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VOLUME 62

(1983)

INDEX

NUMBER 1

COMMENTS

NUMBER 2

PHYSIOLOGY-PATHOPHYSIOLOGY

BOOK REVIEWS

NUMBERS 3—4

PHYSIOLOGY-PATHOPHYSIOLOGY

ABSTRACTS OF THE LECTURES AND POSTERS PRESENTED AT THE MEMBRANE SYMPOSIUM, SÜMEG, HUNGARY, MAY 10—14, 1982

LECTURES

POSTERS

BOOK REVIEWS

AUTHOR INDEX

SUBJECT INDEX

CONTENTS

Acta Physiologica Hungarica, 62 (1), pp. 3—5 (1983)

Obituary

KÁLMÁN LISSÁK 1908—1982

Professor Lissák died in his 75th year on 22 June 1982 in Győr, Hungary. He was the doyen of the Hungarian physiologists, a member of the Hungarian Academy of Sciences, and director of the Institute of Physiology in Pécs for more than three decades, editor in chief of this journal for more than two decades and its praeses consilii until his death. His death is a grievous loss to his students, co-workers and many fellow scientists all over the world. He set three noble goals to himself for a lifelong pursuit: firm establishment of experimental methods in neglected areas of research in his country, introduction of the highest standards of education in physiology, and the strengthening of international scientific connections, cooperation and friendship. His efforts

were crowned with significant success in all his pursuits. He broke ground for solid and internationally respected research work in the fields of neurophysiology, behavioural physiology and neuroendocrinology. His exemplary book of physiological practice served a high level of education for generations of physicians in Hungary. He was among the inaugurators and founders of noted international societies, like the International Brain Research Organization (IBRO) and INTERMOZG, the society of neurophysiologists of the socialist countries. Two weeks before his death with gravely impaired health but unbroken spirits he attended and chaired a festive convention of the latter society in Moscow.

Professor Lissák graduated from the Medical Faculty of the University of Budapest in 1933 and began his scientific career at the Physiology Department of the Medical Faculty of the University of Debrecen in eastern Hungary. His talents, tenacity of purpose and the wise support of his principals rendered possible for him to work in the laboratories of three outstanding neuroscientists of the thirties, Otto Loewi in Graz, Austria, Wilhelm Trendelenburg in Berlin and Walter Cannon in Boston. The two years spent with Cannon and the close friendship which developed between them made an ineffaceable impact on him. He returned home with plans to establish basic neurophysiological research in Hungary. Unfortunately these had to be postponed until after the war. The generous support of the new democratic state created an advantageous background for science. Meanwhile in 1943 he was appointed full professor of physiology and director of the Institute of Physiology at the University of Pécs. His continuous efforts here to create adequate material basis for up-to-date research work in neurophysiology and neuroendocrinology proved successful and reached its climax around the fifties. With a team of freshly recruited young, enthusiastic co-workers he achieved significant results. More than five hundred papers, mostly in noted international journals and several monographs were published during his leadership. His institute in Pécs became a frequented place of cooperative research work for teams composed of researchers from different eastern and western countries. The attractive personality of Professor Lissák helped him to make personal contacts and lasting friendships all over the world. He made use of his connections to obtain scholarships and posts for his co-workers and those of other research institutions in Hungary in laboratories where they could acquire adequate knowledge and the technical skills required by their research.

His achievements were acknowledged by his appointement as vice president of the International Physiological Society. It was mainly due to his efforts that in 1980 the international congress of the society was held in Budapest. His scientific merits were honoured by memberships in many scientific societies all over the world as well as with decorations awarded to him by his country. After his retirement in 1978 he remained active as a member of the Academy of Sciences and as the councillor of the neurophysiological research group of the Academy in Pécs. His death means an irreparable loss for neuroscience in Hungary.

Endre Grastyán

Acta Physiologica Hungarica, 62 (1), pp. 7—17 (1983)

Physiology—Pathophysiology

EFFECTS OF DISTURBANCES OF ACID–BASE EQUILIBRIUM ON THE ACTIVITY OF THE RUMEN

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The effect on ruminal motility of NH_4- or Na-acetate and Na- or K-lactate of various pH and doses infused into the jugular vein (i. v.) or carotid artery (i. a.) was studied in sheep with ruminal fistula

I. v. infusion of NH_4-acetate whenever it was accompanied by a considerable increase of the blood NH_4 level potently inhibited the amplitude and frequency of ruminal contractions. I. a. administration produced more rapid and more apparent responses and a slightly elevated venous ammonia concentration than did the i. v. infusion. Thus the augmented blood ammonia level not only affected the smooth muscles and peripheral nerve endings but also inhibited the vegetative centres of the central nervous system. Infusion of Na-acetate and Na- or K-lactate solutions induced compensated acidosis or alkalosis which reduced the amplitude of ruminal contractions and produced respiratory disturbances. The latter were more apparent in alkalosis. In uncompensated acidosis or alkalosis, ruminal motility was permanently inhibited or abolished. The results appear to show that in not properly fed ruminants the frequently observed and long lasting compensated acidosis or alkalosis might influence the activity of the vegetative centres thus further metabolic disturbances and impaired gastrointestinal activity might follow the shifts in acid–base equlibrium.

Keywords: acid–base equilibrium, ruminal motility, NH_4-acetate, Na-lactate, K-lactate, ruminal fistula, ruminal contraction

Cows with considerable milk production and short fed oxen consume a great amount of carbohydrate and protein with the fodder. Under the action of the ruminal microflora a higher than normal amount of fatty acids (acetic acid, propionic acid, butyric acid), lactic acid and ammonia are formed by fermentation. Whenever there is an imbalance among the components (such as an inappropriate carbohydrate to protein ratio), in most instances the production of ammonia and lactic acid and in particular cases the amount of butyric acid increase in the ruminal juice due to incomplete fermentation.

Earlier we have examined the effect on ruminal motor activity of ammonium compounds and found that the absorbed ammonium ion inhibited the

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motor activity of the rumen by affecting the smooth muscle cells and nerve terminals [4, 5]. Further studies then indicated the absorbed ammonia might alter the gastrointestinal motor activity by interfering with cellular metabolism or through an action exerted on the autonomous motor centres of the central nervous system [10, 11]. Even so the disturbances of the acid–base equilibrium *per se* might also alter the motor activity of the rumen [1, 2, 12, 13, 16]. The large amount of absorbed ammonia attains a high blood level and affects cellular metabolism. This holds true primarily for the neural cells which are sensitive to ammonia. The volatile fatty acids and lactic acid set free may also lead to acute or chronic acidosis depending on the rate of their formation [6, 7, 15]. In this case compensated acidosis develops without overt clinical signs but by exerting an overall noxious effect on the intermediary metabolism it results in decreased milk production and reduced growth rate of the body mass, and later in severe digestive and metabolic disorders [6, 7, 8, 9, 14].

The purpose of the present study was to investigate the effect on ruminal motility of high blood levels of ammonia induced by the infusion of various salts (NH_4- and Na-acetate; Na- and K-lactate), of organic acids (acetic acid, lactic acid) and of compensated and uncompensated disturbances of the acid–base equilibrium.

Materials and methods

Rumen fistula (Jarret) was created in six sheep weighing 40–50 kg. Plastic catheters were inserted into the carotid artery and jugular vein for the continuous infusion of NH_4- and Na-acetate, Na- and K-lactate using an infusion apparatus (Kutesz 5150). The concentration and volume of the infused solutions, the rate of infusion, the route and time of administration and the blood collection periods are shown in the figures. Ruminal motility, changes in amplitude and frequency were monitored by a sensitive Marey's drum connected to a rubber balloon introduced into the rumen via the fistula and recorded on a kymograph driven at a speed of 40 mm/min. (Figures 1–5 are reduced to 2.5 of their original size.) Parameters of the acid–base balance were estaminated by the Astrup technique: in the blood samples collected at appropriate times ammonia was measured [3] and Na^+ and K^+ concentrations were determined by flame photometry. At the commencement of all studies the animals were infused intravenously (i. v.) or intraarterially (i. a) with physiological saline and the resting values were determined.

Results

Earlier we have shown [4, 5, 10, 11] that ammonia reduces the ruminal contractions by its local effect on the mucosa. Considering its toxic effect one may conclude that the high blood ammonia, level by inhibiting the autonomous centres of the medulla oblongata, might suppress ruminal motility [10] through a central site of action. With an increased uptake of protein and NPN (urea) substances the suppression might further aggravate the digestive and metabolic disorder [11]. In addition, a certain shift was observed in the acid–

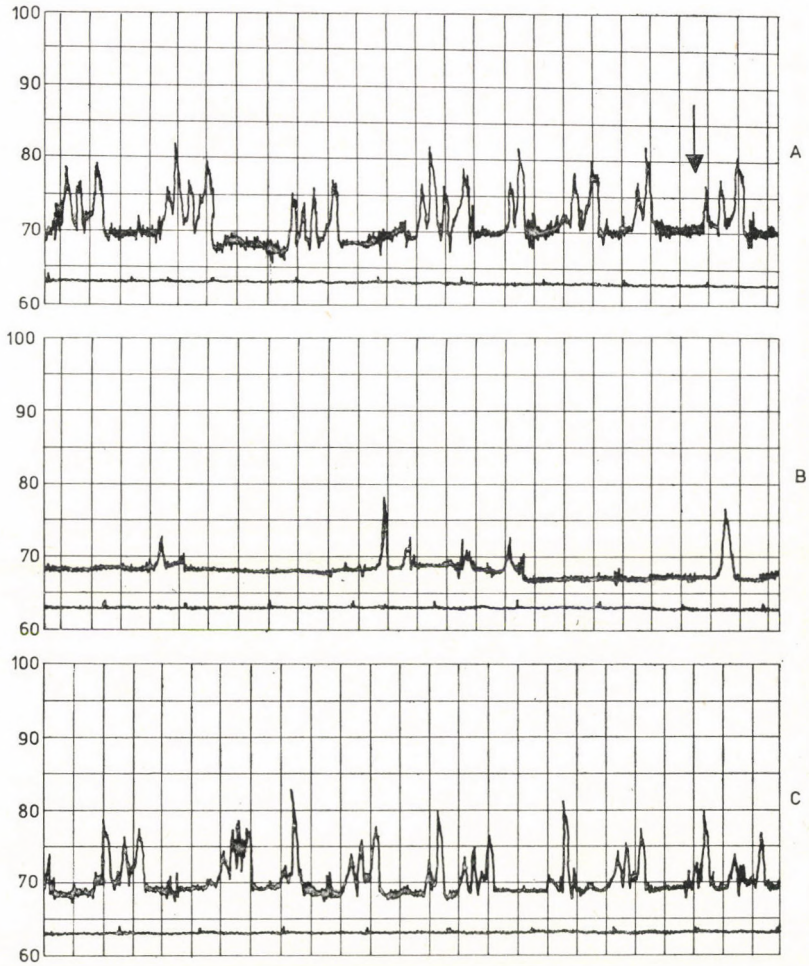

Fig. 1. Infusion of NH_4-acetate solution into the jugular vein (i.v.). pH of the solution: 7.30; concentration of the solution: 0.5 mol/l; infusion rate: 1.36 mmol/min; duration: 9 min; timing: 1 min (bottom line); arrow indicates the start of infusion (A). *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion. NH_4 concentration in carotid blood: 0.10 mmol/l; (*A*); 0.62 mmol/l (*B*); 0.7 mmol/l (*C*)

base balance [10]. Therefore further studies were carried out and now we report on their results.

Figure 1 shows the effect of i.v. administered NH_4-acetate, pH 7.3. It is seen that as soon as the NH_4 concentration had reached 0.60 mmol/l in carotid blood, ruminal motility practically ceased (B). The effect developed in 6–8 min and after termination of infusion the motility was rapidly (9 min) restored (C) with simultaneous normalization of the blood NH_4^+ level [5, 10].

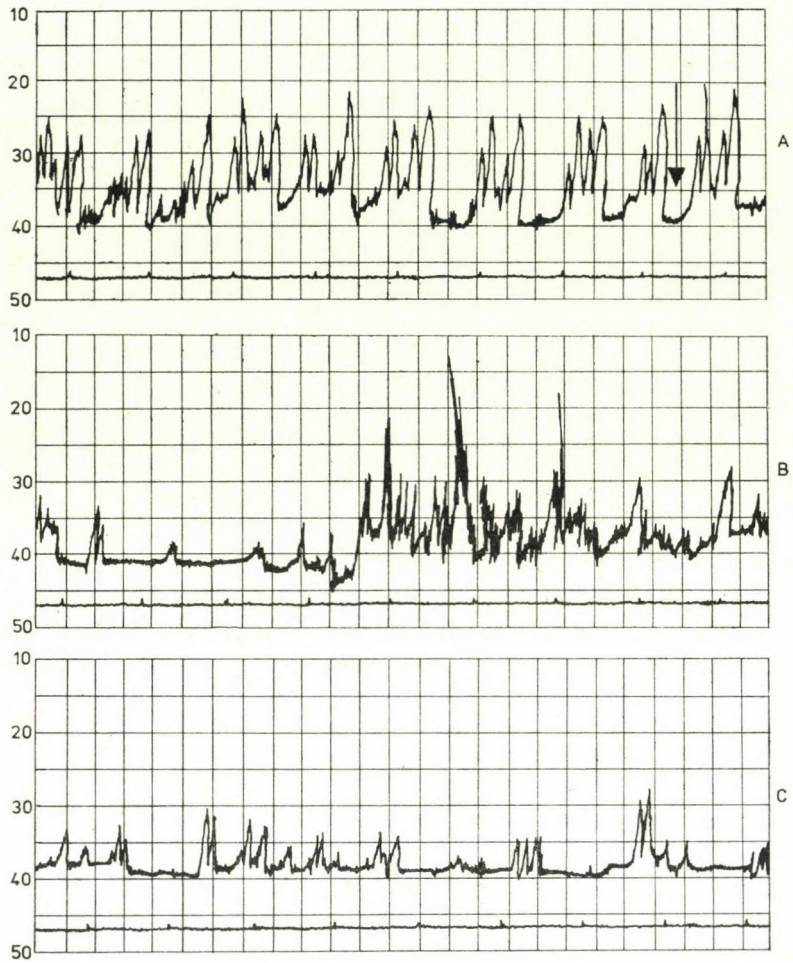

Fig. 2. Infusion of NH_4-acetate solution into the carotid artery (i.a.) pH of the solution: 7.30; concentration of the solution: 0.5 mol/l; infusion rate: 1.36 mmol/min; duration: 9 min. *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion. NH_4 concentration in jugular blood: 0.09 mmol/l (*A*); 0.41 mmol/l (*B*); 0.18 mmol/l (*C*)

Figure 2 represents the effect of an i.a. infusion of NH_4-acetate. Soon (4–6 min) after its start the intensity and frequency of ruminal contractions were markedly reduced, tachypnoea developed and then rapid and deep inspirations appeared *(B)*. The blood ammonia concentration was considerably increased in the jugular blood *(B)* and after its decrease the disturbed motility of the rumen did not cease but persisted for 20–25 min. This period was characterized by a diminution of contractile forces and atypical contractions *(C)*.

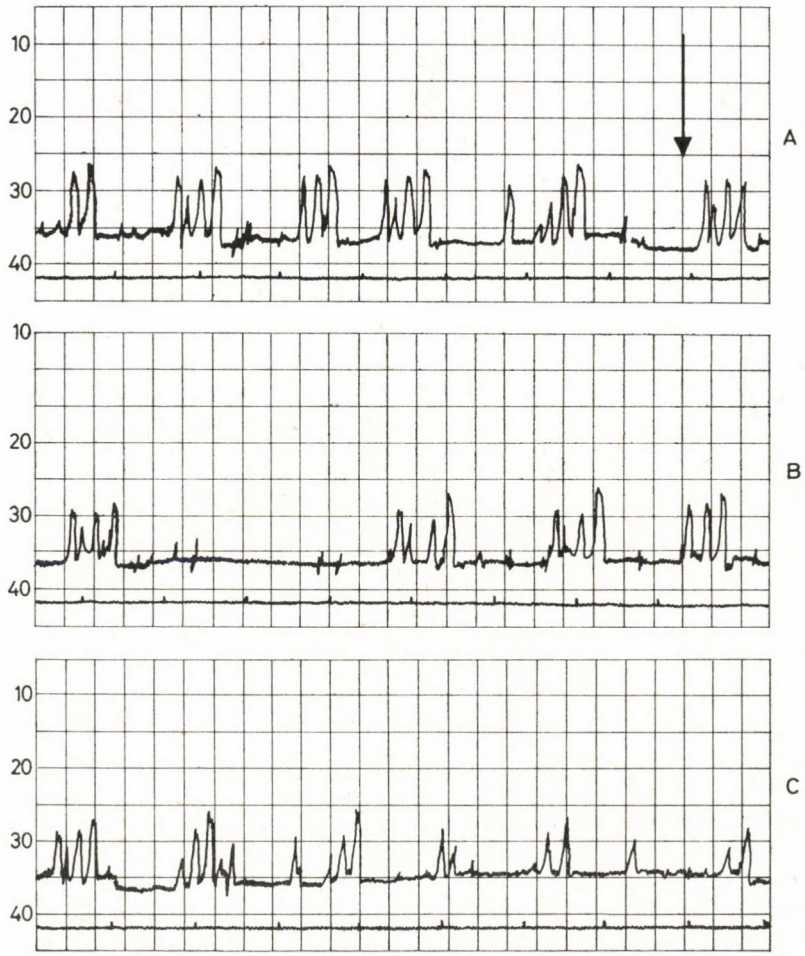

Fig. 3. Infusion of Na-acetate solution i.v.: pH of the solution: 7.30; concentration of the solution: 0.5 mol/l; infusion rate: 1.36 mmol/min; duartion: 18 min. *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion.

Acid–base parameters in carotid blood:

	A	*B*	*C*
pH	7.46	7.39	7.37
St.bic.*	38 mmol/l	32 mmol/l	42 mmol/l
PCO_2	5.23 kPa	4.22 kPa	6.18 kPa
BE**	+ 3.4	+ 1.1	+ 2.8
BB***	33 mmol/l	39 mmol/l	39 mmol/l

* Standard bicarbonate
** Base excess
*** Base buffer

The infusion of ammonium acetate into the jugular vein or the carotid artery reduced the ruminal contractions whenever the blood NH_4 level exceeded 0.4 mmol/l. Upon i. v. administration this effect developed slower but disap-

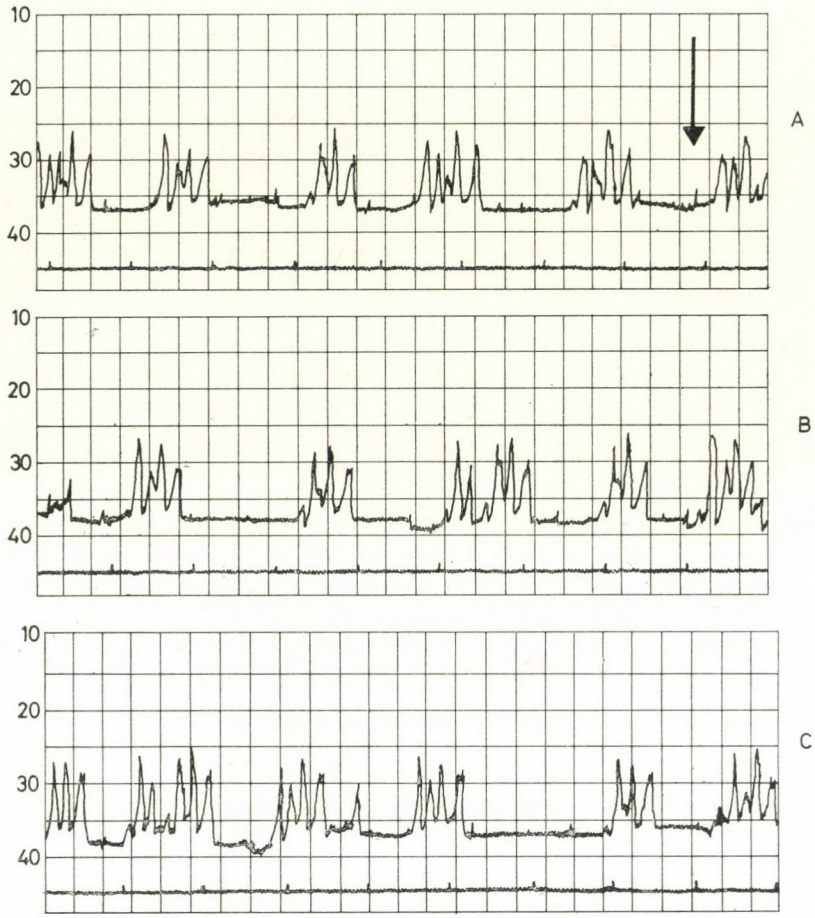

Fig. 4. Infusion of Na-acetate solution i.a.: pH of the solution: 7.30; concentration of the solution: 0.5 mol/l; infusion rate: 1.36 mmol/min; duration: 14 min. *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion.

Acid–base parameters in jugular blood:

	A	*B*	*C*
pH	7.40	7.36	7.46
St.bic.	42 mmol/l	38 mmol/l	44 mmol/l
PCO_2	6.12 kPa	5.10 kPa	5.51 kPa
BE	+6.3	+4.2	+4.2
BB	28 mmol/l	32 mmol/l	37 mmol/l

peared faster; upon i. a. infusion the effect appeared earlier and disappeared later and the respiration was also markedly altered.

Earlier we have studied the imbalance of acid–base equilibrium by sheep [8, 15]. From this experiments appeared to support the view that the shift of the acid–base imbalance might *per se* affect the ruminal contractions. To clarify this problem acidosis or alkalosis was induced by infusions.

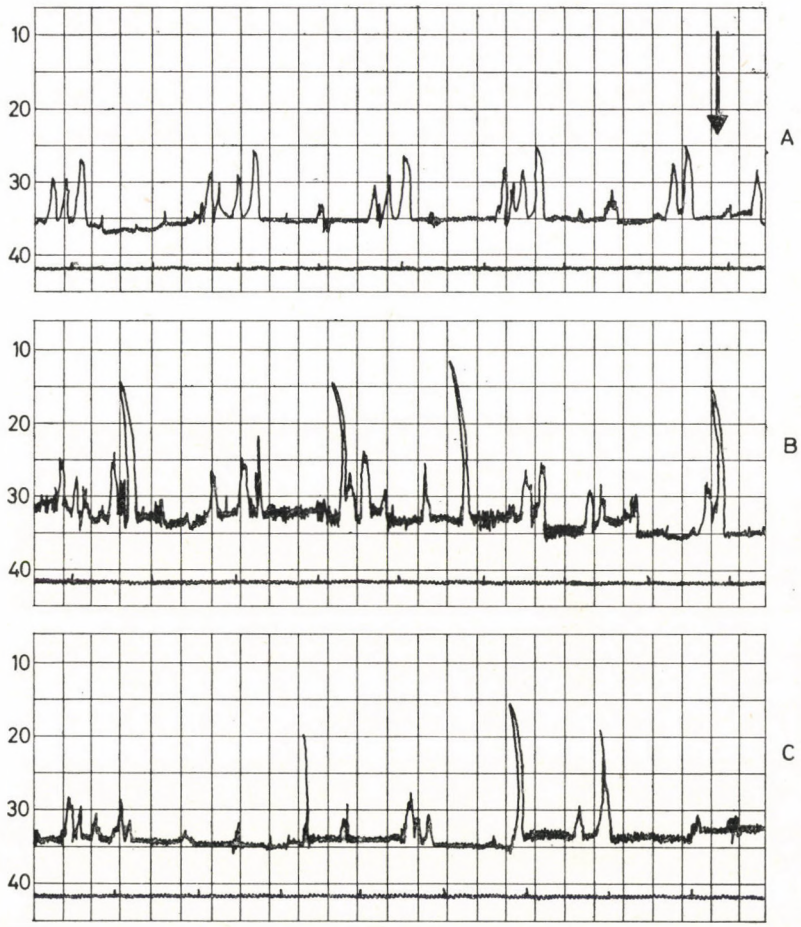

Fig. 5. Repeated infusion of Na-acetate solution i.v.: concentration of the solution: 0.5 mol/l; infusion rate: 1.36 mmol/min; duration: 4×10 min in intervals of 10 min. *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion.

Acid–base parameters in carotid blood:

	A	*B*	*C*
pH	7.49	7.37	7.46
St.bic.	31 mmol/l	24 mmol/l	29 mmol/l
PCO_2	5.45 kPa	4.26 kPa	6.65 kPa
BE	+7.2 mmol/l	+1.7 mmol/l	+3.6 mmol/l
BB	42 mmol/l	54 mmol/l	48 mmol/l

Figure 3 shows the effect of Na-acetate solution pH 7.30 administered by the venous route. Six to seven min after the onset of infusion the amplitudes of the ruminal contractions were slightly, whereas their frequency considerably, reduced *(B)*. After termination of the infusion the effects persisted in mitigated form for 15 min *(C)*. In the blood there was a compensated acidosis with detectable signs even 15–20 min after the infusion.

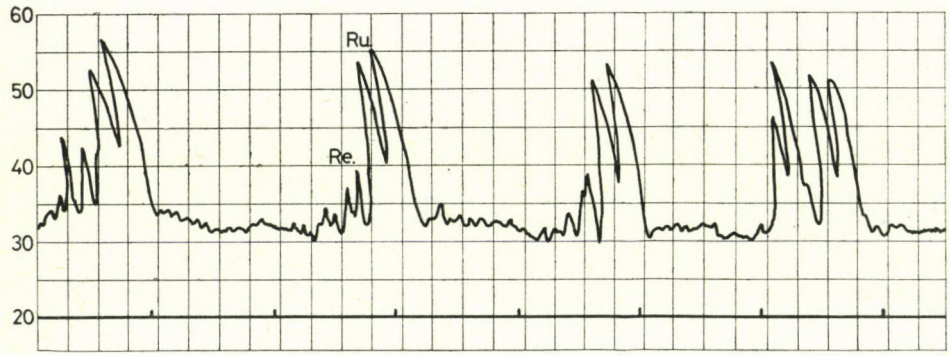

Fig. 6. Ruminal motility before the studies. Timing 1 min
Acid–base parameters in jugular blood:
Normal values: Blood pH: 7.44;
St.bic.: 22.9 mmol/l; PCO_2: 4.44 kPa; BE: +1.4 mmol/l; BB: 42.0 mmol/l

Figure 4 illustrates the effect of the i.a. administration of the same solution. It is seen that the response occurred rapidly (3–4 min; earlier than in Fig. 3) and showed almost the same pattern as after an i.v. infusion. The power of contractions was hardly altered, but their frequency was markedly decreased. 3–5 min after termination of the infusion—i.e. faster than in Fig. 3—the effect vanished *(C)*.

In a further series of studies we examined the effect on the acid–base equilibrium and ruminal motility of i.v. infusions administered repeatedly in short intervals.

Figure 5 demonstrates that in response to the infusions the contractions were markedly decreased and became irregular. Respiration increased both in volume and frequency, and occasionally by deep inspirations occurred *(B)*. A compensated acidosis developed *(B)* and its restoration took a long period. After termination of the infusion (20–30 min), ruminal motility was increased, breathing became quiet and deep inspirations were less frequent. Normal conditions were re-established 35–40 min after terminating the infusions.

Under normal conditions mean Na^+ concentration in the blood was 142 mmol/l, with a range of 134–152 mmol/l. During infusion including the repeated i.v. infusions the blood Na^+ level exceeded the highest normal value in no case. Actually, this was expected since the intake of Na^+ by the infusion was 0.5–0.7 mmol/min considering that during the 10–15 min the total Na^+ intake amounted to 5.0–7.5 mmol. During 4 infusion periods of 10 min each, Na^+ intake was 20–28 mmol. Taking into account the low intake, the fast distribution and excretion of Na^+ it seemed plausible that no hypernatraemia had developed in either study (Figs 3, 4 and 5).

In a further series of examinations the effect of Na- and K-lactate was tested. After a high intake of carbohydrates (cellulose, starch, etc.) a consid-

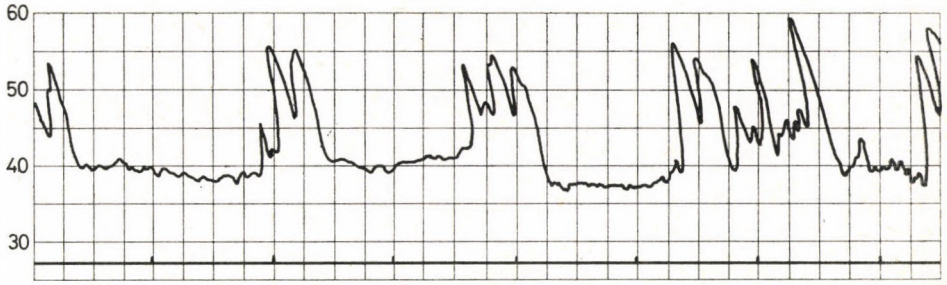

Fig. 7. Infusion of Na-lactate into the carotid artery. pH of the solution: 4.54; infusion rate 1.48 mmol/min; duration: 10 min. *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion. Acid–base parameters in jugular blood on termination of the infusion: pH: 7.36; St.bic.: 22.1 mmol/l; PCO_2: 5.20 kPa; BE: —2.6 mmol/l; BB: 41.2 mmol/l

erable amount of lactic acid can be produced in the rumen. The infusions were administered only by i.a. route.

Figure 6 shows a normal tracing. The complex movements of the rumen which usually consist of 3 or occasionally 4 contraction waves are separated well. They start with a biphasic contraction of the reticulum (Re) followed by the readily separated actions of the dorsal and later by the ventral sac of the rumen (Ru). On changing the location of the balloon the intense contractions of the reticulum are occasionally more apparent (last wave). The interval between the contractions is usually 1–1.5 min (timing lower line). Before the onset of ruminal contractions, breathing had become less frequent and deeper.

Figure 7 indicates the effect of the strongly acidic (pH 4.54) Na-lactate solution (0.5 mol/l) infused i. a. Two to three min after the start of the infusion the movements markedly diminished in amplitude: the contractions became faster and irregular, and the breathing slower. The venous pH decreased and

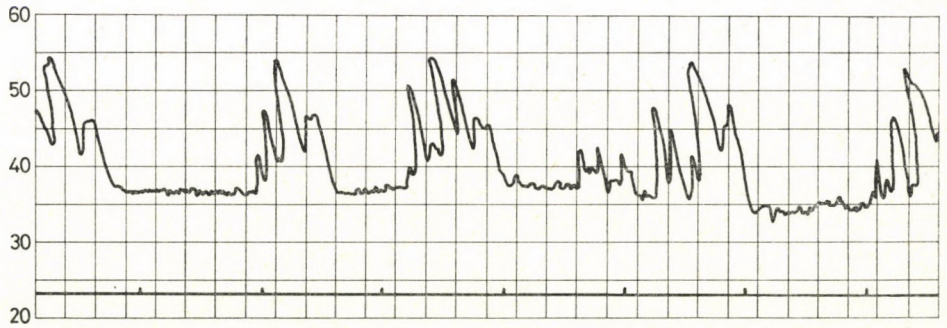

Fig. 8. Infusion of K-lactate into the carotid artery. pH of the solution: 6.95; infusion rate 1.48 mmol/min; duration: 10 min. *A* Ruminal motility prior to infusion. *B* Ruminal motility before terminating the infusion. *C* Ruminal motility 10 min after the end of infusion. Acid–base parameters on the termination of infusion: pH: 7.45; St.bic.: 24.4 mmol/l; PCO_2: 4.00 kPa; BE: +0.2 mmol/l; BB: 46.6 mmol/l

compensated acidosis developed. Serum Na^+-concentration was 143 to 151 mmol/l during the study.

Figure 8 demonstrates ruminal conctractions after i.a. infusion of neutral (pH 6.95) K-lactate (0.5 mol/l) solution. Several minutes after onset of the infusion the breathing became more frequent, the ruminal contractions were in power reduced (wave 1), then irregular responses occurred (wave 3). Breathing became intermittent and slower and high-amplitude inspirations appeared. The tracing reflects the frequent breathing and not the ruminal motility. The normal mean serum K^+ level was 4.5 mmol/l (3.8 to 5.6) during the study. As the blood values show, in response to neutral K-lactate a compensated alkalosis developed which in addition to the inhibition of the ruminal motility potently affected the breathing.

Discussion

Infusion of NH_4-acetate into the carotid artery (i.a.) toward the central nervous system or into the jugular vein (i.v.) was followed by a 5- to 10-fold increase in the blood NH_4 level and a marked reduction of ruminal motility.

The infusion of Na-acetate or Na- or K-lactate solutions i.v. or i.a. also affected the ruminal motility. An analysis of the responses revealed that the compensated acidosis or alkalosis differentially affected the frequency and amplitude of the ruminal contractions depending on the dose, pH, infusion time and route of administration. NH_4^+ whether administered i.v. or i.a. markedly reduced the frequency and amplitude of ruminal motility. After carotid infusion the responses occurred more rapidly and persisted longer than after infusion i.v. The infusion of neutral Na-acetate solution caused mild acidosis and diminished the amplitude of ruminal contractions; carotid infusion elicited less apparent but more rapid responses than the jugular infusion. A severe acidosis potently suppressed the ruminal motility and also markedly affected the amplitude and frequency of contractions. I. a. administration of an acidic Na-lactate solution produced acidosis. The responses were identical with those elicited by Na-acetate. The i.a. infusion of neutral K-lactate solution caused a mild alkalosis, intermittent breathing and weak, irregular ruminal contractions.

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MUSCLE CAPILLARIES IN TENOTOMIZED HUMAN MUSCLES

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Received June 5, 1982
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The capillaries were studied in tenotonized human muscles after tenotomy or spontaneous rupture of the tendon. The mean capillary area was not significantly different in the patients with tenotomy, or with spontaneous rupture of the tendon, as compared to intact muscles. The mean basement membrane area as a percentage of total capillary area was significantly larger and the size capillary lumen significantly smaller in the injured than in the intact muscles. The alterations of the capillaries were time-dependent.

Keywords: muscle capillary, tenotomized human muscle, spontaneous rupture of the tendon, mean capillary area, basement membrane area, injured muscle, intact muscle.

Changes of the capillaries have been studied extensively in diabetes [6, 10, 11], atherosclerosis [1, 8], gout [2], in inflammatory myopathies [3] and in acute anoxic hypoxia [4]. Still, the cause of the changes and especially of the thickening, of the basement membrane has escaped detection.

