Tris-HCl, 5×10^{-6}—10^{-7} M cAMP, pH 7.4). Samples were taken in intervals from 1 to 7 minutes. cAMP concentration was measured according to BROWN et al. [5].

Female rats in ether anaesthesia were ovariectomized 3 weeks prior to the experiments. The steroid hormones in oil were given subcutaneously to intact and ovariectomized rats 48, 24 and 2 hours before sacrifice.

Four analogues of the LH—RH were used in the study. They were as follows. LH—RH (I); D-Phe^2-D-Trp^3-D-Phe^6-LH—RH (II); isophthaloil-bis-(D-Phe^2-D-Trp^3-D-Lys^6-LH—RH) (III); D-Lys^6-des-Gly^{10}-LH—RH-ethylamide (IV); N$^\varepsilon$-(Glp-Phe-D-Trp-Ser-Tyr)-D-Lys^6-des-Gly^{10}-LH—RH-ethylamide (V).

The drugs used in the experiments were oestradiol-17β propionate in oil, testosterone propionate in oil, cycloheximide (Serva), LH—RH (Calbiochem).

The LH—RH analogues II—V were synthetized by NIKOLICS et al. [16] according to the technique of [6] at 1st Institute of Biochemistry of Semmelweis Medical School, Budapest.

Protein content of samples was measured by the LOWRY method [21]; bovine serum albumin was used as standard.

Group means and standard errors were calculated and Student's two-tailed t test was used for statistical evaluation of data.

Results

Incubation of the anterior pituitary tissue of adult male rats in the presence of 10^{-7} M LH—RH for 3 hours led to a marked increase of the cAMP content in the 3rd hour of incubation and there was no significant alteration in the cAMP level during the first 3 hours. The presence of LH—RH in the incubation medium for 20 min was enough to increase the cAMP content in the 3rd hour although the medium was replaced with fresh buffer every 30 min (Fig. 1).

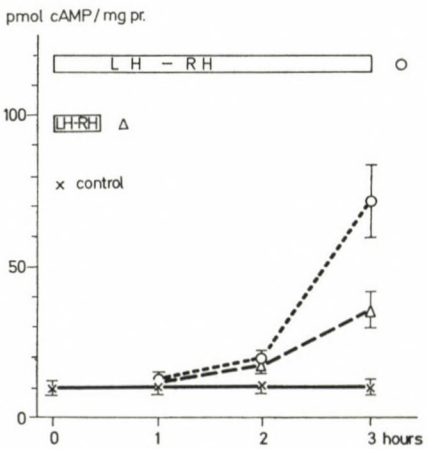

Fig. 1. cAMP content of anterior pituitary of male rats without (×) and with 10^{-7} M LH—RH for 20 min (△) and for 3 hours (○). The buffer was changed every half hour. Horizontal bars represent the presence of LH—RH

Changing the preincubation time from one to 60 min, or shortening it to 8 min did not influence the LH—RH response on the pituitary adenylate cyclase–cAMP system. During preincubation, a decline occurred in the cAMP content. Incubation with LH—RH of anterior pituitary tissue for 8 min did not affect its cAMP content (Fig. 2).

The LH—RH induced increase of cAMP accumulation in the anterior pituitary tissue of male rats showed a striking age dependence, and a signifi-

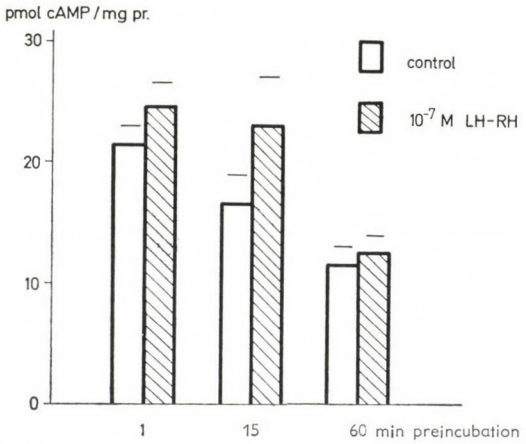

Fig. 2. Short incubation of anterior pituitary of male rats *in vitro* in the presence of 10^{-7} M LH—RH (hatched columns). Preincubation time: time from decapitation to the addition of LH—RH to the incubation buffer. Incubation time 8 minutes. Similar results were obtained after 3 min incubation

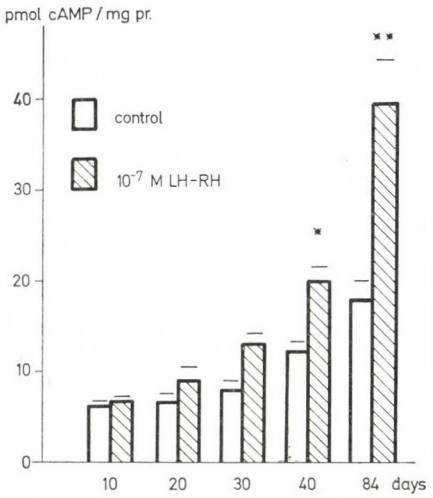

Fig. 3. Age-dependence of *in vitro* LH—RH sensitivity of cAMP content of male rat anterior pituitary. Incubation without (open columns) and with 10^{-7} M LH—RH (hatched columns) for 3 hours. * $p < 0.01$; ** $p < 0.001$

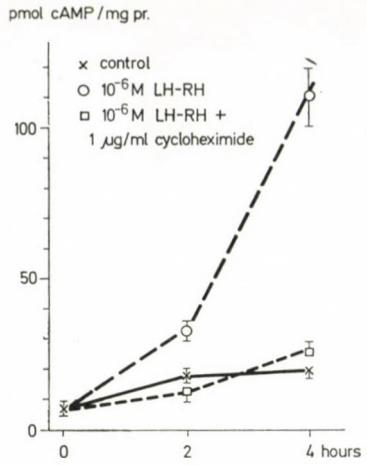

Fig. 4. Effect of cycloheximide on cAMP accumulation in male rat anterior pituitary caused by LH—RH. Control (×), 10^{-6} M LH—RH (○), 10^{-6} M LH—RH plus 1 μg/ml cycloheximide (□)

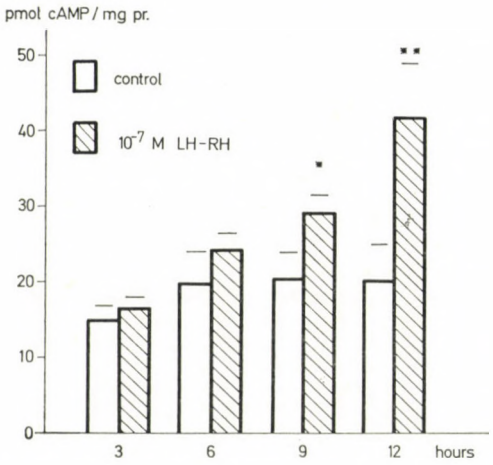

Fig. 5. Incubation of anterior pituitaries of female rats in the presence of 10^{-7} M LH—RH (hatched columns) for different times. * $p < 0.05$

cant increase was observed in male rats of about 40 days of age. These findings were obtained after incubation for 3 hours (Fig. 3).

In studying the mechanism of LH—RH action on the adenylate cyclase–cAMP system, it was found that cycloheximide at 1 μg/ml concentration inhibited the accumulation of cAMP in the pituitary (Fig. 4).

Incubation of the anterior pituitary tissue from female rats in the presence of 10^{-7} M LH—RH resulted in a significant cAMP accumulation after at least 6 hours incubation. The increase of cAMP content in the 12th hour of the incubation was much less than that of the male pituitary after 3 hours

incubation (Fig. 5). This insensitivity to LH—RH of the female pituitary tissue remained unchanged after ovariectomy performed 3 weeks prior to the experiment. Moreover, neither oestradiol treatment (0.2 mg, given s. c. 24 h prior to the experiment), nor testosterone administration (0.4 mg, given s.c. 48 and 24 h as well as 2 h before the experiment) modified the sensitivity to LH—RH of the female pituitary tissue.

The effect of four LH—RH analogues was tested on the adenylate cyclase–cAMP system of the anterior pituitary tissue in male rats after 3 h incubation. Peptides II and III were found to inhibit the LH—RH induced LH release in cell cultures [16]. The peptides IV and V as agonists were effective in inducing LH release. Incubation of the analogues at a concentration of 10^{-7} M with the anterior pituitary tissue of adult male rats was followed by a marked increase in the cAMP content, with the exception of peptide II which did not affect it. Interestingly enough it was irrelevant whether the peptides were agonistic or antagonistic ones (Fig. 6). Peptide II at 10^{-7} to 10^{-6} M concentration significantly inhibited the LH—RH induced accumulation of cAMP (Fig. 7).

Intravenous administration of LH—RH and its analogues caused a significant increase in the cAMP content of male anterior pituitary glands 15 min after the injection. Peptide III did not affect the cAMP level. A delayed but pronounced increase of the cAMP content was found in the 3rd hour after administration of all four analogues but not of LH—RH which induced a short-term rise of the cAMP content (Fig. 8). Peptide III which did not

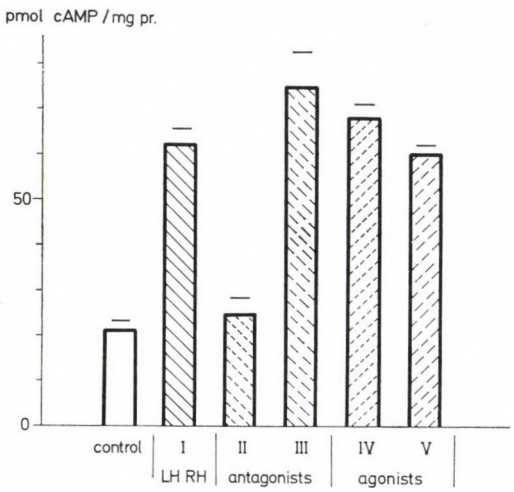

Fig. 6. In vitro effect of LH—RH and analogues on the cAMP level of anterior pituitaries of male rats. Incubation time: 3 hours. I: LH—RH; II: D-Phe^2-D-Trp^3-D-Lys^6-LH—RH; III: isophthalyl-bis(D-Phe^2-D-Trp^3-D-Lys^6-LH—RH); IV: D-Lys^6-des-Gly^{10}-LH—RH-ethylamide; V: N$^{\varepsilon}$-(Glp-D-Phe-D-Trp-Ser-Tyr)-D-Lys^6-des-Gly^{10}-LH—RH-ethylamide

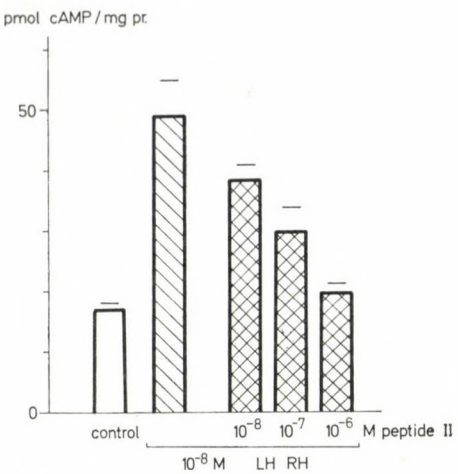

Fig. 7. Inhibition of the effect of 10^{-8} M LH—RH by D-Phe²-D-Trp³-D-Lys-LH—RH (peptide II) at different concentrations

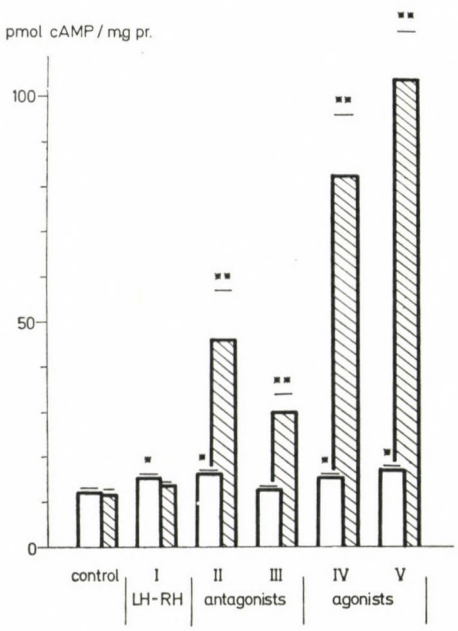

Fig. 8. cAMP content in the anterior pituitaries of male rats 15 min (open columns) and 180 min (hatched columns) after intravenous injection of 10^{-9} mol/100 g peptides. For the name of the peptides see *Fig. 6* or the text

change the cAMP content during 15 min led to a marked cAMP accumulation 3 h later.

LH—RH did not change the activity of adenylate cyclase and its sensitivity to NaF. The increase to NaF without incubation was $238 \pm 13\%$, with

Table I

Adenylate cyclase activity of anterior pituitary homogenate of male rats

	Adenylate cyclase activity pmol cAMP/min, mg protein	
	−NaF	+NaF
Without incubation	63±3	213±7
With incubation*		
Experiment I		
Control	49±5	152±3
LH—RH	45±3	128±4
Experiment II		
Control	30±4	—
LH—RH	24±5	—

* Before homogenization the anterior pituitaries were incubated for 4 hours with or without 5×10^{-7} M LH—RH. In Experiment I, homogenization in Tris-HCl buffer was followed 30 min later by enzyme assay. In Experiment II, homogenization was done in the assay buffer with MgATP

incubation, 210±12% (Table I). In experiment I, the enzyme activity was tested 30 min after homogenization, while in experiment II, it was tested from the very beginning of homogenization.

Phosphodiesterase activity was uninfluenced by the LH—RH at a concentration of 10^{-7} during 3 h incubation. The calculated constants were $K_m = 1.2\pm0.4\times10^{-6}$ mol/liter; $V_{max} = 78\pm42$ pmol/min/mg protein.

Discussion

Involvement of the adenylate cyclase–cAMP system in mediation of the action of hypothalamic releasing hormones on pituitary hormone secretion has been suggested by many investigators. Thus, an activation of adenylate cyclase in response to either crude hypothalamic extract or synthetic LH—RH has been reported [2, 8, 13, 24, 25]. A close parallelism between intracellular cAMP concentration and gonadotropin release was postulated [2, 3, 4, 9, 11], there are, however, observations which indicate that LH—RH induced cAMP accumulation is independent of its effect on LH release [21], and the administration of dibutyryl cAMP to hemipituitaries did not induce LH release [22, 23]. Moreover, there was no detectable rise of the cAMP content in the anterior pituitary up to 10 min after LH—RH administration, but by that time the plasma LH level was already elevated [18]. Other investigations also suggested

a dissociation between the LH—RH action on the cAMP level and gonadotropin release [15, 20].

The present investigation revealed that the LH—RH action on the cAMP level of anterior pituitary is strongly sex and age dependent. In the male pituitary, LH—RH increases the cAMP level at about 40 days of age. On the other hand, the female pituitary tissue showed a lack of sensitivity to LH—RH after 3 hours incubation. The observation seemed to indicate at LH—RH induced cAMP accumulation only in male rats [14, 15], while our data showed that these sex differences should be attributed to differences in the time pattern and kinetics of the response rather than to an insensitivity of the female pituitary tissue. The insensitivity must have developed in the early phase of ontogeny although the mechanism behind the phenomenon is still unclear.

Inhibition of proteins synthesis by cycloheximide prevented the LH—RH induced cAMP accumulation. This supports the view that LH—RH action on the adenylate cyclase–cAMP system requires factor(s) which are synthetized during the long lag of the incubation. This feature of the LH—RH action speaks against the view of cAMP accumulation being causally involved in LH release.

LH—RH caused no change in adenylate cyclase and phosphodiesterase activity although the accumulation of cAMP in the anterior pituitary tissue postulates same similar events. Testing the adenylate cyclase activity in a cell-free system does not exclude the possibility that a slight increase of its activity occurred in intact cells which resulted in cAMP accumulation during the 3 h incubation period. Nevertheless, a sharp rise of cAMP accumulation after 2 h incubation points to an involvement of some other factors.

LH—RH analogues with properties either agonistic or antagonistic to LH release increased the cAMP level of the male pituitary. The analogue peptide II which inhibited the LH—RH induced LH release [16], caused a marked suppression of the LH—RH induced cAMP accumulation *in vitro*. Intravenous injection of this peptide, however, led to a significant increase of the cAMP level in 15 minutes. On the other hand, the antagonist peptide III did not change the cAMP level during the first 15 min, but it did increase the cAMP level in the 3rd hour both *in vivo* and *in vitro*. Since LH—RH produced only a transient increase of the cAMP level if given intravenously but had a prolonged action with a long lag period if tested *in vitro*, the differences in this action of LH—RH and its analogues on adenylate cyclase–cAMP system cannot be attributed simply to different inactivation processes.

The present data do not support the concept that the activation of the adenylate cyclase–cAMP system is closely coupled with biochemical processes necessary for LH release. By all means, the present data and relevant observations in the literature do not exclude the possibility that LH—RH binding to

membrane receptors is not linked directly to activate the adenylate cyclase system, and the consecutive biochemical events play a basic role in controlling LH release and synthesis. This conclusion is in accordance with the observations of others [18] who found that LH—RH administration did not alter the pituitary cAMP and cGMP levels at the same time when LH increased in the blood. Moreover, we must be aware of the difference in the time pattern of LH—RH action upon cAMP accumulation between male and female pituitary tissue *in vitro* which, too, speaks against the assumption of cAMP accumulation being involved in a causal relation to LH release.

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SYNAPTIC INPUTS ON A BIMODAL PACEMAKER NEURON IN *HELIX POMATIA* L.

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1) Synaptic modification of the RPal neuron located in the right parietal ganglion of the snail *Helix pomatia* was investigated by applying single electric pulses to the right pallial, left pallial or anal nerves.

2) Stimulation caused short term changes in the bursting type activity of the neuron: polyphasic synaptic potential including both the depolarizing and hyperpolarizing phases was evoked.

3) The depolarizing phase, probably of polysynaptic origin, proved to be Na^+- and Ca^{2+}-dependent. The hyperpolarizing phase consisted of two components which could be differentiated on the basis of time course, dependence on membrane polarity and sensitivity to ionic environment. The fast component was Mg^{2+}-sensitive while the slow one Ca^{2+}- and Cl^--sensitive. Both of these components seem to be connected to changes in K permeability of the membrane, however, the K channels responsible for the fast and slow components are probably not identical.

The giant RPal neuron identified in the right parietal ganglion of the garden snail [18] is a characteristic example of the bimodal pacemaker type neurons. According to the activity pattern and location it is considered to be homologous [17] to the *Aplysia Br* [1] or R15 [3] neuron, to Cell 11 in Otala [4], F1 neuron in *Helix aspersa* [9] and a neuron of *Helisoma trivolvis* [6].

In the activity pattern of these neurons recorded after isolation of the ganglia postsynaptic potentials occur very rarely, however, by applying biologically active substances into the bath [20] or stimulating peripheral nerves [11, 15, 16] both inhibitory and excitatory postsynaptic potentials can be evoked on it. Even a long term modification of the bimodal pacemaker activity has been shown to occur following stimulation of the pleurovisceral connective in R15 neuron of *Aplysia* [14], stimulation of the left pallial nerve in F1 neuron of *Helix aspersa* [5] and in response to heart stimulation in RPal neuron of *Helix pomatia* [22].

The aim of the present investigations was to clarify

(1) which are the nerves originating from the suboesophageal ganglion the stimulation of which is able to evoke synaptic potentials on the RPal neuron;

(2) how do of these evoked postsynaptic potentials depend on the polarization of the neuron membrane;

(3) what is the effect of changes in the ionic environment on the different components of the postsynaptic potentials.

Materials and Methods

Experiments were carried out throughout the year on *Helix pomatia* L., collected locally and kept out of season in a cold room. The animals hibernating in winter were activated at room temperature by moisty surrounding and feeding.

The whole circumoesophageal ganglion was isolated and placed in an experimental chamber containing 3—4 ml physiological saline (NaCl, 3 g; KCl, 0.35 g; $MgCl_2 \cdot 6\ H_2O$, 2.4 g; $CaCl_2 \cdot 2H_2O$, 1.5 g; $NaHCO_3$, 0.2 g in 1000 ml distilled water). When isolating the ganglion the central 5—10 mm of the right and left pallial as well as of the anal nerves were kept intact. They were placed in suction electrodes and were separately stimulated 2—8 V square wave pulses of 2—5 msec duration. From the RPal neuron membrane potential, spontaneous activity and evoked potentials were recorded intracellularly using 2.5 mol/l KCl-filled glass microelectrodes. A bridge circuit was used for hyperpolarization and depolarization of the soma membrane [24].