In a previous report we have described changes of the capillary density of human muscles after tenotomy [5] and spontaneous rupture of the tendon. The obliteration of the capillaries was particularly pronounced in the amount of anastomoses. To our knowledge, there has been no detailed study of the capillary ultrastructure or of the quantitative changes of muscle capillaries in patients with tenotomy or with spontaneous rupture of the tendon.

Materials and methods

Group I (tenotomized muscles)
Specimens were obtained from the injured muscle and from the surrounding intact muscle in 24 cases during surgical reconstructive procedure 3 to 12 weeks after tenotomy.

Group II (muscles with spontaneous rupture of the tendon)
Specimens were excised from the injured muscle and the identical contralateral intact muscle in 20 cases during reconstructive operations 4 days to 8 weeks after spontaneous rupture of the tendon of the biceps brachii muscle. The clinical data of the patients are summarized in Tables I and II.

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The muscle pieces were immediately trimmed and fixed in 2% osmium tetroxide buffered according to Millonig, and embedded in Durcupan ACM (Fluka). Semithin and ultrathin sections were cut with Reichert OM U2 ultramicrotome, and the semithin sections were stained with toluidin blue and the ultrathin sections with uranyle acetate and lead citrate. The ultrathin sections were examined and photographed by JEOL 100 C electron microscope. The capillaries detected were photographed at a primary magnification of 3800 or 5000 depending on the size. Planimetry according to Williamson et al. [12] was used to measure in each capillary the total capillary area, the basement membrane area, endothelial and luminal areas. The results will be given in percentages of the total capillary area. The number of capillaries in relation to the number of muscle fibre was calculated from semithin sections. In each biopsy, 400 muscle fibres with capillaries in contact were estimated.

Table I

Clinical and pathological data of tenotomized patients

Age	Sex	Time after tenotomy	Injured	Intact
			muscle	
24	F	3 hours	M. flexor carpi rad.	M. palmaris long.
29	M	6 hours	M. flexor pollic. long.	M. flexor carpi rad.
37	F	5 hours	M. flexor carpi rad.	M. palmaris long.
23	M	6 days	M. flexor carpi uln.	M. flexor digit. subl.
27	M	7 days	M. flexor carpi uln.	M. palmaris long.
28	F	28 days	M. gastrocnemius	M. plantaris
27	M	27 days	M. flexor carpi uln.	M. palmaris long.
25	M	25 days	M. flexor digit. subl.	M. flexor digit. prof.
47	M	24 days	M. flexor pollic. long.	M. flexor digit. subl.
33	F	22 days	M. ext. digit.	M. extens. pollic. brev.
27	F	29 days	M. adduct. pollic.	M. extens. pollic. brev.
41	M	23 days	M. biceps brachii	M. brachialis
16	M	28 days	M. extens. digit.	M. extens. pollic. brev.
34	M	26 days	M. flexor digit. subl.	M. flexor digit. prof.
45	M	24 days	M. flexor pollic. long.	M. palmaris long.
45	F	22 days	M. tibialis ant.	M. extens. digit. long.
24	F	8 weeks	M. flexor carpi uln.	M. palmaris long.
31	M	7 weeks	M. flexor carpi uln.	M. palmaris brev.
28	F	9 weeks	M. flexor digit. subl.	M. flexor digit. prof.
45	M	12 weeks	M. flexor pollic. long.	M. palmaris brev.
34	M	6 weeks	M. flexor digit. subl.	M. flexor carpi rad.
41	M	11 weeks	M. flexor digit. subl.	M. opponens pollic.
24	F	8 weeks	M. flexor carpi uln.	M. palmaris long.
42	M	8 weeks	M. flexor carpi rad.	M. parlmaris long.

Table II

Patients with spontaneous rupture of biceps muscle tendon

Age	Sex	Time after spontaneous rupture of tendon
46	M	14 days
51	M	15 days
41	M	10 days
44	M	15 days
31	M	11 days
43	M	4 days
32	F	12 days
28	M	12 days
43	M	13 days
52	M	8 days
41	M	9 days
28	M	18 days
36	F	21 days
31	M	21 days
43	M	23 days
47	M	8 weeks
52	M	6 weeks
52	M	8 weeks
34	F	6 weeks
39	F	7 weeks

Results

The mean total capillary area, consisting of the combined areas of the basement membrane, endothelial cells and capillary lumen, was not significantly different after tenotomy (43.2 $\pm$ 12.4 μm^2) and spontaneous rupture of the tendon (41.9 $\pm$ 13.1 μm^2) from that of intact muscles (mean 42.7 $\pm$ $\pm$ 10.8 μm^2 and 44.2 $\pm$ 11.6 μm^2, respectively).

The mean basement membrane area, as a percentage of the total capillary area was significantly larger, and the capillary lumen was significantly smaller in the tenotomized muscles and in the muscles with spontaneous rupture of the tendon, than in intact muscles. No significant change was found in the area occupied by the endothelial cells. The changes of the capillaries were time-dependent: 1–7 days after tenotomy or the spontaneous rupture of the tendon no differences could be found in the mean basement membrane

Table III

Capillary size

	Mean total capillary area μm^2	Mean basement membrane fraction of capillary area %	Mean luminal fraction of capillary area %	Mean endothelia fraction of capillary area %
Group I				
Tenotomized muscles				
< 7 days (n= 5)	43.8±10.2	30.1±6.5	43.9±7.4	26.0±6.2
> 7 days (n=19)	43.0±12.8	50.5±6.1*	17.8±5.9*	31.7±8.3
Group II				
M. biceps brachii (injured)				
< 7 days (n= 1)	43.2	30.4	46.3	23.1
> 7 days (n=19)	41.8±13.2	46.6±7.7*	21.6±9.3*	31.8±9.2
Intact muscles (n=24)	42.7±10.8	28.2±4.7	44.1±6.5	27.7±3.9
Intact contralateral m. biceps (n=20)	44.2±11.6	29.8±6.7	45.2±7.3	25.0±4.7

* $p < 0.01$ vs. control muscles

Table IV

Time-dependent alterations of capillaries

	Capillary/ muscle fibre ratio	Mean basement membrane fration of capillary area %	Mean luminal fraction of capillary area %	Mean endothelial fraction of capillary area %
Intact muscles (n=24)	1.8 /1	28.2± 4.7	44.1± 6.5	27.7± 3.9
Intact biceps muscles (n=20)	1.74/1	29.8± 6.7	45.2± 7.3	25.0± 4.7
Within 1 day after injury (n=3)	1.76/1	28.9± 7.6	44.9± 6.8	26.2± 6.1
4–7 days after injury (n=3)	1.3 /1	29.8± 9.1	40.2±10.4	30.0± 8.6
8–15 days after injury (n=10)	0.83/1*	38.3± 7.2	33.4± 8.4	30.3± 9.6
18–30 days after injury (n=15)	0.58/1*	50.6± 8.5*	20.6± 6.4*	31.8± 6.6
> 30 days after injury (n=13)	0.51/1*	55.3±11.6*	18.6± 7.7*	26.1±10.1

* $p < 0.01$ vs. control muscles

area and the mean size of the capillary lumen between the intact and the injured muscles (Tables III and IV). In the arterioles and venules no alterations were found histologically and ultrastructurally.

The capillary/muscle fibre ratio decreased rapidly and after two weeks the number of capillaries in the injured muscles was only one half of that in

the intact muscle. Ultrastructural analysis revealed several changes in the capillaries of the injured muscles that were not present in the intact muscles. The most prominent and constant finding was a severe thickening of the basement membrane. Another common feature was an increase in the number of the collagen fibres around the capillaries and also a swelling of endothelial cells. The endothelial cells contained pinocytotic vacuoles in great numbers and frequently protruded into the capillary lumen.

Discussion

According to Regnault et al. [9] a 10 to 15% increase in basement membrane width may develop with age. Thickening of the capillary basement membrane occurs beside diabetic angiopathy [6, 10, 11], in many other conditions such as gout [2], obliterating arteriosclerosis [1, 8, 9], myopathy [7], and inflammatory diseases [3]. In tenotomized muscles not only a decrease of capillary density [5]has been found but also degenerative changes, thickening of the basement membrane and narrowing of the lumen. These alterations develop rapidly in injured muscles, and the cause of the reduced flow might be at the level of muscle microcirculation.

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EFFECT ON BRAIN MONOAMINES IN THE RAT OF SUBSTITUTED AND PROTECTED ANALOGUES OF THE OXYTOCIN FRAGMENT, PROLYL-LEUCYL-GLYCINAMIDE FOLLOWING N-TERMINAL SUBSTITUTION BY HOMOPROLINE

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Prolyl-leucyl-glycinamide (PLG) and its substituted and protected analogues were tested on the steady-state level of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) in various brain areas. The tripeptides were structurally modified at the N-terminal proline residue either by protection with benzoxycarbonyl (Z group) or by tertiary butyroxycarbonyl (BOC), or by substitution with homoproline (HPRO). C-terminal modification was performed by substitution of the amino group of glycine (GLY—NH_2) by methylester (GLY—OMe). The parent molecule (PLG) increased the 5-HT content in the striatum and the NA and DA levels in the dorsal hippocampus. N-terminal protection by Z-group resulted in a loss of these effects. Striatal effects re-appeared if a methylester group was introduced in the C-terminal glycine. Substitution of the N-terminal with HPRO or that of the C-terminal amine group by OMe resulted in a tendency to increase the 5-HT level in the hypothalamus.

Keywords: prolyl-leucyl-glycinamide, noradrenaline, dopamine, serotonin, brain monoamines

PLG is the first hypothalamic peptide which was shown to have direct central nervous system effects. PLG has been isolated by Nair et al. [12] and suggested to be the inhibiting factor of melanocyte stimulating hormone [3]. The tripeptide potentiated the behavioural effects of DOPA and oxotremorine in rats [13]. These and other data led to the hypothesis that PLG might be beneficial in human parkinsonism [7]. In addition to these effects the tripeptide affects learning processes by delaying the extinction of an avoidance task [21], restoring the amnesia induced by puromycin [4] or by electroconvulsive shock [10]. PLG and structurally similar tri- and dipeptides affect also tolerance to and dependence on narcotic analgesics [2, 11, 19, 22]. Since neurotransmitter mechanisms are involved in all the above described CNS processes, the question has been raised whether PLG affected neurotransmission in the

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brain [5, 9, 14, 15]. It seems that the tripeptide facilitates dopamine turnover mainly in the neostriatum [16, 20].

Since the effects of the tripeptide both on learning processes as well as on CNS neurotransmitter mechanisms are rather modest, it has been attempted to improve these effects by modifying the structure of PLG. This strategy has succesfully been applied for other peptide hormones (for review, see Telegdy, [18]). In the present paper the effect of structurally modified and protected analogues of PLG will be described on the steady-state level of NA, DA and 5-HT in different brain parts.

Materials and methods

Male CFY rats weighing 150–200 g were used in all experiments. Five rats per cage were kept under artificial illumination (light between 6.00 a.m. and 6 p.m.) and food and tap water ad libitum. The animals were anaesthetized with sodium pentobarbital (Nembutal® 40 mg/kg) and their skull was exposed by a midline incision. A polyethylene cannula was placed into the right lateral ventricle at least one week before starting the experiments, and fixed to the skull by stainless steel screws and dental acrylic. Treatments were given intracerebroventricularly (icv) via the chronically implanted cannula. Thirty minutes later the animals were sacrificed by decapitation. The brain was removed and dissected immediately by the method of Baumgarten et al. [1] into the following regions: hypothalamus, mesencephalon, striatum and dorsal hippocampus. Localization of the tip of the cannula was controlled at dissection. Brain parts were homogenized in a mixture of 5 ml n-butanol and 700 μl 10^{-2} M HCl and assayed for endogenous monoamines (NA, DA and 5-HT) by the fluorimetric method of Jacobowitz and Richardson [6] as modified by Szabó et al. [17].

The tripeptides were synthetized by one of the authors (L. B.) except for PLG (Richter, Budapest) which was used as a reference substance. Tripeptides were dissolved in physiological saline containing 1% ethanol. An amount of 100 ng dissolved in a volume of 2 μl was injected. Control rats received the same amount of vehicle.

Results were expressed as nmol transmitter/g wet weight tissue. Statistical analysis was performed by Student's *t*-test. A probability of 0.05 was set as the threshold of statistical significance.

Table II

Effect of PLG analogues on the steady state level of dopamine in the striatum

Peptides	Saline	Peptide	p
Pro–Leu–Gly–NH_2	46.50±1.76* (22)	48.31±2.03 (18)	NS
Z–Pro–Leu–Gly–NH_2	44.32±1.04 (9)	48.17±2.02 (10)	NS
Z–Pro–Leu–Gly–OMe	44.59±2.06 (11)	42.39±1.72 (13)	NS
Hpro–Leu–Gly–NH_2	55.94±2.35 (10)	58.68±2.35 (10)	NS
Z–Hpro–Leu–Gly–NH_2	55.94±2.35 (10)	62.30±3.72 (10)	NS
BOC–Hpro–Leu–Gly–NH_2	48.24±2.22 (12)	46.87±2.02 (12)	NS

* mean ±S.E.M. (nmol/g)

Table I

Effect of PLG analogues on the steady-state level of noradrenaline and dopamine in the dorsal hippocampus

Peptides	Noradrenaline			Dopamine		
	saline	peptide	P	saline	peptide	p
Pro–Leu–Gly–NH_2	1.00±0.06* (21)	1.30±0.06 (19)	0.01	3.45±0.18 (21)	4.65±0.28 (20)	0.01
Z–Pro–Leu–Gly–NH_2	1.21±0.06 (10)	1.29±0.09 (9)	NS	4.80±0.37 (10)	5.41±0.42 (9)	NS
Z–Pro–Leu–Gly–OMe	1.01±0.10 (14)	1.15±0.07 (13)	NS	3.37±0.22 (14)	3.86±0.54 (13)	NS
Hpro–Leu–Gly–NH_2	1.30±0.06 (9)	1.60±0.12 (9)	NS	4.44±0.22 (10)	5.01±0.43 (9)	NS
Z–Hpro–Leu–Gly–NH_2	1.30±0.06 (9)	1.56±0.08 (9)	0.05	4.44±0.22 (10)	5.05±0.31 (10)	NS
BOC–Hpro–Leu–Gly–OMe	0.95±0.12 (11)	0.95±0.12 (11)	NS	4.73±0.82 (11)	3.86±0.93 (12)	NS

* mean ±S.E.M. (nmol/g)

Table III

Effect of PLG analogues on the steady state level of noradrenaline and dopamine in the hypothalamus

Peptides	Noradrenaline			Dopamine		
	saline	peptide	p	saline	peptide	p
Pro–Leu–Gly–NH_2	8.98±0.30* (20)	9.57±0.30 (18)	NS	9.73±0.72 (20)	10.31±0.72 (19)	NS
Z–Pro–Leu–Gly–NH_2	8.91±0.45 (10)	8.64±0.32 (9)	NS	9.53±1.05 (9)	8.74±0.80 (9)	NS
Z–Pro–Leu–Gly–OMe	9.16±0.59 (14)	10.40±0.40 (11)	NS	9.24±0.67 (14)	6.70±0.71 (11)	0.02
Hpro–Leu–Gly–NH_2	9.95±0.39 (10)	10.32±0.67 (10)	NS	10.16±0.42 (10)	10.35±0.65 (9)	NS
Z–Hpro–Leu–Gly–NH_2	9.95±0.39 (10)	11.28±0.44 (10)	0.05	10.16±0.42 (10)	11.10±0.37 (9)	NS
BOC–Hpro–Leu–Gly–OMe	9.19±0.48 (11)	9.25±0.48 (11)	NS	9.52±1.25 (12)	9.08±1.42 (12)	NS

* mean ± S.E.M. (nmol/g)

Table IV

Effect of PLG analogues on the steady level of noradrenaline and dopamine in the mesencephalon

Peptides	Noradrenaline			Dopamine		
	saline	peptide	p	saline	peptide	p
Pro–Leu–Gly–NH_2	2.90±0.12* (19)	2.83±0.15 (21)	NS	6.80±0.31 (21)	6.46±0.39 (20)	NS
Z–Pro–Leu–Gly–NH_2	3.72±0.12 (10)	3.64±0.10 (9)	NS	7.44±0.46 (10)	8.68±0.59 (10)	NS
Z–Pro–Leu–Gly–OMe	2.97±0.12 (12)	2.87±0.14 (13)	NS	6.41±0.39 (13)	7.47±0.39 (13)	NS
Hpro–Leu–Gly–NH_2	3.61±0.24 (8)	4.43±0.24 (9)	0.05	7.77±0.20 (8)	7.90±0.20 (9)	NS
Z–Hpro–Leu–Gly–NH_2	3.61±0.24 (8)	3.78±0.18 (10)	NS	7.77±0.20 (8)	8.22±0.26 (10)	NS
BOC–Hpro–Leu–Gly–OMe	4.14±0.59 (11)	3.19±0.24 (11)	NS	6.92±0.83 (12)	6.44±0.74 (10)	NS

* mean ±S.E.M. (nmol/g)

Results

PLG significantly increased both NA and DA levels in the dorsal hippocampus. NA content was also increased by Z-Hpro-Leu-Gly-NH_2, while the other analogues, were ineffective in this region. The increase in the NA level following Hpro-Leu-Gly-NH_2 was not significant (Table I).

The DA content in the striatum was not affected by the tripeptides investigated (Table II).

Table III summarizes the results of NA and DA in the hypothalamus. PLG did not affect the catecholamines in this region. Z-Pro-Leu-Gly-OMe decreased the DA content, while Z-Hpro-Leu-Gly-NH_2 increased the NA content.

In the mesencephalon PLG did not affect the catecholamine levels. Hpro-Leu-Gly-NH_2, on the other hand, increased the NA content (Table IV).

Table V

Effect of PLG analogues on the steady state level of serotonin in the dorsal hippocampus

Peptides	Saline	Peptide	p
Pro–Leu–Gly–NH_2	4.54±0.32* (18)	3.80±0.30 (16)	NS
Z–Pro–Leu–Gly–NH_2	4.50±0.14 (9)	4.03±0.14 (9)	NS
Z–Pro–Leu–Gly–OMe	4.59±0.33 (10)	4.24±0.33 (9)	NS
Hpro–Leu–Gly–NH_2	4.58±0.47 (10)	4.05±0.55 (9)	NS
Z–Hpro–Leu–Gly–NH_2	4.58±0.47 (10)	5.23±0.65 (10)	NS
BOC–Hpro–Leu–Gly–OMe	4.83±0.67 (11)	4.26±0.57 (11)	NS

* mean ±S.E.M. (nmol/g)

Table VI

Effect of PLG analogues on the steady state level of serotonin in the striatum

Peptides	Saline	Peptide	p
Pro–Leu–Gly–NH_2	3.38±0.15* (18)	4.43±0.27 (17)	0.01
Z–Pro–Leu–Gly–NH_2	2.95±0.34 (5)	3.18±0.74 (5)	NS
Z–Pro–Leu–Gly–OMe	3.20±0.12 (11)	3.79±0.23 (9)	0.05
Hpro–Leu–Gly–NH_2	3.71±0.51 (10)	3.35±0.24 (9)	NS
Z–Hpro–Leu–Gly–NH_2	3.71±0.51 (10)	4.11±0.26 (10)	NS
BOC–Hpro–Leu–Gly–OMe	3.61±0.34 (11)	3.07±0.12 (11)	NS

* mean ±S.E.M. (nmol/g)

The 5-HT content of the dorsal hippocampus was not influenced by PLG or its analogues (Table V).

PLG increased the 5-HT content of the striatum and a similar effect was brought about by Z-Pro-Leu-Gly-OMe (Table VI).

On the hypothalamic 5-HT content Z-Pro-Leu-Gly-OMe induced a decrease, while Hpro-Leu-Gly-NH_2 and BOC-Hpro-Leu-Gly-OMe increased it. A slight increase was observed also after Z-Hpro-Leu-Gly-NH_2 (Table VII).

Z-Pro-Leu-Gly-NH_2 increased the mesencephalic 5-HT level, whereas the other analogues were ineffective (Table VIII).

Table VII

Effect of PLG analogues on the steady state level of serotonin in the mesencephalon

Peptides	Saline	Peptide	p
Pro–Leu–Gly–NH_2	4.79±0.36* (17)	3.98±0.34 (16)	NS
Z–Pro–Leu–Gly–NH_2	4.37±0.48 (10)	4.91±0.34 (10)	NS
Z–Pro–Leu–Gly–OMe	4.51±0.21 (10)	4.58±0.35 (9)	NS
Hpro–Leu–Gly–NH_2	3.69±0.23 (9)	4.49±0.40 (10)	NS
Z–Hpro–Leu–Gly–NH_2	3.69±0.23 (9)	5.11±0.45 (10)	0.05
BOC–Hpro–Leu–Gly–OMe	4.85±0.34 (12)	4.87±0.57 (12)	NS

* mean ± S.E.M. (nmol/g)

Table VIII

Effect of PLG analogues on the steady state level of serotonin in the hypothalamus

Peptides	Saline	Peptide	p
Pro–Leu–Gly–NH_2	9.77±0.57* (18)	9.32±0.51 (15)	NS
Z–Pro–Leu–Gly–NH_2	9.37±0.37 (10)	8.42±0.29 (9)	NS
Z–Pro–Leu–Gly–OMe	9.81±0.58 (10)	8.18±0.20 (13)	0.01
Hpro–Leu–Gly–NH_2	9.40±0.30 (9)	12.02±0.57 (9)	0.005
Z–Hpro–Leu–Gly–NH_2	9.40±0.30 (10)	10.79±0.57 (9)	NS
BOC–Hpro–Leu–Gly–OMe	9.11±0.48 (11)	15.00±2.11 (12)	0.05

* mean ± S.E.M. (nmol/g)

Discussion

The present data confirmed earlier findings of other laboratories [5, 14, 20] indicating that prolyl-leucyl-glycinamide affected catecholaminergic

and serotoninergic neurotransmission in various brain regions. In our experiment, the tripeptide increased the NA and DA levels in the dorsal hippocampus and also the 5-HT content in the striatum. It should be emphasized that others [20] as well as our own laboratory [16] reported that PLG facilitates DA turnover in the striatum, but this effect does not seem to be reflected in the steady-state level of striatal DA. PLG also exerts anti-amnesic effects [4, 10] and the dorsal hippocampus plays an essential role in memory formation [8]. In this respect it might be more than a coincidence that PLG affected the hippocampal catecholamine levels.

The effects of PLG on neurotransmission seem to be related to the N-terminal prolin residue of the tripeptide. Accordingly, protection of the N-terminal amino acid by benzoxycarbonyl (Z group) or its substitution by an amino acid structurally similar to prolin (Hpro) resulted in basic alterations in the pattern of neurotransmitter changes. Analogues substituted by homoproline exert their effect predominantly in the hypothalamus and mesencephalon, where the parent molecule had no effect. The striatal effect of PLG, on the other hand, disappeared after substitution with Hpro.

It seems, however, that the C-terminal residue has also some importance in monoaminergic neurotransmission. Accordingly, substitution with methyl-ester instead of the amino group of the C-terminal glycine (Z-Pro-Leu-Gly-OMe) affected hypothalamic DA and 5-HT, which effect was not present after PLG or following Z-Pro-Leu-Gly-NH_2.

Parallel with these experiments we have studied the effects of the same tripeptide analogues on the amnesia induced by electroconvulsive shock in the rat [10]. It seemed, however, that the effects of the above discussed structurally modified peptide analogues on amnesia and on the steady-state levels of monoamines are not very closely related to each other. Since substitution of proline by homoproline resulted in changes in hypothalamic monoaminergic neurotransmission, which were much more pronounced than after PLG itself, it might be of interest to study theeffect of Hpro analogeus on MSH level, which is reportedly inhibited by a local hypothalamic action of PLG [3].

In conclusion, the data indicate that protected and substituted analogues of PLG differentially affect monoaminergic neurotransmission in various regions of the rat brain. Both the N- and the C-terminal part of the tripeptide seems to contribute to these effects.

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PHARMACOLOGICAL CHARACTERIZATION OF POSTSYNAPTIC POTENTIALS EVOKED IN THE BIMODAL PACEMAKER NEURON OF *HELIX POMATIA* L.

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Stimulation of various peripheral nerve trunks evokes very similar compound postsynaptic potentials (PSP) composed of one or more excitatory postsynaptic potentials (EPSP) followed by fast and slow inhibitory postsynaptic potentials (IPSP) on the identified RPal neuron of *Helix pomatia* L.

Evoked EPSPs were reduced or blocked by nicotine, atropine and d-tubocurarine. The two components of IPSP were different in their pharmacological sensitivity. Slow IPSP was partly or totally eliminated by ergometrine and chlorpromazine and was reduced by atropine, nicotine as well as by propranolol. Fast IPSP was reduced only in the presence of ergometrine and could not be blocked by either of the applied drugs.

Participation of cholinergic transmission seems to be essential in the evoked EPSP but its partial involvement in the slow IPSP can also be supposed.

A dopaminergic mechanism may take part in the generation of both components of IPSP but the receptors responsible for the slow IPSP were sensitive to other catecholamine antagonists as well, referring to a more complex origin, or to the involvement of an unknown transmitter.

Comparison of PSPs evoked by stimulation of different nerves shows that presynaptic areas belonging to various peripheral sources are overlapped on the RPal neuron, and they probably act by similar transmitter substances.

Keywords: postsynaptic potential, pacemaker neuron (bimodal), *Helix pomatia* L., excitatory postsynaptic potential (EPSP), inhibitory postsynaptic potential (IPSP), nicotine, atropine, d-tubocurarine, ergometrine, chlorpromazine, propranolol.

By electrical stimulation of peripheral nerve trunks a compound postsynaptic potential (PSP) can be evoked in the identified RPal [9] bimodal pacemaker neuron of the snail *Helix pomatia* L. [17, 23]. In homologous neurons of other gastropods a similar multicomponent PSP was recorded on stimulation of some connectives and peripheral nerves [2, 15]. The evoked potential is usually composed of excitatory postsynaptic potentials (EPSP) as well as of fast and slow inhibitory postsynaptic potentials (IPSP) but on the signal recorded intracellularly from the soma they can partly fuse and diminish each other. Under the effect of PSPs the regular pattern of the cell activity can be

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changed significantly: extra spikes occur or, on the contrary, an inhibition lasting for several ten seconds or a few minutes develops [14, 15, 21]. In the Helix neuron, by changing the ionic composition of the saline or displaying the membrane potential of the soma the components of the evoked PSP were influenced differently and this way they could be recognized separately [10, 22]. The different pharmacological sensitivity of some components was also shown in both Aplysia and Helix neurons [11, 15].

It was also reported that acetylcholine, serotonin and GABA produce on this type of neuron excitatory, while dopamine, noradrenaline and glutamate cause inhibitory effects [1, 13, 20], and it may be supposed that receptors responsible for these effects are similar on homologous neurons.

In the present work we studied the pharmacological properties of the different components of the compound postsynaptic potential evoked by stimulation of various peripheral nerve trunks in the RPal neuron of the snail *Helix pomatia*. We used drugs known as inhibitors of the above transmitters and in this way we have attempted to clarify the nature of transmitters or receptors responsible for the different components of the PSP.

Materials and methods

Experiments were carried out on snails collected locally, stored under laboratory conditions and activated by feeding before the experiment. The suboesophageal ganglionic ring was isolated but 5–10 mm long parts of the right and left pallial and of the anal nerves were kept intact for electrical stimulation. RPal neuron was identified on the basis of its size and location visually as well as according to its typical bimodal activity pattern.

Recording of the intracellular potentials took place using conventional microelectrode techniques as described earlier [22]. The above-mentioned three nerves were stimulated separately in the same preparation using suction electrodes. The parameters of the stimulating rectangular pulse were selected so as to achieve a maximum response on stimulation of each of the nerves, and these values were kept unchanged during the experiment. Stimulation took place every case in a given period of endogenous activity, namely after the burst, at the beginning of the hyperpolarizing phase. Between successive stimulations an interval of at least one minute was inserted to avoid after-effects.

Substances used as inhibitors were dissolved in physiological saline and were added into the perfusion in 10^{-6}—10^{-3} mol/l concentration. Stimulation of the nerve trunks was carried out before and 3, 5, 10 min after drug application and also after washing out. The substances used in the experiments were,

DL-α-aminopimelic acid (Sigma)
Atropine sulfate (NBCo)
Bicuculline (Pierce)
Bufotenine hydrogenoxalate (Fluka AG)
Chlorpromazine hydrochloride (Sigma)
Ergobasine (ergometrine) (Fluka AG)
Nicotine hydrogen tartrate (BDH)
Methysergide bimaleate (Sandoz)
Pentylenetetrazole (Sigma)
Picrotoxin (Fluka AG)
DL-propranolol HCl (Sigma)
Regitin (phentolamine mesylate) (Ciba)
d-tubocurarine dichloride (Suchard)

Composition of the physiological saline was NaCl, 3.5 g; KCl, 0.3 g; $MgCl_2 \cdot 6H_2O$, 2.4 g; $CaCl_2 \cdot 2H_2O$, 1.47 g; Tris, 0.6 g in 1000 ml distilled water; pH = 7.6.

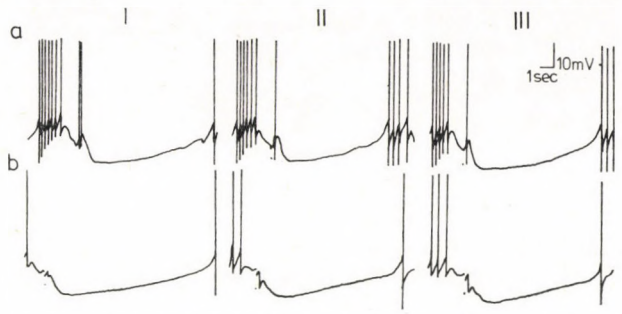

Fig. 1. PSPs evoked by electrical stimulation (dots) in two different preparations (*a* and *b*). Stimulation of left pallial nerve (I), right pallial nerve (II), anal nerve (III). *a* Evoked EPSP resulting in rise of action potential and consequent hyperpolarizing phase. *b* Evoked PSP containing only inhibitory components. The fast and slow IPSP are well separated

Results

On separate activation of the three inputs, very similar compound PSPs were usually recorded intracellularly from the same preparation. Still in different preparations there occurred differences in the appearance and amplitude of the various components. In some cases the EPSP was well expressed consisting of one or more components and evoking even spikes (Fig. 1a), in other

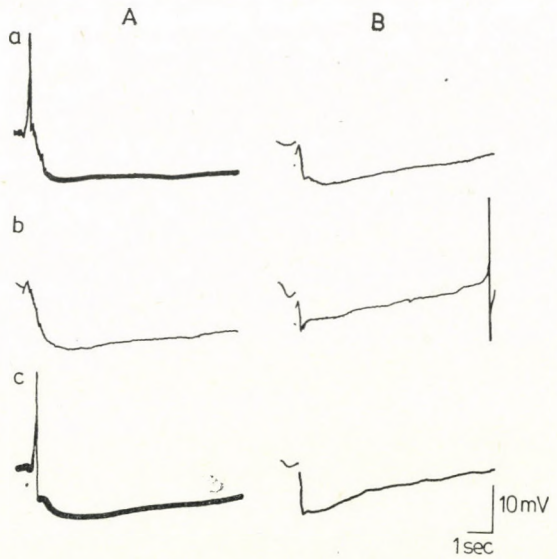

Fig. 2. Effect of nicotine on the evoked PSP (*A* and *B* are different preparations). *A* Stimulation of left pallial nerve (dots). *a* Control, EPSP evokes spike which is followed by IPSP; *b* in 10^{-4} mol/l nicotine EPSP fails to evoke spike; *c* after washing out. *B* Stimulation of right pallial nerve (dots). *a* Control, IPSP components are separated; *b* in 10^{-4} mol/l nicotine (10 min), amplitude and duration of the slow IPSP are reduced; *c* after washing, the amplitude of the slow IPSP increased but did not return to the control

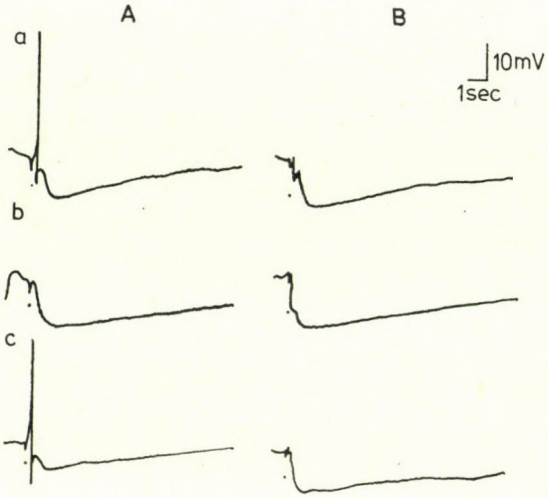

Fig. 3. Effect of d-tubocurarine on the evoked PSP. *A* Stimulation of left pallial nerve (dots). *a* Evoked EPSP followed by IPSP; *b* 10^{-4} mol/l dTc blocks EPSP within 2 min, but does not influence IPSP; *c* after washing out EPSP, spike can again be elicited. *B* Stimulation of right pallial nerve (dots). *a* Control, EPSP appears between fast and slow IPSP; *b* in the presence of dTc only IPSPs occur, fast and slow IPSP are separated; *c* after washing out EPSPs are evoked by stimulation, and similarly to the control they cover the fast IPSP

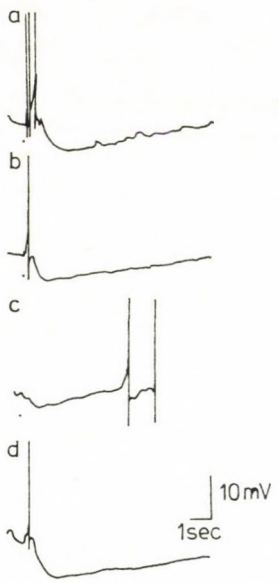

Fig. 4. Effect of atropine on evoked PSP at stimulation of left pallial nerve (dots). *a* Control, evoked spikes are followed by EPSPs and IPSP. EPSPs are superimposed on slow IPSP; *b* In the presence of 10^{-4} mol/l atropine (10 min) a decrease occurs in the number of EPSPs and the amplitude of IPSP; *c* In the presence of 10^{-3} mol/l atropine (10 min) EPSPs are inhibited and a small, fast IPSP appears and the slow IPSP decreases in amplitude and duration. *d* After 20 min washout partial restoration of EPSP occurs

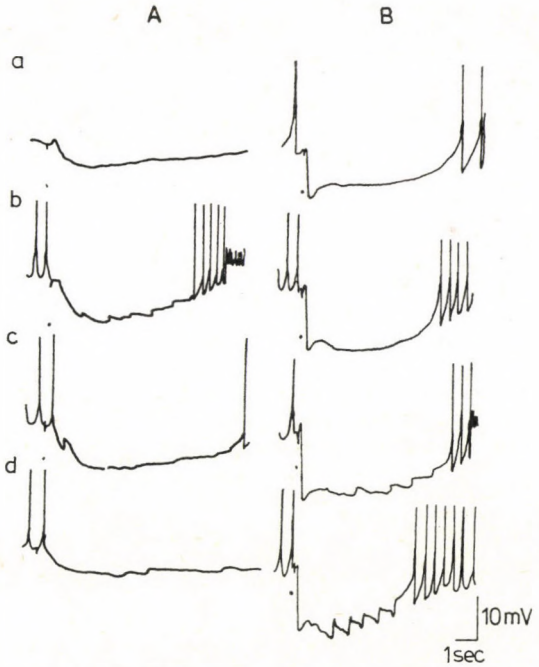

Fig. 5. Effect of bufotenine on evoked PSP. *A* Stimulation of left pallial nerve (dots). *a* Control, EPSPs followed by IPSP; *b* in the presence of 10^{-5} mol/l bufotenine (5 min) EPSPs appear during slow IPSP; *c* after 10 min, EPSP and spike can be evoked, without a change in IPSP; *d* washing out. *B* Stimulation of right pallial nerve (dots). *a* Control, IPSP with fast and slow components; *b* in the presence of 10^{-4} mol/l bufotenine (5 min) a slight depolarization occurs and the amplitude of IPSPs increases; *c* after 10 min no change in IPSP components, and EPSPs occur during IPSP; *d* washing out; abortive spikes during slow IPSP

cases it was present but failed to evoke action potentials (Figs 1*b*, 3*B*). Rarely EPSPs could be seen between fast and slow IPSP (Fig. 6*A*) or superposed on slow IPSP (Fig. 4), while in many cases no EPSP was observed in the compound PSP (Fig. 2*B*).

In contrast, IPSPs were always evoked on nerve stimulation, and on the basis of the time course a fast and a slow component could mostly be separated (Fig. 1*b*) as reported previously [22].

Similar responses were mostly observed on stimulation of the right pallial and anal nerves. On stimulation of the left pallial nerve the fast IPSP was sometimes absent (Fig. 5*A*), while in other cases the EPSP consisted of more components than those evoked by stimulation of the other two nerves.

Pharmacological experiments were carried out indiscriminately on preparations responding differently to stimulation, and it was tried to check the effect of the substances on the EPSP as well as on the fast and slow IPSP.

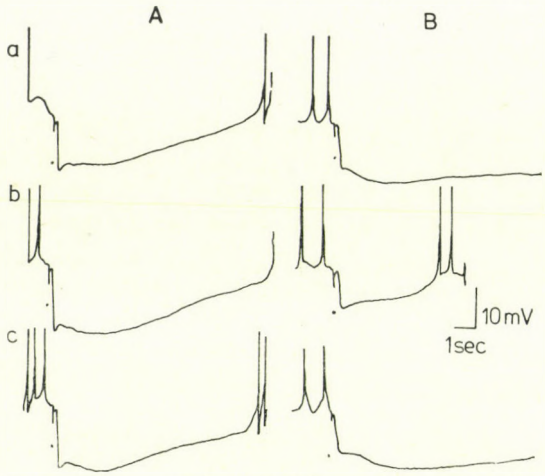

Fig. 6. A Effect of phentolamine on PSP evoked by anal nerve stimulation (dots). *a* Control, EPSPs appear between fast and slow IPSP; *b* in the presence of 5×10^{-4} mol/l phentolamine PSP does not change; *c* after washing out. *B* Effect of propranolol on PSP evoked by anal nerve stimulation (dots). *a* Control; *b* in the presence of 2×10^{-4} mol/l propranolol the slow component of IPSP decreases in amplitude and duration, the fast IPSP is unchanged; *c* restoration after washing out

Nicotine

In the presence of 10^{-4} mol/l nicotine spikes were not evoked by stimulation. Although there occurred EPSPs, both their number and amplitude was reduced (Fig. 2*A*). The fast IPSP became more visible (Fig. 2*B*), partly in consequence of the fact that the slow IPSP decreased both in amplitude and duration. The effect of nicotine could not be totally washed out.

In the presence of nicotine the endogenous pattern of the cell also changed: the amplitude of the interburst hyperpolarization was reduced significantly, the frequency of the spikes decreased and later the generation of action potentials ceased. This effect could not be reversed by washing.

d-Tubocurarine (dTc)

Under the effect of 10^{-4} mol/l dTc, evoked EPSP and spike were inhibited within 2 min (Figs 3 *A*, *B*). IPSP was not influenced and in some cases even the fast IPSP which earlier could not be recognized, became visible, probably because of the elimination of EPSPs (Fig. 3*B*). This effect was reversible; within 5 min after washing EPSP and spikes could again be evoked.

Atropine

In the presence of 10^{-4} mol/l atropine the number of EPSPs was reduced (Fig. 4*b*), at 10^{-3} mol/l they were eliminated (Fig. 4*c*). The fast component of

the evoked IPSP became visible after blocking EPSP, and also the fast and slow IPSPs became separated. Both the amplitude and duration of the slow IPSP were reduced (Fig. 4*c*). After 20–25 min washing both evoked EPSPs and spikes were restored and as a consequence fast IPSP again became unrecognizable. The amplitude and duration of the slow IPSP returned also to the control value. Atropine also influenced the activity pattern of the neuron: instead of series of bursts there occurred single or double spikes with interposed hyperpolarization. This effect could not be washed out.

Picrotoxin, bicuculline, DL-α-amino-pimelic acid, pentylenetetrazole (PTZ) and methysergide

Picrotoxin and bicuculline are known as inhibitors of GABA receptors. They did not influence markedly the evoked fast and slow IPSPs in 10^{-4} and 10^{-3} mol/l concentrations. Neither did cause any of the other drugs (glutamate receptor blockers and 5HT antagonist) changes in the evoked IPSP when applied in 10^{-4} and 10^{-3} mol/l concentration. The slight changes in amplitude and duration of IPSP occurring after their application could be observed in control conditions too after repeated stimulation.

Bufotenine

A transient depolarization of the soma membrane was evoked by 10^{-6} mol/l bufotenine, which became stable (about 10 mV) at 10^{-5} mol/l concentration. As a result, the frequency of spike generation increased. Evoked EPSP was well visible and in some cases the rise of the spike could also be observed (Figs 5*A*, *C*). The amplitude of IPSP increased, its fast and slow components separated (Fig. 5*B*). During slow IPSP, EPSPs occurred (Figs 5*A*/*b* and *B*/*c–d*). The effect of bufotenine could not be eliminated by washing.

Phentolamine and propranolol

In 10^{-4}—10^{-3} mol/l concentration phentolamine did not influence the bimodal activity pattern of the neuron or the evoked PSP (Fig. 6*A*). 10^{-5} and 10^{-4} mol/l propranolol caused 5–10 mV hyperpolarization of the soma and as a result the spike frequency decreased. Evoked EPSPs did not change, the fast component of IPSP remained well visible, while the amplitude of the slow component of IPSP decreased and its duration was shortened (Fig 6*B*). The depression of the slow component was more expressed at higher concentrations of propranolol and it increased with time.

Chlorpromazine

In the presence of 10^{-5}—10^{-4} mol/l chlorpromazine endogenous spike generation became frequent, at 10^{-3} mol/l a decrease of spike amplitudes and later an inhibition of the spikes were observed. The fast component of evoked IPSP remained, however, intact and it was even potentiated (Figs 7*A*/*b–c*) The amplitude of the slow IPSP diminished. Under the effect of 5×10^{-4} mol/l chlorpromazine it disappeared within 10 min. Its recovery after 30 min washing was only partial (Figs 7*A*/c–*d*).

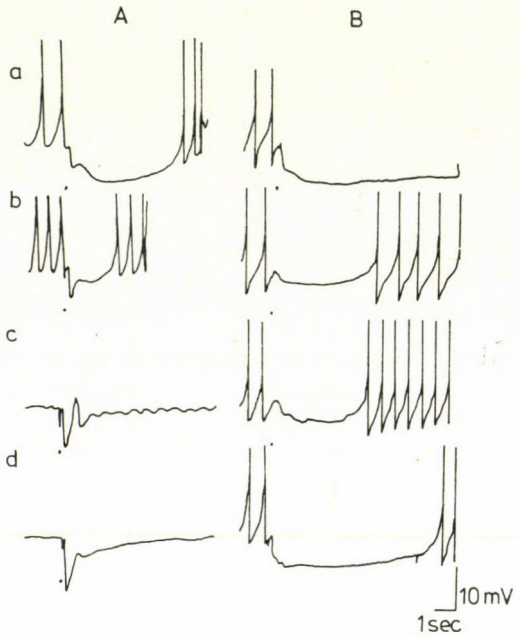

Fig. 7. A Effect of chlorpromazine on PSP evoked by stimulation of right pallial nerve (dots). *a* Control, fast and slow IPSP; *b* in the presence of 5×10^{-4} mol/l chlorpromazine (3 min) the amplitude and duration of the slow IPSP is reduced; *c* after 10 min the slow component of IPSP is eliminated, the amplitude of the fast IPSP is augmented; *d* after 30 min washing, the slow IPSP is par ly restored. *B* Effect of ergometrine on PSP evoked by stimulation of right pallial nerve (dots). *a* Control, EPSP fast and slow IPSP; *b* in the presence of 10^{-5} mol/l ergometrine the amplitude and duration of both IPSP components are reduced; *c* in 10^{-4} mol/l ergometrine EPSPs appear during reduced slow IPSP; *d* restoration of PSP after 50 min washing out

Ergometrine

The effect of 10^{-4} mol/l ergometrine on the activity pattern was similar to the effect of chlorpromazine: beside the increase of spike frequency a decrease of the spike amplitude was observed; later in some cases the generation of action potentials ceased. At 10^{-5} mol/l concentration the duration of the e-

voked IPSP and the amplitude of both components decreased (Fig. 7*B*). In other cases the slow component of the IPSP was intensely depressed while the fast component was not blocked. Frequently, EPSPs were superposed on the depressed slow IPSP. The blocking effect of ergometrine proved to be very strong, evoked PSP similar to the control returned only after 50 min washing.

Discussion

According to the results the three components of the PSP evoked by stimulation of peripheral nerve trunks in the RPal neuron of *Helix pomatia* are different in pharmacological sensitivity (Table I).

Evoked EPSP was eliminated or strongly depressed by the anticholinergic drugs d-tubocurarine, nicotine and atropine but was not influenced by the GABA and 5HT blockers picrotoxin and bufotenine, although 5HT evokes an excitatory response on this and on homologous neurons [5, 13, 20]. The latter effect of 5HT is, however, realized partly through interneurons and partly through permeability changes of the soma membrane itself. ACh evokes also excitation on homologous to RPal neuron by increasing the membrane's Na permeability [3].

In earlier experiments [22] we found that EPSPs cannot be evoked in low Na saline. As they are sensitive to excess Mg and reduced Ca, under such conditions they were also blocked. Taking in consideration these and the present results we may conclude that EPSPs evoked on the RPal neuron are the result of Na-dependent, cholinergically mediated processes.

EPSP recorded from the soma is probably the result of a synchronous activation and summation of a number of excitatory synapses on the cell. This idea is supported by the fact that the EPSP amplitude changes from preparation to preparation, also during the same experiment. If a series of EPSPs is present their polysynaptic origin should also be taken into consideration.

The slow component of the evoked IPSP corresponds to an inhibition of long duration (ILD) recorded from homologous neurons of other species [12, 15]. The several sec long inhibitory potential of the RPal neuron is potassium sensitive: its amplitude increases in K-free saline [23] and decreases in the presence of excess K^+ (unpublished). A similar ion dependency was reported also for the ILD in *Aplysia* and *Helix aspersa* [14, 16].

In our experiments the slow component of the evoked IPSP was sensitive to a number of transmitter blocking agents. Its amplitude was depressed in the presence of the cholinergic antagonists nicotine and atropine. Correspondingly a partial role of cholinergic mediation cannot be excluded, although these drugs in the concentration applied must have influenced also the endogenous pattern of the neuron.

Table I

Effect of drugs on the PSP components evoked in the identified RPal neuron of Helix pomatia L.

PSP component	d-Tc	Nicotine	Atropine
EPSP	inhibited	reduced	reduced
Fast IPSP	resistant	resistant	resistant
Slow IPSP	resistant	reduced	reduced

PSP component	Picrotoxin	Bicuculline	PTZ	DL-α-amino pimelic acid	Methysergide	Bufotenine
EPSP	resistant	—	—	—	—	resistant
Fast IPSP	resistant	resistant	resistant	resistant	resistant	resistant
Slow IPSP	resistant	resistant	resistant	resistant	resistant	resistant

PSP component	Phentolamine	Propranolol	Ergometrine	Chlorpromazine
EPSP	resistant	resistant	—	—
Fast IPSP	resistant	resistant	reduced	resistant
Slow IPSP	resistant	reduced	inhibited	inhibited

DL-α-amino pimelic acid and PTZ which proved to be antagonists of the glutamate receptor in several neurons of *Helix aspersa* [9, 18] did not influence evoked IPSP on the RPal neuron. Similarly, no effect was observed on the evoked IPSP in the presence of methysergide or bufotenine known as 5HT antagonists [7] although bufotenine caused irreversible changes in the spontaneous activity of the neuron. Picrotoxin and bicuculline were also ineffective to the evoked IPSP opposing the possible role of GABA in the transmission process. The α-receptor blocker phentolamine did not cause any change in the evoked PSP, while propranolol, a β-receptor inhibitor caused a decrease of the amplitude of the evoked IPSP together with changes of endogenous bursting. Profound changes of slow IPSP were observed under the effect of the dopamine antagonists ergometrine and chlorpromazine; in the presence of these drugs the amplitude of IPSP was depressed or totally eliminated.

The transmitter substance of the evoked ILD in homologous cells to the RPal neurons is not known. According to literary data dopamine seems to be the first candidate [4, 12, 14] but pharmacological analyses of dopamine receptors did not support this idea [8]. Our experiments showed that even ILD was mediated by dopamine, the receptor response for the slow IPSP could at least partially be blocked with antagonists of other types of transmitter.

The fast component of the IPSP preceding the slow one proved to be resistant to nearly all the applied drugs and it even became expressed when the other two components of the evoked PSP were reduced or eliminated. This points to the fact that within the PSP recorded from the soma several components are fused, and often the fast IPSP is partly or totally masked by EPSP. The amplitude of the fast IPSP was somewhat reduced by ergometrine, indicating thus a possible participation of catecholamines. Nevertheless, the transmitter substance responsible for this component probably differs from the well-known mediators. In this relation further progress can be expected from studies of the peptides and other bioactive agents occurring in the snail brain.

Solitary or compound PSPs occur rarely in the spontaneous activity of the RPal neuron when the CNS is isolated, and even if they are present they disappear in 20–30 min after dissection. In half-intact preparation (heart-intestinal nerve — CNS), however, on adequate peripheral stimulation both excitatory and inhibitory PSPs appear for several 10 s (Salánki et al. [21]) or they may be present without stimulation, "spontaneously". In this case separate EPSPs, fast and slow IPSPs are observed but not consecutively in a manner as in the case of electrically evoked compound PSP. In some cases spontaneous solitary EPSPs or IPSPs occurred under the effect of some drugs such as ergometrine and bufotenine during slow IPSP. They are probably evoked by neurons connected synaptically to RPal cell and activated by the applied drug. For a better understanding of this point, investigation of neurons connected presynaptically to the RPal neuron would be necessary.

PSPs evoked in RPal neuron by stimulation of different nerves are similar, referring to the fact that inputs arriving through these nerves into the CNS may also be similar and they probably overlap each other at the RPal neuron. The transmitter of the similar PSPs must be the same independently of the route by which they had been elicited. Nevertheless, ultrastructural investigations detecting more types of synapse on the RPal neuron than the types of PSP components [6] suggest that various transmitter substances may be responsible for a PSP type which seems to be homogeneous in electrophysiological recording. On the basis of these ultrastructural findings it is supposed that some unidentified substances are taking part in the transmission processes to the RPal neuron which are located in morphologically different synapses and one of these might be responsible for the fast and partly for the slow IPSP.

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EFFECTS OF PHYSOSTIGMINE ON THE VOLTAGE DEPENDENT IONIC CONDUCTANCES OF SKELETAL MUSCLE FIBRES*

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1. The voltage dependent ionic conductances were studied by analysing the phase plane trajectories of action potentials evoked by electrical stimulation of the sartorius muscles of the frog *(Rana esculenta)*. The delayed outward potassium current was measured also under voltage clamp conditions on muscle fibres of either the frog *(Rana esculenta)* or *Xenopus laevis*.

2. On analysing the effect of physostigmine decreasing the peak amplitude, the rate of both the rising and falling phases of the action potentials, it was revealed that the alkaloid at a concentration of 1 mmol/l reduced significantly both the delayed potassium conductance and the outward ionic current values during the action potentials. The inhibition of sodium conductance and inward ionic current was less expressed.

3. The maximum value of delayed potassium conductance measured under voltage clamp conditions was decreased by 1 mmol/l physostigmine. The time constant determined from the development of delayed potassium conductance was increased at a given membrane potential. The voltage vs. n_∞ relationship describing the membrane potential dependence of the delayed rectifier was not influenced by physostigmine.

4. It has been concluded that physostigmine changes the time course of the action potentials by decreasing the value of both voltage dependent inonic conductances and by slowing down their kinetics.

5. It is discussed that results obtained from the phase plane analysis of complex pharmacological effects can only be accepted with some restrictions.

Keywords: skeletal muscle, physostigmine, voltage clamp, phase plane trajectories, ionic conductances

Physostigmine is a well-known inhibitor of cholinesterase enzymes. In addition, together with other cholinesterase inhibitors it is capable of influencing the electrophysiological and transport properties of different excitable membranes. It has been reported that these chemical tools influence the propagation of excitation on the membrane of various neural structures [2, 13, 18]. Varga and Horowicz [22] showed that physostigmine decreased the membrane potential of skeletal muscle fibres. The depolarizing effect was considerably influenced by the quality of the anion forming the physostigmine salt applied in the incubation solution [23]. The alkaloid increased the resting

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membrane resistance of the skeletal muscle fibres by decreasing their resting potassium conductance [23]. Physostigmine definitely inhibited both the influx and efflux of ^{42}K on frog skeletal muscle [22] as well as the ^{42}K uptake of rat heart muscle [21].

In the presence of physostigmine the amplitude and the rate of rise of electrically evoked action potentials decreased and their falling phase was prolonged [14, 16, 20]. It was supposed that the modification of one or both of the voltage dependent ionic conductances underlying the generation of the spike potential may be responsible for the changes in time course of the action potentials.

The method generally used for the investigation of the ionic currents of excitable membranes is the voltage clamp technique. Application of this procedure to skeletal muscle fibres is rendered particularly difficult by the complicated membrane structure, by the necessity to eliminate the movement accompanying depolarizing pulses, etc. [1]. The analysis of phase plane trajectories using simply the action potentials and their first time derivatives yields with some limitations data about the quantity and kinetics of conductance changes and ionic currents taking place during excitation [6, 7]. This method seems particularly suitable for the analysis of pharmacological effects where the use of the voltage clamp technique is more complicated because of the long duration of the experiment, the changing of solutions. etc.

The principal aim of the present work was to investigate the mechanism of physostigmine effect exerted on the action potentials. Analysis of phase plane trajectories was used to demonstrate the actual existence of the previously postulated changes in ionic conductance. In addition the effect of physostigmine on delayed potassium conductance was measured directly by the voltage clamp technique. This allowed to discuss the possibilities and usefulness of the phase plane method.

Materials and methods

Preparations, solutions, chemicals, statistics. The experiments were carried out partly on the sartorius muscles of frogs *(Rana esculenta)* and partly on single skeletal muscle fibres prepared either from the semitendinosus muscles of frog *(Rana esculenta)* or from the ileofibularis muscles of *Xenopus laevis* specimens. Prior to the experiments the muscle preparations were equilibrated in Ringer's solution for 30 min.

The composition of the solutions used in the experiments was as follows (in mmol/l) *Ringer's solution:* NaCl, 115.0; KCl, 2.5; $CaCl_2$, 1.8; TRIS buffer, 2.0. *Sulphate Ringer:* Na_2SO_4, 40.0; K_2SO_4, 1.25; $CaSO_4$, 8.0; TRIS buffer, 2.0; sucrose, 100.0. The special solutions applied in making the cut fibre preparation and at its open end are described elsewhere [9]. Physostigmine (BDH Chemicals Ltd., Poole, England) was applied as the sulphate salt and was dissolved freshly before use. Tetrodotoxin was manufactured by Sankyo Co. Ltd., Tokyo, Japan. The pH of the solution was adjusted to 7.0 ± 0.05, tonicity was checked by an osmometer.

In statistical analysis Student's paired *t*-test, the *t*-statistics for two means and the chi square evaluation were used. The scattering given either next to the means or on the diagrams represents the standard error (S. E.).

Recording of action potentials. Measurements were carried out on the superficial fibres of sartorius muscles at room temperature (20–22 °C). To elicit and record the action potentials, intracellularly located conventional glass microelectrodes filled with KCl solution of 2.5 mol/l were used. The membrane potential was measured by a FET operation amplifier at each penetration. The experiment was continued only if the membrane potential of the fibre exceeded —80.0 mV after impalement of the two microelectrodes. The action potentials were evoked by 0.5–1.0 ms long depolarizing square pulses.

The analog signals reflecting the membrane potential changes were sent to the first channel of a twochannel averaging converter (EMG 32434). The first time derivative of the action potential was simultaneously formed by a differentiating amplifier and this signal was led to the second channel of the averaging converter. The input stage of this equipment is an 8 bit A/D converter which can process analog signals in the range of ± 1 V. During digitizing both signals were sampled 101 times, the sampling time was 40 μs. The digitized signals were recorded by a line printer (EMG, TR 1892) connected to the output of the averaging converter. Further analysis was performed by a microcomputer system (HTSZ, HT-680X) using appropriate programs in BASIC language. The data were sent to the memory of the microcomputer through the keyboard and were stored on magnetic tape. The numerical results obtained were printed out (DZM 180) and the calculated curves were displayed after digital-analog conversion on an X—Y recorder (BRYANS).

Construction and analysis of the phase plane trajectory. Construction and evaluation of the phase plane trajectories of action potentials were performed on the basis of procedure described previously [6, 7, 15]. To obtain the phase plane trajectory we delineated the first time derivative of the membrane potential change (dVm/dt) against the membrane potential (V_m; Fig. 1). Thus, processes exponential in time appeared as linear trajectories. The typical phase plane trajectory shown in Fig. 1 includes three linear segments. The first of these denoted by k_r corresponds to the "foot" of the action potential. The second straight line marked with k represents the period when sodium conductance has been fully activated; its value is constant but sodium inactivation and the outward potassium current are both still negligible. Finally, the third linear part designated by k_f reflects the situation when the delayed potassium conductance is totally activated and unchanging while sodium conductance has already declined to a negligibly low level due to inactivation.

The slopes of the linear parts are equal to the corresponding rate constants. They can be evaluated by linear regression and the maximum values of both sodium (G_{Na}) and potassium (G_K) conductances during the action potentials can be calculated from them. The formulas used in the calculations were derived from the cable equation for a propagating action potential [6].

Consequently

$$G_{Na} = C\,(k/k_r)\;(k + k_r) \tag{1}$$

and

$$G_K = C\;(k_f/k_r)\;(k_f + k_r) \tag{2}$$

where the membrane capacitance C was taken as 5 μF / cm^2 [5]. Moreover, from the slopes of the linear parts it is possible to calculate two time constants reflecting the kinetics of the inward sodium and the outward potassium current, respectively [15]. These are

$$\tau_{Na} = k_r/[k(k + k_r)] \tag{3}$$

and

$$\tau_K = k_r/[k_f(k_f + k_r). \tag{4}$$

The cable equation can be rewritten in a form [7] that permits a point by point evaluation of the total ionic current referred to unit membrane capacitance. Thus

$$I/C = (\mathrm{d}V/\mathrm{d}t)\;(m/k—1) \tag{5}$$

where m is the slope of the phase plane trajectory at the given point. The sodium current was characterized by the peak inward ionic current (I_i) while the peak outward ionic current (I_o) was identified as the measure of the potassium current.

Finally, the maximum rate of rise, the maximum rate of the falling phase, and the peak amplitude (V_s) of the action potentials are obtained directly from their phase plane trajectories.

Voltage clamp measurement of delayed potassium conductance. The experiments were carried out at temperatures of 2–4 °C using the cut fibre technique developed by Kovács and Schneider [8]. Adaptation of the method to our conditions is described elsewhere [9]. In our experiments the length of the intact part [l] was 300–500 μm; its diameter (d) varied between

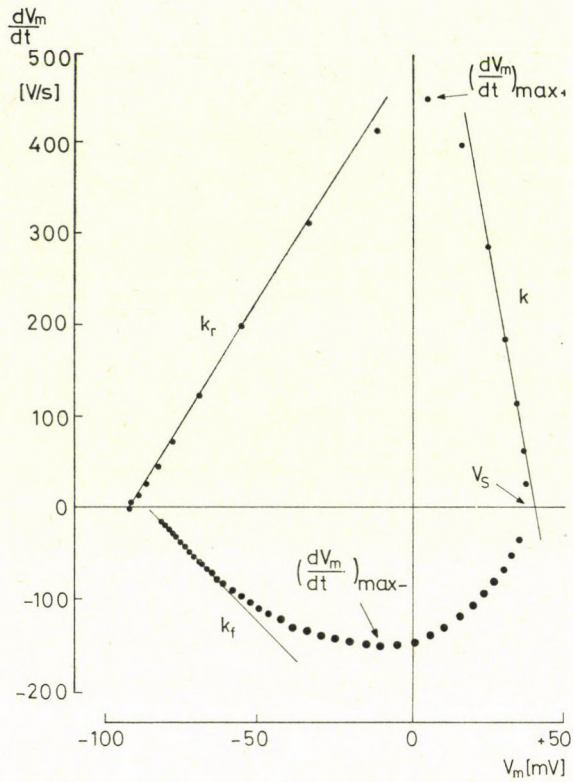

Fig. 1. Phase plane trajectory of the surface action potential of the skeletal muscle fibre. The first time derivative of the membrane potential change (dV_m/dt) is displayed as a function of membrane potential (V_m; dotted line). The k_r, k and k_f linear parts are represented by continuous lines; $(dV_m/dt)_{max+}$ denotes the maximum rate of rise; $(dV_m/dt)_{max-}$ denotes the maximum rate of rise; $(dV_m/dt)_{max-}$ designates the maximum rate of the falling phase of the action potential; V_s marks the peak value of the action potential. For further details see text

70 and 120 μm, while the sarcomere length (s) was 2.4–2.7 μm. The membrane potential of the terminated segment was voltage clamped to —100 mV. The sulphate Ringer applied in the closed end pool as external solution eliminated the chloride conductance, increasing thereby the membrane resistance and preventing the development of a significant voltage drop along the terminated segment. The initial fast inward sodium current was inhibited by 0.31 μmol/l tetrodotoxin added to the sulphate Ringer.

The current signals accompanying the depolarizing pulses after appropriate amplification were sent to the input of the averaging converter working as an A/D converter. Sampling time was 1 ms. The digitized signals were forwarded directly to the memory of the microcomputer through a DMA Controller (on-line connection) then, after finishing the experiment, the data were stored on magnetic tape.

The linear capacitance of the fibres as well as the value of the ohmic current was determined by small depolarizing pulses, and, after appropriate scaling, these components were subtracted from the current signals belonging to larger depolarizing pulses. The difference traces obtained in this way gave the delayed outward potassium current. The potassium conductance values calculated from these current traces are given referred to the linear capacitance of the terminated fibre segments.

Kinetic analysis of delayed potassium conductance. The changes in potassium conductance measured in the experiments were analysed using procedures taken from the literature [1]. The time constant of opening the delayed potassium channels (τ_n) was calculated at the different depolarizing pulses using the equation

$$g_K = g_{K\infty} \ [1—\exp(—t/\tau_n)]^4 \tag{6}$$

where g_K is the value of delayed potassium conductance at a time t while $g_{K\infty}$ is the steady delayed potassium conductance belonging to the given membrane potential. The value of $g_{K\infty}$ is obtained from the equation

$$g_{K\infty} = n^4_\infty \bar{g}_K, \tag{7}$$

where $\bar{g}_K$ is the maximally activated delayed potassium conductance of the fibre segment while n_∞ is a dimensionless variable, the value of which may vary between 0 and 1 depending on the membrane potential. The original membrane potential vs. n_∞ relationship [1] was modified [3] and this corrected form was found to be valid for the cut fibre preparation [8]. Using this relationship the value of n_∞ belonging to the largest depolarizing pulse was determined for each fibre and the $\bar{g}_K$ characteristic of a given fibre was calculated by Eq. 7. The same equation was used to evaluate n_∞ from $\bar{g}_K$ and $g_{K\infty}$ at all other membrane potential levels. The rate coefficients of the delayed rectifier (α_n and β_n) were obtained from the calculated τ_n and n_∞ values using the equations

$$n_\infty = \alpha_n/(\alpha_n + \beta_n) \tag{8}$$

and

$$\tau_n^{-1} = \alpha_n + \beta_n \tag{9}$$

The membrane potential vs. n_∞ relationship reconstructed from our data by means of the equations given above was found corresponding to that reported previously [8]. The possibility of making up outward potassium current records identical with those measured in the experiments proved that the conductance changes obeyed the n^4 kinetics [1] as it was assumed during analysis.

Results

Effects of physostigmine on the action potential parameters

To obtain the phase plane trajectories we had to record action potentials and thus we could reexamine the changes in their time course, described previously by Taylor et al. [20].

The effects of physostigmine are time dependent [14]. The evolving depolarization [22] by itself influences the characteristics of the spikes. In our experiments the action potentials were evoked between the 10th and 40th min. of physostigmine treatment. Both under control conditions and during physostigmine treatment the action potentials were considered only if the resting membrane potential was more negative than —80.0 mV. The restrictions outlined above exclude the possibility that the changes observed in the presence of physostigmine would have resulted simply from depolarization.

The peak amplitude and the rate of rise of action potentials decreased in the presence of physostigmine while the falling phase was prolonged. The changes are evident also in the case of the action potentials displayed in Fig. 3. In Table I it is shown that the 14.1% decrease in peak amplitude (V_s) was significant statistically.

Figure 2 shows that both the maximum rate of rise of the action potentials (max +) and the maximum rate of their falling phase (max —) decreased under the effect of 1 mmol/l physostigmine. The effect exerted on the falling phase (49.2%) was more expressed than the decrease in the rate of rise (31.9%) and this difference manifested itself in the significance levels, too.

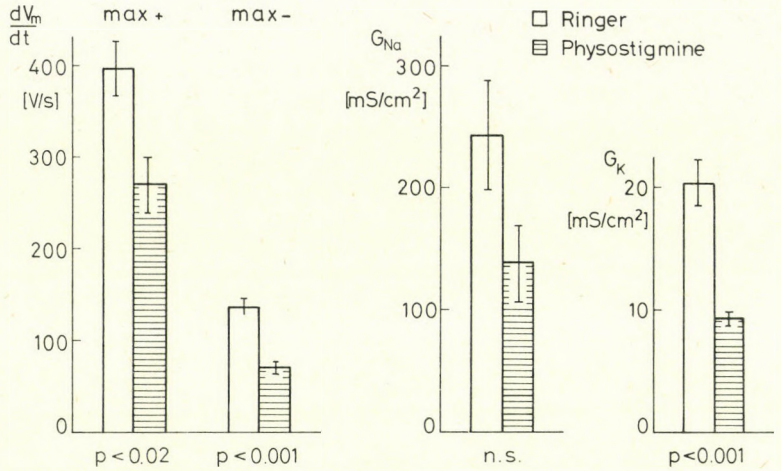

Fig. 2. Effect of 1 mmol/l physostigmine on the maximum rate of rise (max+) and on the maximum rate of the falling phase (max—) of the action potentials as well as on the maximum value of sodium (G_{Na}) and potassium (G_K) conductance. The same fibres as in Table I

The changes described above were reversible: after washing with drug-free Ringer's solution for 30–60 min we could record action potentials identical with those obtained before treatment.

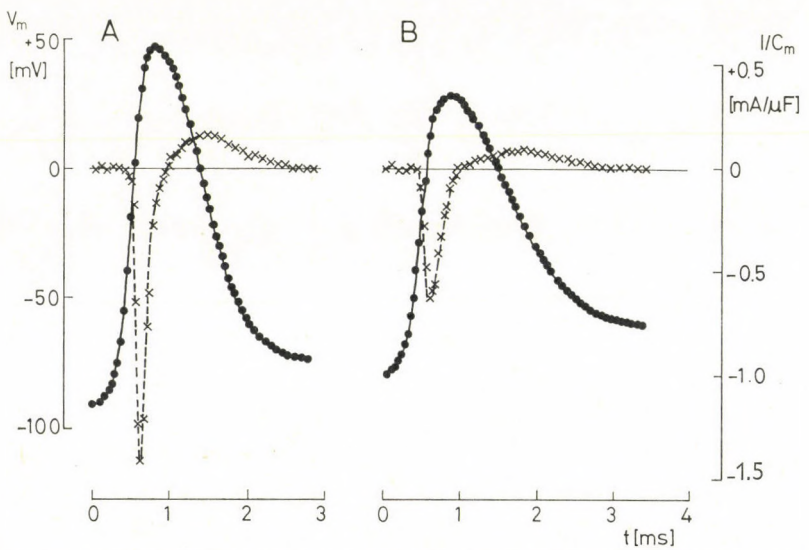

Fig. 3. Action potentials (dots and continuous line, left ordinate) and total ionic current curves referred to unit membrane capacitance (crosses and broken line, right ordinate) displayed as a function of time. *A* Control data measured in Ringer's solution. *B* Traces recorded in the 30th min of 1 mmol/l physostigmine treatment. *A:* fibre 10407; *B:* fibre 10412

Analysis of the physostigmine effect by the phase plane method

The rate coefficients obtained from the phase plane trajectories of the action potentials are presented in Table I. It is seen that the value of all three rate constants decreased under physostigmine treatment. In the determination of k_r, which reflects the rate of the action potential's "foot", the passive electrophysiological properties of the skeletal muscle membrane play an important role. Thus, the increase in resting membrane resistance [23] is presumably an important factor in the decrease of the k_r value due to physostigmine treatment. The significance of the change of k_r will be discussed later.