During the investigations we did not stimulate the intestinal nerve, since the axon of the RPal neuron runs in this nerve [19] and we wanted to avoid antidromic stimulation.

When investigating the effect of ionic environment, the Na^+, Ca^{2+}, K^+ or Cl^- concentration of the saline was changed. Ca^{2+} and Na^+ was replaced by Tris while for changing Cl^--content we used acetate salts. In K^+-free saline the Na^+ concentration was increased equimolarly. For uncoupling the synaptic contacts 50 mmol/l $MgCl_2$ was added into the bath.

Test solutions were added to the chamber by a perfusion system, in many cases the perfusion was kept continuous during the whole experiment, lasting for 30—60 min.

Recording of electric signals was undertaken using a Helcoscriptor technical recorder with continuous oscilloscope monitoring.

Results

By stimulating either of the mentioned peripheral nerves originating from the suboesophageal ganglion a synaptic response including one or more components could be evoked on the neuron. Stimulation evoked both excitatory and inhibitory potentials (EPSP and IPSP). If stimulation was applied during the spontaneous burst of spikes, the activity of the neuron changed because of the appearance of the evoked inhibitory postsynaptic potential (Fig. 1). We could never observe separate EPSP, and whenever there was an initial depolarization (EPSP) it was followed always by hyperpolarizing components (Fig. 1b). IPSP, however, was evoked also without previous depolarizing potential. IPSP consisted of a fast and a slow component independently whether it was preceded by an EPSP or not (Fig. 1c).

The two components of the evoked IPSP could be differentiated easily by polarizing the soma membrane (Fig. 2). At hyperpolarization the amplitude of the fast component gradually decreased and reversed between —50—70 mV. The slow component decreased as well at hyperpolarization and became eliminated at —80—90 mV, however, no reversal could be achieved even with further hyperpolarization. When the amplitude of the components is plotted against the membrane potential values (Fig. 2b) only the curve of the first component

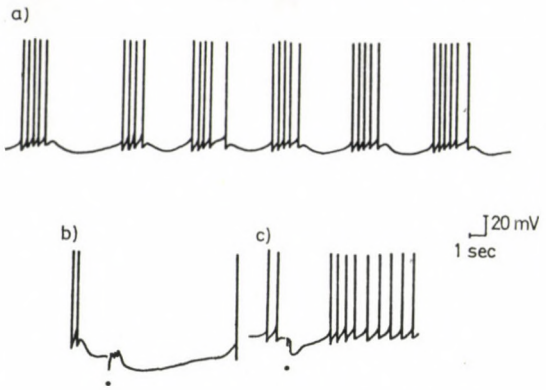

Fig. 1. Effect of stimulation of the anal nerve on the activity pattern of the RPal neuron of *Helix pomatia* L. a) Bimodal pacemaker activity; b) excitatory and inhibitory postsynaptic potentials evoked by a single pulse (dot); c) fast and slow IPSP without preceding EPSP

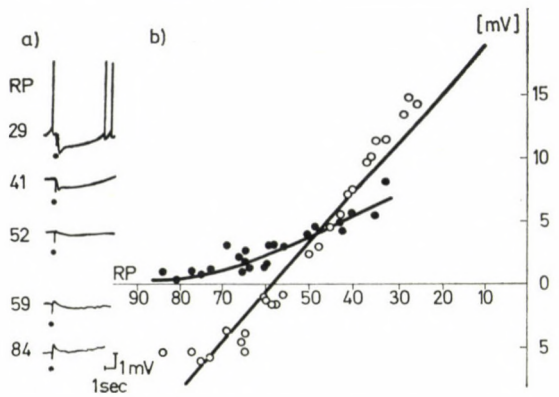

Fig. 2. Changes of the amplitudes of the fast and slow components of the IPSP evoked by stimulation of the right pallial nerve at polarization of the soma membrane. a) Evoked PSPs at different resting potential levels (RP); b) amplitudes (mV) plotted against RP. Open circles: fast IPSP; solid circles: slow IPSP

intersects the abscissa, the curve of the second component only approaching it, without any intersection.

Evoked postsynaptic potentials obtained on stimulation of different nerves were practically the same and changed similarly in various conditions of ion excess and reduction. However, slight differences were seen in different preparations.

Effect of high Mg^{2+}

According to data of others [2], treatment with high Mg^{2+} results in inhibition of the transmission through chemical synapses, therefore we investigated the fate of the evoked synaptic potentials in physiological solution

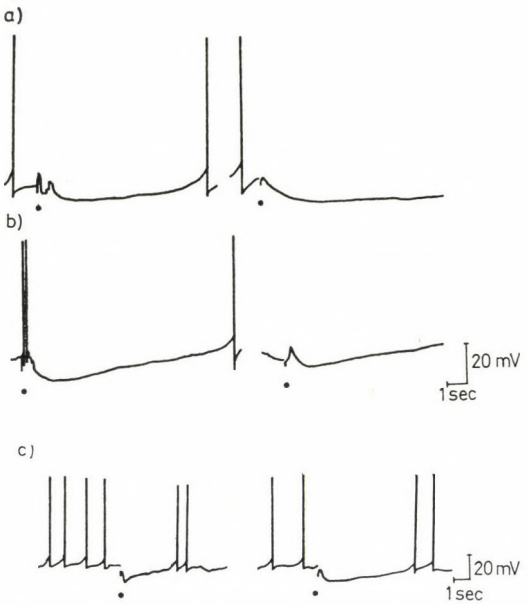

Fig. 3. Effect of high Mg^{2+} on the evoked PSPs. Left: control in physiological saline; right: in 50 mmol/l $MgCl_2$ added to physiological solution (after 5 min). a) Anal nerve stimulation; b) left pallial nerve stimulation; c) right pallial nerve stimulation

supplemented with 50 mmol/l $MgCl_2$. Under such conditions (*i.e.* with increased tonicity of the saline) the spontaneous activity of the neuron remained intact for a long time. The effect of Mg^{2+} on the PSP was time-dependent. Usually the EPSP decreased or was abolished within 5—10 min (Fig. 3a), and after a longer period (20—30 min) the remaining EPSP was further decreased. The effect was well pronounced on the EPSP evoked by stimulation of the right pallial and anal nerves, while the EPSP evoked by stimulation of the left pallial nerve proved to be more resistant (Fig. 3b). The fast component of the IPSP was eliminated as well, however, the slow component usually remained intact (Fig. 3a, b, c). In this respect the IPSPs evoked through different nerves behaved similarly.

Effect of reduced Na^+

In case the Na^+ concentration was reduced to 1/4 of the physiological level in the bath, the EPSP evoked by stimulation of the right pallial or anal nerves disappeared (Fig. 4a), however, the EPSP evoked by left pallial nerve stimulation remained well visible, although did not cause spiking in the soma (Fig. 4b). IPSP was not abolished, the slow component sometimes showing slight decrease or increase. In the absence of Na^+ synaptic potentials could not be evoked. Under such conditions spontaneous activity of the neuron was also blocked, action potentials were not generated, what was still observed

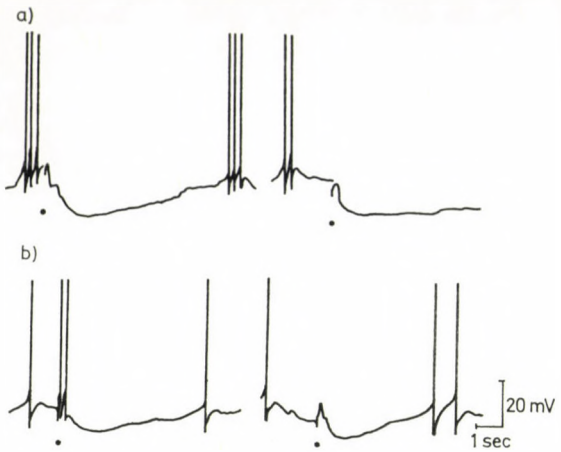

Fig. 4. Effect of reduced Na on the evoked PSPs. Left: control; right: in saline containing 12 mmol/l Na^+. a) Anal nerve stimulation; b) left pallial nerve stimulation

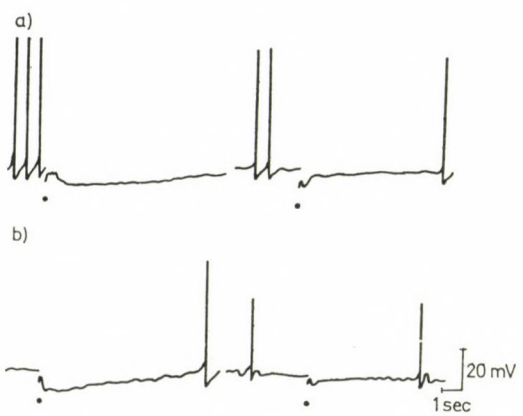

Fig. 5. Fast IPSP is present in Ca^{2+}-free medium. Left: control; right: in Ca^{2+}-free saline. a) In the control the evoked PSP is composed of EPSP and IPSPs; b) in the control the evoked potential is composed of IPSPs only

in the presence of 12 mmol/l Na^+. By replacing for physiological saline both spontaneous activity reappeared and evoked PSPs were also similar to the control.

Effect of lack of Ca^{2+}

In Ca^{2+}-free medium synaptic potentials evoked by the stimulation of either nerve changed in a similar way: EPSP disappeared, the slow component of the IPSP was eliminated or reduced to a large degree, while the fast component of the IPSP was affected only to a slight degree or did not change at all (Fig. 5a, b).

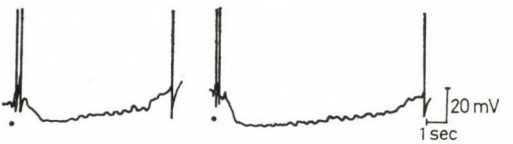

Fig. 6. Effect of K^+-free saline on the PSP evoked by stimulation of the left pallial nerve. Left: control; right: in K-free saline

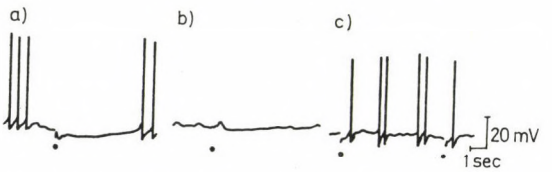

Fig. 7. Effect of Cl^--free saline on the PSP evoked by stimulation of the anal nerve. a) Control; b) in Cl^--free saline soma membrane is hyperpolarized to −120 mV, fast IPSP is reversed; c) in Cl^--free solution after polarization of the neuron back to −50 mV by current injection, only fast IPSP is present

In the absence of Ca^{2+} also the amplitude of the spikes was reduced and after a longer period (30 min) the cell activity was abolished. In this period synaptic potentials could not be evoked either.

When in Ca^{2+}-free saline the concentration of Mg^{2+} was increased to 50 mmol/l, both components of the IPSP were eliminated within 5 min.

Effect of lack of K^+

Evoked PSPs did not change noticeably in K^+-free medium, only the amplitude of the slow IPSP increasing to some degree (Fig. 6). By replacing for physiological saline this component was reduced significantly as compared to the control and was restored to the initial value only after a longer time (5—10 min).

Effect of lack of Cl^-

Cl^--free saline caused significant hyperpolarization (—100—120 mV) of the soma membrane which remained constant for a long period in this milieu.

The amplitude of the postsynaptic potentials became reduced, in other cases some of them were totally eliminated. The fast component of the evoked IPSP was not abolished, however, owing to the high degree of membrane hyperpolarization it became reversed, similarly to that what was observed with hyperpolarization caused by current injection (Fig. 2a). At this level of the membrane potential the slow component of the IPSP was not detected (Fig. 7).

Restoring the membrane potential value to the control by current injection the fast component of IPSP returned to normal, while the slow component was not observed in Cl^--free saline even under such conditions (Fig. 7c).

Discussion

The results obtained in the present investigations show that the neuron RPal receives inputs from a large peripheral area, since complex synaptic responses could be evoked by stimulation of the anal and both pallial nerves.

The natural peripheral origin of the inputs cannot be determined when using isolated ganglia; the converging nerves may include afferents from skin and muscle receptors as well as afferents from various organs. In earlier investigations it was shown that stimulation of the heart and kidney also causes synaptic bombardment on this neuron [22] proving that it receives inputs also through the intestinal nerve. These findings may lead to the suggestion that endogenous bimodal pacemaker activity is less characteristic to this neuron *in situ* than in isolated ganglion preparations.

Synaptic potentials evoked by nerve stimulation with single pulses consisted of several components with a clear dominance of inhibitory character. According to our results EPSP without consecutive IPSP never occurred. The slow component of the IPSP is dominant in influencing the activity pattern of the neuron since it brakes up the burst and/or considerably prolongs the interburst intervals. A similar or even longer inhibitory effect was described on stimulation of the pleurovisceral connective on the R15 neuron of *Aplysia* [15], and at stimulation of peripheral nerves of the F1 neuron of *Helix aspersa* [10] which are homologous with the RPal neuron of *Helix pomatia*.

The compound character of the evoked postsynaptic potential shows that several axons of the same nerve may have a connection on the neuron, however, the role of interneurons in the formation of some components cannot be excluded. This may be valid especially for the EPSP because the appearance of EPSPs on nerve stimulation was rather variable even in the same preparation and, in a number of cases EPSP could not be observed at all. The participation of interneurons in evoking EPSP is supported by the fact that on applying 5HT onto the ganglia EPSPs occur on the cell [20] possibly as a result of activation of other neurons.

The reduction and elimination of PSPs at hight Mg^{2+} concentrations points to the presence of chemical synapses. However, not all of the components were depressed in such environment and also PSPs evoked by stimulation on different nerves in certain cases changed differently with high Mg^{2+} concentration in the solution. The latter might be explained by supposing that different

nerves are connected with the cell in different regions of the ganglia and some of these connections, owing to anatomical characteristics, are not available for $MgCl_2$ even after 20—30 min exposition. On the other hand, the resistance of the slow component of IPSP to high Mg^{2+} is probably due to a general property of the synapse, although it does not seem probable that electrical coupling would be responsible for this component.

The depolarizing component of the evoked PSP may be considered in some cases to be the first phase of a biphasic PSP similarly to that described by PASIC et al. [16], in other cases, however, the independent character of the EPSP is beyond any doubt especially in cases where several EPSPs occur to one stimulus or, in Ca-free solution, when only the EPSP and the slow IPSP disappear while the fast component of the IPSP remains intact (Fig. 5a).

EPSP usually was also abolished at reduced Na^+ concentration while not chaning either in K^+-free nor in Cl^--free saline, indicating that its generation depends either on Na^+- or Ca^{2+}-ions. The resistance of EPSP evoked in low Na^+ by stimulation of the left pallial nerve shows that, in this case, Ca^{2+}-influx could have been responsible for it, since Ca^{2+}-free solution led to its total elimination.

The fast component of the IPSP was very sensitive to high Mg^{2+}. Of the evoked components this was reduced to the greatest extent and was eliminated firstly in the presence of 50 mmol/l $MgCl_2$. At the same time it was intensitive to the absence of Ca^{2+} and Cl^- and to the reduction of Na^+ in the medium. KEHOE [7] suggested that in the *Aplysia* neuron the fast inhibitory component depended on Cl^- permeability, however, this was not supported by JUDGE et al. [5] in the *Helix* neuron. Taking into consideration the value of the reversal potential measured at hyperpolarization of the soma (Fig. 2) it would be reasonable to attribute an important role to the Cl^- ions, however, the results obtained with Cl-free medium argue against this possibility. In voltage clamp experiments LEVITAN et al. [12] investigated the fast inhibitory component, however, they also failed in coming to an unanimous conclusion concerning the ionic basis of this component. It seems most likely that a transient increase in K^+ permeability is responsible for the fast hyperpolarizing phase, however, presently available pharmacological data do not seem to support this possibility.

The slow component is identical with the ILD (inhibition long duration) described by TAUC [23] in *Aplysia*. This component consistently evoked upon stimulation of all the three nerves showing that it is a characteristic response of the RPal neuron to external stimulation. This component was sensitive to Ca^{2+}- and Cl^--free solutions, it was not significantly influenced by high Mg^{2+} level and was enhanced in K^+-free solution. The latter observation indicates that its generation may be explained by assuming an increased K conduct-

ance as it was suggested by KEHOE and ASHER [8]. Ca^{2+} may play a secondary role in the process through regulation of K^+ permeability [13] while Cl^--free solution may depress the slow component by shifting the resting potential towards E_K. The change of K^+ permeability alone, however, is not sufficient to account for the fact that, upon hyperpolarization, the slow wave only disappeared but did not reverse even at a resting potential of —120 mV. Therefore it might be assumed that the effect of the liberated transmitter on the K^+ permeability is voltage dependent and does not activate K^+ channels in the region under E_K.

The absence of PSPs in Na-free saline cannot be interpreted as specific for the synaptic process, since under such circumstances the excitability of the membrane of the RPal neuron is lost, it fails to generate spikes and does not conduct [21]. On the other hand, reduction of Na^+ in the saline to 1/4 of the normal influences only EPSPs.

Our results show that upon stimulation of various nerves the fast and slow components of IPSP, in contrast to the appearance of EPSP, follow each other according to a well determined pattern. Nevertheless, these components exist independently, *i.e.* the appearance of the fast component is not a prerequisite for the generation of the slow component. However, they are not necessarily evoked by different transmitters. It may be assumed that, for the inhibition to occur in two steps, two types of receptors (channels) are required which would react with the same transmitter but in different voltage ranges and with different time courses.

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EXTRAJUNCTIONAL SPREAD OF ACETYLCHOLINE DEPOLARIZATION ON FROG SKELETAL MUSCLE MEMBRANE

By

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1. The extrajunctional spread of end-plate depolarization induced by acetyl) choline (ACh) applied in the bathing solution was studied on frog (*Rana esculenta*-skeletal muscle fibres.

2. The characteristics of this spread were determined on sartorius muscles mounted in a chamber where the end-plate free pelvic part was separated by a rubber membrane from the end-plate containing tibial one. There was no change in membrane potential if ACh was applied selectively on the pelvic part, even at a concentration of 10 mmol/l. However, on applying 1 mmol/l ACh on the tibial part, depolarization spreading over to the pelvic part was observed.

3. The peak values of ACh depolarization were not modified by tetrodotoxin (TTX) at a concentration of 31 nmol/l, whereas it was decreased by 35% in the presence of 3.1 μmol/l TTX.

4. ACh depolarization measured on the extrajunctional membrane in the presence of TTX is the result of the electrotonic spread of end-plate depolarization. The length constant of this spread was about the same as the length constant determined by square pulse analysis.

5. When the generation and propagation of action potentials accompanying ACh depolarization were not blocked either by TTX or Na-free Ringer solution, the depolarization values observed on the pelvic part were slightly higher than what could have been expected on the basis of electrotonic behaviour.

6. The above results imply the absence of ACh receptors from the extrajunctional membrane of twitch fibres. Deviations from the electrotonic spread are due to the action potentials accompanying ACh depolarization.

The membrane of skeletal muscle fibres is much more sensitive to ACh at the end-plates than in the extrajunctional region [2, 3, 4]. The density of special chemoreceptors responsible for this sensitivity decreases rapidly with increasing distance from the nerve endings. When ACh acts selectively only on the end-plates (for example in the case of iontophoretic application or indirect electrical stimulation at low tubocurarine concentrations) depolarization in the extrajunctional region is the result of the electrotonic spread of end-plate depolarization (end-plate potential) [5]. ACh, when applied in the bathing solution, may contact the membrane outside the end-plate, too, allowing an extrajunctional effect to develop. Such an effect may be manifested by changes in the electrotonic spread of end-plate depolarization.

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The existence of extrajunctional ACh effect has been suggested under different experimental circumstances. Cholinomimetic drugs caused depolarization in the extrajunctional membrane of sartorius muscles excised from *Rana pipiens* [14, 15] or from South American frogs [17], however, neither the extent nor the time course of this depolarization could be explained with the cable properties of fibres. KATZ and MILEDI [9] found that succinylcholine depolarization spread from the end-plates with a length constant slightly higher than the usual values measured with the square pulse method. Although no complete comparison was made, they could not exclude the possibility of extrajunctional depolarization being not simply an electrotonic spread. On the extrajunctional membranes of mammalian muscles depolarization induced by suxamethonium and not originating in the end-plates was described [18, 19].

The present experiments were carried out to study the characteristics of ACh depolarization on the extrajunctional membrane of frog sartorius muscles. It was established that ACh depolarization measured in the extrajunctional area was somewhat greater than what could have been expected from the passive character of the spread of end-plate depolarization. The difference was due to action potentials accompanying depolarization, consequently there is no reason to suppose any extrajunctional ACh effect.

Materials and Methods

The experiments were carried out on the sartorius muscles of frogs *(Rana esculenta)*. The actual membrane potential of the muscles was determined as the average of measurements performed on 5 or 10 muscle fibres with the conventional glass microelectrode technique. Only muscles having membrane potentials higher than −90 mV in Ringer solution at the beginning of the experimental procedure were used for further study.

ACh was used throughout the experiments in a concentration of 1 mmol/l since lower concentrations did not evoke measurable depolarization in the extrajunctional area. Due to its electrotonic spread the degree of depolarization on the membrane outside the end-plate decreases, even on the same fibre with increasing distance from the myoneural junction, therefore, the region for the measurements was chosen in accordance with the purpose of the experiments.

On applying ACh in the bathing solution depolarization arose immediately, then the repolarization of muscles started and in the continuous presence of ACh the membrane potential measured in the superficial fibres completely recovered within 20—30 min. Taking this time course into account in comparative measurements the degree of depolarization was always determined in the same period of ACh treatment.

We failed to evoke successive depolarizations with identical amplitude and time course on the same muscle. This is why muscle pairs from the same frogs were used for comparative measurements. ACh depolarizations elicited on the members of the muscle pairs exhibited identical parameters if measured in identical regions of the muscles. We measured for example the depolarizations evoked by 1 mmol/l ACh in the pelvic one-fifth part of the muscle, between the 3rd and 6th minutes of treatment. The corresponding values were 11.03 ± 5.86 and 10.67 ± 7.05 mV, the difference between the members of the pairs being not significant (0.37 ± 1.91 mV; 9 experiments). ACh depolarization showed seasonal variations and the ACh sensitivity of sartorius muscles excised from different frogs varied even during the same season.

The length constant of electrotonic depolarization was measured on whole muscles in the presence of TTX (31 nmol/l). An intracellular microelectrode was used to evoke electrotonic depolarization, two recording microelectrodes were located at 20—50 μm and 2—3 mm from the stimulating one. After measuring electrotonic depolarization, the stimulating micro-

electrode was removed and the muscle was superfused with a bathing solution containing 1 mmol/l ACh, then the degree of the following depolarization was measured. Since the position of the two recording microelectrodes did not change during this procedure it was possible to compare directly the spread of the two types of depolarization. The length constant (λ) was calculated from the equation

$$\Delta V^2_\infty = \Delta V^1_\infty \, e^{-x/\lambda}$$

where ΔV^1_∞ and ΔV^2_∞ are the maximum values of depolarization measured by the recording microelectrodes located at 2—3 mm from each other (x). When required, the end-plates were identified with the indirect stimulation of the sartorius nerve preparation in the presence of 0.003 mmol/l tubocurarine [5], then the muscles were washed carefully to avoid the blocking effect of curarine.

The spread of ACh depolarization was studied in other experiments using a chamber [8] suitable to separate the pelvic end-plate free area of frog sartorius muscle (aneural part) from the tibial one (neural part). The rubber membrane inhibits the diffusion of the solutions in which the two parts of the muscle are separately incubated while not affecting the propagation of action potentials or the electrotonic spread of different depolarizations from one side to the other. The pelvic one-fifth part of the frog sartorius muscle is practically free of end-plates [16], therefore, ACh applied on the neural part cannot evoke depolarization even at a concentration of 10 mmol/l (the average value from 5 experiments: 0.0 ± 0.51 mV). ACh depolarization developing in the neural part spreads over to the aneural part. The characteristics of this spread were investigated in bathing solutions of different compositions. The membrane potential of muscle fibres in the aneural part was measured in each case at 1.5—2 mm from the separating rubber sheet. For comparative measurements pairs of sartorius muscles were used. The muscles stretched to the four-thirds of their slack length were mounted in the separating chamber, taking special care to place the rubber membrane at the same distance from the pelvic end (at the edge of the pelvic one-fifth part). Separate experiments were carried out to decide whether the depolarizations spreading over to the aneural parts of two muscles excised from the same frog are identical. Using 1 mmol/l ACh in the neural parts we obtained values of 7.51 ± 1.87 and 7.81 ± 1.41 mV on the aneural parts incubated in Ringer solution. There was no significant difference in depolarization between the members of the muscle pairs (0.30 ± 1.94 mV; 16 experiments).

The experimental solutions were of the following composition (in mmol/l) = *Ringer solution:* NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85. *Choline Ringer:* Choline Cl, 120; $CaCl_2$, 1.8; K_2HPO_4, 1.08; KH_2PO_4, 0.43. The choline chloride (Reanal Co. Budapest, Hungary) was recrystallized twice before use. ACh was a preparation of Hoffman—La Roche (Basle, Switzerland) TTX was manufactured by Sankyo Co. Ltd (Tokyo, Japan). The pH of the solutions was 7.0 ± 0.05; the experiments were performed at room temperature (20—22 °C).

The scattering of the means represents the standard deviation (SD) and is shown as vertical bars on the diagrams. The significance of differences was calculated by Student's paired *t* test.

Results

Effect of TTX on ACh depolarization

The twitches accompanying the generation of action potentials after the application of ACh rendered the study of the depolarizing effect of ACh more difficult. Therefore, the use of TTX seemed to be reasonable, since this compound does not prevent ACh action on the end-plates [7, 13]. In preliminary experiments we found that TTX decreased the degree of ACh depolarization in the extrajunctional area thus, without aiming at completeness, we tested the effect of two different TTX concentrations (31 nmol/l and 3.1 μmol/l). The concentration of 31 nmol/l was found to be the smallest value to inhibit the generation of electrically evoked action potentials after pretreatment for 1 hour.

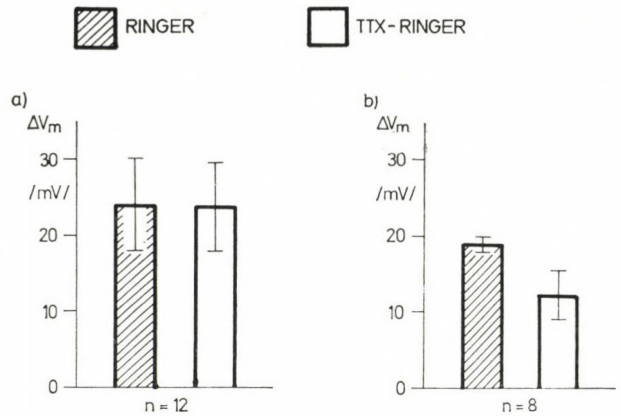

Fig. 1. Effect of 31 nmol/l (A) and 3.1 μmol/l (B) TTX on ACh depolarization. Measurements were performed on sartorius muscle pairs, on the middle one-third part of the muscles. Depolarization was elicited by 1 mmol/l ACh on the control members of muscle pairs (RINGER) and after incubation with TTX for 1 hour (TTX—RINGER). Depolarization was measured between the 1st and 4th minutes of ACh treatment

Results from measurements in the middle one-third part of whole sartorius muscles are shown in Fig. 1. The application of 1 mmol/l ACh depolarized the control members of the muscle pairs by 23.89 ± 6.14 mV on the average. On the other members preincubated with 31 nmol/l TTX for 1 hour, depolarization was 23.75 ± 5.67 mV (Fig. 1A). In experiments with 3.1 μmol/l TTX the corresponding values were 18.83 ± 1.12 mV in Ringer solution and 12.25 ± 3.30 mV in the presence of TTX (Fig. 1B). It is obvious that TTX at lower concentrations did not decrease the degree of ACh depolarization, whereas 3.1 μmol/l TTX gave rise to considerable (35%) inhibition ($p < 0.001$). To avoid this inhibition in further experiments TTX was used in a concentration of 31 nmol/l.

The electrotonic character of extrajunctional ACh depolarization

While studying the characteristics of ACh depolarization we investigated whether the ACh depolarization at the end-plates spreads over to the extrajunctional membrane in a passive way determined by the cable properties, or else the presence of ACh in the bathing solution can modify the features of this process. Our results from experiments performed in the separating chamber in the presence of TTX are presented in Fig. 2. TTX was added to prevent the generation of action potentials. Depolarization was elicited by 1 mmol/l ACh on the neural parts of both muscles and the changes in membrane potential on the aneural parts were measured. The aneural part of the control members of the muscle pairs (muscles A) was bathed in Ringer solution, therefore the depolarization developing here could be regarded as a result of the electrotonic spread of ACh depolarization from the neural part. The

aneural part of the other member (muscles B) was treated with 1 mmol/l ACh before eliciting ACh depolarization on the neural part. In this case the depolarization starting from the end-plate spread over to the extrajunctional membrane pretreated with ACh. The depolarization values of the aneural parts of muscles A and B were the same (8.46 ± 0.85 and 9.06 ± 1.14 mV, respectively), and similarly identical depolarization values were obtained on the neural parts (25.97 ± 2.55 and 24.94 ± 2.39 mV). The difference was not significant in either case. These results show that ACh applied in the bath depolarizes the end-plates and it is only the passive electrotonic spreading of this depolarization that is observed on the extrajunctional membrane.

In our further experiments the length constant of this spread was determined and compared to the length constant of the electrotonic depolarization evoked by constant current stimulation. Figure 3A shows that the two kinds of depolarization had about the same length constant value on the pelvic end of whole sartorius muscles: 3.85 ± 0.93 and 4.25 ± 1.51 mm. The difference is not significant ($0.5 < p < 0.2$) indicating that extrajunctional ACh depolarization decreases with increasing distance similarly to electrotonic depolarization.

In a few experiments performed for comparison one of the recording microelectrodes was located at the end-plate and the other one at 2—3 mm

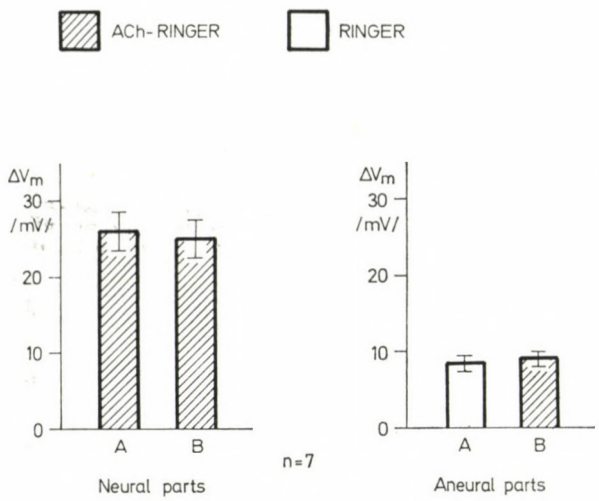

Fig. 2. Spread of ACh depolarization over to the aneural part of sartorius muscles in the presence of TTX. Experiments were performed on sartorius muscle pairs mounted in a separating chamber after pretreatment with 31 nmol/l TTX for 1 hour. Depolarization was evoked on the neural part of both members (muscles A and B) of muscle pairs by applying 1 mmol/l ACh (ACh—RINGER), then measurements were made for depolarizations spreading over to the aneural parts bathed either in Ringer solution (RINGER) or in Ringer solution containing 1 mmol/l ACh (ACh—RINGER). Measurements took place on the aneural parts between the 1st and 3rd minutes of ACh treatment, then on the neural parts between the 4th and 6th minutes of treatment

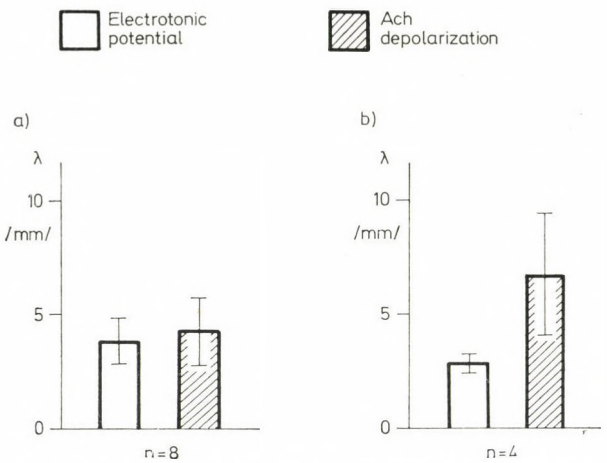

Fig. 3. Spread of ACh depolarization compared to the spread of electrotonic depolarization. The experiments were carried out in the presence of 31 nmol/l TTX on whole sartorius muscle fibres, at the pelvic ends (A) and at the end-plates (B). The length constant of spread (τ) was calculated from the maximum values of the two kinds of depolarization. (See text for details)

from it. The decrease in electrotonic depolarization was found similar to that observed on the extrajunctional membrane, however, the decrease in ACh depolarization was less, *i.e.* the value of $\lambda(6.73 \pm 2.71$ mm) was twice as large as in the case of electrotonic depolarization ($\lambda = 2.87 \pm 0.41$ mm); ($p < 0.02$). The increase in the λ value is well explained by the changes in ionic conductance developing at the end-plate membrane due to ACh treatment.

The role of action potentials

Previous data in the literature suggest that ACh applied in the bathing solution causes depolarization to spread on the extrajunctional membrane to a greater extent than what could be predicted on the basis of electrotonic behaviour [14, 15, 17]. We, therefore, investigated in further experiments whether the presence of action potentials influences the extent of ACh depolarization on the extrajunctional membrane.

The propagation of action potentials was inhibited either by 31 nmol/l TTX or by Na-free (Choline) Ringer solution. The effect of TTX treatment was analysed in experiments using whole sartorius muscles. The degree of ACh depolarization developing on the pelvic end of the fibres was measured in Ringer solution on the control members of the muscle pairs and in the presence of TTX on the other ones. Figure 4A shows that the value of depolarization was 11.36 ± 3.22 mV in Ringer solution and 7.86 ± 2.42 mV after TTX pretreatment, the difference being significant statistically ($p < 0.001$). Consequently, if the generation and propagation of action potentials are not prevented, the degree of depolarization evolving on the extrajunctional area is greater.

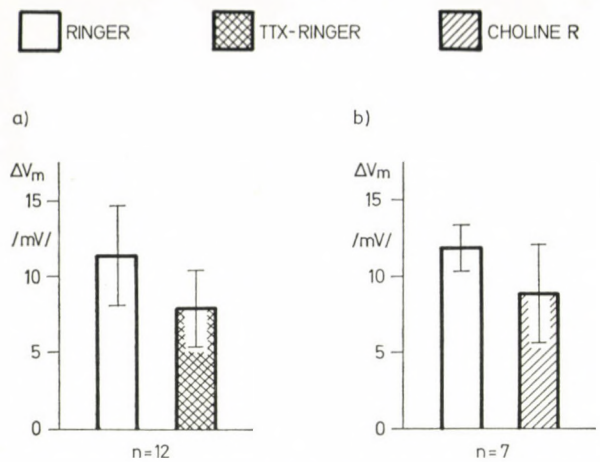

Fig. 4. Spread of ACh depolarization in the presence and absence of action potentials. A: 1 mmol/l ACh treatment was performed on control muscles in Ringer solution (RINGER) and on the other members of muscle pairs in the presence of 31 nmol/l TTX (TTX—RINGER) Depolarization developing on the pelvic end of the whole muscle fibres was measured between the 1st and 4th minutes of ACh treatment. B: 1 mmol/l ACh depolarization was evoked on the neural parts of sartorius muscle pairs mounted in a separating chamber. The degree of depolarization spreading over to the aneural parts bathed either in Ringer solution (RINGER) or Choline Ringer (CHOLINE R) was measured between the 1st and 3rd minutes of ACh treatment. Pretreatment of the aneural part with Choline Ringer changed several times lasted for half to 1 hour, during this period the Ringer solution at the neural parts also was changed

Somewhat different experiments were performed in the separating chamber. In both members of the muscle pairs ACh depolarization was elicited on the neural part bathed in Ringer solution then the value of the depolarization spreading over to the aneural part incubated either in Ringer solution or Na-free Choline Ringer solution was determined. Action potentials were generated on the neural parts of both muscles, but spreading was possible only over to the aneural parts bathed in Ringer solution.

Figure 4B shows that depolarization was found to be 11.83 ± 1.40 mV on the aneural part kept in Ringer solution. Simultaneously, on aneural parts incubated in Choline Ringer we found depolarization of 8.89 ± 3.29 mV, the difference between the members of muscle pairs being significant ($p < 0.01$). It has been concluded that the decrease in the extent of ACh depolarization may be explained by the absence of action potentials since Choline Ringer does not affect the cable properties of muscle fibres.

Discussion

Our results indicate that there are no ACh sensitive receptors on the extrajunctional membrane of the frog skeletal muscle. ACh applied on the end-plate free muscle membrane produces neither depolarization nor modifica-

tion in the electrotonic spreading of depolarization developing at the end-plate. These findings are consistent with the generally accepted view that sensitivity to ACh of twitch muscle fibres decreases rapidly with distance from the myoneural end-plate region. According to MILEDI's [13] investigations performed with iontophoretically applied ACh there is a 10,000-fold difference between the ACh sensitivity of the end-plate and that of the extrajunctional area.

The spread of ACh depolarization was studied in a chamber containing a rubber sheet which allowed the separation of the aneural part of the muscle from the neural one without modifying the physiological properties of the muscle membrane [8]. This fact may explain the discrepancy between our results and those obtained with other separating techniques, furthermore the importance of species differences cannot be excluded either [18, 19].

When the generation and propagation of action potentials are not prevented the value of ACh depolarization on the extrajunctional membrane is greater than it would be expected from the cable properties of muscle fibres. Under our experimental conditions recording of ACh depolarization took place when repolarization had already begun. Therefore, the depolarization increasing effect of action potentials is essentially identical with the delay in repolarization. A possible explanation for this delay is potassium accumulation in the T tubules. Potassium channels responsible for both resting and delayed rectification are present in the membrane of T tubules [1, 11]. Action potentials can increase tubular potassium concentration, and thus, delay repolarization as it was observed subsequent to tetanic stimulation [6]. However, the number and frequency of action potentials propagating on muscle fibres in connection with ACh depolarization are not known, thus, we are unable to prove the above assumption. The number of action potentials is, presumably, not too high, however, it is also necessary to consider the finding that ACh depolarization increases the rate of potassium efflux in the presence of TTX [12]. In summary we conclude that the action potentials can be held responsible for the slight deviation of extrajunctional ACh depolarization from electrotonic spread, so there is no need to suppose any extrajunctional ACh effect.

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A COMPARISON OF EXPERIMENTAL PROCEDURES OF INVESTIGATION OF THE DORSAL ROOT EVOKED VENTRAL ROOT REFLEX IN THE FROG

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Effects of the drug methyl-m-aminobenzoate (MS-222 Sandoz) on the dorsal root evoked ventral root responses were studied by electrophysiological methods in the frog spinal cord. A fairly quick and marked depression of the response was observed from which complete recovery was seen within 60 minutes. Larger doses or repeated injection of small amounts of the drug prolonged the recovery and the monosynaptic discharge component of the ventral root reflex often deteriorated irreversibly. — In further experiments, monosynaptic ventral root discharges were demonstrated in spinal cords isolated from the vertebral canal and kept in Ringer solution. The results are discussed in the light of controversial views about the occurrence of monosynaptic ventral root discharge to stimulation of the primary afferents in the amphibian spinal cord.

The advantage of experimenting on isolated anuran spinal cords have long been recognized [6, 15, 23, 24, see SIMPSON, 18, for further references]. Virtually all supraspinal and spinal input can be eliminated by removing the spinal cord from the vertebral canal, and attention may be concentrated on the responses evoked by stimulation of any of the roots. Drugs are not needed for anesthesia or immobilization, while the effects of various chemical compounds as well as of variations in the ionic environment can be investigated.

At least one important difference emerges from a comparative survey of results obtained from isolated and *in situ* spinal cords [18], namely, the monosynaptic discharge of motoneurons in response to single volleys in primary afferents is known to occur in spinal cords *in situ* [4, 5, 13] while many authors have failed to produce such a monosynaptic reflex in isolated spinal cords [2, 3, 8, 9, 17]. A possible reason for this may lie in the very fact of the spinal neuronal networks being isolated from supraspinal and peripheral inputs. Another likely explanation may be some persisting effect of anaesthetics or relaxants. A third important reason probably comes from species differences since a variety of frogs and towards are used in different laboratories.

Obviously, one needs strict criteria for judging maintenance of the "physiological condition" of the preparation before drawing conclusions on the reasons of differences in experimental findings. A criterion we have found

useful in studying the segmental reflexes in the frog [4] is the occurrence of well synchronized discharge of motoneurons following dorsal root (DR) volleys with an about 4 ms segmental delay. In other words, our criterion is the phenomenon denied to occur in isolated spinal cords. In the present work we have investigated whether monosynaptic discharges can be demonstrated in isolated cords as well as in frogs dissected differently from our usual procedure.