The rising phase of the action potential resulted from the increase in sodium conductance and from the inward sodium current. This process is reflected in the plane trajectory as a linear part whose rate constant (k) was slightly decreased by physostigmine, but the change was not significant statistically. The modification of the τ_{Na} time constant calculated from k and k_r was negligible. It is shown in Fig. 2 that, though sodium conductance decreased from 242.8 mS/cm² to 137.8, this change of 43.3% did not prove to be significant statistically due to considerable scattering.

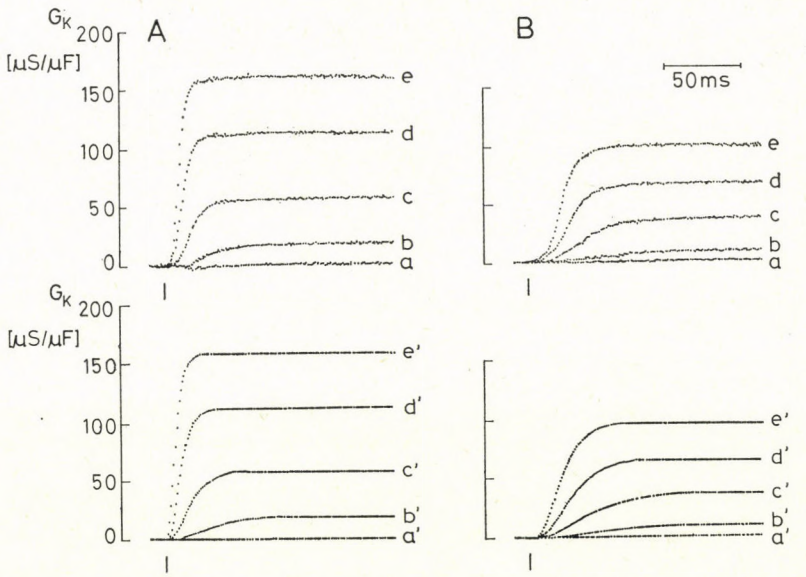

Fig. 4. Delayed potassium conductance traces measured under voltage clamp conditions. The conductance values are referred to unit membrane capacitance. The upper families of curves (*a*—*e*) were recorded under control conditions (*A*) and between the 10th and 30th minutes of 1 mmol/l physostigmine treatment (*B*). Depolarizing pulses of 150 ms duration were applied; their beginning is indicated by vertical bars below the traces. Membrane potential values during depolarization were: *a*, —41.7 mV; *b*, —32.0 mV; *c*, —22.3 mV; *d*, —12.6 mV and *e*, —2.9 mV. Fibre 20217, $l = 350$ μm, $d = 84$ μm, $s = 2.5$ μm. The lower families of curves (*a'*—*e'*) were reconstructed from data obtained by analysis of the experiment

After the inactivation of sodium conductance the delayed outward potassium current plays an important role in the formation of the falling phase of the action potential. It is shown in Table I that the k_f rate constant reflecting this current component was very definitely decreased (47.1%) by physostigmine treatment. The increase of the calculated τ_K time constant was also considerably significant (about twofold). Figure 2 shows that potassium conductance decreased from 20.4 to 9.3 mS/cm².

Table I

Effect of physostigmine (1 mmol/l) on the parameters obtained by phase plane analysis

Fibre	V_s (mV)	k_r	k	k_f	I_i	I_o	τ_{Na}	τ_K
			(ms^{-1})		(mA/μF)		(μs)	
				A. Control				
03251	124.4	5.08	10.80	3.19	0.70	0.14	29.6	192.6
10402	121.2	5.16	16.40	2.62	1.46	0.14	14.6	253.8
10404	129.0	5.55	14.93	3.15	0.97	0.28	18.2	202.5
10405	136.1	8.40	8.47	2.04	0.46	0.16	58.8	394.4
10406	134.5	4.85	16.00	2.96	1.41	0.16	14.5	209.8
10407	136.9	4.51	11.41	2.90	0.73	0.15	24.8	209.9
10408	133.7	5.01	16.73	2.93	1.14	0.17	13.8	215.4
Mean	130.8	5.51	13.53	2.83	0.98	0.17	24.9	239.8
±S.E.	2.3	0.50	1.24	0.15	0.14	0.02	6.1	26.8
				B. Physostigmine				
04026	120.4	3.80	11.61	2.00	0.51	0.11	21.2	327.6
10410	119.6	4.50	14.79	1.68	1.45	0.12	15.8	433.4
10411	126.7	4.30	13.50	1.34	1.03	0.09	17.9	569.0
10412	105.6	4.12	10.25	1.13	0.63	0.08	28.0	694.5
10413	107.1	4.23	7.51	1.63	0.68	0.10	48.0	443.2
10414	91.5	2.80	7.03	1.17	0.28	0.07	40.5	602.8
10415	115.7	3.76	13.86	1.52	0.65	0.09	15.4	468.5
Mean	112.4	3.93	11.22	1.50	0.75	0.09	26.7	505.6
±S.E.	4.5	0.21	1.17	0.12	0.14	0.01	4.9	46.6
p		<0.01	<0.02	n.s.	<0.001	n.s.	<0.01	n.s.	<0.001

In Fig. 3 action potentials and total ionic current records are displayed as a function of time. It can be seen that both the maximum inward and the maximum outward current decreased due to the effect of 1 mmol/l physostigmine, whereas the outward ionic current reached its peak about 0.4 ms later. The data presented in Table I indicate that I_i decreased by 24.0% but this change was not significant statistically. On the other hand, the decrease in I_o was very definite (44.8%).

Voltage clamp analysis of delayed potassium conductance

The potassium conductance curves obtained in a typical experiment are demonstrated in Fig. 4. On comparing the families of traces shown in the upper part of Fig. 4 it is obvious that the maximum value of potassium conductance at a given membrane potential decreased in the presence of 1 mmol/l physostigmine. It is also shown that this smaller conductance value developed more slowly.

From the conductances and time constants determined in the experiments the potassium conductance curves belonging to the individual depolarizing pulses can be reconstructed. As shown in the typical experiment displayed in Fig. 4 the traces measured experimentally (upper families of traces) and the reconstructed ones (lower families of traces) are superimposable both under control conditions and in the presence of physostigmine. According to the considerations presented under "Materials and methods," this strong similarity proves that the potassium conductance changes followed the n^4 kinetics both in the control case and under the effect of physostigmine.

The values of the maximally activated delayed potassium conductance ($\bar{g}_K$) obtained in voltage clamp experiments are presented in Table II. It is shown that physostigmine decreased $\bar{g}_K$ by 32.4%, the change was significant statistically.

The time constants of the delayed rectifier (τ_n) are indicated in Fig. 5. Physostigmine increased τ_n about twofold at each membrane potential. The

Table II

Effect of physostigmine (1 mmol/l) on the maximum delayed potassium conductance ($\bar{g}_K$, $\mu S/\mu F$) of striated muscle fibres

Fibre	Control	Physostigmine
00421	250.60	139.59
00422	169.12	126.99
00423	190.42	155.04
00424	160.08	130.91
00430	190.82	117.15
00505	206.86	132.81
20217*	242.51	150.58
Mean	201.49	136.15
±S.E.	13.02	5.02

The difference between the means is significant statistically ($p < 0.005$). Fibres from *Xenopus laevis* specimens except* *(Rana esculenta)*

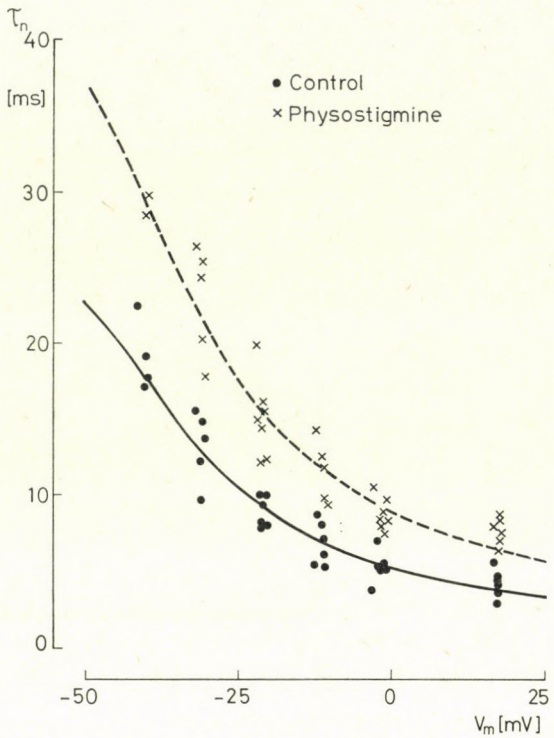

Fig. 5. Time constant of the delayed outward potassium current (τ_n) displayed as a function of the membrane potential. The points shown in the figure were obtained in the experiments carried out on the fibres as represented in Table II. The theoretical curves calculated from the kinetic data obtained by analysis of the experiments (full line, control; broken line, physostigmine) are also shown

difference between the values measured under control circumstances and in the presence of physostigmine was checked by chi square evaluation. It was found that the increase of τ_n was significant statistically ($p < 0.02$).

The kinetic parameters of delayed potassium conductance are listed in Table III. It is clear that the value of n_∞ was the same at the individual membrane potentials before and after physostigmine treatment. This suggests that the alkaloid does not modify the voltage dependence of the delayed rectifier. The rate coefficients (α_n and β_n), however, decreased at each membrane potential in the range under investigation.

Discussion

The data obtained with both methods were in good agreement with those measured previously under similar circumstances [3, 15]. The results presented prove that physostigmine modifies the action potentials of skeletal muscle

Table III

Effect of physostigmine (1 mmol/l) on the kinetic parameters of delayed outward potassium current

Membrane potential (mV)	Number of cases	Control			Physostigmine		
		n_∞	α_n	β_n	n_∞	α_n	β_n
			(s^{-1})			(s^{-1})	
—40.2	5	0.566	28.1	20.6	0.591	16.9	11.0
		±0.083	± 5.6	± 3.7	±0.067	± 3.0	± 1.3
—31.2	5	0.694	55.0	23.0	0.716	32.5	12.4
		±0.046	± 7.9	± 2.2	±0.053	± 4.1	± 1.8
—21.2	7	0.814	92.5	21.0	0.824	56.8	11.7
		±0.022	± 4.6	± 2.6	±0.020	± 4.5	± 0.7
—11.3	5	0.865	137.3	21.8	0.878	78.8	10.6
		±0.013	± 9.1	± 3.3	±0.016	± 7.2	± 0.9
— 2.0	7	0.915	175.8	16.7	0.918	103.4	9.2
		±0.006	±12.1	± 2.1	±0.006	± 4.1	± 0.7
17.7	6	0.971	237.1	7.0	0.972	135.1	4.0
		±0.001	±23.6	± 0.7	±0.001	± 6.5	± 0.2

The numbers indicate the mean ± S.E. Data were obtained on fibres displayed in Table II

fibres by decreasing both sodium and potassium conductances and by slowing down the rate of changes in these conductances. Thus, the experiments supplied direct evidence of the earlier hypothesis [14, 20] based on the alteration in shape of the action potentials.

The results obtained by the phase plane method are decisive mainly in the identification of the effects responsible for the prolongation of the declining part of the action potentials. Physostigmine at a concentration of 1 mmol/l definitely decreased both the delayed potassium conductance and the peak outward ionic current, whereas the time constant characteristic of the outward current was increased. The above modifications may bring about a prolongation of the falling phase of the spike as it has been described in connection with the similar effect of zinc ions [17].

The effect of physostigmine determined by phase plane analysis was confirmed by the data obtained through the voltage clamp technique. A quantitative comparison of the data revealed by the two forms of experimental set-up is rendered difficult by several factors. The experiments were carried out at different temperatures, on various species and in solutions different in composition. Despite the differences mentioned the time constant characteristic of potassium conductance grew about twofold both under voltage clamp circumstances (τ_n, Fig. 5) and when measured by the phase plane method (τ_K, Table I).

As regards the effect responsible for decreasing the rate of rise of the action potential, the experiments failed to supply unambiguous evidence.

On considering the well-known relation between sodium conductance and the maximum rate of rise as well as the peak amplitude of the action potential [4, 24], it was supposed that physostigmine treatment altered the properties of sodium conductance. It is certain that both the sodium conductance and the peak inward ionic current values decreased in the presence of the alkaloid, but these changes were not significant statistically. For the interpretation of the results it is necessary to take into account that these parameters can be derived from the phase plane trajectory only with some uncertainties. The rising phase of the action potential is a fast process and the shortest sampling period that we can use is 40 μs. Thus, only 4–5 points were available to determine the k rate coefficient, and the reliability of these points was decreased by the 8 bit resolution of the A/D converter. It was mentioned in the original description of the method [6] that the formation of the linear part k could not be observed in every case. The explanation for this behaviour is that the conditions responsible for the linearity of this part (maximally activated and constant sodium conductance besides negligibly small sodium inactivation and potassium activation) persist only for a very short time.

Furthermore, it is presumable that physostigmine exerts a weaker influence on the sodium conductance system than on delayed potassium conductance. On the other hand, the limitations of the phase plane method may result in an apparent ineffectiveness of physostigmine on sodium conductance. In the Results section it was shown that the rate constant of the action potential's "foot" decreased under physostigmine treatment. This change modifies the calculation of the conductance values as it is easily comprehensible on the basis of Eqs 1 and 2. On calculating from the mean values presented in Table I we obtained the values of 233.9 and 21.4 mS/cm^2 for G_{Na} and G_K, respectively, under control conditions. In the presence of physostigmine the corresponding values are 216.3 and 10.3 mS/cm^2. If now we use the k and k_f rate constants measured in the presence of the alkaloid with the control value of k_r (eliminating in this way the physostigmine effect exerted on the "foot" of the action potential) the calculated value for G_{Na} and G_K will be 170.4 and 9.51 mS/cm^2, respectively. This rough estimate shows that the diminution of k_r moderates the extent of the decrease in the calculated G_{Na} value which would be probable from the lower value of the k rate constant. The same factor distorts also the change in potassium conductance, but this fact is less manifest due to the more exact measuring conditions or perhaps to the more pronounced physostigmine effect. The considerations outlined above show at the same time the limitations of the applicability of the phase plane method. In the case of complex pharmacological effects, a modification of the different time constants may result in different effects remaining hidden.

As to the mechanism of the physostigmine effect, it is supposed that the alkaloid interacts with the protein molecules forming the ionic channels located

in the membrane of skeletal muscle fibres. Both the maximum value of the activated conductances and the kinetics of the residual conductance have changed due to this interaction. This was supported by the observation according to which development of the physostigmine effect depended on whether the neutral or the protonated form of the alkaloid was present at higher concentrations [14]. The significance of the relative amounts of the two molecule forms has been discussed elsewhere [19]. At any rate the ionic conductance decreasing effect of physostigmine is not specific because it is manifested in the modification of the different electrophysiological and transport properties of different cell types (10–13, 21–23). This complex pharmacological behaviour reminds of the action of local anaesthetics.

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EFFECTS OF PHYSOSTIGMINE ON THE EXCITATION-CONTRACTION COUPLING OF SKELETAL MUSCLE FIBRES*

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1. The effects of physostigmine on the electrophysiological properties of the surface membrane and on the different steps of excitation-contraction coupling were studied on the skeletal muscle of the frog *(Rana esculenta)*.

2. On analysing the phase plane trajectories of electrically evoked action potentials it was found that, similarly to the previously described pH dependence of the depolarizing effect of the alkaloid [14], the physostigmine-induced inhibition of the voltage-dependent sodium and potassium conductances responsible for the generation of the spike was increased in correlation with the decrease of external hydrogen ion concentration (pH 6.4, 7.0 and 8.4).

3. When performing examinations on cut muscle fibres using the single vaseline gap voltage clamp technique [9], physostigmine did not exert any characteristic effect on the strength-duration curve of the contraction threshold determined by short depolarizing pulses even at higher concentrations (2 and 10 mmol/l; pH 7.0). In some cases the value of the rheobase was shifted to a variable extent towards more negative membrane potentials.

4. On using the metallochromic indicator dye antipyrylazo III it was found that the application of 2 mmol/l physostigmine (pH 7.0) decreased the amount of calcium released during the depolarizing pulses. To reach the contraction threshold, a smaller increase in calcium concentration was necessary in the presence of the alkaloid.

5. The relaxing phase of contractions elicited by depolarizing pulses was slowed down due to 2 mmol/l (pH 7.0) physostigmine treatment although the rate of the falling phase of the calcium transients increased simultaneously. The decrease in external hydrogen ion concentration facilitated the development of modifications in the shape of the contractions.

6. The conclusion was drawn that the effects of physostigmine exhibit pH dependence. The alkaloid decreases calcium release from the sarcoplasmic reticulum and increases the calcium sensitivity of the contractile proteins.

Keywords: skeletal muscle, excitation-contraction coupling, intracellular calcium transients, physostigmine, pH dependence.

The cholinesterase inhibitor alkaloid physostigmine is known to influence the contractile properties of skeletal muscles. At a concentration of 1–1.5 mmol/l it was observed to potentiate the twitches of the frog sartorius muscle [17, 22]. One of the causes of twitch potentiation is the prolongation of the surface action potentials [14]. It was not possible, however, to rule out the

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possibility that the alkaloid penetrating the surface membrane of the skeletal muscle and entering the intracellular space may affect other structures, too. The significance of penetration through the membrane is emphasized by the observation that at more alkaline pH values, where an increased amount of neutral molecules is capable of penetrating, both twitch potentiation and prolongation of the action potentials are more expressed [14]. Intracellular sites of action may also account for the data suggesting that physostigmine prevents the development of caffeine contracture [13] and that under appropriate circumstances the alkaloid itself can evoke contractures independent of the surface membrane potential [15]. In addition, physostigmine inhibits the calcium uptake of fragmented sarcoplasmic reticulum vesicles prepared from different species [11, 23].

The experiments to be reported showed that physostigmine inhibits the voltage dependent ionic conductances of the surface membrane in a pH dependent manner. The effect of physostigmine on the properties of the contractions also seems to depend on the pH.

It is concluded, therefore, that the extent of changes developing under physostigmine treatment is determined by the amount of molecules penetrating the muscle membrane. The importance of different possible intracellular acting sites in evoking the mechanical effects is discussed.

Materials and methods

The experiments were carried out on the skeletal muscles of the frog *(Rana esculenta)*. The muscle preparations were equilibrated in Ringer's solution for 30 min before use. The pH of the solutions was adjusted to the required value with an accuracy of 0.05 by adding either HCl or NaOH. Since the effectiveness of the alkaloid may depend on the quality of the anion [25] physostigmine was applied as its sulphate salt throughout (BDH Chemical Ltd., Poole, England). The physostigmine-containing solutions were prepared freshly in every case. The details of evoking and recording action potentials as well as of the analysis of their phase plane trajectories are given elsewhere [21]. Each value for the membrane potential was obtained as the average of 10 impalements performed on different superficial fibres. The scattering given next to the means and on the diagrams represents the standard error (S. E.). The statistical significance of the differences was calculated using the t statistic for two means.

Determination of the strength-duration curve for the contraction threshold. The membrane potentials of the closed ends of cut skeletal muscle fibres was voltage clamped to —100 mV using a single vaseline gap [9]. The detailed description of the method is given elsewhere [10].

The lack of the continuous application of intracellular microelectrodes allows to elicit contractions by depolarizing voltage clamp pulses on the terminated segment without any damage to the preparation. On applying depolarization of different durations we determined the membrane potential values at which the movement just visible at 400× megnification had appeared. The membrane potential belonging to the 100 ms long depolarizing pulse proved to be the rheobase. Physostigmine was applied extracellularly in the incubation solution at the closed end. Since the measurements were performed at temperatures of 2–4 °C, the new solution was cooled down before changing to avoid the possible disturbing effect of a variation on temperature.

Measurement of changes in intracellular calcium concentration.

The experiments were performed using the calcium sensitive metallochromic indicator dye antipyrylazo III [8]. The dye was added in a concentration of 1 mmol/l to the incubation solution of the fibre's open end pool, from where it entered the intracellular space of the closed

end part through free diffusion. The entrance of antipyrylazo III was checked by determining the resting spectrum of the muscle fibre. The calcium transients were recorded at 720 nm wave length where the changes in dye absorbance were not influenced by other factors [18]. The measured changes of absorbance are approximately linear with the changes in intracellular calcium concentration [16]. Since the myoplasmic concentration of the dye increased continuously during the experiments, the calcium transients are given in every case normalized to the resting optical density measured at the wave length of 550 nm. The optical signals accompanying the depolarizing pulses were recorded and analysed using a microcomputer system connected on-line. The sampling time of the analog digital conversion was 1 ms.

Recording of the contrations.

On mounting the fibres a piece of aluminium foil was attached to the tendon of the closed end. The preparations were positioned during the experiment so that the slit of the white light focussed sharply on the level of the fibres was partly obscured by the aluminium foil. Therefore, the movements of the foil relating to the mechanical activity of the fibre were recorded as changes in the intensity of light incident on the photodiode. Recording and processing of the $\Delta I/I$ signals obtained in this way were done as described in the former paragraph.

Results

pH dependence of the depolarizing effect of physostigmine

The results of the experiments concerning the depolarizing effect of physostigmine are presented in Table I. In the course of measurements the muscles were soaked for one hour in Ringer's solution of appropriate pH (6.4, 7.0, 8.4), then their membrane potential was determined. The values

Table I

Depolarizing effect of physostigmine (1 mmol/l)

pH	6.4	7.0	8.4
Depolarization	5.65	9.54	16.42
±S.E.	1.78	3.23	1.76
(mV)			
n	6	7	6
p		<0.05	<0.01

obtained at the three different hydrogen ion concentrations under investigation did not differ significantly either from each other or from the membrane potential of freshly excised muscles. Thereafter the muscles were treated for one hour with 1 mmol/l physostigmine dissolved in Ringer's solution of appropriatep H, and the membrane potential was checked again. Physostigmine concentration and incubation time were chosen to be suitable for the maxi-

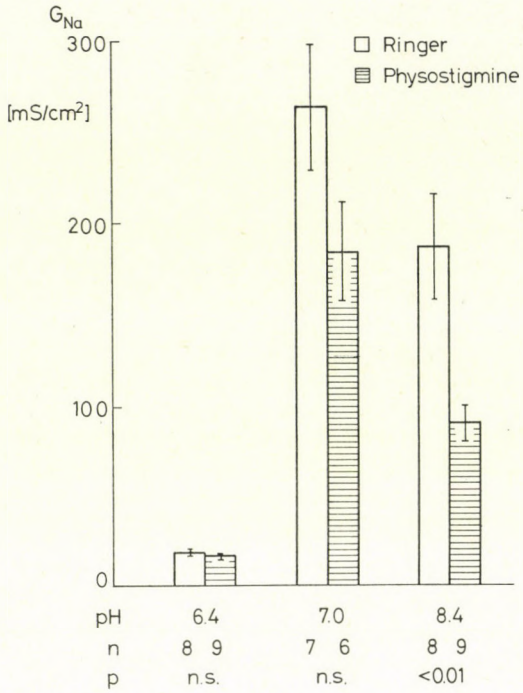

Fig. 1. Effect of 1 mmol/l physostigmine on the maximum sodium conductance during the action potentials at three different pH values

mum physostigmine effect to develop in the case of twitch potentiation [14]. Table I shows the averages of the differences between the values before and after physostigmine treatment. It is seen that the higher the pH of the incubating solution the bigger is the depolarization developed. The differences between the depolarizations measured at pH 6.4 as well as 7.0, and at pH 7.0 as well as 8.4, were significant statistically. The effect of the alkaloid was reversible at all the three pH values, as after washing out with drug-free Ringer's solution of identical pH for one hour the original membrane potential was restored.

Effect of physostigmine on the voltage dependent ionic conductances at different pH values

The effect of physostigmine on the changes in voltage dependent ionic conductances taking place during the action potential was studied by analysing the phase plane trajectories [7, 21]. At the beginning of the experiments the muscles were equilibrated in Ringer's solution of desired pH value for one hour, and then the control action potentials were recorded. Subsequently, 1 mmol/l of the alkaloid was added to the Ringer's solution of appropriate pH.

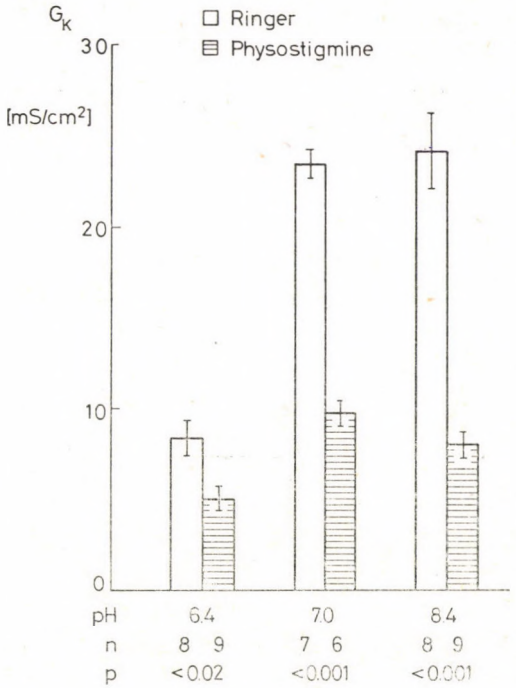

Fig. 2. Effect of 1 mmol/l physostigmine on the maximum value of delayed potassium conductance during the action potentials at three different hydrogen ion concentrations

Action potentials were elicited between the 15th and 45th min of treatment.

The maximum sodium conductance values (G_{Na}) during the action potentials are presented in Fig. 1. It is shown that the magnitude of G_{Na} was modified by the change in hydrogen ion concentration itself. Moreover, it is clear that physostigmine decreased G_{Na} at pH 6.4 to a smaller extent, but more definitely at lower hydrogen ion concentrations. This change was not significant statistically at pH 6.4 and 7.0. Because of the considerable variation of the control values, it is reasonable to express the degree of inhibition in per cent. According to this the alkaloid decreased G_{Na} by 12.7% at pH 6.4, by 29.9% at pH 7.0 and by 52.1% at pH 8.4, i.e., the inhibiting effect was more definite at higher pH values.

The values of delayed potassium conductance (G_K) are shown in Fig. 2. The magnitude of this conductance also changed with the variation of pH. Physostigmine (1 mmol/l) significantly decreased G_K at all three pH values, the extent of inhibition was 40.3% at pH 6.4, 58.8% at pH 7.0 and 67.1% at pH 8.4. Consequently, on decreasing the hydrogen ion concentration the inhibiting effect of physostigmine was intensified in this case, too.

The effect of the alkaloid was reversible, as after washing out for one hour, action potentials identical with the control ones were recorded.

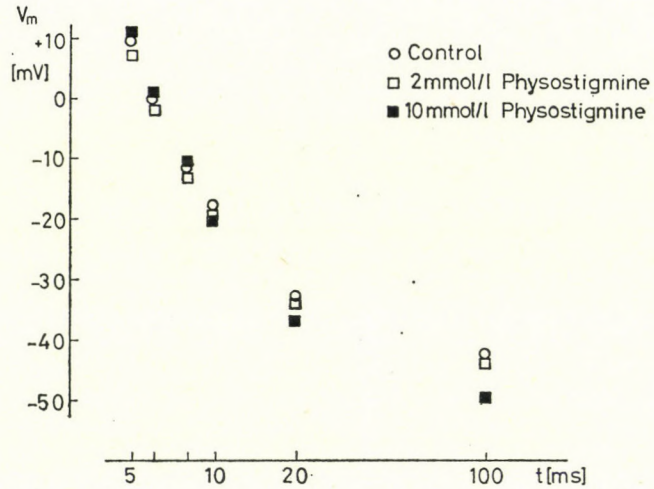

Fig. 3. Effect of physostigmine treatment on the strength-duration curve for the contraction threshold. Durations of the depolarizing pulses are displayed on the abscissa on a logarithmic scale. The membrane potentials during depolarizing pulses are visible on the ordinate. Fibre 20211, $l = 230$ μm, $d = 84$ μm, $s = 2.7$ μm

Effect of physostigmine on the strength-duration curve for the contraction threshold

The experiment shown in Fig. 3 was performed at pH 7.0. Physostigmine in a concentration of 2 mmol/l did not modify the shape of the curve. On increasing the concentration in the extracellular space to 10 mmol/l, the contraction threshold was shifted towards more negative membrane potentials at longer (20 and 100 ms) depolarizing pulses. At shorter pulses no change was found in this case either. In the experiment in question the strength-duration curve was determined between the 10th and 15th min of the treatment. In other experiments with longer treatment periods we did not observe any additional change compared to the effect developing during the first 10–15 min.

As regards the strength-duration curve for the contraction threshold, the sensitivity of the individual fibres to physostigmine was highly variable. In a concentration of 1 mmol/l at pH 7.0 the alkaloid did not cause any change in either case. At shorter pulses physostigmine did not evoke any characteristic alteration at concentrations of either 2 or 10 mmol/l.

The values of the rheobase determined by 100 ms long depolarizing pulses are presented in Table II. It is shown that 2 mmol/l physostigmine did not exert any effect in 50% of the experiments, while in the other four determinations the value of the rheobase was shifted in various degrees (3.9–13.4 mV) towards more negative membrane potentials. The alkaloid, in a concentration of 10 mmol/l, brought about a similar modification (a shift of 5.8–11.1 mV) in all the three presented experiments.

Table II

Effect of physostigmine on the rheobase of voltage clamped skeletal muscle fibres (pH 7.0)

Fibre	Control (mV)	Physostigmine (mV)
A. 2 mmol/l physostigmine		
20 105	—35.6	—49.0
20 106	—33.7	—41.3
20 211	—43.2	—43.7
20 224	—28.2	—28.2
20 315	—40.8	—40.8
20 322	—42.7	—46.6
20 323	—38.5	—43.3
20 331	—40.8	—40.8
B. 10 mmol/l physostigmine		
01 032	—39.4	—46.6
20 204	—45.3	—56.4
20 211	—43.7	—49.5

As regards the pH dependence of the physostigmine effect on the strength duration curve for the contraction threshold, at pH 8.4 the alkaloid even in a concentration of 1 mmol/l elicited a characteristic prolongation of the twitches (see later). In some cases the shift of the rheobase was so definite that during the determination of the strength-duration curve a contracture developed, rendering it impossible to continue the experiment. The phenomenon seemed identical as the observation reported previously, i.e., that physostigmine in high concentration (15 mmol/l)at pH 7.2 is capable of eliciting small contractures independent of the surface membrane [15].

Effect of physostigmine on calcium transients

Intracellular calcium transients are shown in Fig. 4. It is seen that 2 mmol/l physostigmine, pH 7.0, significantly decreased the augmentation of myoplasmic calcium concentration accompanying depolarizing pulses at the three membrane potentials investigated. The decrease amounted to 84.3% at —51.9 mV, 73.7% at —47.1 mV, and 59.9% at —42.3 mV. It was moreover evident, that the change in intracellular calcium concentration developed more slowly in the presence of physostigmine. At —42.3 mV for example the time necessary for the peak amplitude to develop was 29 ms in the control solution, and 58 ms under the effect of physostigmine.

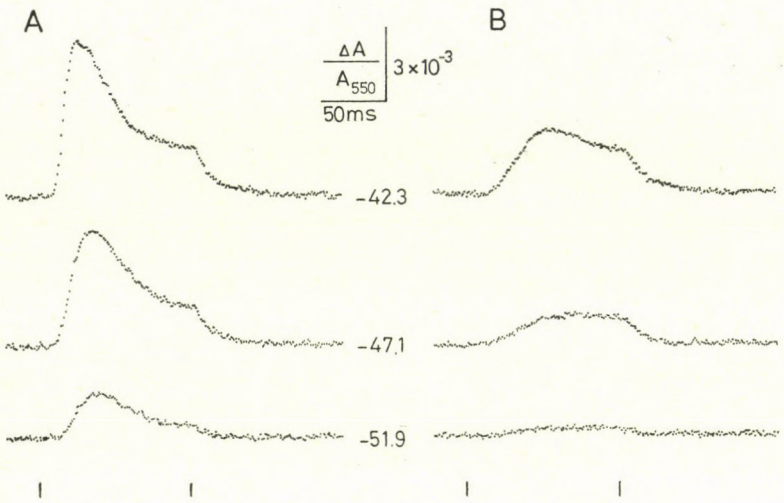

Fig. 4. Calcium transients evoked by 100 ms long depolarizing pulses (indicated by vertical bars at the bottom) in control solution (*a*) and in the presence of 2 mmol/l physostigmine (*b*). The membrane potentials during depolarizations are shown between the corresponding traces. Fibre 20 323, 6 sweeps averaged

Calcium transients elicited by 5 and 10 ms long depolarizing pulses are shown in Fig. 5. The value of depolarization was just necessary to reach the contraction threshold in every case. It is clear that at the same pulse duration the depolarization had to be higher to reach the contraction threshold on this

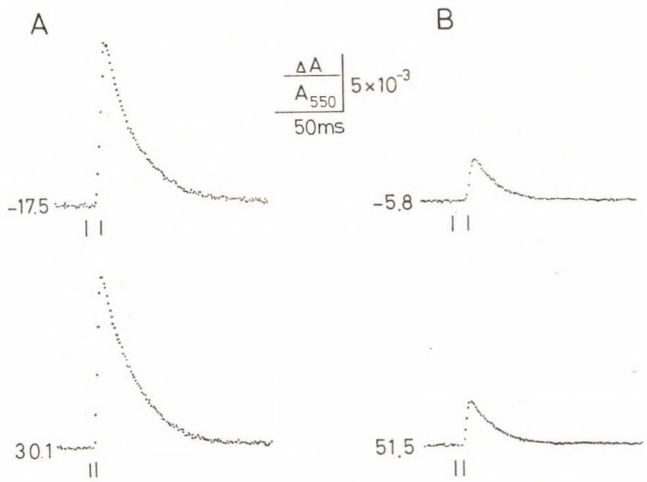

Fig. 5. Threshold calcium transients evoked by depolarizing pulses (indicated by vertical bars below each curve) of 10 ms (upper part) and 5 ms long (lower part) in control solution (*a*) and in the presence of 2 mmol/l physostigmine (*b*). The membrane potentials during depolarizations are shown next to the individual traces. Fibre 20 322, $l = 400$ μm, $d = 112$ μm, $s = 2.7$ μm. 4 sweeps averaged

fibre in the presence of physostigmine. In accordance with our previous observation the peak amplitude as well as the rate of the falling phase (γ) of the threshold calcium transients were independent of the value of the membrane potential during depolarization both in the control solution (Fig. 5*a*) and in the presence of physostigmine (Fig. 5*b*). On the other hand, it is evident that, due to the effect of 2 mmol/l physostigmine (pH 7.0) an increase in calcium concentration smaller by 75.7% was enough to reach the contraction threshold. The γ rate constant increased simultaneously by about 45%, i.e. the decrease in intracellular calcium concentration took place faster.