Materials and Methods

Spinal cords in situ. Adult frogs (*Rana esculenta*) weighing 80—100 g were selected, anaesthetized by ether, spinalized by a mid-medullar section and the lumbar segments were exposed by dorsal laminectomy. Either the 10th DR, or the de-efferented sciatic nerve was prepared for stimulation. Ventral root (VR) 9 and 10 were cut in the vertebral canal and prepared for recording. The contralateral hindlimb was denervated and the frog was mounted on a heavy metal frame. The effect of the ether was usually over within 25—30 min, as indicated by spontaneous movements or vigorous flexions in the forelimbs to pinching the skin. Most of the body surface was immersed in a water bath oxygenated and maintained at 17—19 °C.

Isolated spinal cords. Smaller weighing 50—70 g frogs were kept in icy cold water for 20—40 min, then decapitated and quickly eviscerated. The vertebral column together with the lumbar spinal nerves were cut out from the body and put in cold, oxygenated Ringer solution. In this solution the spinal cord was removed from the vertebral canal, all roots were cut next to the cord except the roots of segment 10, which were prepared for mounting on hook electrodes. The cord was then moved into a recording chamber where it was continuously superfused with oxygenated Ringer solution (NaCl, 114 mM; KCl, 2 mM; $NaHCO_3$, 2 mM; $CaCl_2$, 2 mM; glucose, 1 g pro liter; pH, 7.2; temperature, 9—15 °C). The roots of segment 10 were mounted on bipolar platinum electrodes and sealed with a mixture of petroleum jelly and liquid paraffin.

Stimulation and recording. Rectangular pulses of 5 V or less and 0.1 ms were applied through isolation transformers to the primary afferents. Responses from the 9th or 10th VR were recorded by a differential preamplifier and led to one channel of an oscilloscope. Another preamplifier connected to the other channel monitored the incoming DR volley on the dorsal surface of the 10th segment by a glass microelectrode.

Results

Figure 1 shows a typical example of the DR evoked reflex in a frog prepared as in our previous experiments, described in the first section of Methods. A slow potential component (called VRP) underlied the faster peaks attributed to discharges in the motoneurons. This slow component could only be detected if the recording leads were close enough to the cord to pick up a significant portion of the electrotonically spreading postsynaptic depolarization of the ventral horn cells [6]. We considered a reflex good enough to meet our criterion when the first fast component took off from the slow VRP with a segmental delay no longer than 4.5 ms at 17—19 °C, and reached a peak amplitude at least twice as big as the subsequent largest peak [4]. Segmental delay was defined as the time from the negative peak of the afferent wave to the onset of the VR discharge. We put the recording electrodes as distant as possible from the cord for ease of separating the discharge component from the rising

phase of the slow VRP, which we found quite slow and small at distance of 6—7 mm.

The DR evoked VR discharge in the amphibian spinal cord is generally known as different from those shown in Fig. 1. According to BROOKHART et al. [3] the typical segmental delay was 6.0—8.9 ms, and they described the

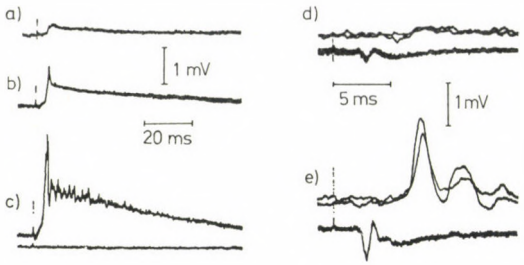

Fig. 1. Typical VR responses in lumbar segments of the frog to primary afferent volleys. Left: responses in the 10th VR to volleys in the 10th DR. The intensity of stimulus shock is increased from a to c. The early discharge as well as the slow VRP grows with stronger stimulation and becomes maximal with about 3 times threshold intensity. DC recordings. Right: pairs of simultaneously recorded DR and VR responses to volleys in the sciatic nerve. All lumbar VRs were cut distal from the recording electrodes. d: near threshold intensity of stimulation for VR response. e: Responses to 3 times stronger pulses

response as asynchronous, without a well delineated early component. SIMPSON [18] listed a number of subsequent works which confirmed the original description. We have also seen such type of VR responses (Fig. 3), more often in frogs with rather poor circulation in the dorsal blood vessels of the spinal cord. Thus sufficient oxygen supply appears a very important, although likely not the only prerequisite for successful demonstration of the monosynaptic discharge. Asynchronous late components followed the large monosynaptic peak regularly in our material as well.

The monosynaptic peak of the VR response could regularly be elicited during the 6—10 hours of recording sessions. If often changed in amplitude while not in latency and the first peak was always larger then the late components. Furthermore, similar well synchronized discharges could be recorded also from the VR adjacent to the stimulated segment, from the deafferented spinal nerves 10 or 9, and from the de-afferented peripheral nerves of the hindlimb extensors. These findings contradict earlier beliefs [7, 12] that synchronous firing to DR volleys in the frog motoneurons be a rare, transient phenomenon without any significant role in the motor performance of hindlimb musculature.

Effect of MS-222 upon the discharges of motoneurons

Ethyl-m-aminobensoate (MS-222 Sandoz, Tricaine, Finquel Ayrest) is a widely used drug for immobilization of cold blooded animals. Many authors use it for ease of handling and dissection of the frog, thus one wonders how this drug affects the DR evoked responses of the motoneurons.

A group of frogs (n=13) was prepared as usual under ether anaesthesia and left to recover completely. Five to eight hours after cessation of ether treatment, control records were taken from the VR responses to primary afferent volleys and MS-222 (E 1626 Sigma), 0.1—0.3 mg/kg body weight was injected intraperitoneally or intravenously.

Doses of 0.1—0.2 mg/g depressed the VR discharges remarkably within 8—10 min. The depression was reversible and complete recovery occurred within 60 min in 10 out of 13 cases (Fig. 2).

Recovery from the depression induced by 0.3 mg/g was longer (about 3 hours) and often incomplete in several aspects (Fig. 3). For example, the amplitude of the first component of the discharge remained relatively small, the latency of the discharges became longer, and the reflex decreased more

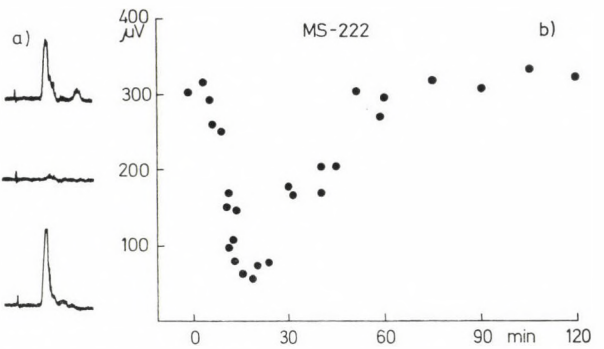

Fig. 2. Effect of MS-222 on the VR discharge. a: VR responses from a spinal cord *in situ*, after 4 hours of initial ether anesthesia. Top.: control, taken before the drug injection. Middle: response to the same DR stimulus when the effect of 0.1 mg/g MS-222 was most pronounced. Bottom: recovery, the record was taken about 60 min after the drug injection. b: peak amplitudes of the VR discharges plotted against time after the MS-222 injection. Sample records from this experiment are shown in a

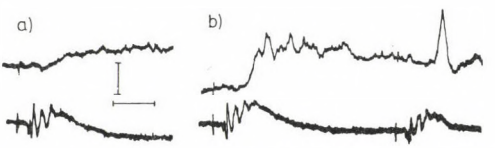

Fig. 3. Example of persisting effect of MS-222. a: VR response recorded about 20 minutes after the injection of 0.3 mg/g MS-222. b: 3 hours later, responses to a pair of DR volleys of the same intensity. Upper trace, VR recording; lower trace, monitoring of the DR volley, Calibration: 1 mV and 10 ms

quickly with repetition of testing, than in the control experiments prior to MS-222 treatment.

A second 0.1—0.2 mg/g injection at 20—30 min after the first 0.1 mg/g revealed some potentiation: the recovery from the effect of the second injection was sometimes extremely slow. Since 2 out of the 6 frogs treated with a total of 0.3 mg/g MS-222 showed incomplete recovery even at 4 hours after the injection, larger doses were not tested.

Textbooks recommend 0.1—0.4 mg/g MS-222 for surgery [14], or simply to put the animal into 1:5000 solution of the drug until movements stop. We do not know to what extent were the experiments interfered with by the fact that we gave the MS-222 to frogs which apparently recovered from ether anesthesia. However, it seems unlikely that ether could enhance the elimination of MS-222.

We conclude therefore that the early, well synchronized discharge of the motoneurons often, but not always, recovers from the deep depression produced by MS-222. Although individual sensitivity seems to vary considerably, monosynaptic DR—VR reflex can usually be evoked about one hour after the administration of small doses of MS-222. However, neither a second small dose, nor a single larger one can safely be given in the hope of certain, complete, and reasonably rapid recovery. These observations are supported by experiences from another series of experiments [20], where about 50 frogs were dissected under without initial ether treatment.

Experiments on isolated spinal cords

Methodology of experiments on isolated spinal cords seems to be similar in different laboratories except some less important technical details. We have adopted the technique used for years at the Institute of Physiology, Czechoslovak Academy of Sciences, Prague [20, 21].

Our own, rather limited experience in this field allows three statements so far: (i) VR responses to DR volleys occurred characteristically (in 12 out of 15 cases) without an early, well synchronized discharge component. In other words we confirm the results of other authors (see 18) on this point. (ii) In 3 cases, however, well synchronized early discharge could be demonstrated. (iii) In these latter groups, the discharge component decreased in amplitude soon, and after 10—30 min, the early large component become similar to, or even smaller than, the late components of the response.

Figure 4 shows a reflex characteristic for the isolated spinal cords. The latency is about 7 ms, the first peak is smaller than the second one (Fig. 4a). A few minutes later the same stimulus evoked the response shown in Fig. 4b. The deterioration of the discharge components in general was clear, the slow VRP component dominated. This type of response persisted for hours.

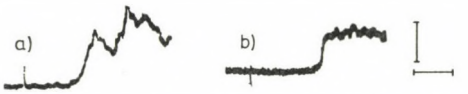

Fig. 4. Typical VR responses in isolated spinal cords. a: in the first minutes of the recording and b: about 10 minutes later. Note that the early component of the response in a fails completely in b. Calibration: 1 mV and 5 ms

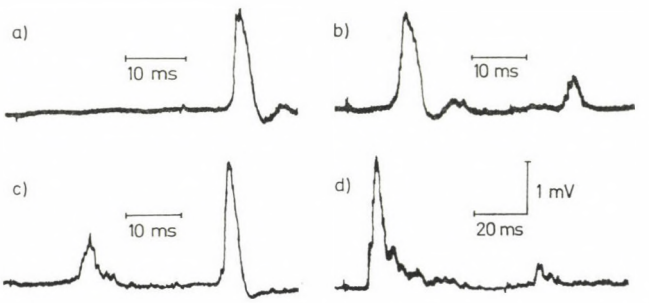

Fig. 5. Demonstration of large monosynaptic VR response in isolated spinal cords. a: response from the 10th VR to a single volley in the 10th DR at 9 °C. b: the same DR volley induces a much smaller response after a large response evoked by a conditioning DR 10 volley. c and d: another spinal cord. c: responses to a pair of DR 10 volleys when the conditioning stimulus is weak, the test response when the intensity of the first shock is increased to evoke maximal VR response. The more synchronous wave in the test response in c as compared to the first response in d is evaluated as indicating facilitation of the monosynaptic discharge of the motoneurons

Figure 5 shows responses recorded in two spinal cords during the initial period when large early discharge could be induced in them. The latency (8 ms at 9 °C) was not too long in view of a Q_{10} of about 2. Cooling by 10 °C increased the latency of VR responses by a factor of about 2 in the spinal cords *in situ* well [22].

Double volley experiments revealed another phenomenon which was not typical in isolated cords, but was often found in spinal cords *in situ* [4]. When the conditioning DR volley produced a near maximal VR response, the discharge induced by a second, similar volley given 20—40 ms after the first one was quite small (Fig. 5b and d). DR evoked VR discharges are known to be facilitated by a conditioning DR volley in the frog [13, 18]. We confirmed this observation by using low intensity conditioning volleys inducing submaximal VR responses (Fig. 5c) [4].

Discussion

The main conclusion of the present experiments is that monosynaptic discharge of motoneurons can be evoked by single DR volleys after MS-222 treatment as well as in isolated spinal cords. The phenomenon may thus be used to judge the "physiological condition" of the preparation.

Monosynaptic discharge occurs in about 70% of the spinal cords studied [4] when using our original method (*i.e.* ether anesthesia) and *in situ* spinal cords with fair blood supply). Only about 50% of the spinal cords treated with MS-222 give good monosynaptic discharges, nevertheless this ratio is apparently influenced by the preceding ether anesthesia in these experiments. We have failed to detect monosynaptic VR discharges in 80% of the isolated spinal cords and the remaining 20% gave monosynaptic response for only a relative short initial period of investigation. The high percentage of failures indicates that much remains to be improved in the experimental procedures in using isolated spinal cords. On the other hand, these observations emphasize the importance of technical factors and suggest that physiological and biochemical events involved in the primary afferent motoneuron coupling in the frog deteriorate much easily than for example the those involved in the polysynaptic excitation or antidromic invasion of the motoneurons.

Deterioration may occur either pre- or postsynaptically. The primary afferents branch densely when enter the segment [1, 19] and part of the fine preterminals may fail to conduct impulses, resulting in a decrease of excitatory inputs of the motoneurons. In the terminals the amount of transmitter actually released may decrease because of poor efficiency of the releasing mechanism or depletion of the transmitter available. Factors which influence these processes have been studied in detail for example in the neuromuscular junction [11]. On the postsynaptic side, dendrites which receive the majority of the DR terminals, may fail to generate depolarizing current sufficient to initiate monosynaptic action potentials. Relative decrease of the background level of excitation in the motoneurons can also make DR—EPSPs insufficient to fire the cell. Persisting effect of MS-222 and/or interruption of sensory and supraspinal pathways may result in a considerable decrease of spontaneous activity of the interneurons and a consequent loss in background excitation of the motoneurons.

We do not use the term deterioration to imply irreversible block of responsiveness in a part of the neuronal elements. If the silent interneurons are activated by a presynaptic volley the level of background excitation will probably increase in the motoneurons for a period during which a subsequent similar presynaptic volley can induced monosynaptic firing [13] and Fig. 5c. Of course, mechanism of this facilitation may have additional factors *e.g.* an increase in transmitter release caused by the conditioning [10, 16].

We are reluctant to assume that failure in finding good monosynaptic discharge in other laboratories is entirely due to technical reasons. However, these technical reasons may become crucially important when one defines what is typical in a series of experiments. The likely involvement of species differences remains to be demonstrated.

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OF POST-DENERVATION HYPERTROPHY IN CHICK SLOW MUSCLE FIBRES AFTER COMPLETE ELIMINATION OF STRETCH AND TENSION

By

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For the slow muscle fibres in the pure slow muscle ALD as well as in two mixed muscles, flexor metacarpi ulnaris and sartorius, it is demonstrated that the phenomenon of post-denervation hypertrophy still occurs under conditions preventing stretch of muscle after denervation or even in tenotomized muscles in shortened state relieved of tension. Striking differences between the slow fibres of the ALD and those of the mixed muscles are seen, both with respect to their histochemical property and to the degree of development of post-denervation hypertrophy. The slow fibres in mixed muscles show the phenomenon of post-denervation hyperthrophy in a much more striking form than do the ALD fibres, and should be used in further studies of the phenomenon.

The phenomenon of long-lasting post-denervation hypertrophy in the slow muscle, anterior latissimus dorsi (ALD) of the chick first described by FENG et al. [2] has since repeatedly been observed by a number of other investigators. In their studies on the phenomenon, FENG et al. were aware from the outset that the incidental drooping of the wing and consequent stretch of the muscle caused by the denervation operation, might be an important factor in the development of hypertrophy, and they made careful studies to establish whether denervation would still cause hyperthrophy in the chick slow muscle fibres if the influence of stretch was excluded. They adduced two lines of evidence to show that this was so. First, in the case of ALD, they showed that the wing drooping occurred only during the first 1—2 weeks after the operation, while the post-denervation hypertrophy persisted for more than 8 weeks, and that the prevention of wing drooping did not abolish but only reduced the development of post-denervation hypertrophy. Secondly, they showed that the post-denervation hypertrophy was not unique to ALD, but was apparently a general phenomenon shown by the slow muscle fibres of the chick which outside the ALD, were found scattered in various muscles of mixed fibre composition. FENG et al. in their first report on the subject demonstrated this with the biventer cervicis and later [3] also with the sartorius muscle, this time using botulinus poisoning instead of denervation. With the mixed muscles there was no apparent incidental stretch resulting from their denervation, so the striking hypertrophy shown by their slow fibres following

denervation seemed to be clear evidence of it being a specific effect of denervation.

In spite of the evidences adduced by FENG and his collaborators for the specific nature of post-denervation hypertrophy in the chick slow muscle fibres, we find the assertion repeatedly made in the literature [7, 10, 11, 12] that stretch is apparently an essential factor in the development of post-denervation hypertrophy. One piece of evidence often quoted in support of this view is the report by JIRMANOVÁ and ZELENÁ [7] that in tenotomized ALD denervation caused atrophy instead of hypertrophy. We have therefore performed a further study of post-denervation hypertrophy in the chick slow muscle fibres with particular reference to the question whether stretch is really essential to its development. Our work includes a repetition of JIRMANOVÁ and ZELENÁ's tenotomy experiment on the ALD and further observations on another mixed muscle of the chick, the flexor metacarpi ulnaris, in which denervation naturally produces very little change in length and thus, a control of muscle length is especially easy. The histochemical method for myosin ATPase was used throughout; it allowed the relation between the type of muscle fibres and the type of trophic response to denervation to be seen more directly than in previous works using non-specific histological staining.

Materials and Methods

Adult white Leghorn chicks weighing about 1.5 kg were used in the experiments Operations were done under pentobarbital anaesthesia (usually 30 mg/kg intraperitoneally) The following types of operation were performed.

(1) Simple denervation of the ALD by sectioning the common nerve trunk to ALD and PLD, with the wing restrained from drooping.

(2) Tenotomy of the ALD at its spinal insertion on one side and such tenotomy plus denervation of the ALD on the other side. Adherence and reattachment of the tenotomized ALD to the underlying muscles was prevented by inserting under it a thin silicon rubber sheet.

(3) Simple denervation of the flexor metacarpi ulnaris (FMU) with the ulnar-metacarpal joint fixed in a flexed position and so with the muscle fixed in a shortened position.

(4) Tenotomy of the flexor metacarpi ulnaris on one side and tenotomy plus denervation of this muscle on the other side.

(5) Simple denervation of the sartorius muscle.

The myosin ATPase histochemical method was used as described by GUTH and SAMAHA [5] and JAFFE et al. [6].

Results

1. *Hypertrophy of the slow fibres in ALD, FMU and sartorius muscles after simple denervation under conditions excluding stretch*

Figure 1a and b show the histological cross section of normal ALD and 3-week denervated ALD demonstrating two histochemically distinct types of fibre in each case. Comparing Fig. 1a and b, the hypertrophy of a considerable

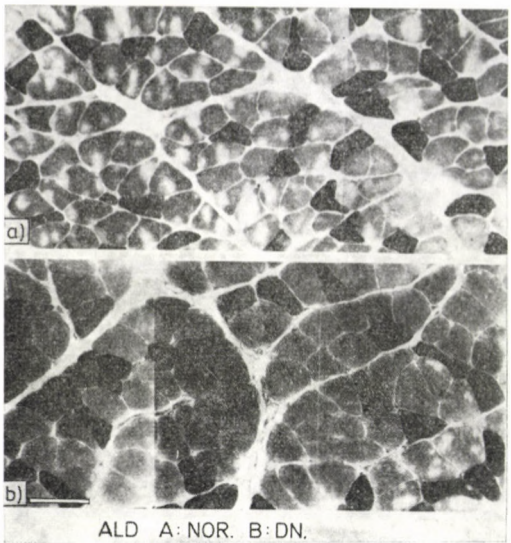

Fig. 1. Post-denervation hypertrophy of the lightly stained fibres of ALD under conditions excluding stretch. a, control. b, 3 weeks after denervation. In this and all subsequent histochemical pictures: myosin ATPase staining; scale bar, 100 μ

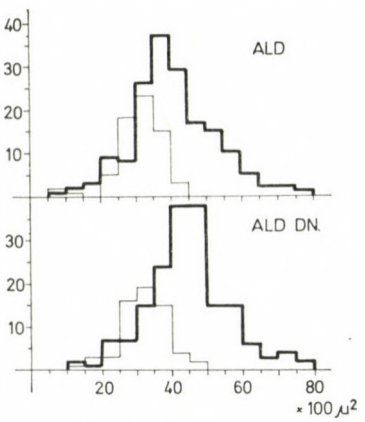

Fig. 2. Histograms corresponding to Fig. 1 showing distribution of the cross sectional areas of the two types of fibre. In this and all subsequent histograms: thick line, lightly or more lightly stained fibres; thin line, deeply stained fibres

proportion of the fibres of the more lightly stained type after denervation is quite evident.

Figure 2a and b plot the distribution of the sizes of the two types of fibre in Fig. 1a and b. With the muscle length fixed, the volume and hence the weight of each fibre is proportional to its cross section area, and the average cross section area of the fibres may be taken as an index of their average weight

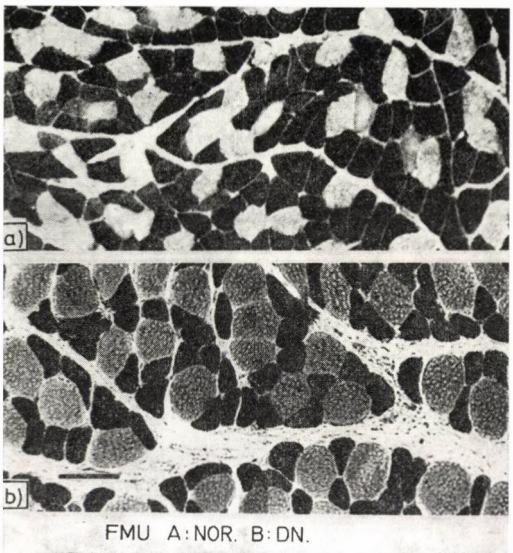

Fig. 3. Post-denervation hypertrophy of the slow (lightly stained) fibres of FMU. a, control; b, 3 weeks after denervation. Muscle kept at somewhat shortened state corresponding to flexed position of the ulnar–metacarpal joint

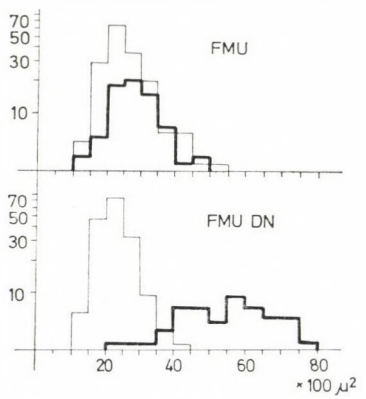

Fig. 4. Histograms corresponding to Fig. 3

and may be used as a quantitative measure when comparing which type of fibres of the experimental and the control muscle to judge the occurrence and degree of hypertrophy or atrophy. For the two types of fibre in ALD, the lightly stained ones may be arbitrarily designated as alpha fibres and the deeply stained ones as beta fibres. For the experiment in Figs 1 and 2 after 3-week denervation, the alpha fibres show a definite hypertrophy of 10.9% while the beta fibres show practically no change. This amount of post-denervation hypertrophy is small for ALD, but it is to be remembered that in this experi-

ment the wing was restrained from drooping and the effect of stretch was excluded. Under such conditions, post-denervation hypertrophy of the ALD is known to be much reduced. The different behaviour of alpha and beta fibres in respect of post-denervation hypertrophy has already been observed by others [8]. The purpose of showing the ALD experiment here is mainly for comparison with the FMU experiment shown in Figs 3 and 4 and the sartorius experiment shown in Figs 5 and 6. It is a striking fact indeed that the post-denervation hypertrophy in the slow fibres of the FMU and sartorius muscles is so much greater than in those of ALD under experimental conditions excluding stretch. Actually the post-denervation hypertrophy of the FMU and sartorius slow fibres is much greater than that of ALD even under conditions permit-

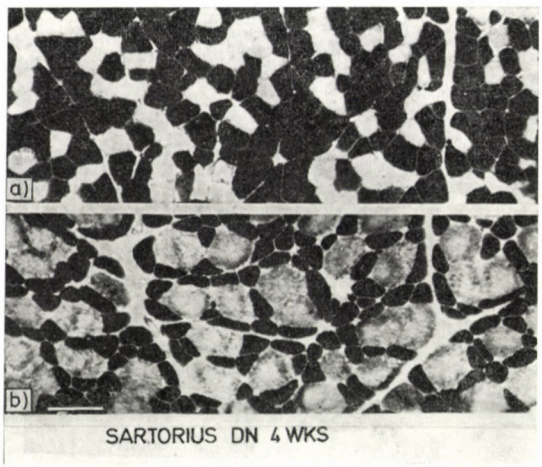

Fig. 5. Post-denervation hypertrophy of the slow (lightly stained) fibres of the sartorius muscle. a, normal control; b, 4 weeks after denervation

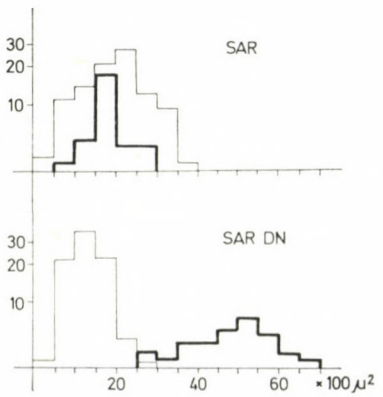

Fig. 6. Histograms corresponding to Fig. 5

ting the wing to droop and to stretch the ALD during the first 1—2 weeks following the operation. The incidental circumstance of wing drooping and consequent of the ALD in simple denervation experiments performed on the ALD had tended to make these authors to stress this factor of stretch in considering the causation of post-denervation hypertrophy. It seems that in the study of this phenomenon of post-denervation hypertrophy we should now shift our attention from ALD to the slow fibres in the mixed muscles, not only for the reason that we can thereby liberate ourselves from distraction by the factor of stretch, but also for the phenomenon being so much more striking. The post-denervation hypertrophy of the slow fibres in FMU after 3 weeks denervation and in sartorius after 4 weeks denervation shown in Figs 3—6, as calculated from the increase in their average cross section areas, amounts to 93.6% and 168%, respectively. It may be noted that in the case of slow fibres in the sartorius muscle the phenomenon still persists in a conspicuous form 12 weeks after denervation.

2. *Post-denervation hypertrophy of the slow fibres in tenotomized ALD and FMU*

The experiments described above seem to be sufficient for showing that under conditions excluding stretch of the muscle beyond its normal resting length, the phenomenon of post-denervation hypertrophy in the slow fibres still occurs. The phenomenon occurs even in tenotomized muscles relieved of tension and shortened considerably below their normal length. The experiment on tenotomized ALD is shown in Figs 7 and 8, and that on tenotomized FMU in Fig. 9. Comparing the two types of fibre in the tenotomized control ALD with the corresponding types of fibre in the simultaneously tenotomized and denervated contralateral ALD in Fig. 7, one notices a striking differential response to denervation of alpha and beta fibres: the alpha fibres show hypertrophy while the beta fibres atrophy. Figure 8 shows the corresponding histograms. The post-denervation hypertrophy of the alpha fibres amounts to 29%, while the post-denervation atrophy of beta fibres reaches 34%. It is to be noted that the degree of atrophy of the beta fibres varies in such experiments, being usually less than that shown in Figs 7 and 8. In this experiment the control ALD after simple tenotomy had shortened from the normal length of 3.8 cm to 2.84 cm, that is, by 25.3%; the contralateral ALD after simultaneous tenotomy and denervation had shortened from 3.8 cm to 2.72 cm, that is, by 28.5%. The average gross weight in 11 experiments of the ALD after combined tenotomy and denervation exceeded that after simple tenotomy by 23.2%.

In the ALD both alpha and beta fibres are presumably multi-innervated slow fibres. Their different behaviour seems to make it impossible to make

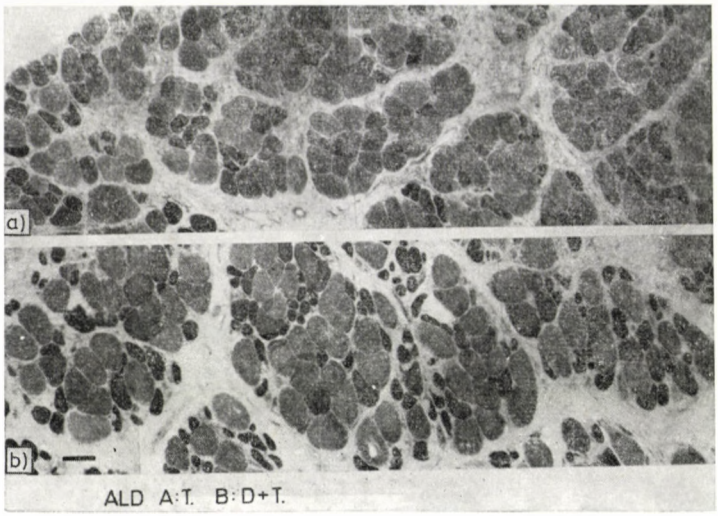

Fig. 7. Post-denervation hypertrophy of the more lightly stained (alpha) fibres in tenotomized ALD. a, control, 3 weeks after tenotomy; b, after tenotomy plus denervation

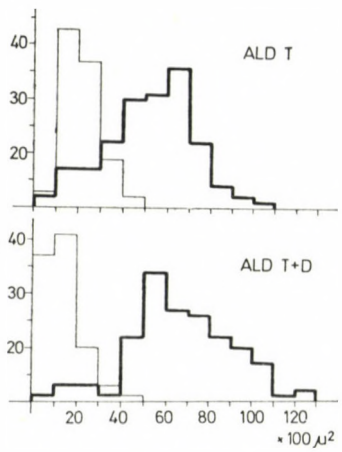

Fig. 8. Histograms corresponding to Fig. 7

a statement about the slow fibres in general. With respect to the hypertrophy after denervation, and for the purpose of the present paper, it may be understood that we use the term slow fibres to refer specifically to alpha fibres in the case of ALD.

In the experiment on tenotomized FMU in Fig. 9 it is immediately obvious that with additional denervation the unstained slow fibres show striking hypertrophy while the deeply stained fast fibres show no obvious change. In this muscle the relative shortening after tenotomy is of much less extent

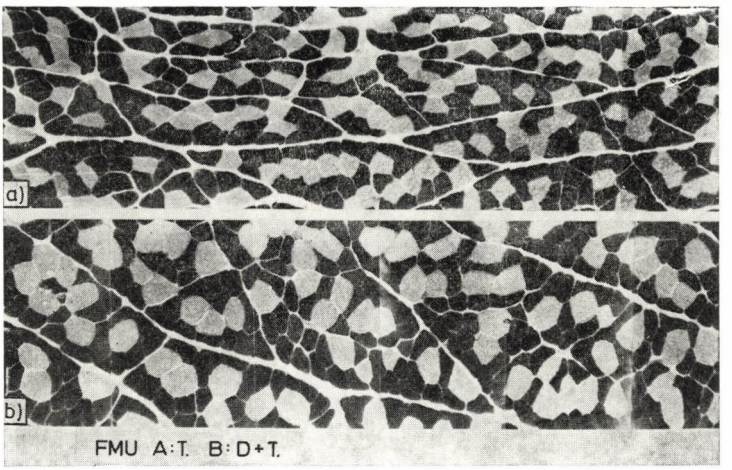

Fig. 9. Post-denervation hypertrophy of the slow (lightly stained) fibres in tenotomized FMU. a, control, 3 weeks after simple tenotomy; b, after tenotomy plus denervation

than in the ALD. The control side after simple tenotomy shortened from a normal length of 7.4 cm to 6.6 cm, while the experimental side after simultaneous tenotomy and denervation shortened from 7.4 cm to 6.5 cm. We have not made measurements of the cross section areas of the fibres in this experiment, but it was clearly seen that the degree of post-denervation hypertrophy in normal and tenotomized FMU was of a similar order.

Discussion

The results presented above prove beyond any possible doubt that the chick slow muscle fibres still show the phenomenon of long-lasting post-denervation hypertrophy under conditions which exclude any incidental stretch of the muscle following its denervation of in fact even under conditions of tenotomy which place the muscle in an abnormally shortened state throughout. A number of points, however, need to be discussed further for special notice and further study.

(1) *Heterogeneity of the slow muscle fibres in the chick*

There are two histochemically distinguishable types of fibre in the ALD. They show different propensity to develop hypertrophy after denervation and they also respond differently to tenotomy or to tenotomy plus denervation. Although the alpha fibres of the ALD and the slow fibres in mixed muscles like FMU and sartorius all consistently show post-denervation hypertrophy

both in normal and in tenotomized muscles, and a striking difference in the magnitude of the post-denervation hypertrophy attained. And they differ also in their histochemical property: the slow fibres of ALD do not show the same differential pH stability in their myosin ATPase reaction as the slow fibres of FMU do. The latter, like mammalian twitch fibres, show a reversal of the staining reaction with a change from preincubation at pH 10.4 to preinacubation at pH 4.3, while the former do not [1]. A detailed study of the histochemical, biochemical and fine structural differences among the subtypes of the slow muscle fibres in correlation with their respective tendency to develop post-denervation hypertrophy may yield some clues to the mechanism underlying the phenomenon of post-denervation hypertrophy. SHAFIQ et al. [9] have shown that the two types of ALD fibres, which they called tonic I and tonic II fibres, have different Z band widths. Possibly their tonic I fibres with thicker Z bands correspond to our alpha fibres, while their tonic II fibres with thinner Z bands correspond to our beta fibres. It is of obvious interest to determine the Z band widths of the slow fibres of the FMU and sartorius muscles.

(2) *Specificity of the phenomenon of post-denervation hypertrophy*

Having excluded such extraneous factor as stretch as the cause of post-denervation hypertrophy, we may try to probe into the basic nature of the phenomenon. Earlier, FENG [4] had shown that tenotomy, immobilization of disuse of the sartorius muscle through isolation of the spinal cord segment containing its motor nucleus, did not cause hypertrophy of its slow fibres: their hypertrophy only followed denervation. The inability of tenotomy to make the slow fibres hypertrophic is confirmed by the present study. One is tempted to suppose that the post-denervation hypertrophy of the slow muscle fibres in the chick may be the result of a loss or depletion of some specific neurotrophic agent normally supplied by the nerve to regulate or check the growth of the fibres. Various experiments bearing on this supposition may be designed. One is to cross-innervate the slow fibres with a nerve normally supplying fast fibres and then after the lapse of sufficient time perform denervation to see whether or not the slow fibres will show post-denervation hypertrophy. Such experiments are now under way.

(3) *Occurrence of post-denervation hypertrophy of the slow fibres in tenotomized muscle*

This point requires some special comments. JIRMANOVÁ and ZELENÁ [7] drew from their experiments the conclusion: "In contrast to denervation alone, simultaneous denervation and tenotomy of the ALD muscles results in atrophy".

In their experiments, in comparison with normal ALD, the simultaneously tenotomized and denervated ALD indeed showed atrophy. But is this the proper comparison? If instead of making such comparisons, one compares tenotomy plus denervation with tenotomy alone in JIRMANOVÁ and ZELENÁ's data, one finds that by muscle weight the tenotomy plus denervation group is slightly less than the simple tenotomy group, while by fibre diameter the former is slightly greater than the latter, both differences being statistically non-significant as pointed out by the authors themselves. Therefore the conclusion quoted above should not be construed as meaning that in tenotomized ALD, denervation results in atrophy. These authors used non-specific histological staining in their work. The present work, using the fibre type-specific myosin ATPase method, brings out the differential response to denervation in the two types of fibres in the ALD, which seems more evident in tenotomized than in normal ALD. In tenotomized ALD, denervation causes the alpha fibres to hypertrophy and may cause the beta fibres to atrophy. As far as the alpha fibres are concerned, the hypertrophy following denervation seems to be no less striking in tenotomized and shortened ALD than in ALD kept at normal length.

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NEW METHOD FOR MEASURING CAPILLARY FILTRATION COEFFICIENT AND POSTCAPILLARY VESSEL COMPLIANCE IN DIFFERENT ORGANS AND TISSUES UNDER CONSTANT PERFUSION

By

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A new method has been developed for measuring the rate of capillary filtration. By the method capillary filtration coefficient (CFC) can be estimated in any organ. CFC in anaesthetized cats was 0.055 and 0.129 ml/min/mm Hg/100 g tissue in the lungs and in the small intestine, respectively.

Among the experimental methods for measuring the capillary filtration coefficient (CFC) and the changes of capacitance function of the vessels, plethysmography [4, 6] and gravimetry [3, 5] are widely used. Application of these methods is, however, rather difficult because during surgical preparation the function of some organs and tissues cannot be preserved. This paper presents the estimation of CFC and postcapillary vessel compliance that can be used in practically every region of the cardiovascular system without major surgical interference.

Materials and Methods

The principle of the method for measuring CFC and vascular compliance of the lungs has been described in detail elsewhere [1, 2]. The organ under study is perfused by a pump set for constant flow rate. Venous outflow is directed through a catheter into an extracorporeal glass reservoir, from which the blood is returned by a second tube of the pump into the venous system of the animal. Initial venous pressure of the organ is set within the range of normal values for the given vascular area by adjusting the distal end of the catheter to a proper height. The temperature of the blood is kept at 37 °C by a thermostat. Outflow from the reservoir at steady state is equal to the inflow to the organ. In this situation the level of blood in the reservoir does not change. When the distal end of the catheter is raised, venous pressure in the organ increases resulting in a change of CFC and vascular compliance. These two parameters are measured by an electromanometer and recorded on a polygraph indicating the changes in hydrostatic pressure as a function of time. Each measurement lasted 7.5—9 min.

An original recording of CFC and vascular compliance due to venous hydrostatic load in the small intestine of an anaesthetized cat is shown in Fig. 1. It can be seen that the curve ABCD is similar to the gravimetric and plethysmographic curves presented in the literature [3, 5]. The first relatively rapid phase (AB) is caused mainly by a distention and filling of the venous vessels. The second slow phase (BC) is a result of outward capillary filtration.

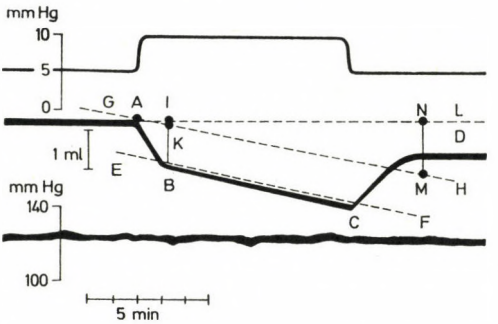

Fig. 1. Fluid filtration and blood volume in the small intestine of the cat under hydrostatic load. Upper tracing venous outflow pressure; middle: blood level in extracorporeal blood reservoir; lower tracing: perfusion pressure to the organ

Since blood volume (ΔV) in the extracorporeal reservoir can be measured and calibrated (BI), and an increase of venous blood pressure in the organ (ΔP_v) is also determined, compliance (C) of the postcapillary vascular bed can be expressed by the equation,

$$C = \frac{\Delta V}{\Delta P_v} \text{ ml/mm Hg}$$

As fluid filtration occurs also during the AB phase, we assume that correction of ΔV can be done by subtracting IK from BI.

Determination of CFC is carried out according to the equation,

$$CFC = \frac{k \times h \times 100}{t \times w \times \Delta P_c}, \text{ where}$$

t(min) arbitrary time period (AN); A is determined by the intercept of the base line AL and GH; the latter is parallel with the slope (EF) of the filtration phase; h(cm) length of the perpendicular drawn from point M to N; w(g) = weight of the investigated organ; it is measured at the end of each experiment; ΔP_c (mm Hg) = gain of capillary hydrostatic pressure; k = conversion coefficient for h in ml; it is determined during calibration.

Results and Discussion

In this method ΔP_c is substituted by ΔP_v as it has been described by THULESIUS [7]. This is done in view of the uncertainty of the transmission coefficient of the blood pressure from veins to capillaries.

In experiments on anaesthetized cats the method yielded mean values for CFC in the small intestine and lungs of 0.129 ± 0.006 and 0.055 ± 0.008 ml/min/mm Hg/100 g tissue, respectively.

CFC values measured by elevating the venous outflow pressure may differ in the same organ depending on whether constant flow or constant pressure is used for perfusion. This, however, is no obstacle in determining the changes of CFC in organs and tissues under neurogenic or humoral control.

The necessary preconditions while applying the method are,

(a) exclusion of vascular anastomoses between the investigated organ and other organs and tissues;

(b) the use of anticoagulants;

(c) steady state of vasomotor activity in the perfused area during the hydrostatic load.