Effect of physostigmine on the contractions evoked by depolarizing pulses

During determination of the strength-duration curve for the contraction threshold, the character of the movement elicited by the voltage clamp depolarizing steps had changed due to physostigmine treatment, i.e. the rate of relaxation decreased. The phenomenon is presented in Fig. 6. The movements of the terminated muscle segment were recorded in the form of light intensity changes. Voltage clamp pulses of 10 ms were applied, the depolarizations exceeded by 10 mV the magnitude of the depolarizing pulses necessary to reach the contraction threshold. Under the effect of 2 mmol/l physostigmine (pH 7.0) the mechanical threshold was shifted towards a more positive membrane potential in this case, too. The peak amplitudes of the light intensity changes proportional to the contractions were about the same, neither did change the time necessary for the peak amplitude to develop (40 and 38 ms, respectively). On the other hand, relaxation took place more slowly, the widening of the contraction curve was 25.9% at 80% of the peak amplitude, 28.6% at 50%, and 29.2% at 20%.

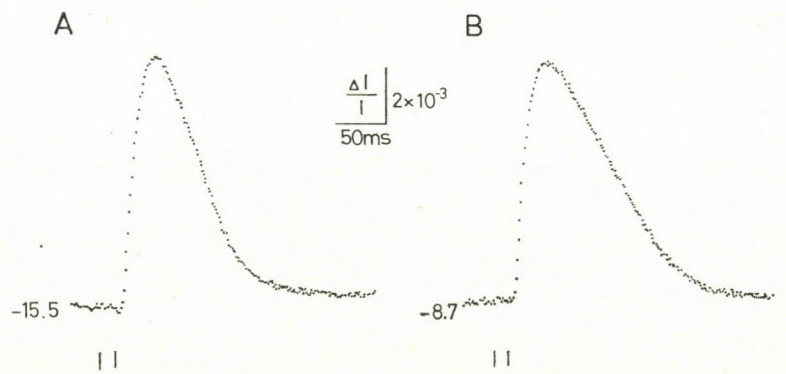

Fig. 6. Contractions elicited by 10 ms long depolarizing pulses (indicated by vertical bars below the curves) under control circumstances (*a*) and in the presence of 2 mmol/l physostigmine (*b*). The membrane potential values during the depolarizations are shown next to the individual traces. Fibre 20 331, $l = 280$ μm, $d = 112$ μm, $s = 2.7$ μm. The records displayed in the figure are single sweeps

Discussion

The results presented indicate that the twitch potentiating property of physostigmine is a resultant of various effects exerted on the different steps in excitation-contraction coupling.

We found in our experiments that on increasing the pH of the incubation solutions, the inhibiting effect of physostigmine on the ionic conductances of the surface membrane was also intensified. Both the depolarization and the decrease in the voltage dependent ionic conductances responsible for the action potentials became more definite. It has been pointed out previously that the cause of depolarization was the decrease in resting potassium permeability [24], while physostigmine influenced the time course of the action potentials by inhibiting both the sodium and the delayed potassium conductance [21].

There is a connection between the physostigmine effects exerted on the surface membrane and the amount of neutral molecules, but the relation is not a linear one. The alkaloid has a pK value of 8.2 [12]. The concentration of the neutral form is, therefore, 0.016 mmol/l at pH 6.4, 0.059 at pH 7.0 and 0.613 at pH 8.4, in the case of 1 mmol/l total physostigmine concentration. Thus, on decreasing the hydrogen ion concentration the ratio of increase in the amount of this molecule form is 1 : 3.7 : 38.3, but this relation does not exist in any of the physostigmine effects.

The parameters of the action potentials and the calculated ionic conductances were themselves dependent on the pH. This is supported by data in the literature according to which alterations in pH change the different ionic conductances of the surface membrane of both neural and muscle cells [3, 5, 6, 19]. The observed extent of conductance changes related to the modifications of pH must be considered with some restrictions because, due to the complex pharmacological effect of hydrogen ions, the conductance values obtained with the phase plane method are not sufficiently reliable [21].

The effect of physostigmine on the contraction threshold has been studied previously using potassium contractures [14]. It was found that the alkaloid practically did not modify the threshold at different pH values. In the case of the mechanical responses elicited by potassium depolarizations it had, however, to be taken into account that physostigmine decreases the magnitude of depolarization, too [24]. In our experiments we investigated the behaviour of the contraction threshold in voltage clamp conditions, excluding therefore the physostigmine effect exerted on the electrophysiological properties of the surface membrane. The value of the rheobase determined by 100 ms long depolarizing pulses was either unchanged or shifted towards more negative membrane potentials in the presence of 2 and 10 mmol/l physostigmine, respectively. In the case of contractions evoked by shorter pulses (5–10 ms),

the effect of physostigmine varied considerably from fibre to fibre as for its existence, direction and magnitude. Consequently, the effect of physostigmine on the contraction threshold is not characteristic and higher concentrations are necessary to elicit the occasional modifications than to change the electrophysiological properties of the surface membrane.

In our experiments 2 mmol/l physostigmine decreased the peak amplitude of the calcium transients related to the given membrane potential level. From the change in shape of the transients the conclusion has been drawn that the release of calcium was inhibited, whereas the reuptake seemed to have been modified to a lesser extent. The calcium release inhibiting effect of physostigmine explains well the previous finding that the alkaloid is able to prevent the caffeine contracture under appropriate circumstances [13]. This behaviour reminds of the similar effect of local anaesthetics [4].

In addition to potentiating the twitches, physostigmine evokes a prolongation of contraction time [14]. This fact was explained mainly by the widening of the surface action potential. A similar alteration in the character of the movement was observed in our experiments, but in this case a possible change in the time course of the surface electrical activity could not be a causal factor. According to data in the literature, physostigmine decreases the calcium uptake of the sarcoplasmic reticulum [11, 23]. Such a modification would be suitable to explain the persistence of the active state for prolonged period. In our experiments, however, the declining phase of the threshold calcium transients was faster in the presence of the alkaloid, i.e. the decrease in intracellular calcium concentration took place more quickly.

The present experiments have shown that although the alkaloid did not characteristically modify the strength-duration curve for the contraction threshold, in the presence of 2 mmol/l physostigmine a considerably smaller increase in intracellular calcium concentration was enough to evoke a movement of the same (threshold) magnitude than under control conditions. We suppose that the alkaloid increases the calcium sensitivity of the contractile apparatus and this change is manifested in its slowing relaxation. This is supported by data according to which physostigmine accelerates the superprecipitation of actomyosin prepared from rabbit skeletal muscle [20], and together with neostigmine it increases the speed of contraction and retards the relaxation of glycerol treated fibres prepared from the rabbit's skeletal muscle [1].

The question arises whether the described effects of physostigmine are based on its cholinesterase inhibiting property. The effect of the alkaloid depends on the amount of neutral molecules able to penetrate the membrane. It has, however, to be taken into account, that the pH of the intracellular space follows the pH changes in the extracellular space only to a small extent [2]. Therefore, the entering neutral physostigmine molecules are protonated in a degree regulated by the actual myoplasmic pH. This fact suggests

the possibility that in the described physostigmine effects the inhibition of different intracellularly located cholinesterase activities may play an important role. Neostigmine, which is impermeable at the pH values tested exhibits the above-mentioned properties of physostigmine only slightly. The effects of diisopropyl-fluorophosphate capable of entering the intracellular space [12] is very similar to those of physostigmine [14].

Consequently, the twitch potentiating properties of physostigmine might be regarded as the resultant of different effects exerted on various structures. In the potentiating of the twitches evoked by action potentials the dominant factor is the increase of the mechanically effective area of the spikes. At the same time the inhibition of calcium release and the increase in calcium sensitivity of the contractile apparatus, are contradictory effects and, therefore, they may eliminate each other.

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PIRACETAM MODIFIES MORPHINE AND RELATED DRUG-INDUCED ELEVATION OF SERUM CORTICOSTERONE CONCENTRATION IN RATS

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Piracetam (2-oxo-l-pyrrolidine acetamide, UCB 6215) or physiological saline solution was injected intravenously to female rats; after 60 min the animals were decapitated and blood was collected. Piracetam in doses of 100, 300 and 600 mg/kg resulted in a progressive suppression of serum corticosterone concentration (Cpd B) as compared to the controls. Morphine (5 and 10 mg/kg), nalorphine (5 and 10 mg/kg) and naloxone (0.5 mg/kg) induced a significant rise of Cpd B 30 min after subcutaneous injection, however, this could be prevented by 300 mg/kg piracetam given intraperitoneally 60 min prior to decapitation. Piracetam was ineffective in reducing the effects of high doses of morphine (20 mg/kg) and nalorphine (20 mg/kg). The drug had no effect on either ether stress or electric footschocks induced activation of the pituitary-adrenocortical system. In vitro the drug had no effect on pituitary ACTH release following exposure to crude hypothalamic extract. It is concluded that the effect of piracetam on the pituitary-adrenocortical axis is mediated through hypothalamic or extrahypothalamic brain structures and influences one of the effects of morphine and related drugs.

Keywords: piracetam, morphine, nalorphine, naloxone pituitary-adrenocortical activity.

Certain biological and pharmacological actions of piracetam (2-oxo-1--pyrrolidine acetamide, UCB 6215) became known from the work of Giurgea and his coworkers [10–15, 23] who showed that the drug facilitated learning mechanisms, improved memory storage and retrieval, and increased resistance of the central nervous system to hypoxia and drug intoxication in experimental animals. This has been corroborated by others [27]. Buresova and Bures [5] demonstrated that piracetam facilitated interhemispheric transfer of visual information in the rat. Recently, Vlahov et al. [35] showed that it augmented local cerebral blood flow in the cat.

The substance has many clinical applications, namely, in geriatric medicine, in resuscitation and intensive care, and pediatry [6, 32, 33]. It is known that piracetam has no stimulant properties [37] and there is also evidence that it has anxiolytic action [8]. Earlier, we published that piracetam diminished ACTH and adrenocortical functions [19] which suggested that the

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previously revealed piracetam-induced facilitation of learning, memory storage and retrieval mechanisms were not necessarily based on the involvement of hypothalamo-pituitary ACTH functions. The intimate mechanisms responsible for the effects of piracetam are not completely understood, therefore at the present stage any experimental finding on the interaction with other CNS active substances may be valuable.

The present paper is devoted to study the effect of piracetam on the pituitary-adrenocortical system, and its mutual or reciprocal action with morphine, nalorphine, naloxone and certain stressor agents.

Materials and methods

Female rats of R-Amsterdam strain weighing 200–250 g were used. Animals were housed 7 to a cage and had unlimited access to food and water. The ambient temperature was approx. 22 °C, and the room was light controlled with a 14 h light, 10 h dark cycle, light on at 06:00 h.

Piracetam was dissolved in isotonic saline and given intravenously or intraperitoneally in a volume of 0.15 ml/100 g body weight. Morphine (Morphinum hydrochloricum®, Biogal, Debrecen, Hungary), nalorphine (Nalorphin®, Chinoin, Budapest, Hungary) and naloxone hydrochloride (Endo Laboratories, Inc. N. Y., U. S. A.) were injected subcutaneously. Control animals received identical volumes of physiological saline solution either i.v., i.p. or s.c.

To minimize the effects of circadien variables on the pituitary-adrenocortical system [16], all experiments were started at 09:00 h (rat-time) when saline or piracetam was injected. This was followed 30 min later by a second injection of saline, as control, or the drugs given s.c. The first group of animals was decapitated 60 min after piracetam administration.

Ether stress: piracetam pretreated or saline injected control rats were placed into an ether-jar for 1 min and were killed by decapitation 30 min after the exposure to ether. Electric footshocks: ten unavoidable electric footshocks of 2 mA intensity were given over 30 s in a modified jumping-box and rats were killed 40 min afterwards. Blood was collected, centrifuged and sera were stored in a deep-freezer at —20 °C.

The action of piracetam on the pituitary-adrenocortical axis was assessed by in vitro experiments. The technical details have been published elsevhere [28]. Briefly, anterior pituitary tissue and adrenocortical slices of control, untreated rats were incubated in Tyrode solution in oxygen and carbondioxide atmosphere at 37 °C. Mediobasal hypothalami including the median eminence were homogenized in a buffer solution, centrifuged and the supernatant was discarded. The residue was extracted with 0.1 M HCl. To test CRF activity, different volumes of the neutralized extracts were added and the corticosterone output of adrenal slices into the incubation medium was measured. Corticosterone concentrations were determined with competitive protein binding radioassay [25]. Large numbers of samples were assayed simultaneously to minimize interassay variation. The data were analyzed using ANOVA and this was followed by *t*-statistics (HP-97 programs).

Results

Thirty min prior to the s.c. injection of the saline solution, piracetam was given i.v. to rats which were killed by decapitation 30 min after the second injection. Piracetam suppressed serum corticosterone concentration within this interval. The suppression was weak with a dose of 100 mg/kg, while significantly greater with doses of 300 or 600 mg/kg (Table I).

Single s.c. injections of morphine in doses of 5, 10 or 20 mg/kg resulted in a significant elevation of corticosterone concentration which started to re-

Table I

The effect of piracetam on corticosterone production in vivo

Group	Treatment		Corticosterone, μmol/l 0.5 h
	—0.5 h	0 h	
1	Saline 0.3 ml i.v.	Saline 0.1 ml s.c.	0.28±0.02 n=21
2	Piracetam 100 mg/kg i.v.	Saline 0.1 ml s.c.	0.18±0.06 n=7
3	Piracetam 300 mg/kg i.v.	Saline 0.1 ml s.c.	0.07±0.01 n=7
4	Piracetam 600 mg/kg i.v.	Saline 0.1 ml s.c.	0.03±0.01 n=7

Results are given as the mean±S.E.M.
Anova: $p < 0.01$
1 vs. 2: N. S.
1 vs. 3: $p < 0.001$
1 vs. 4: $p < 0.001$

turn to the normal range 2 hrs after the injection. This elevation was prevented by piracetam injected 30 min before morphine administration. However, even in high dose (600 mg/kg) piracetam failed to prevent the effect of 20 mg/kg morphine injection.

Nalorphine was injected s.c. in doses of 5, 10 and 20 mg/kg and naloxone in doses of 0.5 and 5 mg/kg. Both substances resulted in an increase of serum corticosterone concentration, although the time courses were somewhat different from that of the morphine induced changes. Nalorphine showed the shortest lasting hormonal effect, while the corticosterone concentration in the 0.5 mg/kg naloxone treated group returned to the normal range after 2 h of injection. Piracetam administration 30 min prior to the injection of these drugs significantly inhibited the activation of the pituitary-adrenocortical axis. However, it should be noted that piracetam failed to reduce the effect of high doses of nalorphine (20 mg/kg), moreover, 5 mg/kg naloxone injection did not cause any consistent increase of corticosterone concentration at the time of samplings (Table II).

When compared to the controls, piracetam did not modify the effect of ether stress on serum corticosterone concentration. Rats were killed by decapitation 30 min after the exposure. In addition, no significant difference could be demonstrated between piracetam and saline treated groups in serum corticosterone concentrations 40 min after the unavoidable electric footshocks (Table III). It is important to note that no difference in gross behavior could be observed between piracetam treated rats and matched controls.

In vitro experiments were conducted to determine whether piracetam directly influenced the pituitary or adrenal gland, or its effect was mediated

Table II

The effect of piracetam pretreatment on morphine and related drug-induced changes in serum corticosterone concentration

Group	Treatment		Corticosterone μmol/l		
	—0,5 h	0 h	0.5 h	1 h	2 h
1	Saline 0.3 ml i.p.	Saline 0.1 ml s.c.	0.28±0.02 n=21	—	0.10±0.06 n=7
2	Piracetam 300 mg/kg i.p.	Saline 0.1 ml s.c.	0.07±0.01*** n=7	0.03±0.01 n=7	0.05±0.01 n=7
3	Saline 0.3 ml i.p.	Morphine 5 mg/kg s.c.	0.62±0.14 n=14	1.26±0.06 n=12	0.57±0.13 n=7
4	Piracetam 300 mg/kg i.p.	Morphine 5 mg/kg s.c.	0.24±0.07* n=14	0.07±0.01*** n=7	0.24±0.05 n=7
5	Saline 0.3 ml i.p.	Morphine 10 mg/kg s.c.	0.88±0.12 n=7	0.88±0.09 n=7	0.63±0.18 n=7
6	Piracetam 300 mg/kg i.p.	Morphine 10 mg/kg s.c.	0.34±0.09** n=7	0.17±0.04*** n=7	0.35±0.05 n=7
7	Saline 0.5 ml i.p.	Morphine 20 mg/kg s.c.	1.30±0.18 n=7	1.07±0.07 n=7	0.62±0.10 n=7
8	Piracetam 600 mg/kg i.p.	Morphine 20 mg/kg s.c.	1.17±0.13 n=7	0.68±0.17 n=7	0.62±0.08 n=7
9	Saline 0.3 ml i.p.	Nalorphine 5 mg/kg s.c.	0.62±0.08 n=7	0.19±0.06 n=7	0.08±0.03 n=7
10	Piracetam 300 mg/kg i.p.	Nalorphine 5 mg/kg s.c.	0.23±0.07** n=10	0.15±0.01 n=7	0.09±0.02 n=6
11	Saline 0.3 ml i.p.	Nalorphine 10 mg/kg s.c.	0.85±0.11 n=7	0.40±0.05 n=7	0.22±0.11 n=7
12	Piracetam 300 mg/kg i.p.	Nalorphine 10 mg/kg s.c.	0.20±0.03*** n=7	0.14±0.04** n=7	0.05±0.01 n=7
13	Saline 0.5 ml i.p.	Nalorphine 20 mg/kg s.c.	1.07±0.14 n=7	0.76±0.13 n=7	0.41±0.13 n=7
14	Piracetam 600 mg/kg i.p.	Nalorphine 20 mg/kg s.c.	1.04±0.13 n=7	0.81±0.06 n=7	0.38±0.14 n=7
15	Saline 0.3 ml i.p.	Naloxone 0.5 mg/kg s.c.	0.69±0.09 n=7	0.28±0.05 n=7	0.06±0.01 n=7
16	Piracetam 300 mg/kg i.p.	Naloxone 0.5 mg/kg s.c.	0.24±0.02*** n=7	0.10±0.02** n=7	0.05±0.02 n=6
17	Saline 0.3 ml i.p.	Naloxone 5 mg/kg s.c.	0.17±0.06 n=6	0.14±0.03 n=6	0.08±0.02 n=6

Results are given as the mean±S.E.M.
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

through hypothalamic mechanisms. Experiments revealed that neither 2 μg nor 10 μg piracetam, dissolved in 20 μl saline solution and added to the medium, influenced the corticosterone production of the in vitro preparations (Table IV).

Table III

The effect of piracetam pretreatment on ether stress and electric footshock-induced corticosterone production

Group	Treatment		Corticosterone, μmol/l
	—0.5 h	0 h	0.5 h
1	Saline 0.3 ml i.p.	Ether stress 1 min	1.40±0.21 n=18
2	Piracetam 300 mg/kg i.p.	Ether stress 1 min	1.22±0.17 n=17
	—0.5 h	0 h	0.67 h
3	Saline 0.3 ml i.p.	10 Footshocks 2 mA over 30 sec	0.29±0.13 n= 8
4	Piracetam 300 mg/kg i.p.	10 Footshocks 2 mA over 30 sec	0.31±0.09 n= 7

Results are given as the mean±S.E.M.
Anova: N.S.
1 vs. 2: N.S.
3 vs. 4: N.S.

Table IV

The effect of piracetam on corticosterone production in vitro

	In vitro medium	Corticosterone production in μmol/100 mg adrenal tissue/h
1	A + P	0.053±0.010 n=6
2	A + P + 0.1 ml MBH	0.077±0.01 n=6
3	A + P + 0.3 ml MBH	0.090±0.011 n=6
4	A + P + 1.0 ml MBH	0.123±0.011 n=6
5	A + P + 0.3 ml MBH + 2 μg PIRA	0.075±0.010 n=6
6	A + P + 0.3 ml MBH + 10 μg PIRA	0.087±0.005 n=6

Results are given as the mean±S.E.M.
Anova: N.S.
3 vs. 5: N.S.
3 vs. 6: N.S.
Abbreviations: A = adrenal slices; P = pituitary tissue; MBH = hypothalamic extract; PIRA = piracetam

Discussion

The short-term administration of morphine has profound effects on hypothalmic-pituitary activity in a number of animal species. Morphine has been reported to be a potent stimulus of ACTH, prolactin and growth hormone release and inhibitor of FSH, LH and TSH [18, 22, 24, 29–31, 39, 40].

Nalorphine and naloxone predominantly antagonizes the depressant but not the stimulant actions of morphine. Nalorphine also has certain agonist actions of morphine, therefore it is not surprising that it increases serum corticosterone. Naloxone has none of the agonist properties of morphine, yet in itself also results in an elevation of corticosteroid level in rats, cats and men [9, 18, 36]. The results are corroborated in our present experiments on rats using low dose of naloxone.

Our in vitro experiments indicate that piracetam does not possess a direct action on the pituitary or adrenal gland, and this implies that the effect is mediated through hypothalamic structures or extrahypothalamic pathways.

Recently, Wurtman et al. [38] have reported that piracetam resulted in a reduction of regional brain acetylcholine concentration in the hippocampus, and hypothesized that this may reflect an accelerated release of the transmitter which, in turn, could be associated with increased hippocampal cholinergic transmission. Data from recent experiments [2, 3, 4] indicate that the cholinergic system may exert tonic effects on neuronal excitability which is functional in the potentiation of hippocampal responses. Our electrophysiological findings [20] showed that piracetam resulted in a consistent increase of the frequency of amplitude discriminated spikes in the dorsal hippocampus which coincided with a moderate depression of multiunit activity in the mesencephalic reticular formation. Moreover, we have also found that low doses of morphine resulted in increased neuronal activity in the brainstem reticular formation, and the onset of the high frequency firing rate and general motor activity could be significantly delayed by piracetam pretreatment. As to the locomotor activity, this is in agreement with previous findings that morphine and morphine derivatives induce biphasic effects on the general motor activity in rats. The excitatory effects are prevalent if the drug is administered in low doses, while with high doses the stimulatory action occurs following the initial depression [1, 25]. The above data may suggest that the action of piracetam and morphine on CNS mechanisms underlying the pituitary-adrenocortical events are different, and led us to assume that they are not necessarily competing on the same brain mechanism.

Although piracetam is a cyclic derivative of gamma-aminobutyric acid, it should be emphasized that the substance is completely devoid of GABAergic properties and does not modify brain GABA levels and turn-over rate, nor is it capable of being converted into GABA [11]. Moreover, it does not influence

ether stress or electric footshock induced activation of the pituitary-adrenocortical system. The latter finding is not surprising, since the drug does not influence the threshold to pain (Giurgea, personal communication). It is unlikely that the drug occupies receptors to antagonize morphine actions. Data presented here are unequivocal, but the mechanisms by which piracetam diminishes morphine- and related drug-induced elevation of serum corticosterone concentration is not certain at this time. It is known that a few polypeptides, i. e. desglycineamide-lysine vasopressin, prolyl-leucyl-glycineamide (MIF) and cyclo-leu-gly can influence the acute and chronic effects of opiates in a variety of ways [7, 17, 21, 34]. Piracetam may bind to receptors that have an affinity for the latter peptides. Another possible mode of action for the piracetam modification of morphine effects on certain endocrine events would be a neural substrate(s) that is distinctive from the known opiate antagonists. In other words, two independent mechanisms are coinciding which result in the marked modification of the function of the final common pathway, the hypothalamo-pituitary-adrenocortical axis. Further study is required to clarify these hypothetical mechanisms of action.

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EFFECT OF IMMOBILIZATION ON THE ENZYMES OF GLYCOGEN METABOLISM*

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Activity of the enzymes regulating glycogen metabolism was determined for glycogen phosphorylase (Ph), phosphorylase kinase (PhK) and glycogen synthetase (GS) in fast extensor digitorum longus (EDL) and slow (soleus) muscles of rats following immobilization by plastering.

It was shown that the activity not only of Ph but also of PhK was about 8 times higher in the EDL than in the soleus muscle in correlation with the higher glycogenolytic activity of fast muscles. The activity of GS was approximately similar in the two muscles.

PhK activity decreased significantly in the immobilized EDL muscle while in the soleus an early reduction of GS activity ensued. Reduction of the activity of the glycogenolytic enzymes caused dedifferentiation in the muscles with anaerobic metabolism and containing large amounts of glycogenolytic enzymes. The similar rate of Ph and PhK in fast and slow muscles, as well as the similar rate of their reduced activity, during immobilization indicated a close correlation between the two enzymes and raised the possibility of a combined regulation of their turnover.

Keywords: immobilization, m. soleus, m. extensor digitorum longus, glycogen metabolism, glycogen phosphorylase activity, phosphorylase kinase activity, glycogen synthetase activity.

Prolonged immobilization may cause atrophy in skeletal muscles along with a decrease in their mass and strength. Earlier it has been shown that as a consequence of immobilization a change occurs in the composition of sarcoplasmic and contractile proteins [31] as well as the ATPase activity and contractile parameters of the muscles [29, 30]. MacDougall et al. [22] and Booth and Seider [3] showed a decrease of creatine phosphate, ATP and glycogen content in immobilized muscles. These data indicate changes in the metabolism of immobilized muscle which may be in correlation with changes in amount or regulation of enzymes participating in the synthesis and decomposition of glycogen. Significant changes were shown in the different enzymes of

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immobilized muscles [28] but the enzymes of glycogen metabolism have not been studied.

In the present experiments, the effect of immobilization of an extremity was studied on the activity of glycogen phosphorylase (Ph), phosphorylase kinase (PhK) and glycogen synthetase (GS) enzymes in fast and slow muscles of rats.

Materials and methods

The right hind-limb of male CFY-type rats weighing 200–250 g was fixed in plaster in a moderately flexed state. The left hind-limb was used as a control. Enzyme activities of intact animals of the same age were also determined. Four groups of five rats were studied 1, 2, 3 and 4 weeks after immobilization. They were anaesthetized by 5 mg/100 g body weight pentobarbital sodium and their extensor digitorum longus (EDL) as well as the soleus muscles showing fast and slow glycolytic metabolism, respectively, were prepared from the limbs. After weighing a group of similarly treated muscles was chilled in liquid nitrogen, powdered in a dish and homogenized in a Potter—Elvehjem homogenizer. The powder was diluted five-fold in buffer containing 0.1 M NaF, 0.04 M Na_2-β-glycerophosphate, 0.01 M mercaptoethanol and 0.004 M EDTA pH = 6.8, then centrifuged at 10 000 *g* at 4 °C for 10 min and the supernatant was used as muscle extract. After proper dilution the enzyme activities and protein content were determined.

The activity of glycogen phosphorylase (EC 2.4.1.1.), phosphorylase kinase (EC 2.7.1.38) and glycogen synthetase (EC 2.4.1.11) were estimated by the method of Illingworth and Cori [20], Krebs and Fischer [21] and Thomas [32] respectively. Protein content was measured by the biuret reaction according to Gornall et al. [17]. In the case of Ph and GS a unit transformed 1 μmol substrate in 1 min, and in the case of PhK an activity which was able to activate a unit amount of Ph *b* in one min. Enzyme activities were compared on the basis of the extract's protein content. Ph *b* of rabbit skeletal muscle was prepared in crystallized form according to Fischer and Krebs [10]. Significance was calculated on the basis of Student's *t* test.

Results

The activities of Ph, PhK and GS of the non-treated control rats are shown in Fig. 1. It can be seen that the activities of Ph and PhK were nearly equally about 8 times higher in EDL than in the soleus muscle. Thus, the ratio of Ph and PhK was principally the same in fast and slow muscle alike. The activity of GS was higher in the soleus than in EDL muscle, but the difference was not significant.

Changes in the weight of fast and slow muscles are shown in Fig. 2. It can be seen that immobilization resulted in a more significant loss of weight in the soleus than in the EDL muscle, in agreement with our previous experience [29]. The difference was more pronounced after the 2nd week.

Extracts of immobilized and control muscles showed no difference in protein content. This situation did not change with time and therefore enzyme activities based on the amount of solubilized protein were in good agreement with the enzyme concentrations calculated from muscle weight.

Changes in the activity of Ph and PhK enzymes are shown in Fig. 3 as compared to the controls. During immobilization Ph and PhK activity

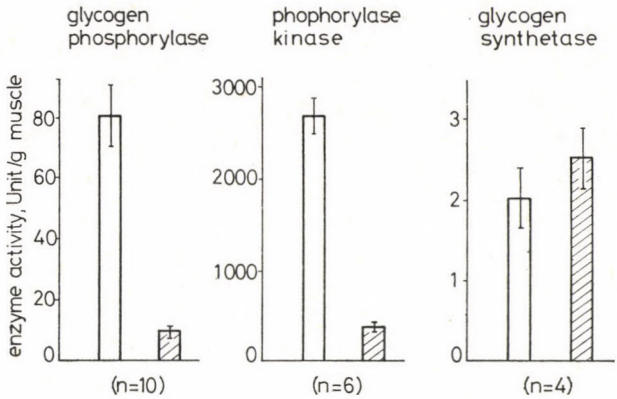

Fig. 1. Ph, PhK and GS activity in slow and fast muscles of untreated rats. Means ±S. D. are indicated; n = number of determinations; empty blocks m. EDL; striated blocks, m. soleus

decreased gradually in EDL muscle in comparison with the control. In the 4th week, both enzyme activities were reduced to 40–50% of the control side. At the same time, in the soleus muscle, Ph and PhK activities did not differ in the immobilized muscle from those on the control side.

GS activity in immobilized muscles is shown in Fig. 4. It can be seen that in the fast muscle there was no change in GS activity during immobilization, while on the 7th day in the immobilized slow muscle GS activity was much lower than in the control. From this time on a gradual increase occurred from 55 to 75% of the control. Considering the loss of weight (Fig. 2), during the whole period of immobilization the total amount of enzyme in the soleus muscle was reduced but after the 7th day the rate of decrease was less in comparison to the loss of weight.

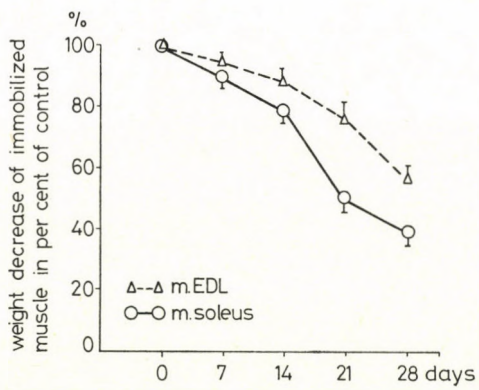

Fig. 2. Reduction of weight of EDL and soleus muscle during immobilization. Data are given in per cents of the values measured before immobilization. Each value represents the mean of 5 measurements

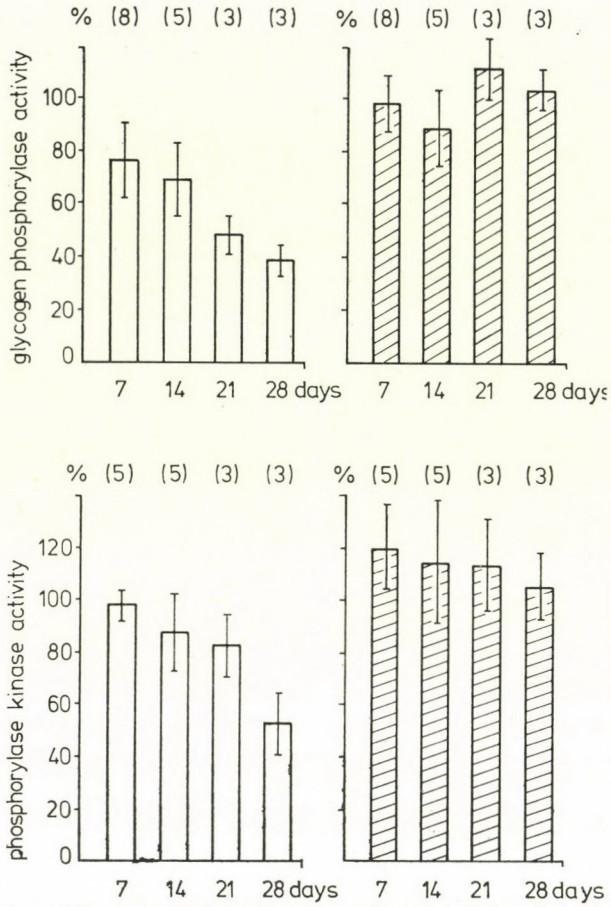

Fig. 3. Ph and PhK activity in immobilized EDL and soleus muscles in comparison with data for the contralateral untreated (control) side. In the picture means ±S. D. are given. Numbers in brackets indicate the number of determinations; empty blocks, m. EDL; striated blocks m. soleus

Discussion

It has long been known that the intensity of glycogen phosphorylation is different in the different types of skeletal muscle [33] and that glycolytic and oxidative metabolism is also different in fast (tetanic) and slow (tonic) muscles [7]. Glycogen content and phosphorylase activity are higher in fast than in slow muscles which, on the contrary, are characterized by a large amount of mitochondria and by an excess of oxidative metabolism [5, 25]. Muscle fibres differing in metabolism can be differentiated [8] by the histochemical study of Ph, the enzyme responsible for the first step of glycogen decomposition. In contrast, data are few concerning the quantitative conditions

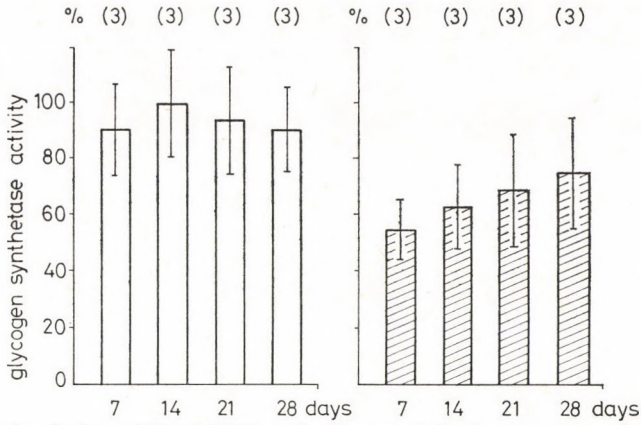

Fig. 4. GS activity in immobilized EDL and soleus muscles in per cents of the contralateral control values. In the picture means ± S. D. are given. Numbers in brackets indicate the number of determinations; emply blocks, m. EDL; striated blocks, m. soleus

of PhK and GS. According to our results, the activity of PhK similarly to that of Ph is 8 times higher in the EDL muscle possessing mainly a fast (tetanic) anaerobic energy supply, than in the soleus which is slow (tonic), indicating the role of PhK during glycogenolysis (Fig. 1). The distribution of activities pointed to a similar ratio of the two enzyme concentrations in both the tetanic and the tonic muscle. Generally, the ratio of the activities of Ph and PhK are independent of the type of muscle and the rate of change in both enzyme activities, which is also the same in immobilized muscles (Fig. 3). This fact indicates a narrow functional connection and a co-ordinated regulation of the intracellular turnover of the two enzymes.