The method described may be termed extracorporeal blood volumetry.

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EFFECT OF THE BETA-RECEPTOR BLOCKER PINDOLOL ON SURVIVAL IN $HgCl_2$ INDUCED ACUTE RENAL FAILURE IN DOGS

By

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The effect of the beta receptor blocker pindolol on survival was investigated in $HgCl_2$ intoxicated dogs. A single injection of 100 μg/kg b.w. pindolol intravenously (i.v.) caused a significant rise in urinary sodium excretion and a significant decrease of plasma renin activity (PRA) and urinary norepinephrine (NE) and epinephrine (E) excretion in control dogs. A single injection of 3 mg/kg $HgCl_2$ i.v. resulted in death of the animals within 3—5 days. Pretreatment with the above dose of pindolol increased length of survival to 4—8 days, two dogs recovering from acute renal failure (ARF). The degree of azotemia was smaller in the pretreated group than in the control dogs given $HgCl_2$ only. Pindolol prevented the $HgCl_2$ induced marked increases of urinary catecholamine excretion and PRA. These findings support the hypothesis that increased activity of the sympathetic nervous system is involved in the pathomechanism of the nephrotoxic model of ARF. Pindolol pretreatment decreases the severity of ARF though it can not prevent it.

The role of the sympathetic nervous system in the pathomechanism of acute renal failure (ARF) has been studied extensively. It is well known that increased adrenergic activity may cause ARF during shock or surgery, while in the nephrotoxic forms of ARF the exact role of the sympathetic nervous system has not yet been completely cleared. The fact that the administration of beta receptor blockers reduces the severity of different models of experimental ARF in the early stage [7, 13, 15, 18] has prompted us to study the effect of the beta blocking agent pindolol on survival of dogs in mercuric chloride induced ARF.

Materials and Methods

Male dogs weighing 12—18 kg were used in the experiments. All animals were kept in metabolic cages with free acces to food and water. The animals were divided into 3 groups. The first group was given 100 μg/kg b.w. pindolol intravenously (i.v.). In our earlier experiments this dose of pindolol consistently reduced the severity of $HgCl_2$ induced ARF in rats [15]. The second group was used as a control and received 3 mg/kg b.w. mercuric chloride i.v. In the third group 100 μg/kg b.w. pindolol was injected i.v. 1 hour prior to $HgCl_2$ injection.

The following parameters were determined daily from peripheral venous blood samples: blood urea-N (BUN), plasma creatinine, plasma sodium and potassium, and plasma renin activity (PRA). Urine was collected in 24-hour portions, and the concentrations of sodium, potassium, creatinine and catecholamines were determined.

BUN was measured by the Azur test kit (Galenopharm). Plasma and urine creatinine levels were determined by absorption on Lloyd's reagent purified Fuller's earth) and direct elution of the alkaline picrate [7]. Plasma and urine sodium and potassium contents were measured by flame photometry (Zeiss, Flaphokol).

Haematocrit was measured by a Janetzky Hematokrimeter. PRA was determined by radioimmunoassay of angiotensin I using a New England Nuclear kit. Urinary catecholamine levels were determined by the fluorimetric method. Daily urine samples were collected over hydrochloric acid (5—10 ml 5 N HCl) and kept at —20 °C until analysis. Urinary catecholamines were absorbed on an aluminium oxide column and eluted with 0.1 mol/l perchloric acid. Norepinephrine and epinephrine were oxidized at pH7 and pH2 by potassium ferricyanide [5].

Statistical analysis was performed using Student's paired "t" test having found at least six animals alive. All numerical results were expressed as means ± S.E.

Results

Single i.v. injection of 100 μg/kg b.w. pindolol did not alter either plasma sodium or potassium concentrations, BUN or plasma creatinine levels. PRA decreased on the first day from a control value 3.86±0.57 nmol/l on to 2.18± 0.40 nmol/l ($p < 0.001$), returning after 48 hours to the control level. Table I presents the effect of pindolol on the rate of sodium water and urinary catecholamine excretion. The urine flow and endogenous creatinine clearance (GFR) were not different from controls, the urinary sodium excretion increased significantly ($p < 0.01$) after the first day. Within this time the rejected quantity of tubular fraction of sodium expressed in per cents of the filtrate (TRF_{Na}) rose about 67%. Urinary catecholamine excretion decreased significantly in the first 24 hours norepinephrine (NE) by 35%, ($p < 0.05$) and epinephrine (E) by 22% ($p < 0.05$).

Figure 1 demonstrates the survival of dogs. A single i.v. dose of 3 mg/kg $HgCl_2$ produced ARF, and the animals succumbed within 3—5 days. Pretreatment with pindolol prolonged the survival to 4—8 days, moreover, two dogs recovered from ARF. The mean blood urea N concentration was significantly higher in the $HgCl_2$ treated control group than in the group receiving also pindolol pretreatment (Fig. 2).

Table I

Effect of 100 μg/kg b.w. pindolol on kidney function

Time day	Urine flow ml/min	$C_{creat.}$ ml/min	$U_{Na}V$ μmol/min	TRF_{Na} %	Urinary catecholamine excretion	
					NE mmol/24 h	E mmol/24 h
C	0.46±0.25	41.5±2.30	40.62±4.04	0.74±0.13	0.51±0.06	0.18±0.02
1	0.52±0.24	43.35±6.70	71.61±6.15**	1.24±0.09**	0.33±0.02***	0.14±0.01**
2	0.53±0.19	46.24±7.11	51.05±6.34	0.82±0.10	0.48±0.04	0.17±0.04
3	0.48±0.18	36.43±6.30	41.63±6.74	0.86±0.16	0.47±0.05	0.18±0.03

n = 6
** $p < 0.01$ *** $p < 0.001$. Changes with references to controls

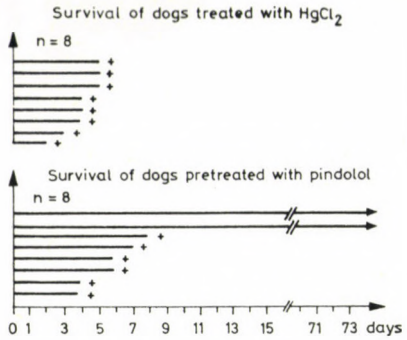

Fig. 1. Survival length of $HgCl_2$ intoxicated dogs with and without pindolol pretreatment; n = number of animals

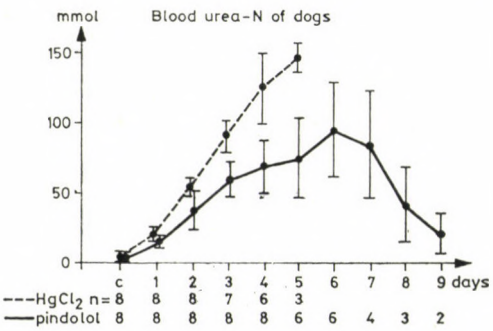

Fig. 2. Changes in blood urea N levels of $HgCl_2$ treated and pindolol pretreated and intoxicated dogs; n = number of animals

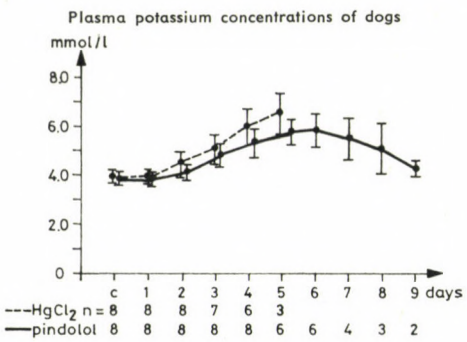

Fig. 3. Changes in plasma potassium concentrations of $HgCl_2$ treated and pindolol pretreated, intoxicated dogs; n = number of animals

The plasma potassium concentration increased in both groups, the rise of the control $HgCl_2$ treated dogs was from control value of 3.9 ± 0.15 mmol/l to 4.46 ± 0.23 mmol/l ($p < 0.001$). In the pretreated animals this change was not significant (from 3.85 ± 0.20 mmol/l to 4.04 ± 0.18 mmol/l ($p < 0.1$) after the second day (Fig. 3).

A marked haemoconcentration was observed during the first 48 hours in both groups. Mean haematocrit rose from 46.8±1.3% to 57.1±0.8% ($p < 0.001$) in the $HgCl_2$ treated control group, and from 45.3±0.9% to 55.8±1.1% in the pindolol pretreated group ($p < 0.001$). The increased haematocrit level returned to the control value after the 3rd day. At this time the plasma sodium level had already decreased in both groups due to excessive diarrhoea. Following $HgCl_2$ administration, within the first 24 hours PRA increased to about

Table II

Changes in plasma sodium and plasma renin activity (PRA) in the $HgCl_2$ treated control group and in the pindolol pretreated group

Time day	Dogs treated with $HgCl_2$ alone			Dogs pretreated with pindolol		
	plasma sodium concentration mmol/l	PRA nmol/l	n	plasma sodium concentration mmol/l	PRA nmol/l	n
c	140.2±1.91	1.46±0.33	8	141.1±1.96	2.28±0.65	8
1	149.8±2.83**	6.87±1.91***	8	138.1±5.41	3.22±0.90	8
2	136.4±1.02*	5.77±1.43***	8	134.0±6.89*	3.62±2.16	8
3	128.4±4.90***	6.25±1.51***	7	131.0±7.48*	3.80±1.44	8
4	127.2±3.65***	4.92±1.58**	6	132.0±9.94*	3.67±1.56	8
5	132.3±2.59	4.60±.032	3	134.0±4.67*	2.92±1.01	6
6				133.5±6.16*	2.70±1.15	6
7				133.0±2.14	2.78±0.95	4
8				132.5±2.52	2.52±0.48	3
9				139.0	1.85	2
				132.0	2.88	

n = number of animals
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Changes with references to controls

Table III

The effect of 3 mg/kg b.w. $HgCl_2$ on kidney function

Time day	n	Urine flow ml/min	$C_{creat.}$ ml/min	$U_{Na}V$ μmol/min	Urinary catecholamine excretion	
					NE mmol/24 h	E mmol/24 h
c	8	0.36±0.04	36.1 ±3.31	32.94±5.23	0.36±0.05	0.14±0.02
1	8	0.57±0.09**	4.42±1.85***	71.74±8.95***	0.79±0.17**	0.28±0.04**
2	8	0.15±0.06**	0.29±0.13***	10.10±2.25**		

n = number of animals
** $p < 0.01$; *** $p < 0.001$. Changes with references to controls

Table IV

The effect of $HgCl_2$ administration with pindolol pretreatment on kidney function

Time day	n	Urine flow ml/min	$C_{creat.}$ ml/min	$U_{Na}V$ μmol/min	Urinary catecholamine excretion	
					NE mmol/24 h	E mmol/24 h
C	8	0.32±0.07	40.37±4.45	40.54±9.15	0.41±0.05	0.13±0.02
1	8	0.60±0.14***	5.04±0.86**	61.43±8.15***	0.49±0.03	0.19±0.05
2	8	0.37±0.12	0.89±0.41***	20.57±7.70***		
3	4	0.56±0.30	1.01±0.21	6.10±2.13		
4	2	0.60	3.05	9.6		
		0.20	0.97	8.8		

n = number of animals
** $p < 0.01$; *** $p < 0.001$. Changes with references to controls

5 times the control level and remained high throughout (Table II). Pindolol pretreatment prevented this marked increase of PRA. As a result of $HgCl_2$ intoxication, urine flow increased in both groups after the first day, then decreased markedly (Table III) and in the dogs treated with $HgCl_2$ alone anuria ensued after 48 hours. The pindolol pretreated dogs developed anuria only after 72 hours (Table IV) the two surviving dogs maintaining adequate urine flow.

The $HgCl_2$ intoxication caused a progressive fall in edogenous creatinine clearance, in the control group from 36.10±3.31 ml/min to 4.42±1.85 ml/min ($p < 0.001$) and in the pindolol pretreated dogs from 40.37±4.45 ml/min to 5.04±0.86 ml/min ($p < 0.001$) during the first 24 hours (Tables III and IV). While the GFR of died animals stopped after the second or third day, the GFR of two survivors began increase slowly after the 4th or 6th day. Both NE and E excretion increased about twofold without pretreatment (Table III) while pretreatment with pindolol prevented this rise (Table IV).

Discussion

According to our results, pindolol has a modifying effect on the development and course of ARF, though it can not prevent it. Four pretreated dogs died later than the control animals given $HgCl_2$ only, and two dogs recovered from ARF. The severity of azotemia was found to be less in the pretreated dogs than in the control ones.

In previous studies [2, 10] it has been shown that marked decreases of renal blood flow (RBF) and glomerular filtration rate (GFR) and/or tubular damage occur during the initial and late phases of ARF both in man and

in animals models. According to SOLOMON and HOLLENBERG [25] $HgCl_2$ injected into the renal artery causes a reduction in RBF and GFR, while the administration of phenoxybenzamine reduces the vascular response to $HgCl_2$. They have suggested that $HgCl_2$ results in a decrease of RBF *via* release of endogenous catecholamines.

The mercury ion affects the active transport sites or enzyme systems in the cell membrane of the tubular cell [23]. In our experiments the impairment of membrane transport functions was apparent in the decreased sodium and water reabsorption despite the low GFR (Tables III and IV).

In our earlier experiments [15] pindolol pretreatment decreased the severity of $HgCl_2$ induced ARF in rats, however, the effect was dose-dependent, because pindolol in doses of 1—5 μg/100 g b.w. i.p. was less effective than in doses of 10—20 μg/100 g b.w. The mechanism by which pindolol alleviates the severity of ARF in dogs and rats is still unknown. Pindolol is a beta-receptor blocker with some chinidine-like effect and intrinsic sympathomimetic activity [1].

In the first 24 hours following $HgCl_2$ treatment the urinary catecholamine excretion rose about twofold (Table II). This means that the activity of the sympathetic nervous system increased in the early stage of $HgCl_2$ intoxication, probably as a result of significant losses of salt and water. Pindolol pretreatment inhibited the rise of NE and E excretions (Table IV).

The effect of pindolol may be mediated also by intrarenal beta receptors. Numerous beta receptors have been demonstrated along the nephron [6]. SHIBUTA et al. [24] showed that beta receptor blockers given i.v. increased the urinary sodium excretion without affecting inulin clearance. In the present experiments administration of 100 μg/kg pindolol increased sodium excretion in face of unchanged endogenous creatinine clearance. The TRF_{Na} increased significantly, which indicates decreased tubular reabsorption (Table I). The protective action of pindolol may involve metabolic effects in the tubular cells resulting in less cellular damage. According to LEWIS [20] pindolol may penetrate across the cell membrane and has an anaesthetic effect, the tolerance of tubular cells being increased to noxious agents.

CHEVALIER and FINN [9] observed that the beta receptor blocker propranolol prevented the rise in proximal tubular pressure and this effect was accompanied by a significant improvement of inulin clearance. In our experiments the decrease of GFR was the same in both $HgCl_2$ treated groups (Tables III and IV), thus the protective effect of pindolol cannot be explained by such a mechanism. It is well known that beta receptor blockers inhibit renin release and decrease renal renin content [17, 22, 29]. SARUTA et al. has been suggested that the effect of pindolol is less than that of the other beta blockers, presumably as a result of its weak membrane stabilizing action and marked intrinsic sympathomimetic activity [22]. In the present study a single injection of

pindolol significantly reduced the PRA by about 43% within the first 24 hours and 48 hours later it returned to the control level.

The role of the RAS in the pathomechanism of ARF is still a matter of debate. A transient increase in peripherial renin levels occurred within 6 to 24 hours and PRA returned to the control level within one or two days after induction of ARF [12, 21]. In our experiments the increased PRA of the intoxicated control dogs failed to return to the control value after 24 hours due to excessive diarrhoe (Table II). According to our previous observations the renal renin content (RCRC) remained unchanged during the first day and by the 48th hour it increased to several times of the control value [16, 21]. Extracellular volume expansion with protracted saline loading decreases plasma renin and renal renin activity, wich has been found to protect against $HgCl_2$ or glycerol induced ARF [11, 28]. BEAUMONT et al. [3] found that $NaHCO_3$ loading failed to protect against $HgCl_2$ induced ARF and they suggested that the Cl^- ion might have been responsible for the reduction of RCRC induced by long term saline loading. Nevertheless THIEL et al. [27] and BIDIANI et al. [4] have shown that protection in the nephrotoxic model of ARF can be achieved without suppression of plasma renin and renal renin content. According to KIRSCHBAUM et al. [19] saline loading significantly decreases renal cortical Hg^{++} levels and probably as a result of this, saline loading has a protective effect in $HgCl_2$ induced ARF. In our experiments the beta receptor blocker pindolol inhibited the marked increase of PRA (Table II), however, ARF developed in all cases despite of low PRA. It appears from these data that increased PRA *per se* is not responsible for development of ARF while it may be an aggrevating factor.

Summing up, the beta receptor blocker pindolol decreased the severity of $HgCl_2$ induced ARF, however, the mechanism by which this agent modify the effect of $HgCl_2$ still remains to be cleared.

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A BALLOON CATHETERIZATION MODEL FOR THE SUPERSELECTIVE CATHETERIZATION OF CEREBRAL VESSELS

By

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Model experiments have been conducted in dogs in an attempt to simulate balloon catheterization of human cerebral blood vessels. We have developed balloon catheters suitable for temporary occlusion of blood vessels, superselective angiography and for therapeutic vascular occlusion. The advantage and disadvantages of the catheters and balloons used are described. The present haemodynamic observations may serve as a basis for practical conclusions concerning similar procedures in man.

The first endovascular therapeutic intervention on the blood vessels of the brain was made as early as 1930 by BROOKS [2]. The basis for these trials had been supplied by the observation that materials of appropriate specific gravity and size are carried by the blood stream in the direction of the highest flow following injection into the arterial circulation. Pieces of muscle were introduced into the common carotid artery of patients suffering from carotid-cavernous fistula that entered the cavernous sinus as a result of a shunting mechanism and occluded the fistula in fortunate cases. The method was, however, not reliable because the direction of the muscular embolism could not be controlled.

The lack of solution of radical treatment for certain forms of cerebral vascular diseases, *e.g.* arterio-venous fistula, certain aneurysms, extensive arterio-venous angioma, required further research and development of the endovascular methods.

Transfemoral catheterization has made feasible the endovasal approach of the branches of the external carotid artery, as well as the manipulation inside the internal carotid artery up to the level of the syphon [6, 7, 8]. However, the second or third order cerebral blood vessels could not be reached this way, owing to the small calibre and branching pattern of these vessels.

Attempts have also been made to use electromagnetically controlled catheters [1, 11, 13, 24]. However, this technique is extremely complicated, requires special equipment and is difficult to employ in clinical practice.

The so-called balloon catheterization method has been developed by SERBINENKO [20, 21, 22]. A flexible microcatheter much smaller in diameter than the cerebral vessels with an intra-arterially inflatable balloon attached

to it is allowed to be carried by the blood stream to the desired site. In addition to its diagnostic value, the method makes also efficient therapeutic interventions feasible [5, 10, 16, 17, 19, 23].

The accessories required for this superselective catheterization of cerebral blood vessels are not available commercially, therefore those working in this field make them themselves. Many details of the technique are not yet clarified and far from being uniform.

Although the clinical usefulness have not yet been outlined clearly, the method has perspectives in many respects.

Making use of the technical data described in the literature [14, 15, 18] we have designed various puncturing needles, reflux blocking systems and balloon catheters. Serial experiments have been conducted in dogs with the aim of working out a reliable method for balloon catheterization which is suitable for multipurpose clinical use.

Materials and Methods

Ten mongrel dogs of either sex weighing 20 to 25 kg were anaesthetized with Nembutal (50 mg/kg) and subjected to 20 experiments (Table I). It is a peculiar feature of the canine brain vascular system that from the common carotid artery, 5 to 7 mm in diameter each 2 to 4 branches of internal carotid arise, 0.5 to 1 mm in diameter almost at rectangles, which cannot be subjected to balloon catheterization by the present technique because of their size and pattern of arborization. On the other hand, the external carotid artery and its branches reached *via* the common carotid artery as well as the arteries of the lower extremities *via* the femoral artery have proved to be suitable for this purpose. Percutaneous puncture of the common carotid and vertebral arteries being difficult to perform, we have prepared

Table I

Classification of animal experiments according to the blood vessels catheterized

Experimental animals	Common carotid artery		Vertebral artery		Femoral artery	
	right	left	right	left	right	left
1	+	+				
2	+	+				
3	+	+				
4	+				+	
5	+				+	
6	+				+	
7	+				+	+
8				+		
9	+			+		
10			+	+		
Total	11		4		5	

Table II

Classification of balloon types tested in the experiments according to the blood vessels catheterized

Blood vessels catheterized	Balloon with calibrated leak	Balloon with wall weakened at one point	Balloon with metallic marker	Double stemmed balloon (one with double lumen catheter)	Detachable balloon
Common carotid artery	6	19	4	3	10
Vertebral artery		6	10		
Femoral artery	4	4	2	5	10
Total	10	29	16	8	20

them surgically. After puncture cotton swabs soaked in papaverine were placed on the exposed vessels to diminish eventual vascular spasm. The femoral artery was punctured percutaneously in each case. After puncture, one side branch of the needle was used for heparin administration throughout the intervention, while the other lumen was used for passing the balloon catheter pulled through the reflux blocker into the needle. The position of the balloon was controlled by radiography. When a detachable balloon was used, it was filled with polymerizing silicone, detached from the catheter, then angiography was performed through the lumen serving for heparin administration.

Two types of balloon catheters were designed. One type served for temporary occlusion of a vessel, or for angiography through the catheter only. The other type possessed a detachable balloon for therapeutic purposes. The balloons were prepared by us of latex, with diameters of 0.3 to 1.0 mm, 5 to 15 mm in length and wall thickness of about 0.1 mm. Depending on the purpose, we made various kinds of balloons: (1) one with the wall weakened at one point; (2) one with its wall perforated in advance; (3) one provided with a metallic marker; (4) detachable balloon. The balloons were fixed to the catheters by sticking or by binding, using a nylon thread 0.12 mm in diameter.

In the course of 20 experiments a total of 83 balloon catheters (4 to 5 per experiment) were tested (Table II).

Results and Discussion

So far dogs [3, 6], rabbits [15] and goats [15] have been used as models for superselective catheterization using the balloon catheter. On the basis of our own experiments we have found that the external carotid, basilar artery and femoral artery of the dog were useful models for balloon catheterization. The catheterized vessels are similar in diameter to the second and third order human intracranial blood vessels.

The needle and the designed reflux blocker used in our experiments proved to be suitable for use also for endovascular interventions in man (Fig. 1).

Reflux of blood is prevented most reliably by the reflux blocker consisting of two metallic parts which can be screwed together and sealed with heparinized cotton, which at the same time allows also for free mobility of the catheter.

Among the catheters those softening on heating have been found the most suitable, since they are rigid enough to be passed across the needle and

are at the same time flexible enough to overcome vascular curvings. They are also pliable enough to allow for secure fixing of the balloon and for attachment to the syringe by adequately shaping their proximal tip (Figs 2, 4). The double stemmed balloon catheter (Fig. 3) is ideal for superselective angiography [12, 17], for which purpose the balloon catheters provided with balloons of weakened or preperforated wall can also be employed [10, 14] (Fig. 4). A disadvantage of the weakened balloon is that on filling the weakened site does not always rupture, the balloon becoming elongated instead like a sausage. Normal blood

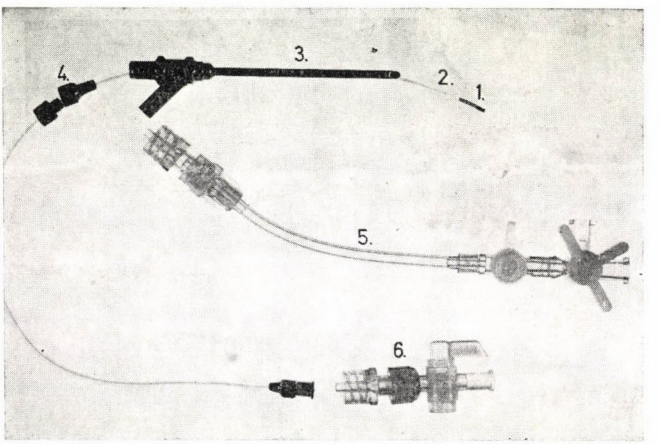

Fig. 1. System for balloon catherization: 1. Balloon with metallic marker. 2. Catheter tip of funnel shape. 3. Double inlet needle. 4. Reflux blocker for use with one catheter. 5. Side branch with stopper device for rinsing. 6. Stopper device attachable to catheter

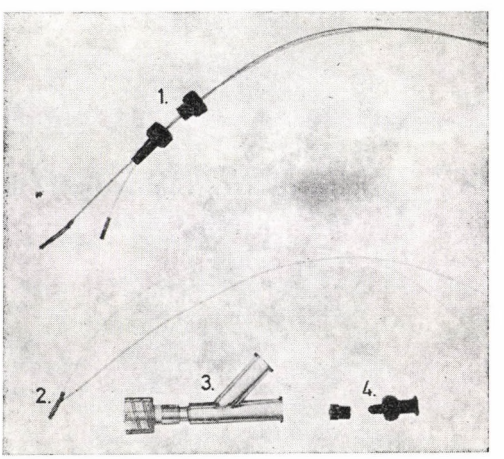

Fig. 2. Component part variants used with balloon catheterization: 1. Reflux blocker suitable for introduction of two catheters. 2. Balloon catheter with double polyethylene stem. 3. Y-shaped connecting piece. 4. Device for attacheent to syringe of polyethylene catheter

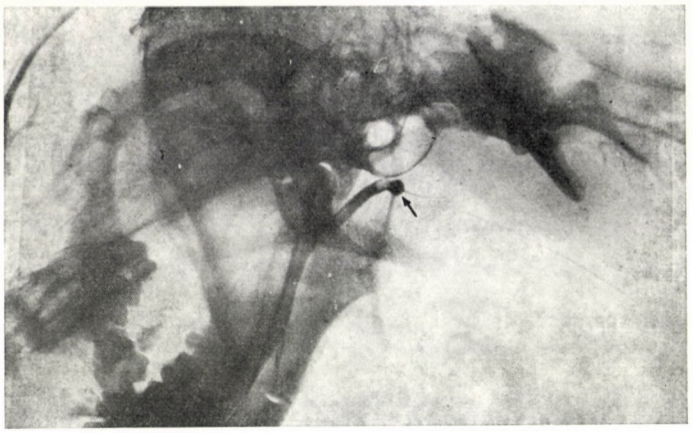

Fig. 3. Superselective angiography of the external carotid artery of the dog by means of double lumen catheter. The arrow points at the balloon introduced into the external carotid artery and filled with contrast medium. Through the other lumen of the catheter selective angiography can be performed above the occluded vessel

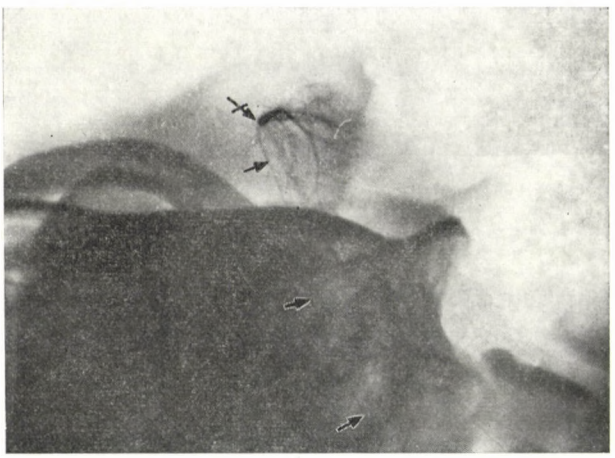

Fig. 4. Superselective angiography of the artery of a dog's ear by means of balloon catheter with its wall weakened. The microcatheter was passed up through the external carotid artery (arrows) to one of the small calibre arteries of the ear (crossed arrow, the balloon is seen here). Angiography was done by causing the weakened balloon wall to rupture

vessels withstand this pressure (we have not observed vascular rupture in our experiments), however, the possibility of vascular rupture is obvious in the case of pathological blood vessels. The balloons with the being perforated wall in advance have been examined following angiography; it was found that the contrast medium administered under high pressure had ruptured the balloon at the site of perforation, it proved excellent for superselective angiography, while it was unsuited for further catheterization of another blood vessel

Fig. 5. Superselective angiography of the dog's basilar artery by means of a balloon catheter provided with a micro-size metallic marker. The tip of the balloon catheter (arrow) inserted into the vertebral artery exposed in the neck is shown. The rami of the basilar artery are clearly demonstrated by superselective angiography

in the same experiment. Likewise, the balloon provided with a micro size metallic marker was suitable for angiography (Fig. 5), since the marker has a small leak, the tractive force of the balloon cannot always be increased during the experiment. The detachable type balloon devised by us could be readily detached after filling up with polymerizing silicone and has proved to be suitable for vascular occlusions.

Dynamic studies carried out during the experiments have shown that the balloon attached temporarily or permanently to the catheter of appropriate diameter and physical properties acts as a special kind of embolus in the blood stream. The balloon continues to rise with the blood stream in the vessel, pulling the catheter with it until the tractive force of the balloon exceeds the frictive force acting on the catheter. The extent of this friction depends on the pressure exerted by the reflux blocker, on the length and quality of the needle on the angle of the ramifications of the catheterized vessel as well as on the number of its curvatures. By increasing the capacity of the balloon its tractive force can be increased until the balloon wall does not press against the wall of the vessel being catheterized.

We have also found that the balloon catheter does not necessarily move in the direction of the largest vessel at the ramification sites. We assume that the behaviour of the balloon catheter is influenced, beside the angle of ramification and diameter of the vessel, also by the shape, length and relation to the vascular wall of the balloon. Further flow dynamic studies are required to elucidate these problems.

As a result of our series of experiments we have created balloon catheters, needles and reflux blocking systems which can also be used in the clinical practice for diagnostic and therapeutic purposes. The experience obtained in the course of the experiments made it possible to employ reliably the method also in patients.

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Recensiones

Siegmund J. BAUM, and G. David LEDNEY

Experimental Hematology Today 1979

Springer Verlag, New York—Heidelberg—Berlin. 267 pages with 123 illustrations. Price DM 118,—; US $ 64.90

This well illustrated book reviews the most important discoveries of recent years on the basis of the papers given at the 7th Meeting of the Society of Experimental Haematology held in Chicago, Ill.

The first part summarized the knowledge on the haemopoetic stem cell, including the latest data on its physiology. The second part discusses the physiological properties of granulocytes and macrophages, and the functional and anatomical heterogeneity of these cells. The third part contains some new data on erythropoiesis and megakaryopoiesis. Part four deals with the most important immunological relations of transplantation and part five with bone marrow transplantation, first of all with its clinical value. In the 6th part the animal models of some haematological diseases are discussed. The book contains practically all important issues interesting for the clinical and experimental haematologists. The volume is not easy reading, but can still be recommended to every haematologist dealing with leukaemia and anaemia.

Ibolya NAGY

H. Y. ELDER and E. R. TRUEMAN (Eds)

Aspects of animal movement
Seminar Series 5

Cambridge University Press, Cambridge 1980. 250 pages, with 95 figures and 12 tables. Price £ 18.00 A H/C (SBN 0 521 23086 1) and £ 6.95 A P/B (SBN 0 521 29795 8)

The book contains 12 papers given at the Society for Experimental Biology meeting held at Reading in December, 1978, with an additional paper presented at a subsequent session. The volume is concerned with animal movement and, like previous books of the series, it reviews current research done by a number of workers of international reputation.

Included are discussions of muscle contraction, coordination and energy requirement involved in locomotion in different environments. The main types of locomotion are peristaltic, jet propulsion, swimming of beetles and fish, insect jumping and flight of insects and birds. There are chapters about the principles of coordination in invertebrates, the mechanisms of walking and a final discussion of the usefulness of the concept of mechanical efficiency as applied to terrestrial locomotion.

The book gives a balanced presentation of the principal advances in the study of animal movement. It will be useful to scientists interested in the research of locomotion.

T. KUKORELLI

H. HEIMPEL, E. C. GORDON-SMITH, W. HEIT and B. KUBANEK (Eds)

Aplastic anemia. Pathophysiology and approaches to therapy

Springer Verlag, Berlin—Heidelberg—New York 1979. 292 pages, with 81 figures and 71 tables

This 24th volume of the series Haematology and Blood Transfusion deals with a very important question of clinical haematology.

It contains the material of a conference held in Reisenburg, that was organized with international participation by the University of Ulm. One of the main purposes was to interpret aplastic anaemia instead of the usual clinical and experimental description of the disease, with the aid of the data obtained by culturing the haemopoietic cells.

After the introduction, the clinical symptoms of aplastic anaemia are summarized, discussing the possible role of viruses and transformation of the disease into leukaemia. The data obtained in the last 10 years by bone marrow transplantation and cell culture indicate that aplastic anaemia is a disease of the haemopoetic stem cells. We do not know exactly either the first damaging cause, whether it is a virus or a drug, or the exact process of the damage, because in man there may be a long interval between the action of the damaging factor and the appearance of clinical symptoms. Studies of stem cell functions for which there are now great possibilities since the advances in cell culture and cell kinetic methods, with probably help to detect the exact pathogenesis of aplastic anemia. More and more data speak for the functional importance of immunological processes, though in the opinion of THOMAS its role can be assumed in not more than every fifth patient.

The chapter on a prospective study of 352 patients is of a considerable practical interest, especially as regards the data on the therapeutic value of androgens and steroids.

In the detection of aplastic anaemia the main problem is that one can see the illness only at the end of the whole process, when the original pathogenetic factor is not present any longer.

The volume is easy to read and understand and is instructive for every clinician who deals with haematological diseases, but it gives many data and ideas to experimental haematologists, too. The summarizing remarks contribute to the understanding of the problems and the discussions reflect the living questions of clinical haematology.

Ibolya NAGY

J. KNOLL (general editor) and F. DARVAS (editor):

Chemical Structure–Biological Activity Relationships Quantitative Approaches

Third Congress of the Hungarian Pharmacological Society Vol. 3, Akadémiai Kiadó, Budapest 1980, 378 pages

This symposium one of the six held in Budapest on August 22—25, 1979, was a direct continuation of the symposium series started in Prague in 1973 and continued in Suhl (GDR) in 1976. The 100 participants came from 11 countries. The volume contains 38 selected presentations in six Chapters dealing with all aspects of QSAR.

In the Preface the editor explains that the main aim in the selection of contributions was to point out the methodological aspects of QSAR and to inform the reader about the research done in Middle European countries.

This symposium appears to be the first where a section was devoted to prediction studies, *i.e.* estimation of the activity of compounds before their synthesis. A number of papers dealt with the presentation of new methods and algorithms to improve the effectiveness of QSAR studies and how the biological activity can be influenced by the hydrophobicity and the steric properties. A separate section discussed the QSAR aspects of peptides, given first of all a specific QSAR model for peptides, then the empirical energy calculations of encephalin analogues, and the stereoselectivity of opioid receptors.

The book will be of interest not only to pharmacologists, but also to chemists and other experts who are closely concerned with the biological activity of natural and chemical compounds.

E. MINKER

M. KOGAN, D. C. HERZOG (Eds)

Sampling Methods in Soybean Entomology

Springer-Verlag, New York—Heidelberg—Berlin 1980

This monograph of the Series in Experimental Entomology published under the editorship of Thomas A. MÜLLER (Department of Entomology, University of California, Riverside, California, USA) and edited Marcos KOGAN (Economic Entomology, Illinois Natural History Survey and Agricultural Entomology, University of Illinois, Urbana, Illinois, USA) and Donald C. HERZOG (Department of Entomology and Nematology, University of Florida, Quincy, Florida, USA) was written by 32 experts from USA, Japan, India and Canada. The 587-page book contains 8 sections included in 28 chapters with 252 figures.

In the preface of the series Thomas A. MILLER writes: "Many specialized techniques are confined to one specific research laboratory. Although methods may be considered commonplace where they are used, in another context even the simplest procedures may save considerable time. It is the purpose of this series (1) to report new developments in methodology, (2) to reveal sources of groups who have dealt with and solved particular entomological problems, and (3) to describe experiments which might be applicable for use in biology laboratory courses.

The volume describes a variety of methods for the collection and analysis of data on arthropod populations in a row crop and discusses their use in basic ecological research and in application to pest management programs. Many practical procedures for acquiring relative and absolute samples for each developmental stage of over 50 species of phytophagous insects, their parasitoids, predators and diseases, are described in great detail. Specific chapters explain how to build many of the sampling devices, how to select the best device and technique based on the characteristics of the species to be studied, and how to analyse the sampling data. Complete sampling programs and sequential sampling plans are presented for several species.

The book is organized into eight major sections. Section I includes descriptions of the **crop as** the substrate from which arthropods are extracted; the most common sampling procedures in population and in injury estimation; techniques and basic concepts in sampling theory, and sequential sampling programs for parameter measurement and for management decisions. The reader is referred back to this section throughout the entire volume. Sections II through VIII are devoted to major species or complexes of pests or natural enemies. Within each of the sections there are from one to five chapters on species or species complexes. The contributors have attempted to follow a uniform structure within each chapter by providing the necessary spatial (geographical), temporal (phenological), and biological (life history) background necessary for the development of an intelligent sampling program. Some chapters on species complexes include practical keys for identification of the most common genera or species within the complex. These keys, however, should be used with caution; and workers, particularly those outside the USA, should always consult a taxonomist to confirm their identifications.

The contents of the monograph is as follows.

Section I. Concepts and Techniques:

1. Soybean Growth and Assessment of Damage by Arthropods (M. KOGAN, S. G. TURNIPSEED),
2. General Sampling Methods for Above-Ground Populations of Soybean Arthropods (M. KOGAN, H. N. PITRE, Jr.),
3. Introduction to Sampling Theory (W. G. RUESINK),
4. Sequential Sampling Plans for Soybean Arthropods (M. SHEPARD),
5. Sequential Estimation of Soybean Arthropod Population Densities (W. G. RUDD).

Section II. Lepidopterous Defoliators:

6. Sampling Velvetbean Caterpillar on Soybean (D. C. HERZOG, J. W. RODD),
7. Sampling Soybean Looper on Soybean (D. C. HERZOG),
8. Sampling Green Cloverworm on Soybean (L. P. PEDIGNO).

Section III. Coleopterous Defoliators:

9. Sampling Mexican Bean Beetle on Soybean (S. G. TURNIPSEED, M. SHEPARD),
10. Sampling Bean Leaf Beetles on Soybean (M. KOGAN, G. P. WALDBAUER, G. BOITEAU, C. E. EASTMAN).

Section IV. Other Foliage Feeders:

11. Sampling Aphids in Soybean Fields (M. E. IRWIN),
12. Sampling Leafhoppers on Soybean (C. G. HELM, M. KOGAN, B. G. HILL),
13. Sampling Phytophagous Thrips on Soybean (M. E. IRWIN, K. V. YEARGAN),
14. Sampling Whiteflies on Soybean (S. M. VAISHAMPAYAN, M. KOGAN),
15. Sampling Mites on Soybean (S. L. POE),

Section V. Underground Feeders:

16. Sampling Phytophagous Underground Soybean Arthropods (C. E. EASTMAN),

Section VI. Stem and Axil Feeders:

17. Sampling Coleopterous Stem Borers in Soybean (W. V. CAMPBELL),
18. Sampling Epinotia aporema on Soybean (B. X. CORREA FERREIRA),
19. Sampling Threecornered Alfalfa Hopper on Soybean (J. A. MUELLER),
20. Sampling Stem Flies in Soybean (G. A. GANGRADE, M. KOGAN),

Section VII. Pod Feeders:

21. Sampling Heliothis spp. on Soybean (R. E. STINNER, J. R. BRADLEY Jr., J. W. VAN DUYN),
22. Sampling Lepidopterous Pod Borers on Soybean (T. KOBAYASHI, T. OKU),
23. Sampling Phytophagous Pantatomidae on Soybean (J. W. TODD, D. C. HERZOG)

Section VIII. Natural Control Agents:

24. Sampling Parasitoids of Soybean Insect Pests (N. L. MARSTON),
25. Sampling Predaceous Hemiptera on Soybean (M. E. IRWIN, M. SHEPARD),
26. Sampling Ground Predators in Soybean Fields (J. F. PRICE, M. SHEPARD),
27. Sampling Spiders in Soybean Fields (W. H. WHITCOMB),
28. Sampling Pathogens of Soybean Insect Pests (G. R. CARNER).

The life cycle, host, and geographical range data provided make this book an invaluable source of information on many insect pests, not only for soybean but for other row crops such as cotton, rice, maize, and tobacco.

This excellent monograph is highly instructive not only for researchers directly interested in entomology, but for anyone in the world working with breeding or growing of soybean

E. KURNIK and L. SZABÓ

David, T. LOWENTHAL, Krishan BHARADWAJA and Wilbur W. OAKS (Eds)

Therapeutics through exercise. The fifty-first Hahnemann symposium

Grune and Stratton Inc., New York 1979, 222 pages. Price US $ 19.50.