The concentration of GS, an enzyme responsible for the limitation of glycogen synthesis, was higher in the soleus than in the EDL muscle (Fig. 1). Earlier similar difference was observed in the fast and slow muscles of rats and monkeys [1]. The significant concentration of GS may ensure the regeneration of glycogen content, the turnover of which in slow muscles was shown the exceed that in fast muscles [23].

Reduction of the muscle mass is well-known to occur during immobilization. According to data in the literature [3] and our earlier results [29], the decrease of mass is larger in slow than in fast muscles (Fig. 2). Similarly, changes of the protein content of the sarcoplasmic reticulum [31] and of the contractile properties [30] of muscle fibres is also more expressed in tonic muscles. Significant changes were found to take place also in fast muscles, and these were associated with a significant decrease in the concentration of glycogenolytic enzymes. The reduction of the concentration of glycogenolytic enzymes under the effect of immobilization results in a decrease of the differences between slow and fast muscles and simultaneously in a dedifferentiation

of metabolism. This was shown by the fact that, while Ph activity was 8 times more intensive in the control EDL than in the soleus muscle, the difference was reduced to about 3fold by the 28th day. Thus, the immobilization caused a dedifferentiation in the glycogenolytic enzyme groups, too, in agreement with previous observations [2, 9, 27, 31].

The early significant decrease of GS activity in the soleus muscle (Fig. 4) may play a role in the decrease of the glycogen level [3], and indirectly in the contractility [30].

The reduction of the concentration of the functionally significant proteins is attributed to increased proteolysis [12, 13]. According to Goldspink [14–16] proteolytic and hydrolytic enzyme activity increases under the effect of immobilization and changes occur also in protein turnover. Among the lysosomal enzymes, an increase in acid phosphatase activity has been observed [28], as well as an increase in protease activity after denervation (18). Factors which help the preferential proteolysis of an enzyme are rather variable [11]. The allosteric sensitivity of Ph against different proteases has been shown [4, 26], and in this way changes in the concentration of the different metabolites may also play a role in its proteolytic inactivation. PhK [6, 19] and GS [24] are rather sensitive to proteolytic effects in experiments in vitro, while the sensitivity to cell proteases of the enzymes of glycogen metabolism as well as their degradation in vivo are still unknown.

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Book Reviews

J. W. KEBABIAN and J. A. NATHANSON (Eds)

Cyclic Nucleotides, I and II

Handbook of Experimental Pharmacology. Vol. 58, G. V. R. Born, A. Farah, H. Herken and and A. D. Welch (eds) Springer Verlag, Berlin, Heidelberg, New York 1982. 1444, pp. Price: Part 1, DM 390,—; approx. US $ 173.20, Part 2, DM 560,—; approx. US $ 248.70

This book which covers an area of great current interest the biochemistry, pharmacology and physiology of cyclic nucleotides consists of two volumes and each volume contains two sections.

In Section I the authors discuss the chemistry and biochemistry of cyclic nucleotides in 8 chapter. They give detailed information about the chemistry, formation and degradation of cyclic nucleotides and their analogue compounds. The receptors to adenylate cyclases and the modulation of the responsiveness of receptor-associated adenylate cyclases are also discussed in this chapter.

Section II gives an excellent overview of protein phosphorylation in 5 chapters.

Section III discusses 9 different areas of cyclic nucleotide research related to physiological and pharmacological events of cellular regulatory processes.

Section IV deals with the physiology and pharmacology of organ systems in 13 chapter. Cyclic nucleotide research is such a large field that the authors could only summarize the general principles.

In the first section an excellent overview is given by T. W. Rall on the history and present status of cyclic nucleotides. The chapter on the chemistry of cyclic nucleotides has been written by Ravenkar and Robin and it gives an outstanding summary of the advances achieved in the last decade in the synthesis of cAMP. They describe a number of potent cyclic nucleotide derivatives which may have significant effects on different cellular mechanisms such as cellular proliferation, immune response, asthma, gastrointestinal functions, etc. One of the most important chapters of this section is concerned with the modulation of the responsiveness of receptor associated adenylate cyclases written by J. P. Perkins, T. K. Harden and J. F. Harper. Antagonist-induced adaptive phenomena that occur in hormone-stimulated adenylate cyclase systems as well as the acute inhibition of adenylate cyclase by a number of neurohumoral agents are discussed in this chapter.

The second section is introduced by P. Greengard, one of the pioneers of the cAMP-dependent phosphorylation studies. He gives an outstanding review of protein phosphorylation, summarizing the role played by protein phosphorylation in mediating the biological effects of the hormones and neurotransmitters that act through cyclic cAMP. This chapter deals also with the pioneer work of Kuo and Greengard on the cyclic AMP-dependent protein kinases.

The third section deals mainly with the role of cyclic nucleotides in the regulation of different physiological and biochemical processes. The wide scale of these events shows the exclusive importance of these substances as second messengers in several physiological events. In intercellular communication these substances may however also play an important role as first messengers. The chapter on cyclic nucleotides as first messengers, written by R. Van Driel, deals almost exclusively with cAMP-mediated communications between special cells. He describes the generation of the cAMP signal, reception of the signal by cell surface receptors, transfer of information from the cell membrane to various intracellular systems that are responsible for chemotaxis and also the signal destruction by phosphodiesterase. This chapter is of considerable interest for those who are seriously involved in the molecular aspects of intercellular communications.

For pharmacologists, the section on the physiology and pharmacology of organ systems seems to give the most important information. This section deals with the role of cyclic nucleotides in several tissues such as the nervous system, the eye, the thyroid gland, bones, the gastrointestinal tract, the vascular system, the pineal gland, the epithelium and platelets, and also with their importance in the immune response. The role of cyclic nucleotides in the nervous system is described by Dunwiddie and Hoffer and they present an excellent and detailed information on the second messenger role of cyclic nucleotides in the peripheral and central nervous system. The direct and indirect messenger role of these substances for a variety of transmitters, the dynamics of cyclic nucleotide synthesis and degradation, the pre- and postsynaptic modulation by cAMP and the role of cAMP in intermediary metabolism are summarized in this review, as well as the several effects of cyclic GMP.

Both volumes of the book contain a collection of excellent reviews which will be meaningful for almost all biochemists, physiologists and pharmacologists. Its clarity, the balance of presentation and the division of the chapters (each chapter begins with a detailed summary) allow the reader to enjoy the book and pick up the most up-to-date information.

K. MAGYAR

L. NOVER, M. LUCKNER and B. PARTHIER (Eds)

Cell Differentiation. Molecular Basis and Problems

Springer Verlag, Berlin, Heidelberg, New York 1982. 650 pp. 228 figures and 65 tables. Price DM 118.—; approx. US $ 55.00

A book with the same title and the editors was published in 1978 by Fischer Verlag, Jena. Jena. The authors of both the earlier and the present volume are exclusively from the German Democratic Republic. This new English edition is not only a translation of the earlier volume but its extended and modified version. Some of the earlier contributors are new missing and the new contributors either reorganised the old chapter or added new topics. Nover took the greatest share who apart from the editorial work wrote alone or with coauthors almost half (about 280 pages) of the volume.

The mechanism of differentiation is a very exciting problem but has not yet been fully elucidated despite the many data quoted. During differentiation a single cell, the fertilized ovum, gives rise to myriads of cells with different morphology and function, forming a common system in which there is an interdependence of the function of cells in the multicellular organism. Thus, differentiation occurs at cellular level, therefore multicellularity is not a criterion but, based on the aforementioned correlations, also expressed and can be studied at the level of the organs and the organism.

Differentiation as a concept is not a new idea; its scientific exploration started with Roux in the last century and was continued later by Hertwig and Spemann. These studies seem to be highly relevant even now and have rendered available a great deal of information with regard to the events and stages of differentiation. Still, they could not comprehensively unravel the regulatory aspects. Therefore, a confrontation of these results and facts with the achievements of the age of molecular biology became absolutely demanding. The volume is intended to fulfill this goal when it surveys the events of differentiation both in prokaryotic and eukaryotic (plant and animal) cells based on the accomplishments of molecular biology and replaces the terms of developmental mechanics and developmental physiology by those of molecular biology. It is of course unavoidable that the volume contains more or less fundamental elements of molecular biology which in turn are essential in understanding the pertaining regulatory problems.

The contributors who otherwise performed superb work were recruited from a single country. This fact appears to extend and restrict the content. Thus, new chapters have been included dealing with specialized topics such as the effect of insulin and oestrogen, but curiously enough the effect of progesterone, testosterone and of the catecholamines are not discussed. This and other chapters of the volume of course provide exceedingly useful materials and are not to be criticised. Within the same framework a very good chapter deals with the determination and function of the immune system and with the possible role of errors of cellular differentiation in tumour genesis.

The format is pleasing, the figures are illustrative and didactic. The electron micrographs and autoradiographic illustrations presented are, however, not of the best quality. The

immense literature cited is up-to-date, the detailed and well-arranged subject index is of great help to the reader.

This volume will be of interest not only to researchers interested in differentiation but also to physicians, biologists and those involved in related areas.

Gy. CSABA

J. SALÁNKI (Ed.)

Physiology of Excitable Membranes

Advances in Physiological Sciences. Vol. 4. Pergamon Press, Oxford and Akadémiai Kiadó, Budapest 1981

This is the 4th volume of a series of 36 books which contains the papers presented at the 28th International Congress of Physiological Sciences held in Budapest in 1980.

In five large chapters the reader finds a number of interesting papers on the 360 pages of this book completed with preface and subject index. Subjects of the five chapters differ widely from each other because the chapters cover independent symposia organized in the Section on General Cell Physiology. Since, however, all the papers deal with various aspects of cell excitability, it is not only justified to collect them into a single book, but it is also a gain to the readers to find traces and results of so many approaches of that important, basic problem of neurobiology in the same book.

The first paper is a survey of works made by Kostyuk and his colleagues in Kiev during the last 5–6 years to elucidate the ionic mechanisms of excitability of nerve cells. This paper serves well as a general introduction to the rest of the book.

Meves, co-editor of the first chapter on "Charge Movement in Nerve Membrane" points at the problems associated with studies an "gating currents". The problems are numerous not only because of difficulties involved in research of living membranes at the molecular level but also because the processes studied are extremely complicated. In his concluding remarks Meves notes that a generally accepted hypothesis concerning charge movements and Na permeability changes is still missing. Still, as we see from the nearly 100 pages of this chapter, considerable efforts have been made at finding one.

The second chapter, co-edited by H. D. Lux, deals with calcium electrogenesis. It seems clear now that Ca^{++} can be the main current carrier during action potential in some membranes, in others Ca current associates with Na current during the process of depolarization. Once initiated, Ca currents activate other currents carried for example by K ions. It is therefore important to consider that Ca has powerful but different kinds of effects in various excitable membranes.

A comparatively new field of research uses optical methods to study the excitability. Under the chairmanship of N. Chalazonitis, an interesting symposium has revealed evidence of structural, macromolecular changes within the membranes and/or in the axoplasm underneath. An important advantage comes from utilization of optical methods, namely the possibility of simultaneous recording from large numbers of individual units to follow up various cells of a network involved in the processing of an output from a system.

The last two chapters deal with synaptic transmission. First we get recent data on modulation of synaptic transmission mostly by catecholamines (coeditor, E. S. Vizi), and then the topic is focussed on transmission in autonomic ganglia (co-editor, V. I. Skok). Thus the papers in both chapters elucidate further some rather slow but widespread chemical effects the released transmitters. These effects are powerful and they differ considerably from the other faster, shorter, and fairly localized effects of synapses like those well known to influence the motoneurons in the spinal cord.

On the whole, this is an excellent volume of the series, and since it contains quite new data and well written views of top experts of the subjects, it will be much welcomed by a fairly large number of readers. The quality of typesetting is good, the text is easy to read and the illustrations are good and clear.

G. CZÉH

GY. SZÉKELY, E. LÁBOS and S. DAMJANOVICH (Eds)

Neural Communication and Control

Advances in Physiological Sciences. Vol. 30 Pergamon Press, Oxford and Akadémiai Kiadó, Budapest 1981. 340 pp

The book is a collection of papers delivered at a Satellite Symposium of the 28th International Congress of Physiological Sciences held in Debrecen, Hungary in 1980. Looking at the table of contents the first impression one gets is a bewildering diversity of topics and style of presentation. This thematical richness makes it impossible to register and evaluate systematically the topics dealt with in the book. A series of papers of analytic type presenting experimental data, and theoretical essays follow each other in a rather random sequence. In the former group one finds papers dealing with the dynamics of macromolecular membrane processes, synaptic interaction, dendrite topology, locomotor coordination and perceptual processes at different levels of complexity. In the second theoretical group of lectures, concepts of comparative neurobiology, mathematical models of neuronal networks and problems of consciousness in the context of interhemispherical information exchange are presented.

The preface of the editors and the key-note speech given by MacKay reveal that the divergence and abundance of topics were the result of willful planning with the expectation that disciplinary disparity and confrontation of experimental and theoretical approaches would result in an effect of "interfertilization". This "metascientific" aim as MacKay calls it, is justified in principle and might be a good invitation to read the book. It promises an exciting intellectual adventure. I readily admit that the reader will not feel deceived indeed in this respect. The quality of the papers and the originality of information offered makes the book a very useful piece of reading. As to the expectation of a mutual interfertilization, however, the reader remains uncertain and left to his own judgement since the (allegedly lively) discussions are not included in the book. MacKay thinks that a most fruitful interaction could be observed between theories and new experimental data on cerebellar function (Ito, Marr, Alben). Szentágothai finds Bullock's propositions, based on comparative morphological findings, as well as the new discoveries concerning dendritic branching patterns and topology (Székely) as very promising areas of research. According to the reviewer, a mutually fertilizing interaction could have been further promoted by the inclusion of further disciplines. This mainly holds for the approach of the extremely complex processes of consciousness and interhemispheric information transactions.

On the whole the symposium (and the book) is a very interesting and valuable undertaking, even if its lasting effects will only be reflected by future results and changed research strategies in the related fields. Nevertheless, the book will convince its readers that the initiative is worth of continuation. And it is a good guide to become adapted to such new, catalyzing forms of scientific information exchange.

E. GRASTYÁN

J. SZENTÁGOTHAI, J. HÁMORI, M. PALKOVITS (Eds)

Regulatory Functions of the CNS

Advances in Physiological Sciences, Vols 1 and 2. Pergamon Press, Oxford and Akadémiai Kiadó Budapest 1981

These are the first 2 volumes of a series of 36 books which contain the papers presented at the 28th International Congress of Physiological Sciences held in Budapest in 1980.

Volume 1, which has the subtitle Principles of Motion and Organization, starts with one of the oldest and most difficult problem of modern neurophysiology, the mechanism of transmission in the monosynaptic reflex pathway in the spinal cord. To be short, the mechanism seems to be better understood in terms of chemical transmission. The latest experimental findings from the mammalian spinal cord argue against the electrotonic mediation of transmission, an idea which has received apparently strong experimental support during the late seventies. In submammalian species like frogs, the transmission has a dual electronic and chemical mechanism.

The next chapter, on the neuronal mechanism of voluntary movements and precentral motor area is unfortunately restricted to the opening and closing remarks of the chairmen completed only with quite short summaries of the presented papers. One feels that it is too high a price for the speed of publication to drop almost the whole material on that topic. Omission of papers from other chapters because of failure in receiving the manuscripts in time aroses similar feelings of want when the chairman refers to talks not to be found in the book.

Two chapters deal with a symposium split up into a part on locomotion and another on the principles of motor organization. One of the central problems of the control of locomotor movements remains how far we can get keeping Graham Brown's old proposal on the alternating activity of half centres in the spinal cord. More recent ideas share the weakness of the old one, namely neither is complete enough to be in full harmony with all experimental results. More agreement emerges on the existence and role of supraspinal command systems, called "locomotory region", in the brain stem. The fact, that part of the papers in the chapter "Principles of Motor Organization" deal also with locomotion may illustrate how premature at present it is to try to formulate such highly general principles of the funcions of the CNS.

The last two chapters badly need the convenient guidance of chairmen for many of us who feel less at home in the field of eye movements and pursuit control system or sleep and unitary activity of the brain. Although both chapters contain excellent papers on some specific problems, it seems difficult to find out which are currently the most important questions in these areas of neurobiology.

The Editors stress that the subtitle of Volume 2, Subsystems, should be taken in the functional rather than in the anatomical sense. This volume has chapters on ontogenic development, cortex, cerebellum, striatal mechanisms, and on subcortical sensory processing in addition to two papers on plasticity and on functional units of the cerebellum.

Tsukahara summarizes a great body of recent work which provides evidence for enduring plastic changes in the electrophysiological behaviour of the red nucleus neurons due to lesion-induced collaterals routing of afferent systems in mammals. Other, loosely related papers deal with maintained alterations of neuronal connections induced in various experimental models by deprivation of function, by axotomy or crushing the afferent systems.

The symposium on modular organization principles in the CNS received particular attention and one regrets to see that a number of interesting papers have been omitted. Szentágothai's concluding remarks suggest that the theory on principles of modular organization is more and more widely accepted in works on cortex and subcortical systems alike.

Perspectives in cerebellar physiology apparently aim at the basic, elementary membrane processes in the cerebellar neurons, to the further details of neuronal circuits, and to the functional aspects of the cerebellum and its clumbing fibre input. This large chapter contains many interesting papers written by the best experts on the cerebellum.

There is another large chapter on striatal mechanisms, the length of which may reflect the "quiet revolution in the thinking about striatal mechanisms" which has taken place in the last few years according to the Pasiks. Detailed morphological, physiological, and developmental studies are reported together with papers on pharmacological and behavioural aspects of the striatum. The results support the conclusion that the simple view on the purely motor function of the striatum should be replaced by more complex theories which include even some cognitive function of the striatum.

The last chapter on subcortical sensory processes seems to be restricted to the visual sense, since three papers deal with the retina, two with the superior colliculus, and one discusses the lateral geniculate as an interface between the eye and the brain. In this chapter the reader again misses the guidance of the chairmen.

In summary, these two volumes are giving an appropriate impression about the proceedings of the Congress in Budapest. They both bear the advantages and drawbacks of rapid publication: few of the papers are loosely typed, some of the illustrations are poor in quality as compared with other excellent ones. The fact that the books appeared on the market within about half a year after the Congress should, however, be appreciated.

G. Czéh

Acta Physiologica Hungarica, 62 (1), pp. 99—101 (1983)

Comments

ON THE PAPER OF J. HERCZEG

[ACTA PHYSIOL. ACAD. SCI. HUNG. 58, 147—155 (1981)]

D. G. PORTER

PRE-CLINICAL VETERINARY STUDIES, DEPARTMENT. OF ANATOMY, THE MEDICAL SCHOOL, UNIVERSITY OF BRISTOL, ENGLAND

(December 11, 1982)

This paper is primarily a rebuttal of Porter and Challis [10] which in principle is perfectly legitimate but in fact embodies a number of unjustified criticisms which can be refuted by published data to which the paper makes no reference. In Downing, Lye, Bradshaw and Porter [2] we had (a) shown that the dosage of oestradiol which Dr. Herczeg criticises (p. 48) as 5X physiological could be reduced 10 fold without altering the results and provided plasma titres showing that the dosage was within "physiological" limits; and (b) that oestradiol treatment maintained the myometrial progesterone receptor population thus avoiding a likely criticism that the lack of effect of progesterone treatment was due to receptor depletion. Furthermore the allegation is made without evidence that our recording technique induced stress yet the first 30 minutes of recordings made in Dr. Herczeg's own experiments had to be discarded "because the animals were occasionally restless initially". No account is taken of the fact that in our laboratory with this same technique we have been able to demonstrate the progesterone "block" in sheep (Lye and Porter, [5], rabbits (Porter, [7]) and pigs (Watts and Porter [14] unpublished) and even more pertinent, the delicate inhibitory actions of both relaxin (Porter, Downing and Bradshaw [11]) and of oestradiol-17β (Downing et al [2]) in rats.

On page 149 Dr. Herczeg compares the stability of his intra-uterine pressure recordings to the "intermittent" recordings of Porter and Challis [10]. Again the criticism ignores the clear data in Downing et al. [2] which demonstrates (a) stable recordings unaffected by a progesterone treatment regimen which had been shown to be adequate to maintain normal pregnancy in ovariectomized pregnant rats and (b) that the "intermittent" nature or "variability" criticised on p. 151, was explicable on the basis of the surprising inhibitory action of *oestradiol* itself (first reported by Burns [1]).

On page 153 Dr. Herczeg claims that his results are consistent with all the literature cited in his introduction except Porter and Challis [10]. Indeed they were, but none of the papers cited actually demonstrated that progesterone inhibits the myometrium of the rat! Moreover Dr. Herczeg chose to ignore papers which had attempted but failed to demonstrate the progesterone "block" in this species (e.g. Melton and Saldivar, [6], Fuchs, [4]). He also failed to cite other data that progesterone does not "block" the guinea pig uterus (Ehrhardt and Kneip, [3]; Marrow, Anderson and Callantine, [15]; Schofield, [13], Porter, [8, 9]; Porter, Yoshinaga and Ford, [12], which gives precedents for our results in the rat.

Finally it is offensive to read that "investigators can be justifiably expected to scrutinize their findings with extreme care before challenging a basic regulatory principle" carrying as it does the implication that we had challenged the progesterone "block" hypothesis irresponsibly. Our 1978 paper was based on the use of treatment regimens, demonstrated to be physiological, in 10 virtually unrestrained rats from each of which intra-uterine pressure recordings had been taken continuously for 100 h. These extensive records had been subjected to complete quantitative analyses. Furthermore plasma steroid and myometrial receptor concentrations were measured as corroborative evidence. Dr. Herczeg's experiments measured intra-uterine pressure for one hour per day of which 30 min of each recording had to be discarded!

I contend that Dr. Herczeg's paper represents an ill-justified condemnation of our work and by omitting reference to our more recent papers, as well as those of others with which they are consistent, it suggests that his criticisms are biased beyond the normal limits of scientific objectivity.

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Acta Physiologica Hungarica, 62 (1), pp. 103—105 (1983)

RESPONSE TO THE COMMENTS OF PROF. D. G. PORTER ON THE PAPER OF J. HERCZEG, [ACTA PHYSIOL. ACAD. SCI. HUNG. 58, 147–155 (1981)]

J. HERCZEG

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, WHO COLLABORATING CENTRE FOR RESEARCH IN HUMAN REPRODUCTION, UNIVERSITY MEDICAL SCHOOL, SZEGED, HUNGARY

(January 10, 1983)

Professor Porter's comments on my paper raises the important question of the general validity of progesterone (P) "block" as a basic principle in uterine physiology.

In fact our debate on the issue started much earlier in the scientific literature (Biology of Reproduction, *14*. 190–193, 1976) to which Professor Porter made no reference, but without knowing that the scientific community might be confused about the exact nature of our points of views.

To my best knowledge at present the view is generally upheld by scientists working in the field that the uterus is an inhibited organ and parturition commences when the uterus is released from inhibition. It is also generally accepted that progesterone is the main inhibitory factor of the myometrium and a great number of papers cited by Professor Porter also support this basic premise.

P "block" was elegantly and succesfully demonstrated in vitro by Professor Currie (1982) in his most recent set of experiments.

However, there are also highly respected investigators who believe that there are species differences in this respect, thus challenging the validity of the basic regulatory principle of P "block". An example of such attempts aimed to find an alternative factor is the paper of Porter (1974), in which a "new" Myometrial Inhibitory Factor (MIF) was introduced. It was reported that, in addition to the steroidal suppressor progesterone, the circulating blood of late pregnant rabbits contains a nonsteroidal "myometrial inhibiting factor". This conclusion was derived from the observation that cross-circulation of blood from a pregnant donor to a nonpregnant recipient suppressed both the frequency and magnitude of cyclicin trauterine pressure in the recipient rabbit.

This report prompted us to repeat these experiments as an initial preparatory step for the attempted isolation of MIF. Using a similar setup we were unable to find evidence for a "new" MIF. (Herczeg and Török, 1976)

and we expressed our opinion: ". . . that conclusions based on studies in which the conditions of animals are known to be unphysiological, must be evaluated with extreme caution".

This time I appreciate Professor Porter's valuable comments which contribute significantly to the advancement of our understanding on myometrial regulation.

Professor Porter in his comments on my findings first suggests that oestradiol pretreatment would have been necessary and he cites their own work to support his claim. However, we found earlier in Professor Csapo's Reproductive Physiology Laboratory that oestradiol pretreatment induced a special regulatory condition which was characterized by periodic outbursts of high level cyclic activity (Fig. 1): similar phenomena could also be clearly

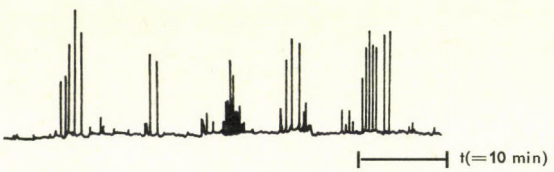

Fig. 1. Intrauterine pressure cycles of a post-partum ovariectomized rat pretreated with oestradiol-17β (1 μg/day for 3 days). Note the high amplitude pressure cycles, representing a special regulatory condition which we could not interpret as an " inhibition". Paper speed: 1.25 mm/min (Beckman Polygraph)

identified on Professor Porter's own recordings (see Text-fig. 1, upper tracing: Porter and Challis, 1974). This type of activity could hardly be interpreted as "inhibition" since the periodic contraction cycles emerge suddenly from the quiet baseline with a high rate of rise of pressure. We have concluded that in this type of regulatory condition oestradiol ensures maximum working capacity of the uterine muscle characterized by "rhythmic" and periodic activity cycles.

Moreover in the cited paper the authors themselves emphasize their opinion that " . . . the possibility existed that the failure of progesterone to inhibit the myometrium of the rat post partum was due to the lack of cytoplasmic receptors for progesterone. Our results show that this explanation is improbable. High affinity binding sites for progesterone were found in the myometria from all four experimental groups . . . " (Downing et al. 1978).

Concerning the recording technique we have been able to demonstrate that continuous recording for several hours definitely alters the characteristics of the contractility pattern: the amplitude of pressure cycles tend to increase over prolonged periods of time, thereby preventing the effects of a blocking agent to be clearly identified. In our own experiment — as Professor Porter pointed out — we used shorter recording time and analysed only the second half of our records, as a perfectly justifiable precaution.

Professor Porter also criticized us for not having mentioned that they had been able to demonstrate the P "block" in some animals, however, this was beyond the scope of the re-investigation. The interesting "intermittent" activity pattern (". . . the nature of the inhibition is of interest for it is brought about primarily by an increase in the periods of quiescence": Downing et al. 1978) in the experiment of Porter and Challis, 1974 was — in my opinion — induced by oestradiol pretreatment which overshadowed the inhibitory effect of progesterone.

The last set of Professor Porter's remarks centered on the necessity of references: however, in my debated second paper — just as in our earlier work — I have restricted myself to the re-examination of their particular experiment and have come to the conclusion that progesterone "block" is probably a basic regulatory mechanism in reproductive physiology.

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CONTENTS

PHYSIOLOGY — PATHOPHYSIOLOGY

BOOK REVIEWS

Acta Physiologica Hungarica, Volume 62 (2), pp. 107—112 (1983)

CELLULAR ENERGY SYSTEMS AND RESERPINE ULCER IN RATS

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Gastric ulcer was elicited in rats by reserpine (5 mg · kg^{-1} sc.) administration. Ulcer formation (number and severity) was measured 6, 12, 18 and 24 hr after reserpine administration. At the time of killing of the animals, tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic adenosine monophosphate (cAMP) were measured enzymatically and by radioimmunoassay in the gastric fundal mucosa. The sum of ATP + ADP + AMP (adenylate pool) and the ratio of ATP · ADP^{-1} were calculated.

It was found that (1) the tissue levels of ATP, AMP, cAMP, sum of ATP + ADP + AMP (adenylate pool) and ratio of ATP · ADP^{-1} increased significantly in the gastric fundal mucosa 6 hr after reserpine administration, thereafter these values decreased gradually and significantly; (2) the tissue level of ADP decreased significantly in the gastric fundal mucosa 6 hr after reserpine administration, meanwhile its level increased significantly at 18 and 24 hr; (3) the value of energy charge (ATP + 0.5 ADP · ATP + ADP + AMP^{-1}) remained unchanged; (4) the peaks of biochemical alterations in the gastric fundus mucosa preceded the appearance of ulcers.

It was concluded that (1) reserpine ulcer appears after an active metabolic response in the rat gastric fundal mucosa; (2) hypoxaemic damage in the gastric fundal mucosa can be excluded as a possible underlying mechanism of ulcer formation produced by reserpine administration; (3) before the appearance of reserpine ulcer, significant changes in the feedback mechanism, system, i.e. between the ATP — membrane ATPase — ADP and the ATP — adenylate cyclase — cAMP energy systems, can be observed in the rat gastric fundal mucosa.

Keywords: ATP-dependent energy systems, feedback mechanism, reserpine, gastric ulcer, hypoxaemic damage of gastric mucosa, metabolism of gastric fundal mucosa, reserpine-induced adaptation in the tissue metabolism.

Peptic ulcer disease represents a very complex pathological phenomenon, in which the gastric mucosal erosions (lesions) appear as a consequence of different pathological factors (burns, stress, drugs, different diseases and other unidentified factors). Some (about 20 to 30 per cent) of ulcers is provoked by different drugs, as antirheumatic and antihypertensive agents, steroids, etc.

In the last years, numerous biochemical studies have been carried out to study the possible background of the different types of gastric lesions and ulcers in the gastric and small intestinal mucosa [5—11, 13, 14]. The possible role of the membrane and ATP-dependent energy systems (ATP — membrane ATPase — ADP and ATP — adenylate cyclase — cAMP) of the gastric and small intes-

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tinal mucosa, and the functional equilibrium between them, were studied and evaluated in pylorus-ligated [5, 6, 10, 12, 13], pylorus-ligated plus epinephrine-treated [10], pylorus-ligated plus salicylate-treated [10], stressed by swimming [10], indomethacine-treated [3] rats, and in other types of gastric fundal mucosal lesions produced by intragastric administration of 0.2 M NaOH, 25% NaCl, 0.6 M HCl and 96% ethanol, and on the other hand, in patients with chronic gastric, duodenal and jejunal ulcers [5, 7, 8, 10, 11].

The gastric mucosal lesions (ulcers) can be provoked by reserpine administration in a dose of 5 mg · kg^{-1} subcutaneously in rats. The lesions (ulcers) appear in the rat gastric fundal mucosa 12, 18 and 24 hr after reserpine administration. In this paper, the biochemical background of the ulcer development was studied in rats, provoked by reserpine administration, and the tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic adenosine monophosphate (cAMP) were measured in the rat gastric fundal mucosa 0, 6, 12, 18 and 24 hr after reserpine administration.

Materials and methods

The experiments were carried out on CFY strain rats of both sexes, weighing 180 to 210 g. The animals were fasted for 24 hr before the experiments, but they received water and libitum.

The animals were treated with reserpine (Rausedyl®, Gedeon Richter, Budapest, Hungary), in a dose of 5 mg · kg^{-1} subcutaneously in 1 ml volume. The animals in the control group were treated with 1 ml saline solution subcutaneously. The animals were killed at 6, 12, 18 and 24 hr after reserpine administration. The animals in the control group were killed 24 hr after application of saline.

The number of ulcers was noted. The severity of ulcers was expressed by the following scale: 1, ulcer is less than 1 mm; 2, ulcer size is between 1 and 2 mm; 3, the ulcer size is between 2 and 3 mm; 4, ulcer size is between 3 and 5 mm, and 5, the ulcer size is greater than 5 mm. The scored values were summarized and expressed as ulcer severity computed for one rat stomach. The number and severity of gastric ulcers were calculated for each individual animal (means ± SEM).

The tissue levels of ATP, ADP, AMP were measured enzymactically (Boehringer, Ingelheim FRG), while tissue level of cAMP was estimated by radioimmunoassay (Becton, Dikinson, Orangeburg, USA). The protein content was assayed by biuret reaction [2]. The adenylate pool in the gastric fundal mucosa was calculated as the sum of ATP + ADP + AMP

Table I

Biochemical characterization of the rat gastric fundal mucosa in the control group of animals treated with physiological saline solution only. The number of animals was 15 in every case

ATP	12.22±1.70	nanomoles · mg protein^{-1}
ADP	14.47±2.00	nanomoles · mg protein^{-1}
AMP	10.47±1.50	nanomoles · mg protein^{-1}
ATP+ADP+AMP	37.20±5.6	nanomoles · mg protein^{-1}
cAMP	4.39±0.15	picomoles · mg protein^{-1}
ATP · ADP^{-1}	0.33±0.01	
ATP+0.5 ADP	0.52±0.04	
ATP+ADP+AMP*		

* value of energy charge was calculated according to the formula of ATKINSON [1]

[1]. The extent of phosphorylation and/or dephosphorylation was estimated by the formula of energy charge (ATP + 0.5 ADP. ATP + ADP + AMP^{-1}) [1]. The values of ATP, ADP, AMP, adenylate pool were expressed in nonomoles · mg mucosal $protein^{-1}$ (means ± SEM), while the cAMP expressed in picomoles · mg mucosal $protein^{-1}$ (means ± SEM).

Statistical analysis of the results was carried out using Student's *t* test, except in the case of ulcer severity when the Mann and Withney's method was applied.

The biochemical constituents of the normal gastric fundal mucosa in rats are presented in Table I.