The editors have done their work well indeed. The contributors appear to have followed precise instructions so they could avoid overlapping nearly completely. In comparison to several other multiauthor volumes this one excels in the unity of the contributors in approaching their subject.

The volume consists of four main parts: i. Applications of Exercise Physiology; ii. The Individual in this Environment; iii. The Effects of Exercise on Disease; and iv. Exercise and Environmental Stresses.

A good description of the physiological and pathophysiological processes dominates not only the first part devoted explicitly to physiology, but also the subsequent ones. Even Part iii deals with the theoretical bases of exercise therapy rather than with practical routine

And this is the real value of the book: it presents a clear and well-readable material on the possible relationships between medicine in general and exercise physiology proper to almost all of the diverse disciplines within medicine. Beyond and over the strictly medical problems the material extends to psychological and biomechanical aspects. Topics of actual importance for competitive sports are also included, such as overtraining, the questions related to drugs or the relations of sexuality and performance. The last chapter discusses the role of the physician in giving positive guidance in exercise and points out in an apparently humble but good-humoured way the challenge therapy through exercise, and the possibility of an active style of life for the physicians in setting a positive example for the patients and their colleagues alike without spending any large sums of money, yet perceptibly improving their standards of life.

In regarding the book as a whole one cannot but regret that in some chapters the proportions of space and content are not always well-balanced.

Good and comprehensive lists of references are attached to every chapter.

R. FRENKL

T. A. MILLER (ed.)

Neurohormonal Techniques in Insects

Springer-Verlag, New York—Heidelberg—Berlin 1980, 282 pages with 90 figures and 23 tables. Price DM 79,—; approx. US $ 46.70

The existence of specific insect hormones differing from those of vertebrates was assumed a long time ago and their physiological significance in individual development and metabolism was described before many years. Still, successful isolation and exact chemical determination of a few insect hormones were achieved only in the past two decades when appropriate methods for handling and testing small quantities of substances have been worked out.

The book contains detailed and comprehensive reviews on endocrine organs and hormones discovered in various insects. The chapters have been written by specialists who are working with the given hormone and are familiar with the whole process of isolation, bioassay, chemical identification and also with the anatomy of producing structures. In most cases a description of the isolation of the organ is given and the idea to give as much technical details as possible is characteristic of the volume.

The chapters on proctolin, adipokinetic hormone, diuretic hormone, bursicon, as well as on hormones regulating carbohydrate metabolism and different stages of individual development are to some degree different due not only to the approach and style of the different authors but also to the varying amount of knowledge on the subject and precision of the methods applied.

The book with its up-to-date references and a good subject index is a very useful manual for everybody who is interested in insect endocrinology, and can also be recommended for university teaching.

J. SALÁNKI

K. OOTA, T. MAKINODAN, M. IRIKI and L. S. BAKER

Aging phenomena. Relationships among different levels of organization

Volume 129 of Advances in Experimental Medicine and Biology. Plenum Press, New York, London. Price $ 37.50

Humanity has been concerned with the problem of aging for several thousands of years, but a scientific approach dates back only to a few decades. During this time a vast knowledge has been accumulated giving rise to several theories that try to trace back the process of aging to somatic mutations, programmed events, a catastrophic accumulation of errors in protein synthesis, and to immunologic and physicochemical alterations. All of the symposia and meetings held on this topic provide new data to support or overthrow previous

theories and setting new ones. This volume contains the material of the Gerontology Symposium held in Tokyo in 1978, and it reshapes our old views in several fields. Thus, among others, it makes probable that in aging, somatic mutations are less important than genetic programming (MERTINI et al.). Based on the results presented, the decrease of intracellular communication with age which was observed both in the nervous system and in cells with hormone receptors, appears to be very important. The immunotheory of aging is becoming more and more predominant and the application of up-to-date techniques (*e.g.* genetic engineering) favours this (Makinodan). Without interrogating the existence of the thymus-clock proposed previously by BURNET, the results of EVERITT, and HIROBAWA and HAYASHI suggest the presence of a hypothalamic "clock".

These and other results included in the volume do not of course suffice for understanding the problem of aging. Several new data and aspects are nevertheless provided despite that Europe was represented among the authors only by two French scientists, thus achievements in gerontology achieved in this part of world are found at most in the references cited.

G. CSABA

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РЕЗЮМЕ

ВЛИЯНИЕ ЭЗЕРИНА И ОУАБАИНА НА ПРИЕМ МЫШЦАМИ КАЛИЯ, РУБИДИЯ И ЦЕЗИЯ

ДЬ. ПФЛИЕГЛЕР, Т. КОВАЧ и Б. САБО

Авторы в экспериментальных условиях провели сравнение приема ^{42}K, ^{86}Rb и ^{131}CS быстрой *(m. extensor digitorum longus — EDL)* и медленной *(m. soleus* — SOL) мышцами крысы. Они определяли, в каком отношении тормозит полное вхождение *(influx)* оуабаин (10^{-4}моль/литр) и подавляющий обменную диффузию K : K эзерин (10^{-3}моль/литр).

Было показано, что инфлюкс калия в изолированную быструю мышцу (EDL) больше, чем в медленную (SOL), но разницы между входом рубидия и цезия в условиях *in vitro* в мышцы разных типов не отмечали.

Оуабаин в одинаковой степени (25%) тормозил прием K мышцами обоего типа крысы и быстрой мышцы лягушки. Эзерин ингибировал 65% всего инфлюкса ^{42}K в мышцу лягушки, 26% в быструю (EDL) и всего 18% в медленную (SOL) мышцу млекопитающего.

Остающийся после одновременного введения оуабаина и эзерина т. наз. «резидуальный» инфлюкс в мышцах EDL и SOL крысы составил половину всего инфлюкса, в то же время в портняжной мышце лягушки резидуальный инфлюкс составил всего 10%.

Эзерин не оказывал ингибирующего действия ни на вхождение ^{86}Rb в медленную, ни на вхождение его в быструю мышцу крысы.

Результаты настоящих экспериментов согласуются с различными морфологическими свойствами тубулярной и саркоплазматической систем быстрых и медленных мышц.

УЧАСТИЕ ПЕДАЛЬНЫХ НЕЙРОНОВ В РЕГУЛЯЦИИ КАРДИО-РЕНАЛЬНОЙ СИСТЕМЫ И ИХ СВЯЗЬ ИДЕНТИФИЦИРОВАННЫМИ КЛЕТКАМИ ОСТАЛЬНЫХ ГАНГЛИЕВ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ ВИНОГРАДНОЙ УЛИТКИ

КАТАЛИН Ш.-РОЖА и Д. Б. ЛОГУНОВ

В центральной нервной системе виноградной улитки (**Helix pomatia L.**) изучалось участие педальных ганглиев в регуляции сердечной деятельности и их связь другими ганглиями. Было установлено, что

1. Большое число клеток педальных ганглиев получает входы из сердца. Ответ педальных нейронов к афферентам сердца был характерным: перивчное интенсивное увеличение частоты разрядов сменялось угнетением, затем наблюдалось вторичная менее интенсивная активация. Отдельные клетки активировались только задержкой.

2. Многие клетки висцерального и правого париетального ганглиев активировались синхронно педальными клетками во время раздражения сердца. В разных ганглиях ответы нааффиренты сердца могут быть одинаковым или противоположным, но в разных клетках их продолжительность совпадает.

3. Один нейрон левого педального ганглия (LP3) создаёт моносинаптические связи многочисленными висцеральными клетками. Отростки этих висцеральных клеток выходят в интестинальный нерв. Нейрон LP3 вызывает ВПСП во висцеральных клетках. Связь LP3 вызывает ВПСП во висцеральных клетках. Связь LP3 с висцеральными клетками осуществляется через химические синапсы.

4. Один из возбуждающих мотонейронов сердца (V41) синхронно несколькими педальными клетками получает сензорные входы из сердца. Это возбуждающий мотонейрон сердца вовлечён и в афферентные и эфферентные пути нейронной сети.

5. Один мотонейрон (V43) был иденитфицирован, вызывающий расслабление сердца и посылающий отросток в интестинальный нерв и подобно другим мотонейронам активирующийся афферентами сердца.

ИЗМЕНЕНИЯ СОДЕРЖАНИЯ cAMP, ВЫЗВАННЫЕ ЛГ—РГ, В ПЕРЕДНЕМ ГИПОФИЗЕ САМЦОВ И САМОК КРЫС *IN VIVO* И *IN VITRO*

Г. САБО и Э. ЭНДРЕЦИ

Инкубирование в течение трех часов передней доли гипофиза крыс-самцов в присутствии 10^{-7}М ЛГ– РГ имело результатом значительное накопление cAMP. Для повышения запаздывающего изменения уровня cAMP была достаточной 20-минутная инкубация вместе с ЛГ– РГ. Добавленный в инкубирующую среду циклогексимид затормаживал увеличение содержания cAMP, индуцированное через ЛГ—РГ. Накопление cAMP в гипофизе крыс-самок, вызванное ЛГ—РГ, определялось только после 6-часового инкубирования, и степень повышения уровня cAMI тоже была значительно меньше, чем в гипофизе крыс-самцов. Кастрация или введение половых стероидов не влияли на реактивную способность гипофиза крыс-самок.

Мы изучали аналогичное влияние двух агонистов и двух антагонистов ЛГ—РГ с точки зрения высвобождения ЛГ на увеличение содержания cAMP в переднем гипофизе ***in vivo*** и ***in vitro***. Один из антагонистов тормозил увеличение содержания cAMP, индуцированного посредством ЛГ– РГ, в условиях ***in vitro***, в противоположность этому, другой повышал содержание cAMP. Внутривенным введением ЛГ—РГ и — за исключением одного из антагонистов – аналогов за 15 мин увеличили уровень cAMP в переднем гипофизе. Только ЛГ– РГ не индуцировал продолжительного увеличения содержания cAMP, тогда как аналоги как агонистов, так и антагонистов значительно повышали уровень cAMP спустя 3 часа после внутривенного их введения.

ЛГ– РГ не оказывал влияния на активность аденилат-циклазы и фосфодиэстеразы в переднем гипофизе, а также не изменял активность чувствительной к NaF аденилат-циклазы.

На основании экспериментальных данных можно предположить, что для увеличения количества cAMP посредством ЛГ—РГ требуется синтез какого-то фактора (белка). Далее, оказалось, что половая разница, обнаруживающаяся в действии ЛГ—РГ на систему аденилат-циклаза-cAMP, может быть приписана скорее разной временной модели и кинетике реакции, чем полной ареактивности гипофиза крыс-самок. Изучая формирование аккумуляции cAMP, индуктированной ЛГ—РГ, установили, что значительное повышение сначала отмечается примерно в возрасте 40 дней. Настоящие результаты тоже подтверждают представление, по которому повышение уровня cAMP, индуктиванное благодаря ЛГ—РГ, является результатом комплексного биохимического процесса.

СИНАПТИЧЕСКИЕ ВХОДЫ В НЕЙРОНАХ БИМОДАЛЬНОГО ПЕЙСМЕКЕРА У *HELIX POMATIA L.*

Я. ШАЛАНКИ и А. ВЕХОВСКИ

1. Мы изучали возможность влияния на синапсы клеток RPal правого париетального ганглия в изолированном ганглионарном кольце ***Helix pomatia***, раздражая одиночными импульсами пвавые и левые паллиальные, а также анальные нервы.

2. Раздражение кратковременно изменяет активность клетки, обнаруживающей деятельность Br-типа: вызывается синаптический сигнал, включающий в себя также многофазный, деполяризационный и гиполяризационный участок.

3. Участок деполяризации является Na- и Ca-зависимым, и, вероятно, представляет собой результат полисинаптических процессов. Волна гиперполяризации может быть разложена на два компонента на основании крутизны наклона, зависимости от полярности мембраны и ионной чувствительности. Для первого компонента характерна чувствительность к Mg, для второго — к Ca и Cl. Оба компонента могут быть связаны с изменением проницаемости к K, калиевые канальцы, однако, играющие роль в возникновении быстрого компонента, отличаются от калиевых канальцев, ответственных за появление медленного компонента.

РАСПРОСТРАНЕНИЕ АЦЕТИЛХОЛИНОВОЙ ДЕПОЛЯРИЗАЦИИ В ЭКСТРАЮНКЦИОНАЛЬНЫХ МЕМБРАНАХ ПОПЕРЕЧНО-ПОЛОСАТЫХ МЫШЦ

Л. КОВАЧ, Г. СЮЧ и И. ТЕРЕК

1. На волокнах поперечно-полосатых мышц лягушки *(Rana esculenta)* изучали экстраюнкциональное распространение деполяризации, возникающей в ответ на добавление 1 ммоль/литр ацетилхолина к раствору.

2. Для измерения особенностей распространения эксперименты выполняли на таких портняжных мышцах лягушки, у которых тазовую часть, лишенная конечной пластинки, изолировали от тибиальной части, содержащей конечные пластинки, резиновой мембраной. В отделенной тазовой части ацетилхолин, даже в концентрации 10 ммоль/литр, не вызывал возникновение деполяризации, в то же время деполяризация, вызванная ацетилхолином в тибиальной части, распространялась на тазовую часть.

3. Тетродоксин в концентрации 31 нмоль/литр не оказывал влияния на величину деполяризации, вызванной ацетилхолином в портняжной мышце, в концентрации же 3,1 мм моль/литр уже вызывал 35%-ое торможение.

4. В присутствии тетродоксина деполяризация, измеряемая на экстраюнкциональной мембране, является результатом электронического распространения ацетилхолиновой теполяризации, возникающей на конечной пластинке. Пространственная константа ацедилхолиновой деполяризации, распространяющейся на экспраюнкциональную область, тождественна пространственной константе, определенной с помощью прямоугольно-волнового анализа.

5. Если возникновение и распространение потенциала действия не подавляют гетродоксином или лишенным натрия раствором Рингера, то величина деполяризации, распространяющейся на экстраюнкциональну тазовую часть, несколько больше, чем деполяризация, которая последовала бы при электротоническом распространении.

6. Результаты настоящих опытов указывают на то, что на экстраюнкциональных мембранах *twitch*-волокон отсутствуют ацетилхолиновые рецепторы. Отклонение от лектротонического распространения вызывает появление серии потенциалов действия, опровождающей ацетилхолиновую деполяризацию.

СРАВНЕНИЕ ЭКСПЕРИМЕНТАЛЬНЫХ МЕТОДИК, ПРИМЕНЯЕМЫХ ДЛЯ ИЗУЧЕНИЯ РЕФЛЕКСА С ПЕРЕДНЕГО КОРЕШКА, ВЫЗЫВАЕМОГО РАЗДРАЖЕНИЕМ ВОЛОКОН ЗАДНЕГО КОРЕШКА СПИННОГО МОЗГА ЛЯГУШКИ

ДЬ. ТЭГЗЕШНЕ и Г. ЦЕХ

Авторы изучали с помощью электрофизиологических методов влияние, оказываемое препаратом метил-м-амнобензоатом (МЗ – 222, *Sandoz)* на потенциалы с переднего корешка, вызываемые раздражением заднего корешка спинного мозга лягушки. Спустя несколько минут отмечалось полное угнетение рефлекторной реакции. Эффект бесследно исчезал приблизительно через 60 мин, если препарат применяли в малой дозе. В ответ на введение большой дозы или повторное введение малой дозы препарата продолжительность депрессивного действия возрастала, а моносинаптический компонент переднекорешкового рефлекса часто необратимо нарушался.

Во второй части сообщения авторы демонстрируют моносинаптический рефлекс с переднего корешка изолированного переживающего спинного мозга лягушки, помещенного в раствор Рингера. Результаты экспериментов сравниваются с установившимся в литературе взглядом на моносинтаптические рефлекторные связи спинного мозга лягушки.

О ПОСТДЕНЕРВАЦИОННОЙ ГИПЕРТРОФИИ В ВОЛОКНАХ МЕДЛЕННЫХ МЫШЦ ЦЫПЛЕНКА ПОСЛЕ ПОЛНОГО ЛИШЕНИЯ РАСТЯЖЕНИЯ И НАПРЯЖЕНИЯ

ФЕНГ ТЭ-ПЕИ, ВИ ВАНГ-ЮНГ и ЛУ ДА-КСИНГ

В экспериментах на медленных мышечных волокнах одной чисто медленной мышцы *(m. anterior latissimus dorsi)* двух смешанных мышц *(m. flexor metacarpi ulnaris* и *m. sartorius)* авторы показали, что явление последенервационной гипертрофии наблюдается также в тех случаях, когда после денервации предотвращается растяжение мышцы, более того — оно наступает даже в состоянии укорочения теномизированных мышц, которые освобождены и от напряжения. Поразительные различия обнаруживаются между медленными волокнами *m. latissimus dorsi* и смешанных мышц, как в отношении гистохимических свойств, так и с точки зрения степени выраженности гипертрофии после денервации. В медленных волокнах смешанных мышц после денервации обнаруживают гораздо более выраженную гипертрофию, чем в волокнах чисто медленной *m. anterior latissimus dorsi*. Необходимо дальнейшее изучение этого явления.

НОВЫЙ МЕТОД ДЛЯ ОПРЕДЕЛЕНИЯ КАПИЛЛЯРНОЙ ФИЛЬТРАЦИИ И ПОСТКАПИЛЛЯРНОЙ КОМПЛИАНЦИИ В РАЗЛИЧНЫХ ОРГАНАХ И ТКАНЯХ В УСЛОВИЯХ ПЕРФУЗИИ С ПОСТОЯННОЙ СКОРОСТЬЮ

Д. П. ДВОРЕЦКИ

Нами был разработан новый метод для измерения капиллярной фильтрации. Коэффициент капиллярной фильтрации (CFC) можно определить в любом органе с помощью этого метода. Коэффициент капиллярной фильтрации в легких и тонкой кишке у наркотизированных кошек составлял 0,055 и, соответственно, 0,129 мл/мин/мм рт. ст. на 100 г ткани.

ВЛИЯНИЕ БЛОКАТОРА БЕТА-РЕЦЕПТОРОВ ПИНДОЛОЛА НА ВРЕМЯ ПЕРЕЖИВАНИЯ У СОБАК С ОСТРОЙ ПОЧЕЧНОЙ НЕДОСТАТОЧНОСТЬЮ, ВЫЗВАННОЙ ХЛОРИСТОЙ РТУТЬЮ

Ю. ШИКЛОШ и К. ГААЛ

В экспериментах на собаках авторы изучали влияние, оказываемое пиндололом на острую почечную недостаточность, вызванную сулемой ($HgCl_2$). После однократного внутривенного введения пиндолола (100 мкг/кг веса тела) достоверно увеличивалось количество выделяемого с мочой натрия, достоверно уменьшалась активность плазменного ренина (PRA) и количество выделяемых с мочой катехоламинов — адреналина (A) и норадреналина (NA).

Острая почечная недостаточность вызывалась однократным внутривенным введением $HgCl_2$ в дозе 3 мг/кг веса тела. В другой группе животных внутривенная инъекция 100 мкг/кг пиндолола давалась за час до введения такой же дозы сулемы. После введения только сулемы животные погибали на 3 – 5-й день, предварительное же введение пиндолола увеличивало время переживания на 4 – 8 дней, две же собаки полностью оправились после острой почечной недостаточности. Степень азотемии была значительно меньше в группе, где вводился пиндолол, чем в группе только с отравлением хлористой ртутью. Пиндолол предотвращал вызываемое $HgCl_2$ уменьшение активности плазменного ренина и увеличение выделения в моче катехоламинов. Обобщая наши наблюдения можно сказать, что предварительное введение пиндолола уменьшало тяжесть протекания острой почечной недостаточности, хотя и не могло предупредить ее развитие. Основываясь на результатах наших экспериментов, мы приходим к выводу, что повышенная активность симпатической нервной системы играет существенную роль в патомеханизме острой почечной недостаточности.

МОДЕЛЬ КАТЕТЕРА С БАЛЛОНОМ ДЛЯ СУПЕРСЕЛЕКТИВНОЙ КАТЕТЕРИЗАЦИИ МОЗГОВЫХ СОСУДОВ

Ш. ЦИРЬЯК, Э. ПАСТОР, Л. ЛАЗАР, ДЬ. ДЕАК и Ф. ЛАНИ

С целью катетеризации мозговых сосудов человека мы выполнили серию модельных экспериментов на собаках. Разработали метод изготовления катетеров с баллончиком, пригодных для облитерации сосудов, суперселективной ангиографии и терапевтической закупорки сосудов. Описали достоинства и недостатки применяемых катетеров и баллонов. На основании наблюдений над динамикой кровотока сделали выводы, которые могут быть использованы также и при клинических вмешательствах.

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