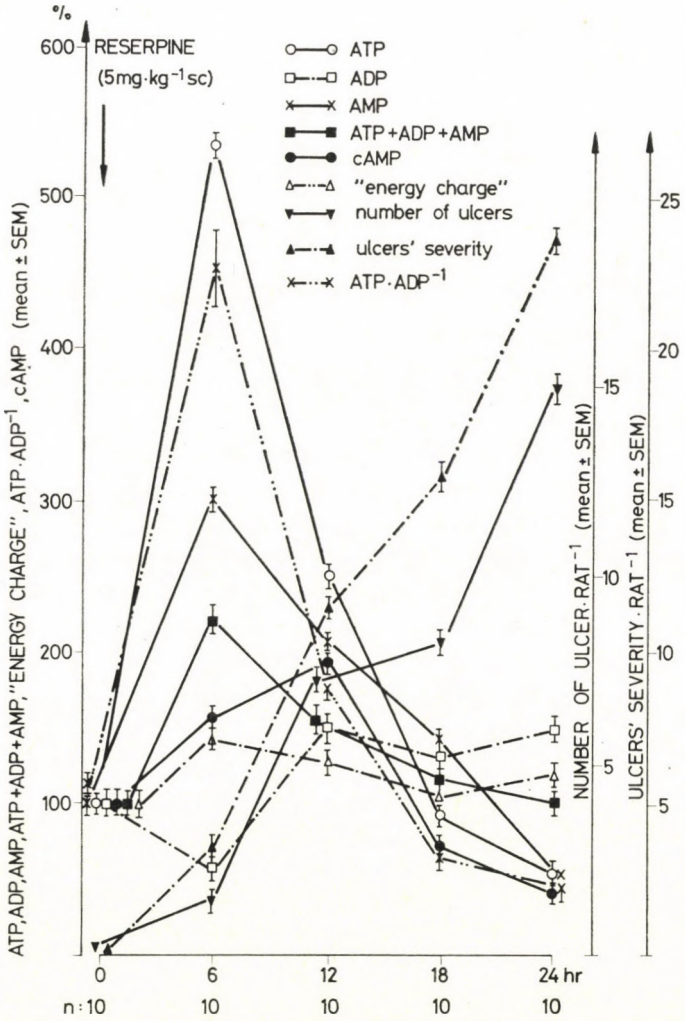

Fig. 1. Biochemical alterations in the rat gastric fundal mucosa during the development of gastric ulcer produced by reserpine administration. The results are expressed in per cent values, except of the number and severity of ulcers, which vere expressed in absolute numbers (means ± SEM). The value of energy charge was calculated by the formula of ATKISON [1] : ATP + 0.5 ADP. ATP + ADP + AMP^{-1}. The number of animals was 15 in each group

The tissue levels of ATP, cAMP, AMP, sum of ATP + ADP + AMP, ratio of ATP · ADP^{-1} increased significantly 6 hr after the administration of reserpine, thereafter the quantities of these compounds and calculated values decreased significantly and gradually until the 24th hr (Fig. 1). ADP significantly decreased 6 hr after reserpine administration, thereafter its quantity increased significantly. The peaks of these biochemical changes preceded the development of gastric ulcers. The value of the energy charge [ATP + 0.5 ADP · ATP + ADP + AMP^{-1}) remained unchanged in the gastric fundal mucosa after administration of reserpine.

Results

In our previously published papers we have shown that very complex and by neural, hormonal and pharmacological influences well regulated feedback mechanism system exists between the cellular ATP — membrane ATPase — ADP and the ATP — adenylate cyclase — cAMP energy systems in the rat and human gastric fundal mucosa [5, 6, 10, 12, 13]. It has also been shown that this functional equilibrium between the two energy supply systems is disturbed during the development of gastric lesions [ulcers] in pylorus-ligated, pylorus-, ligated plus epinephrine- and salicylate-treated rats and of different gastric mucosal damages produced by intragastric administration of 0.2 M NaOH, 25% NaCl, 0.6 M HCl and 96% ethanol in rats [10, 13, 14].

During the development of stress ulcer produced by 5 hr swimming, the tissue level of cAMP increased significantly concomitantly with the appearance of ulcer [10]. When the development of stress ulcer was prevented the tissue level of cAMP in the rat gastric fundal mucosa remained at the same level as in the control animals [10].

Reserpine is known to liberate the adrenergic mediator substances which may cause a hypoxaemic damage in the rat gastric fundal mucosa [15, 16, 17]. The presence of the hypoxaemic damage in the gastric fundal mucosa was suggested by physiological observations [15, 16, 17]. In our study, biochemical methods were used to prove [or to exclude] the possibility of hypoxaemic damage in the rat gastric fundal mucosa after the application of reserpine.

According to basic biochemical knowledge, there are two main indications of the hypoxaemic damage of tissue metabolism: [1]. a significant decrease of tissue ATP level resulting from impaired resynthesis of ATP; [2]. a significant increase of tissue lactate level.

These biochemical changes represent the cross section of tissue metabolism. In this study the tissue level of lactate was not measured, meanwhile the ways of ATP breakdown by membrane ATPase and adenylate cyclase were together measured with the changes in the tissue level of ATP.

Our results indicate a significant increase of ATP, AMP, sum of ATP + ADP + AMP, ratio of ATP · ADP^{-1} in the rat gastric fundal mucosa 6 hr after the administration of reserpine. During this time the tissue level of ADP decreased significantly. Thereafter the tissue levels of ATP, AMP, cAMP, sum of ATP + ADP + AMP, ratio of ATP · ADP^{-1} decreased gradually and significantly, meanwhile the tissue level of ADP increased significantly. These results indicate a significant increase of ATP transformation into cAMP (and of cAMP into AMP), associated with a significant decrease of ATP transformation into ADP 6 hr after reserpine administration. When the rate of ATP transformation into cAMP decreased significantly, the rate of ATP transformation into ADP increased significantly (at 18 and 24 hr). The changes in the ATP-dependent energy supply systems can be explained as follows:

1. The $Na^+ - K^+$-dependent ATPase activity can be directly inhibited by adrenergic mediators, consequently the extent of ATP transformation into ADP can be directly decreased by adrenergic mediators [4, 5, 10, 12, 13],

2. The ATP transformation into cAMP can directly stimulated by adrenergic mediators [4, 5, 10, 12, 13],

3. cAMP and AMP inhibit the $Na^+ - K^+$-dependent ATPase activity [4 5, 12, 13].

The significantly higher level of cAMP indicates a significantly increased rate of ATP breakdown by adenylate cyclase in the rat gastric fundal mucosa. In contrast to the increased ATP breakdown, a significantly higher level of ATP was measured after reserpine administration than in the control group. The significant elevation of ATP substrate can only be the result of significantly increased ATP resynthesis in the rat gastric fundal mucosa after reserpine administration, as compared to the control animals. Increased ATP resynthesis in tissue specimens can occur only under circumstances of sufficient oxygen supply. These results exclude the possibility of a hypoxaemic damage in the rat gastric fundic mucosa after reserpine administration.

The formula of Atkinson (ATP + 0.5 ADP · ATP + ADP + AMP^{-1}) is a suitable way to estimate the extent of phosphorylation and/or dephosphorylation [1]. Our results indicate that the extent of phosphorylation and/or dephosphorylation remained unchanged during the development of reserpine ulcer.

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LOCAL EFFECT OF ANAESTHESIA ON CEREBRAL BLOOD FLOW IN THE RAT

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Local blood flow was measured in 81 cerebral structures of the paralysed rat during nitrous oxide, sodium pentobarbital and glucochloralose anaesthesia. With a ventilatory mixture of 75% nitrous oxide and 25% oxygen the flow values in comparable structures were slightly lower than those reported in awake animals. Pentobarbital and chloralose depressed the local flow by an average of 32 ± 2% and 39 ± 2%, respectively, as compared to values measured during nitrous oxide administration. In some areas the flow was reduced below 40% of the control, whereas other regions showed no significant change at all. Based on the coefficients of correlation, the distribution of local flow values is similar under pentobarbital and chloralose, but differs considerably from the pattern observed during nitrous oxide anaesthesia. The results indicate that the effect of anaesthesia on cerebral blood supply depends a great deal on local factors, and experimental data collected under different types of anaesthesia are not directly comparable.

Keywords: anaesthesia, cerebral blood flow, nitrous oxide, sodium pentobarbital, glucochloralose.

It was realized early in the history of brain blood flow measurements that anaesthesia has a profound effect on the cerebral circulation. It is very often assumed that the influence of an anaesthetic agent is uniform throughout the brain since data on the magnitude of local changes are scarce. In most studies either total blood flow was measured, which gives no information on possible regional differences, or a small number of localized measurements was performed, which is inadequate to characterize the overall situation.

Many studies on cerebral blood flow have been carried out on awake animals, but in some situations the experimental stress or the applied surgical procedure may cause a greater distortion of the results than a well-controlled anaesthesia. Accordingly, many studies require the use of anaesthetics, and a thorough knowledge of their effects is a basic criterion of the proper interpretation of the data. We therefore examined the effect of three frequently employed anaesthetics, nitrous oxide, sodium pentobarbital and chloralose, in a large number of areas of the rat brain.

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Materials and methods

Preparation of experimental animals

The experiments were carried out on male rats anaesthetized either with a ventilatory mixture of 75% nitrous oxide and 25% oxygen, with 50mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories) i.p., or with 100 mg/kg glucochloralose (alpha-chloralose, Fisher Scientific Co) i.v. In the nitrous oxide group, the tracheal tube was inserted under halothane anaesthesia (4% to 5%), and additional surgery was carried out under nitrous oxide supplemented with 0.5% halothane when necessary. In the chloralose group, one femoral vein was exposed and catheterized under ether anaesthesia maintained until the infusion of the chloralose solution.

Both femoral arteries and both femoral veins were cannulated with polyethylene catheters (PE-50) for continuous recording of arterial blood pressure, collection of blood samples and infusion of different substances. The electroencephalogram (EEG) was recorded from two brass screws inserted biparietally into the skull.

The animals anaesthetized with pentobarbital or chloralose were ventilated with 30% oxygen and 70% nitrogen. Immobilization was accomplished in all groups with 30 mg/kg gallamine triethiodide (Flaxedil, Specia) i.v. The frequency of ventilation was adjusted for an arterial CO_2 tension between 35 and 40 mm Hg. The CO_2 content of the expired air was monitored with an infrared analyzer (Beckman Instruments), blood gases and pH were checked with a microanalyzer (Radiometer) immediately before the determination of cerebral blood flow. With the aid of a temperature controller (Yellow Springs Instrument Co) the rectal temperature of the animals was prevented from dropping below 37 °C. The animals were left undisturbed for at least 30 minutes before the cerebral blood flow determination.

Determination of local cerebral blood flow

The blood flow in different areas of the rat brain was measured with the chemically inert diffusible tracer iodoantipyrine (4-[N-methyl-^{14}C]-iodoantipyrine, New England Nuclear). Approximately 100 μCi/kg of the tracer dissolved in 1 ml physiological saline was infused into the femoral vein over a 1-minute period. If the tracer infusion is started at time 0, the amount of indicator accumulated in any given point of the tissue can be calculated according to the equation [8].

$$C(T) = \lambda \cdot K \int_0^T \{C_a(t) \cdot \exp[-K(T - t)]\} dt \quad (1)$$

where C(T) is the concentration of tracer in a given anatomical structure at time T, $C_a(t)$ is the time course of the arterial tracer concentration, and λ is the brain : blood partition coefficient, which was shown to be 0.8 for iodoantipyrine [9]. For a tracer that is not diffusion limited, the factor K is defined as the blood flow (F) per unit mass of tissue (W) divided by λ, that is,

$$K = F/(W \cdot \lambda) = 1CBF/\lambda \quad (2)$$

where 1CBF is the local cerebral blood flow, usually given in ml/(min · g).

At the 60th second of the iodoantipyrine infusion the animal was decapitated in order to cut off instantaneously the blood supply to cerebral structures. The brain was quickly removed from the skull and frozen in liquid Freon 12. Until sectioning, which was done within 48 hours, the specimen was stored on dry ice.

For the reconstruction of the arterial iodoantipyrine concentration during the infusion, 20 μl samples were collected from the femoral artery into calibrated microcapillaries approximately every 3 seconds. Blood coagulation was prevented by 1000 IU/kg heparin (Panheprin, Abbott Laboratories) administered intravenously just prior to sampling. After the infusion the samples were transferred to chromatography paper. The dried blood spots were cut out and placed in scintillation vials containing 1 ml of distilled water and 10 ml of a dioxane-based scintillator (Bray's solution, New England Nuclear). Determination of the ^{14}C concentration was performed by utilizing a liquid scintillation counter (Packard Instrument Co) with external standard counting for quench correction.

The frozen brain was cut into 20 μm sections with a microtome (American Optical Corp) at −18 °C. Every 15th section was picked up with a cover glass and dried on a hot plate at 60 °C. The brain slices and a set of carbon-14 standards for autoradiography (Poly-[^{14}C]-

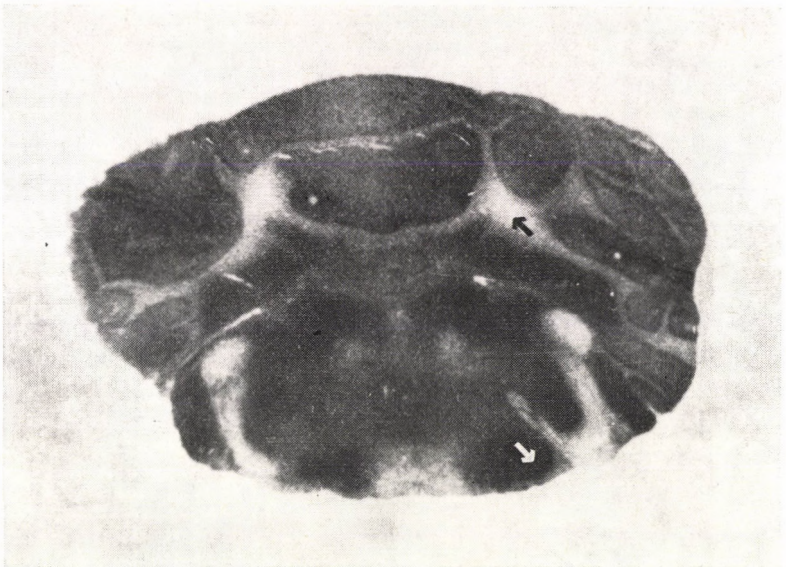

Fig. 1. Autoradiographic representation of local cerebral blood flow produced by ^{14}C-labelled iodoantipyrine. Darker areas correspond to higher flow, but the relationship is not linear. Arrows point to the extreme values of 0.11 ml/(min · g) and 2.05 ml/(min · g)

-methyl methacrylate, Amersham) were placed on X-ray film (SB-5, Eastman Kodak Co) for 10 days. The plastic radiation sources had previously been calibrated for brain tissue of 20 μm thickness, so that differences in absorptance could be taken into account.

Optical density of the autoradiographs was measured with a microdensitometer (Gamma Scientific) connected to a PDP-11 computer (Digital Equipment Corp). The area of analysis measured 200 μm in diameter, small enough as compared to the structures of the rat brain and large enough to compensate for the grain size of the film. From the arterial iodoantipyrine concentrations and the ^{14}C concentration vs. optical density curve, which was determined individually for each film, the computer program calculated the local blood flow value corresponding to the film darkness in the area of interest on the autoradiograph. Figure 1 shows an actual autoradiograph illustrating the resolution of the technique. Anatomical identification of the cerebral structures was made with the aid of a stereotaxic atlas of the rat brain [5].

Statistical evaluation of data

One-way analysis of variance was performed on the general physiological variables listed in Table I. For the determination of significant blood flow and vascular resistance changes over all cerebral structures between different experimental groups, values were paired according to the anatomical localization and two-way analysis of variance was applied. When the analysis of variance indicated significant differences for a given variable, pairwise comparisons between control and each experimental group were carried out with modified t-statistic. The critical values were calculated by the method of Bonferroni [12].

Results

The general physiological parameters of the animals in this study are shown in Table I. The differences between blood pCO_2 and mean arterial blood pressure values were not significant according to the analysis of variance. Oxy-

gen tension of arterial blood was significantly lower in the nitrous oxide group (89 mm Hg) due to the lower oxygen concentration in the inspiratory gas mixture (25% versus 30%). This value was, however, still high enough to have no influence on the cerebral circulation.

Average cerebral blood flow (CBF) in each experimental group was calculated from the mean values of 81 individual anatomical areas according to the formula

$$CBF = \left(\sum_{i=1}^{81} 1\,CBF_i\right) \Big/ 81 \tag{3}$$

with

$$1CBF_i = \left(\sum_{j=1}^{n} 1\,CBF_{i,j}\right) \Big/ n \tag{4}$$

where $1CBF_{i,j}$ is the local cerebral blood flow of structure i in experiment j from a total of n experiments that compose the series in question. These average CBF values, which are included in Table I, are not true brain blood flows since the structures were not weighed according to their actual volume, but they are useful for a comparison of the different experimental groups.

Local cerebral vascular resistance was estimated in each experiment as the quotient of mean arterial blood pressure and local cerebral blood flow for each structure. Average resistances were calculated in the same way as the average flow values and are also given in Table I. According to the parameters defined above, the average blood flow was 1.28 ± 0.05 ml/(min · g) in the nitrous oxide, 0.83 ± 0.03 ml/(min · g) in the pentobarbital, and 0.75 ± 0.03

Table I

General physiological variables, cerebral blood flow and vascular resistance after the administration of different anaesthetics[a]

	75% N_2O + +25% O_2	50 mg/kg pentobarbital	100 mg/kg chloralose
Number of experiments	12	11	7
Arterial CO_2 tension, mm Hg	36.9±0.3	35.9±0.7	38.0±0.6
Arterial O_2 tension, mm Hg	89±3	112±5***	133±4***
Arterial pH	7.39±0.01	7.45±0.01***	7.39±0.01
Mean arterial blood pressure, mm Hg	127±4	114±7	124±9
CBF[b], ml/(min · g)	1.28±0.05	0.83±0.03***	0.75±0.03***
CVR[c], mm Hg · min · g/ml	1.27±0.05	1.65±0.05***	2.02±0.08***

[a] All values reported are mean ± SEM
(*** significantly different from nitrous oxide anaesthesia with $P < 0.001$)
[b] Average cerebral blood flow (see text)
[c] Average cerebral vascular resistance (see text)

in the chloralose anaesthetized group. The decrease of flow in the last two groups was the consequence of an increased vascular resistance since the differences of the arterial blood pressure values were not significant statistically.

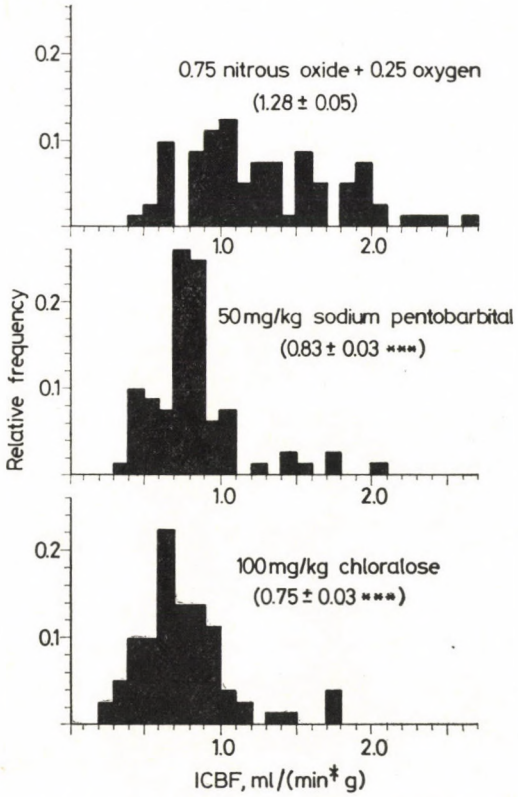

Fig. 2. Relative frequency histogram of local cerebral blood flow (1CBF) values in ml/(min · g) after the administration of different anaesthetics based on measurements in 81 anatomical structures. Numbers given in parentheses represent the average blood flow (see text). The significance of change from control was calculated after two-way analysis of variance by modified t-statistic with critical values according to Bonferroni (***: $P < 0.001$)

In Figure 2 the histograms constructed from the 81 mean 1CBF values are shown for each experimental group. It is evident from this Figure, based on absolute values, that blood flow to the brain was generally higher and more heterogeneous under nitrous oxide anaesthesia than in the pentobarbital or chloralose series. Whereas the coefficient of correlation between the latter two was 0.88 ($P < 0.001$), the distribution of flow values under nitrous oxide anaesthesia was much less coupled to those under pentobarbital ($r = 0.66$, $P < 0.001$) or chloralose ($r = 0.75$, $P < 0.001$).

Figure 3 shows the same data expressed in per cents of the nitrous oxide controls, that is, the mean flow of structure i in equation (3) is replaced by

$100 \cdot 1CBF_i/1CBF_{ic}$, where $1CBF_{ic}$ stands for the mean flow of structure i in the control group. It can be seen from these plots that the magnitude of flow responses exhibited large topographical variations, even though almost all

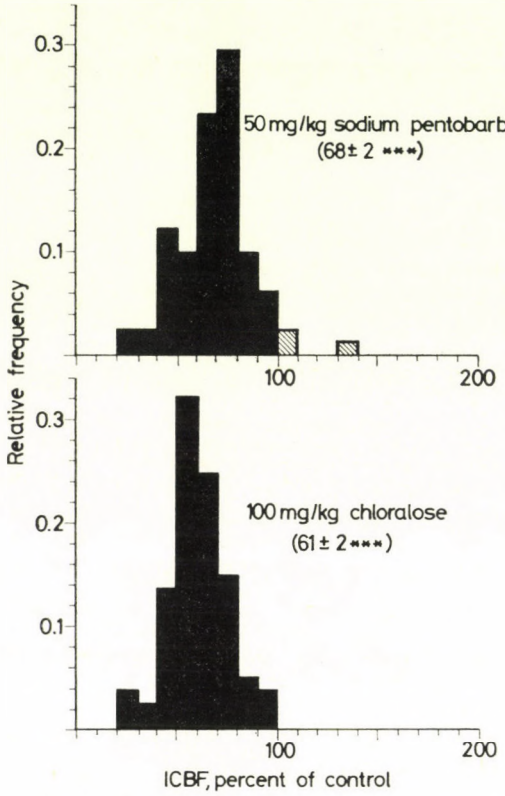

Fig. 3. Relative frequency histogram of local cerebral blood flow (1CBF) values under pentobarbital and chloralose anaesthesia expressed in per cent of nitrous oxide controls. Numbers given in parentheses represent the average blood flow. Statistical significance was calculated as in Figure 2

structures displayed a decrease in blood flow. There were three structures out of 81 that showed an increased flow under pentobarbital anaesthesia, but only the 1CBF increase in the area of the spinocerebellar tract was significant statistically. Under chloralose anaesthesia the blood flow was reduced in all areas.

The most severe depression of local blood flow was observed in the following areas: cingulum ($-67 \pm 3\%$, $-78 \pm 2\%$), optic chiasma ($-69 \pm 3\%$, $-58 \pm 6\%$), radix tractus spinalis n. trigemini ($-69 \pm 2\%$, $-69 \pm 2\%$), parietal region of the cerebral cortex ($-59 \pm 2\%$, $-62 \pm 4\%$) and the temporal region of the cerebral cortex ($-71 \pm 2\%$, $-70 \pm 4\%$), where numbers in

Table II

Local cerebral blood flow in some anatomical areas after the administration of different anaesthetics[a]

	75% N_2O + 25% O_2	56 mg/kg pentobarbital	100 mg/kg chloralose
Regio parietalis corticis cerebri	1.50±0.14 (10)	0.69±0.06 (11)***	0.81±0.10 (7)***
Regio occipitalis corticis cerebri	1.26±0.05 (9)	0.73±0.06 (9)***	0.69±0.06 (7)***
Corpus geniculatum mediale	1.39±0.09 (11)	0.91±0.07 (10)***	0.91±0.09 (7)**
Corpus geniculatum laterale	1.32±0.08 (10)	0.81±0.05 (11)***	0.99±0.15 (7)*
Nucleus lateralis thalami	1.58±0.23 (11)	0.85±0.06 (10)***	0.85±0.09 (7)*
Nucleus ventralis thalami	1.29±0.09 (9)	0.91±0.05 (10)**	0.76±0.07 (6)***
Nuclei hypothalami	0.97±0.07 (11)	0.76±0.05 (11)*	0.73±0.08 (7)*
Corpus mamillare	1.06±0.05 (10)	0.72±0.05 (9)***	0.68±0.06 (6)***
Amygdala	1.08±0.06 (11)	0.67±0.05 (11)***	0.61±0.05 (6)***
Gyrus dentatus	0.92±0.07 (11)	0.73±0.06 (11)	0.60±0.06 (7)**
Nucleus caudatus/Putamen	1.43±0.15 (11)	0.88±0.06 (10)**	0.81±0.06 (7)**
Nucleus accumbens	1.15±0.10 (10)	0.89±0.06 (9)	0.81±0.09 (7)*
Globus pallidus	1.53±0.06 (9)	0.73±0.06 (11)***	0.87±0.05 (7)***
Colliculus superior	1.65±0.16 (10)	1.06±0.09 (11)**	1.33±0.17 (7)
Cortex cerebelli	1.03±0.08 (11)	0.72±0.05 (10)**	0.78±0.07 (7)

[a] All values reported are mean ± SEM (ml/(min·g))
Figures in parentheses represent the number of experiments where the blood flow of the particular structure was determined
(*, **, ***: significantly different from nitrous oxide anaesthesia with $P < 0.05$, $P < 0.01$ or $P < 0.001$)

parentheses represent the values in the pentobarbital and the chloralose group, respectively. The average flow of all five investigated cerebrocortical areas was reduced by 56 ± 5% of the control in both experimental groups, whereas the decline of flow over all structures amounted to 32 ± 2% and 39 ± 2%. Table II presents the absolute flow data of selected cerebral areas.

Discussion

One of the very first investigations employing the diffusible indicator technique reported the heterogeneous effect of anaesthesia on local cerebral blood flow [6]. In this study, thiopental induced flow reductions ranging from 22% to 53% of awake control values were observed in different brain structures of the cat. In later experiments investigating the effect of anaesthesia antipyrine was used as tracer [4, 10], which underestimates the flow because of the diffusion limitation of this substance at the blood–brain interface.

Since iodoantipyrine was proposed as a tracer for localized blood flow measurement in the brain [9], an increasing number of experimental studies have reported quantitative data in various cerebral areas. Most studies employed nitrous oxide anaesthesia since it changes the cerebral hemodynamics substantially less than do other anaesthetic agents [2]. In our laboratory the majority of earlier experiments were performed under pentobarbital or chloralose anaesthesia. We have therefore been interested in gathering information concerning the relationship between these data and the flow values measured under nitrous oxide.

The present experiments have demonstrated that both pentobarbital and chloralose depress the blood flow substantially. In addition, their effect exhibits regional variations, that is, the reactivity of different cerebral areas to these anaesthetics is highly heterogeneous. The calculated coefficients of correlation imply that only 44% of the pentobarbital and 56% of the chloralose data are linearly related to the nitrous oxide values. It can therefore be concluded that 1CBF measurements carried out under different anaesthetics are in general not directly comparable since these agents may significantly change the pattern of local flow distribution.

The areas with higher flow exhibited a tendency of being depressed to a greater extent than the low flow areas, but the correlation between the absolute flow values under nitrous oxide and the per cent flow reductions of the same area due to pentobarbital or chloralose were low (-0.55 and -0.38, respectively).

The close coupling between cerebral blood flow and metabolism (Sokoloff, 1981) suggests that the flow reduction due to both pentobarbital and chloralose is the consequence of a decrease in metabolic activity. Crane et al. [1] measured the glucose utilization of the cerebral cortex in rats after pentobarbital injection and found a 56% reduction as compared to awake controls. The pentobarbital induced flow decrease in the present experiments ranged from 41% to 71% in different cortical regions. Our data compare well also with the local changes in glucose consumption measured by Dudley et al. [3] after chloralose injection. The severe reduction of cortical blood supply observed with the use of non-gaseous anaesthetics is especially noteworthy as the physiological importance and the easy accessibility of the cortex have made this part of the brain very important in cerebral circulatory research.

Since our flow values under nitrous oxide are in general only slightly lower than the values measured in the awake rat by Sakurada et al. [9], this type of anaesthesia most probably reflects a more physiological condition than the application of the two other agents. On the other hand, we have observed considerably higher animal to animal variations in the former group, which probably resulted from external factors that can easily alter the cerebral circulation in specific areas under light anaesthesia. This may create difficulties in the de-

tection of low magnitude effects if the measurements cannot be repeated in the same animal as is the case with autoradiographic techniques. Nevertheless, since the more uniform response of the cerebral circulation under pentobarbital and chloralose is presumably the consequence of reduced cerebrovascular reactivity (Marin et al., 1981), the use of nitrous oxide could facilitate the detection of physiological changes that might be totally suppressed by other anaesthetics.

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EXOCRINE FUNCTION OF THE PANCREAS IN REGULARLY SWIMMING RATS

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The exocrine function of the pancreas was investigated in the course of adaptation to regular physical activity. In albino rats exercized by swimming for six weeks it has been observed that — in response to an identical physiological stimulation (liquid food via gastric tube) and under ad libitum feeding the rate of secretion and enzyme activity exceeded control values by 100 to 150%; — in pair-fed rats the values of the exercized animals were 50 to 70% higher, and — vagotomy suspended this effect even when food was offered ad libitum.

On the basis of these results adaptation to regular exercise involves the exocrine function of the pancreas. In this functional increase both the increased food intake after training sessions and the direct effect of muscular work have a share. For adaptation an intact innervation is necessary.

Keywords: Exocrine pancreas, adaptation to regular exercise, exercise and diet, vagotomy, enzyme induction, innervation in exercise adaptation.

Adaptation to regular physical exercise in the exocrine function of the pancreas has not yet been studied.

Hilsted et al. [8] reported on the hormonal control of the alimentary tract and the pancreas in humans. They found an increase in the peripheral plasma concentration of a vasoactive intestinal peptide (VIP) along with rising levels of secretin, somatostatin and pancreatic polypeptides after three hours of bicycle exercise. The latter agents were suggested to have arisen by vagal stimulation elicited by the decreasing blood glucose level [11].

In earlier experiments focussed on the metabolic adaptation to muscular work, microsomal enzyme activity of the liver was found to be increased [4, 5]. No influence could be attributed to hepatic innervation in this adaptive phenomenon which because of its similarity to pharmacological enzyme induction was regarded as a kind of endogenous enzyme induction [6].

Investigation into the relationship between regular muscular activity and exocrine pancreatic function was stimulated mainly by the unresolved question of whether or not the observed increase in enzyme activity in various organs,

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an apparently enhanced functional capacity, was a sign of general adaptation. Among the adaptive processes of growing physical fitness, a further problem was the locus of control, because its importance seemed to differ depending on the organ studied. While innervation may be of minor significance for the liver, the intact nervous control of the pancreas has long been known to be essential.

Accordingly, the points studied in the present rat experiments were

1. the nature of changes in pancreatic secretion occurring during regular swimming exercise, both under ad libitum feeding and in pair-fed animals; and

2. the role of the vagus nerve in this context.

Materials and methods

Two experiments were performed using adult Wistar rats, ten in each group. Both experiments lasted six weeks.

Experiment 1

Pancreatic secretion was studied under different feeding regimens combined with regular exercise. Dietary regimens started concurrently with the swim training. Food intake was measured in the groups fed ad libitum. In order to match energy uptake, exercized, but pair-fed rats were given only as much food as consumed by the control group. The techniques and procedures employed in the swimming exercise and in the studies of pancreatic function agreed with those used previously [3, 15, 16]. There were 32 sessions of 60 min swimming exercise in a bath of 29 °C in the course of the six weeks of training. The excess weight to be carried was 4 g per 100 g b.w. In order to induce pancreatic secretion both the experimental and control rats were fasted for 18 hours at the end of the experiment, then through a gastric tube they were given 1.5 ml of liquid food (casein, starch, and sunflower oil). Immediately thereafter the rats were anaesthetized by urethan, the common duct was cannulated and pancreatic juice was collected under ice cooling for 120 min. The amount of the collected juice was measured; its total protein content was determined by the Bürgi method [2]. Trypsin, chymotrypsin and lipase activity was estimated by the method of Schön et al. [9] while amylase activity according to Smith and Roe [12]. The enzyme activities were related to the amount of pancreatic juice collected for 120 min, then means and standard deviations were calculated. Differences were analyzed by the *t* test.

Experiment 2

Pancreatic function was studied under vagotomy, feeding ad libitum and regular swimming exercise. Procedures were the same as in Experiment 1. The vagus nerve was transected immediately under the diaphragm. A corresponding technique was employed in the sham operations [1]. Three weeks after the intervention swimming exercise and dietary regimens were started and continued for six weeks.

Results

Food intake of the respective experimental groups are shown in Table I. When food was not restricted, the exercizing rats consumed in general 2 g per 100 g b.w. more than the controls, but they did not differ in weight. Though food intake in pair-fed animals was the same, the exercized animals weighed about 20% less than the reference animals.

Though provocation by liquid food was the same, all the studied functional parameters of the pancreas were 100 to 150% higher in the swimming

Table I

Food intake and weight increase in Experiment 1

	Ad libitum groups		Pair-fed groups	
	1 Control	2 Swim-exercized	3 Control	4 Swim-exercized
Mean fodder uptake				
g/day/rat	13.8	18.0	19.3	18.6
g/100 g b.w.	7.0±1.1	9.0±1.3	9.0±1.0	9.0±1.3
Body weight				
initial in g	150±8	155±7	168±7	170±6
final in g	245±20	246±18	260±21	243±16
change/6 w. in g	95	91	92	73

group fed ad libitum and consuming more food (Fig. 1). In pair-fed animals, in which not only pancreatic stimulation, but food intake was also the same, swimming exercise was observed to increase enzyme activity to levels 50—70% higher than in the controls (Fig. 2).

In order to decide if the phenomenon was brought about via the vagus nerve or else by some other mechanism, a group of rats was subjected to vagotomy and another group to a sham operation. Table II demonstrates that the swimming exercise after vagotomy or the sham operation was again associated with a greater food intake and smaller weight increase.

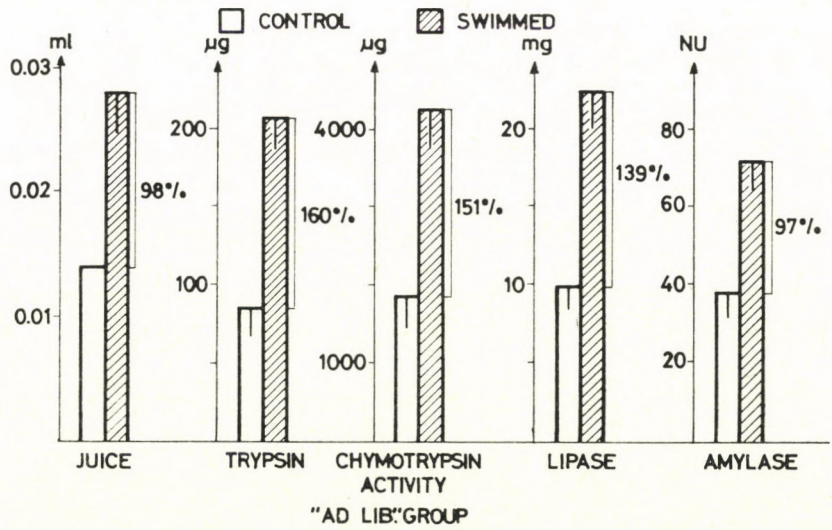

Fig. 1. Effect of swimming exercise on pancreatic secretion under feeding ad libitum

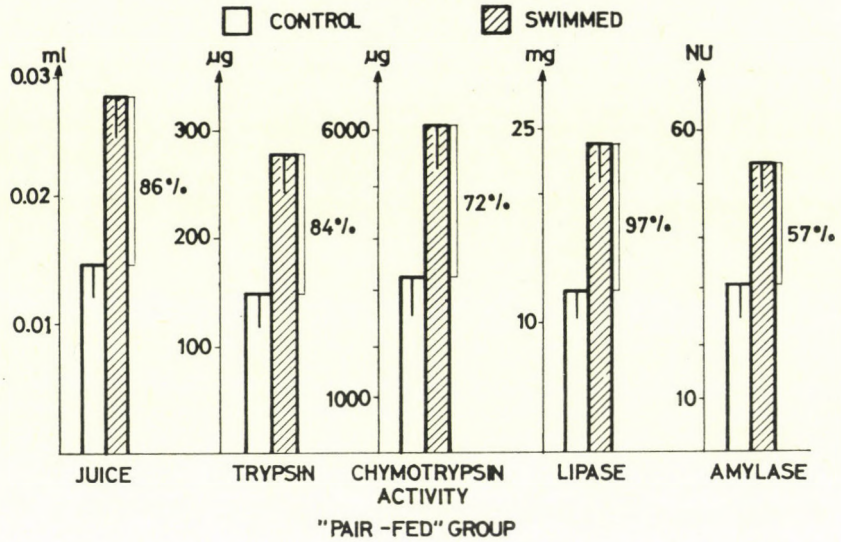

Fig. 2. Effect of swimming exercise on pancreatic secretion in pair-fed animals

As shown in Fig. 3, the amount of pancreatic juice did not change appreciably after vagotomy. In the sham-operated rats the secretion rate was higher when they were exercized. In general, neither the secretion rate, nor the enzyme activity differed from those of the sham-operated controls, except trypsin activity which decreased significantly. In sham-operated swimming rats the activity of all studied enzymes rose significantly whereas such a rise failed to develop in the vagotomized rats (Figs 4 and 5).

Table II

Food intake and weight increase in Experiment 2

	1 Sham operation	2 Sham operation swimming	3 Vagotomy	4 Vagotomy swimming
Mean fodder uptake				
g/day/rat	22.4	27	21.8	27.1
g/100 g b.w.	7.1±1.2	9.0±1.4	7.0±1.1	9.2±1.3
Body weight				
initial in g	250±11	249±9	248±10	255±12
final in g	383±26	352±36	374±34	336±33
change/6 w. in g	133	103	126	81

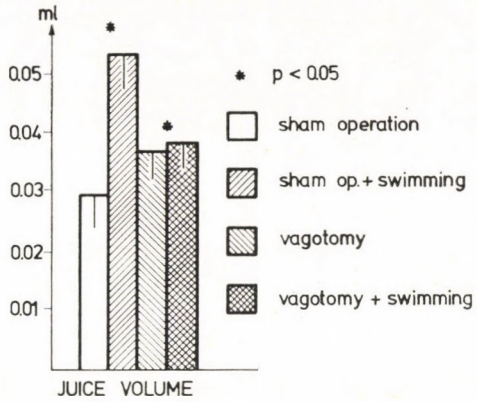

Fig. 3. Postvagotomy pancreatic secretion rate in exercized and control rats

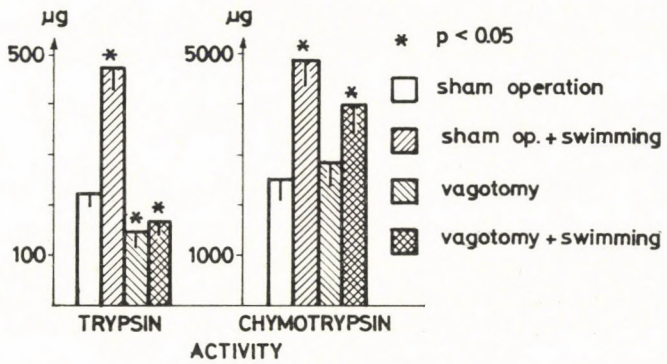

Fig. 4. Postvagotomy activity of trypsin and chymotrypsin in exercized and control rats

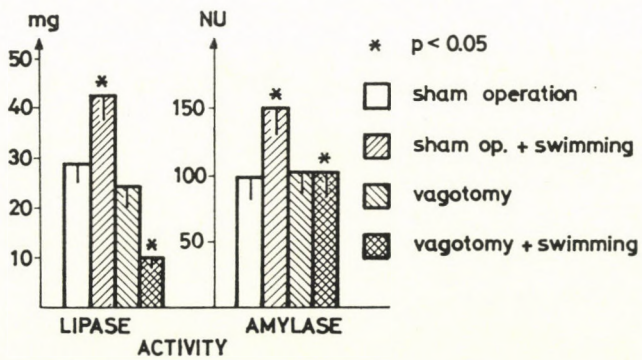

Fig. 5. Postvagotomy activity of lipase and amylase in exercized and control rats

Discussion

Pancreatic secretion markedly increased in Experiment 1 in the rats exercized regularly by swimming under ad libitum feeding, which meant an increased food intake, despite that pancreas stimulation corresponded fully in the compared groups. This accentuated response to the same physiological stimulus suggests, on the one hand, an adaptation to the former ampler food intake. Despite the fact that by the increased pancreatic secretion and increased absorption a greater part of the energy requirement of physical training could be covered, the slower weight increase indicated a faster overall energy turnover. On the other hand, the observed high level of enzyme activity may be regarded as an indicator of enhanced pancreatic function elicited by the regular exercise. This assumption is supported by the observation that both the amount and the enzyme activity of the pancreatic juice rose by 60—70% in the pair-fed (isocaloric supply) rats.

In the vagotomized group pancreatic secretion was essentially normal in the ninth postoperative week except a significantly lower trypsin activity. This agrees with the now classical rat experiment of Crider and Thomas [13] in which pancreatic function decreased after vagotomy only temporarily and was later restored to normal, but with some decrease in protein content.

Pancreatic secretion increased also in the sham-operated and ad libitum fed animals when they were subjected to regular swimming. After vagotomy this functional increase failed to develop despite the swimming exercise, i.e. every studied level was significantly lower than in the exercized but sham-operated rats. This finding stresses the role of the vagus nerve in enlarging the functional capacity in response to physical exercise. This issue corresponds to the view [14] that the neural factor is the mediator of the intestinal control of pancreatic secretion. In animal experiments, Schwartz [10] demonstrated a postprandial increase of pancreatic polypeptides in peripheral blood. A similar result was obtained by Guzman et al. [7] following hormonal provocation. In this latter experiment increased secretion was elicited by gastrin, CCK octapeptide, cholecystochinin or secretin, while it was significantly reduced by vagotomy. Accordingly, it was suggested that cholinergic and hormonal stimuli might interact in the liberation of pancreatic polypeptides and in the control of the exocrine pancreas. Relying on the above mentioned report of Hilsted et al. [8] as well as on our present results, it is concluded that cholinergic and hormonal factors are equally responsible for the exercise-induced increase of pancreatic secretion.

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INTERSTITIAL FLUID PRESSURE CHANGES IN PREGNANT RATS

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In pregnant rats significant interstitial fluid pressure changes could be detected by means of capsules chronically implanted into the subcutaneous tissue. The capsular pressure increased significantly from a control value of -4.3 ± 0.5 mmHg to -0.7 ± 0.5 mmHg during the first period of pregnancy. Immediately before parturition the capsular pressure returned to the control level. During lactation the pressure rose as high as $+0.5 \pm 0.9$ mmHg. After lactation the pressure returned again to the control value. By determining the extracellular fluid and plasma volume, as well as protein concentration in plasma and capsular fluid, the hydrostatic and colloid osmotic forces operating in the extracellular space could be analysed. It has been concluded that the observed capsular pressure changes during pregnancy are not solely of volumetric or colloid osmotic origin.

Keywords: extracellular fluid volume, colloid osmotic pressure, capsule technique, interstitial pressure, pregnancy.

A great number of physiological and pathological experiments [6, 7, 9, 17] has convincingly shown that the values for interstitial fluid pressure, measured by the chronically implanted capsule technique [6], correspond to those predicted from the Starling concept of pressure equilibrium at the capillary membrane [14]. In all these experiments the changes of interstitial fluid pressure were interpreted as being volume-dependent.

Recently, more attention has been paid to the physicochemical properties of the extracellular matrix [5, 10, 11] and to its role in regulating interstitial pressure [4, 8]. Several forces contribute to the net matrix pressure: some are mechanical in nature, others reflect osmotic behaviour of the matrix. The van't Hoff and Donnan osmotic pressures represent the osmotic forces. The Donnan osmotic pressure is due to the large number of anionic groups on each mucopolysaccharide molecule. The mechanical forces include elastic recoil of the matrix, hydrostatic pressure of the fluid located within the matrix, and electrostatic repulsion of neighbouring anionic moieties.

Thus, it might be expected that electrostatic forces play a significant role in the changes of the interstitial pressure if the structure and thereby the physi-

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cochemical properties (permeability, ion-binding capacity, etc.) of the extracellular matrix alter. Such changes in the structure and physical properties of connective tissue may be elicited, e.g., by pregnancy and different hormones [12, 1, 13]. Since changes in the extracellular fluid volume are accompanied by structural alterations of the ground substance during pregnancy this condition may serve as a suitable in vivo model to examine the relative contribution of different (volume-dependent and molecular) components to interstitial pressure changes.

The aim of this work was to asses whether there is any change in the interstitial pressure during pregnancy and, if so, whether this change can be explained by Starling forces alone or other factors are involved as well.

Materials and methods

Experiments were performed on female rats weighing 200—250 g. The animals had free access to food and water and were maintained under conditions of controlled light (from 6AM—6PM) and temperature (26 ± 2 °C). Prior to the measurements the animals were fasted for 24 hours. Anesthesia was induced by i.p. injection of sodium pentobarbital, 5 mg/100 g body wt, during the measurements.

Measurement of interstitial fluid pressure [6]

A cylindrical plastic tube of 5 mm in diameter, and 15 mm length, closed at both ends and perforated by 100—110 of 1 mm holes was implanted subcutaneously on the back of the rats. After a healing period of 4—6 weeks, the value of control pressure within the capsule was measured by inserting transcutaneously a 26 gauge syringe needle through one of the perforations into the cavity of the capsule. The needle was connected with a Statham pressure transducer (23Db) by means of a polyvinyl tube. The system was filled with physiological saline solution.

In *series I* (n = 9) of the experiments, the interstitial fluid pressure was measured during pregnancy and lactation. After measuring the control values of interstitial pressure female rats were caged with fertile males for 24 hours to know the exact date of pregnancy. The pregnancies resulting were normal, all of the animals were prima gravidae. The interstitial pressure was measured on the first, second and third week (4th and/or 7th, 10th and/or 15th, 18th and/or 20th day) of pregnancy, further on the first week (1st and/or 3rd and/or 5th and/or 8th day) of lactation and, finally, after the 15th day of lactation and after cessation of lactation. The measurements were performed in the same hour of the day.

Measurement of plasma and extracellular fluid volume [17]

In *series II* (n = 69) of the experiments, the plasma and extracellular fluid volumes were determined in control and 15th and 20—21st day pregnant rats. Plasma and extracellular fluid volumes were determined with Evans blue (T-1824) and sodium thiocyanate, respectively, given into the saphenous vein of anesthetised animals. Blood was drawn from the carotid artery for determination of plasma concentrations of Evans blue and thiocyanate. The fluid volumes were calculated by extrapolating to zero time and using the dilution formula.

Haematocrit and analytical determinations

In *series III* (n = 9) of the experiments, packed cell volume, total sodium concentration in plasma and protein concentrations in plasma and interstitial fluid were determined in control and pregnant rats. For this purpose blood samples from the carotid artery and interstitial fluid from the capsule were collected. Arterial haematocrit was estimated by a micromethod.

Sodium concentration was determined by flame photometry (Flapho 4, Zeiss), and the total protein concentration was assayed by the biuret method, using bovine serum albumin as a standard.

Statistical analysis

Data are presented as means ± SEM. Statistical significance was determined by Student's paired *t* test; $p < 0.05$ was considered significant.

Results

Figure 1 shows the typical changes of interstitial pressure during pregnancy and lactation. The interstitial pressure increased significantly from −4.5 mmHg to +0.3 mmHg during the first 15 days of gestation but decreased progressively thereafter reaching the initial value at the time of delivery. Post partum, the interstitial fluid pressure increased again and remained above atmospheric pressure during the whole period of lactation. At the end of this period, the interstitial pressure returned to its initial negative value.

In Table I, the average capsular pressure changes are shown in control, pregnant and lactating animals. The average data show the same trend of the interstitial pressure during pregnancy as the individual curve in Fig. 1. The mean (±SEM) capsular pressure on the 2nd week of pregnancy (−0.7 ± 0.5 mmHg, n = 8) was significantly ($p < 0.001$) higher than the control value (−4.3 ± 0.5 mmHg, n = 9). On the 3rd week of pregnancy, pressure decreased significantly ($p < 0.05$) from −0.7 ± 0.5 mmHg (n = 8) to −2.6 ± 0.5 mmHg (n = 5). Mean pressure on the 3rd week was still higher than the control value, in contrast to the pattern of the individual pressure curve shown in Fig. 1. This was due to the rapid pressure changes in this period and to the fact that the

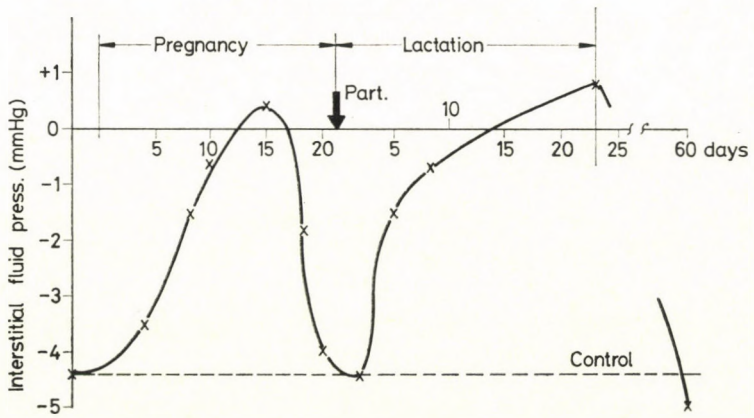

Fig. 1. Representative recording of the characteristic changes in subcutaneous interstitial pressure during pregnancy and lactation

Table 1

Mean values ± SEM for subcutaneous interstitial pressure during pregnancy and lactation

Groups	Control	Pregnant			Lactating		3 weeks after cessation of lactation
Time (week)		1st	2nd	3rd	1st	3rd	
Interstitial pressure (mmHg)	−4.3±0.5 (n = 9)	−3.3±0.6 (n = 9)	−0.7±0.5 (n = 8)	−2.6±0.5 (n = 5)	−2.1±0.5 (n = 9)	+0.5±0.9 (n = 5)	−4.7±0.5 (n = 9)
p_1		N.S.	<0.001	<0.02	<0.01	<0.001	N.S.
p_2				<0.05			

p_1 against control
p_2 against 2-week-pregnant

Table II

The plasma and extracellular fluid volume in control and pregnant rats

	Control	Day of pregnancy	
		15	20—21
Extracellular fluid volume ml/kg body wt	270±9 (n = 24)	300±29 (n = 9)	308±12* (n = 12)
Plasma volume ml/kg body wt	35.9±1.5 (n = 8)	40.7±1.0 (n = 8)	44.3±1.2** (n = 8)

*: $p < 0.02$; **: pp < 0.001 against conrol

Table III

Mean values ± SEM for body weight, haematocrit and total sodium concentration in plasma during pregnancy

	Control	Day of pregnancy	
		15	20—21
Body weight (g)	233.8±9.0 (n = 9)	272.6±10.0* (n = 9)	296.8±19.0* (n = 6)
Haematocrit (%)	43±1 (n = 9)	37±1** (n = 9)	35±1** (n = 8)
Na^+_{plasma} mmol/l	143±1 (n = 9)	148±2 (n = 9)	142±1 (n = 9)

*: $p < 0.01$; **: $p < 0.001$ against control

mean pressure for the 3rd week was derived from several individual pressure curves by pooling all the data obtained on the 18th and the 20th day of pregnancy. During lactation capsular pressure was significantly ($p < 0.001$) higher ($+0.5 \pm 0.9$ mmHg, $n = 5$) than the control value. Three weeks after the cessation of lactation the average capsular pressure did not differ significantly from the control value.

Table II shows the values for plasma and extracellular fluid volume in the control and pregnant rats. Both plasma and extracellular fluid volumes were increased during pregnancy until delivery. On the 20—21st day of pregnancy the plasma volume (44.3 ± 1.2 ml/kg) and the extracellular fluid volume (308 ± 12 ml/kg) increased significantly compared to the control values ($35.9 \pm \pm 1.5$ ml/kg and 270 ± 9 ml/kg, respectively). Consequently, the interstitial fluid volume — which was approximated by subtracting the plasma volume from the total extracellular fluid volume — was also increased at the end of pregnancy.

In Table III, the average (±SEM) body weights, haematocrit values and sodium concentration in plasma in the control and pregnant rats are shown. Body weight increased progressively during pregnancy until delivery. The haematocrit values in the pregnant rats were significantly ($p < 0.001$) lower than

Table IV

Changes in effective protein concentration between plasma and interstitial fluid in control and pregnant rats

Groups	$c_i - c_{pl}$ g/l	$\Delta(c_i - c_{pl})$ g/l	p
Control	-32.8 ± 2 (n = 8)	0.0	—
15th day of pregnancy	-32.4 ± 1 (n = 6)	+0.4	N.S.
20—21st day of pregnancy	-23.5 ± 3 (n=7)	+9.3	<0.02

c_i total protein concentration in the interstitial (capsular) fluid
c_{pl} total protein concentration in the plasma
$\Delta(c_i - c_{pl}) = (c_i - c_{pl})_{\text{pregnant}} - (c_i - c_{pl})_{\text{control}}$
p: against control

those in the controls. There was no difference in the plasma sodium concentrations between pregnant and control animals.

Table IV shows the changes in the protein concentration difference between plasma and interstitial fluid (called osmotically effective protein concentration) as calculated from the total protein concentration of plasma and interstitial fluid. The effective protein concentration remained unchanged until the 15th day but decreased (in absolute value) on the 20—21st day primarily as a result of the decreased plasma protein contents. The difference between the effective protein concentration of pregnant and control rats reflects the trend of the colloid osmotic pressure gradient during pregnancy. According to $\Delta(c_i - c_{pl})$, the colloid osmotic pressure gradient between plasma and interstitial fluid was zero on the 15th day, and positive on the 20—21st day of pregnancy.

Discussion

We observed significant changes in the interstitial fluid pressure during pregnancy. To explore the pressure changes recorded by chronically implanted capsules in subcutaneous tissue, it is necessary to consider different factors operating in extracellular space.

Effect of the extracellular volume

It is known that the extracellular fluid volume expands during pregnancy. This volume effect is manifested in the second period of pregnancy as a result of increased fluid and sodium retention due to the enhanced aldosterone and oestrogen secretion. In our experiments we found that both extracellular fluid and plasma volumes were increased. In addition to the measured extracellular volume expansion, the increased body weight and decreased haematocrit values,

in line with the practically constant sodium concentrations, can also be considered as indicators of isosmotic hypervolaemia. If the interstitial fluid pressure response were due solely to the extracellular volume expansion, it ought to have continously increased during the whole period of pregnancy. Contrary to this, in the second period of pregnancy pressure rather decreased. Consequently, the return of the pressure to its control value before delivery can not be interpreted in these terms.

Osmotic effect

Another factor responsible for the changes in interstitial fluid pressure may be a shift in osmotic equilibrium. A pressure difference develops between two fluid spaces separated by a semipermeable membrane if there is a concentration gradient of the solutes. A concentration gradient occurs when the membrane is not freely permeable to the diffusing substances, or it is permeable, but the secretion and excretion rate of the diffusing substances are not the same. In our experiments osmotic pressure changes might have occurred both (i) between plasma and interstitial fluid and (ii) between interstitial and capsular fluid. Since other studies [15, 2, 3] have failed to find a significant difference in effective protein concentration between the capsule and tissue fluids, we assumed that the osmotic gradient between the interstitial and capsular fluid was zero, i.e. the composition of these fluids is identical and there is no semipermeable membrane between them. Therefore, we analysed only the equilibrium between plasma and interstitial space for protein-like osmotically active components. The osmotic pressure of the proteins is a colligative property which is determined by the molar concentration of proteins and the degree of their dissociation. The degree of protein dissociation depends on the ionic strength of the aqueous and on the chemical nature of small ions present.

In our calculation, the effects of the osmotic forces were not considered in their full complexity. It is clear that all the small ions participating in osmotic equilibrium should be analysed, and the protein fractions as well as the dissociation of proteins together with the quantity of free ions (not only the total concentration, which was measured) should be taken into account.

Nevertheless, it is obvious from the calculated data of the colloid osmotic pressure changes (see Table IV) that the protein dependent tendency of osmotic pressure changes did not coincide with the experimentally measured pressures. If the changes in interstitial fluid pressure were caused by colloid osmotic forces, then they should remain unchanged on the 15th day and increase on the 20—21st day of pregnancy. On the contrary, we found an increase on the 15th and unchanged (compared to control) pressure on the 20—21st day of pregnancy. Consequently, the capsular pressure changes observed during pregnancy are not of colloid osmotic origin.

The final conclusion may be drawn from these investigations that the characteristic change in the interstitial pressure during pregnancy can not be the sole consequence of either the effect of volume or colloid osmotic pressure changes.

This suggests that other factors are involved beside Starling forces in the interstitial pressure changes occurring during pregnancy. Looking for a plausible interpretation of the measured pressure changes, the mechanical properties of the tissue, i.e. its elasticity can be considered as such a non-Starling type factor, if any. The elasticity of tissue is governed by, among others, the electrostatic repulsion between the electrical charges present in the polyelectrolyte gel matrix. Thus, indirectly the elasticity depends on many parameters of the connective tissue (composition, structure etc.) and type and quantity of small ions in the interstitial fluid. In order to obtain an insight into the relation between the tissue characteristics and the interstitial pressure, further work is needed.

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STEP-LIKE SUPERPRECIPITATION OF ACTOMYOSIN

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The superprecipitation of natural actomyosin prepared from rabbit skeletal muscle shows a step-like character under physiological conditions (KCl 0.14 M, ATP 0.001 M).

1. Alkali metal cations applied in the same (0.14 M) concentration cause a delay according to the Hofmeister sequence, while in their presence the step-like character of superprecipitation is retained.

2. Reduction of the Ca^{2+} concentration also causes a delay in the development of superprecipitation, but its step-like character is retained in this case, too.

3. Increasing the Mg^{2+} concentration in the medium causes a delay or disappearance of superprecipitation and modifies it to occur in a single step.

It is assumed that the actions studied exert their effect in a combined way actin directly or indirectly on the interaction of actin and myosin.

Keywords: actomyosin, superprecipitation, inorganic monovalent and divalent cations, contraction model.

The phenomena of superprecipitation and clearing of skeletal muscle actomyosin were first described by Szent-Györgyi [10]. Under the effect of lower (10^{-4}M) or higher (10^{-2}M) ATP concentrations the actomyosin suspension shows an abrupt superprecipitation or a delayed one preceded by clearing, respectively. In both cases the abundantly hydrated protein is transformed to a dehydrated form. It is generally accepted that clearing corresponds to muscle relaxation, and superprecipitation to contraction in vivo.

The state of actomyosin, the interaction between actin–myosin–ATP, the rate and extent of the development of superprecipitation are substantially influenced by the ionic strength of the medium [3] (Table I). An increase of KCl concentration results, for example, in a more extensive clearing [7, 14]. This shift in the character of superprecipitation can be explained by a reduced tendency for association and dehydration of actin and myosin.

In the experiments described below the characteristics of superprecipitation were studied at nearly physiological (0.14 M) KCl concentration. Under the effect of different inorganic ions and changes in ATP concentration the superprecipitation showed a step-like character.

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Table I

Actin–myosin interaction

	ATP present	No ATP	Low ATP
High KCl 0.6 M	Myosin monomers Actin filaments Completely dissociated	Myosin monomers bound to actin filaments (arrowheads)	Same as ATP present
Low KCl 0.2 M	Myosin filaments and actin filaments dissociated during "clearing"	Actomyosin gel. Myosin may be short filaments	Actin and myosin filaments interacting during superprecipitation

Materials and methods

Actomyosin was prepared from skeletal muscle of New-Zealand rabbits weighing 2.5—3 kg. During its preparation, which was based with slight modifications on the method of Ebashi [1] the temperature was held between 0 and +4 °C. Minced rabbit skeletal muscle was mixed with three volumes of 0.05 M KCl, homogenized in a Waring blendor and centrifuged for 10 min at 5000 *g*. After the extraction of sarcoplasmic proteins with 0.05 M KCl the residue was suspended in three volumes of Weber—Edsall solution (0.6 M KCl, 0.04 M $NaHCO_3$, 0.01 M Na_2CO_3) and stored for 24 hours at 2 °C, being stirred occasionally.

The suspension was then centrifuged at 20,000 *g* for 1 hour, and the supernatant was squeezed through several layers of cheesecloth to remove fat. The filtrate was diluted by adding slowly three volumes of water and gently stirred thereafter for 5 min. The resulting turbid solution was centrifuged at 8000 *g* for 10 min and the pellet (crude actomyosin) was suspended in an equal volume of 1.2 M KCl solution. It was centrifuged again at 20,000 *g* for 1 hour to remove denatured actomyosin and other insoluble materials. The same precipitation and solubilization procedures were repeated two more times to gain purified actomyosin. The resulting solution was dialysed against 0.6 M KCl using cellophane tubing for 5—6 hours and centrifuged at 20,000 *g* for 1 hour. The resulting supernatant was used in the experiments as purified actomyosin solution. Its protein content was determined with the biuret reaction, calibrated by the micro-Kjeldahl method.

The rate and extent of superprecipitation were determined by turbidimetry as described by Ebashi [1]. The suspension was continuously stirred and its turbidity recorded by Zeiss (Jena) UV VIS spectrophotometer at 660 nm in a 1 cm thick cuvette. The final volume of the reaction mixture was 3 ml. Further quantitative details of the procedure were published previously [11].

All reagents used were of analytical grade.

Results

The time course of superprecipitation of actomyosin induced by the addition of ATP is mainly determined by the ionic strength of the reaction mixture. In the presence of 0.06 M KCl, superprecipitation immediately begins after the addition of ATP. At higher KCl concentration ATP first induces a clearing of the suspension the duration of which depends on the ATP concentration. In our experiments under different conditions the superprecipitation of actomyosin showed a step-like character. The parameters of the phases changed in dependence on the KCl concentration (Fig. 1). On changing the monovalent cation, its

effect in retarding the superprecipitation corresponded to the Hofmeister sequence, but after clearing the increase of turbidity always proved to be step-like (Fig. 2). If the concentration of ATP was increased from 0.0001 M to 0.0013 M, the development of superprecipitation slowed down but its step-like character was retained or even became more expressed (Fig. 3).

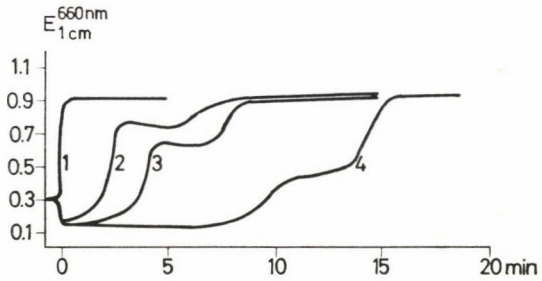

Fig. 1. Effect of the changes in KCl concentration on the superprecipitation of actomyosin. The incubation medium was composed of: $MgCl_2$, 0.001 M; $CaCl_2$, 0.0001 M; protein, 1 mg/dm³; tris-maleate buffer pH 7.0, 0.02 M; ATP, 0.00013 M. The KCl concentration was varied as follows: 1: 0.06 M; 2: 0.12 M; 3: 0.13 M; 4: 0.14 M

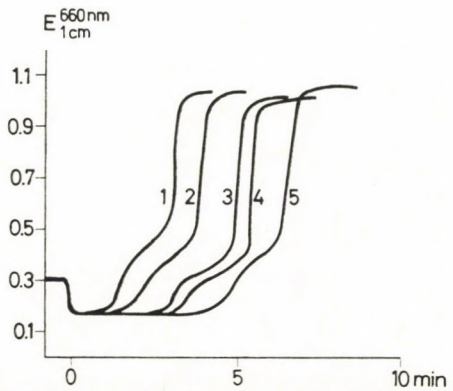

Fig. 2. Effect of monovalent cations on the superprecipitation of actomyosin. The composition of the incubation medium was the same as in the case of Fig. 1 except for ATP which was 0.0006 M. The concentration of the different salts was 0.14 M. Curve 1: CsCl; 2: RbCl; 3: NaCl; 4: KCl; 5: LiCl

On reducing the concentration of ionized Ca^{2+} by adding EGTA to the reaction mixture, delayed the development of superprecipitation without changing its step-like character (Fig. 4).

On increasing the concentration of Mg^{2+} in the solution caused a delay in the development of superprecipitation and its step-like character gradually disappeared (Fig. 5).

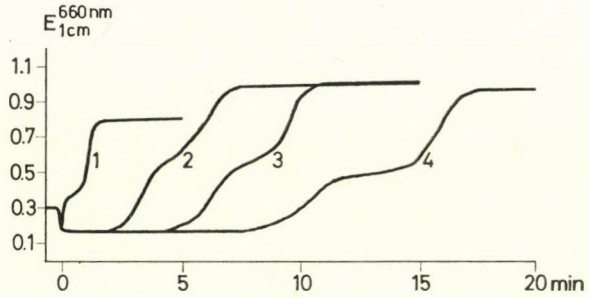

Fig. 3. Effect of changes in ATP concentration on the development of superprecipitation. The composition of the medium was as before except for KCl which was 0.14 M and ATP which in curve 1 was: 0.0001 M, in 2: 0.00066 M; in 3: 0.001 M; and 4: 0.00133 M

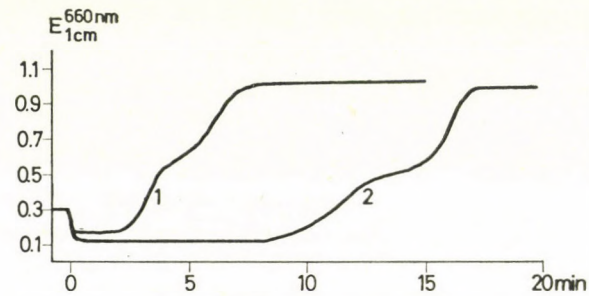

Fig. 4. Effect of changes in Ca^{2+} concentration on the superprecipitation of actomyosin. The incubation medium contained 0.14 M KCl, 0.001 M $MgCl_2$, protein and buffer as before, and 0.0006 M ATP. Ca^{2+} concentration was changed by adding EGTA to the incubation medium. Curve 1: 0.0001 M $CaCl_2$; 2: 0.0001 M $CaCl_2$ + 0.0001 M EGTA

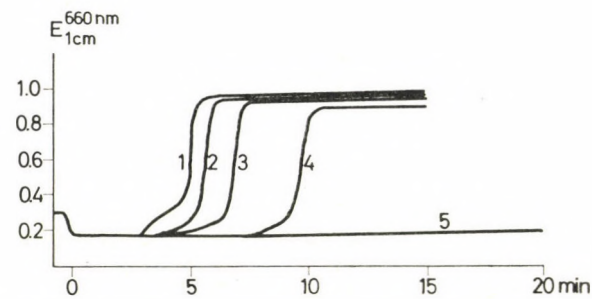

Fig. 5. Effect of increase in $MgCl_2$ concentration on the superprecipitation of actomyosin. The composition of the incubation medium was as before, ATP was 0.0006 M, $MgCl_2$ was in curve 1: 0.001 M; 2: 0.003 M; 3: 0.005 M; 4: 0.01 M; 5: 0.02 M

Discussion

The colloidal state as well as the turbidity of the actomyosin suspension are highly dependent on the ionic strength of the solution. At high salt concentration ($\mu = 0.6$) the myosin monomers are attached to the actin filaments but the complex is macroscopically in a dissolved state. In the presence of ATP, the myosin monomers are detached from the filamentous actin, while the solution shows changes in its character and viscosity. At low ionic strength ($\mu = 0.06$) actomyosin is in gel form which in the presence of ATP dissociates into myosin and actin, dissolves and shows clearing. If, however the ATP concentration is low, the dispersity and turbidity of the suspension immediately and extensively increases ("super"-precipitation) (13).

At physiological KCl concentration ($\mu = 0.14$) the nucleotide-free actomyosin is also in gel form; it dissociates in the presence of a large amount of ATP, then as its concentration decreases, superprecipitation develops.

The phenomenon of superprecipitation was studied by most authors by the method of Ebashi [1] or that of Watanabe [14] in 0.06 M KCl which is favourable for the fast development of superprecipitation. At nearly physiological concentrations of KCl and ATP (0.14 and 0.001 M, respectively) superprecipitation develops step-wise. This type of superprecipitation is not characteristic of K^+ because in the presence of other alkali metal ions applied in the same concentration superprecipitation differs mostly in the length of clearing, but its step-like character is retained. The ATPase activity of actomyosin is high at low Ca^{2+} concentration, and its elevation inhibits enzyme activity [2, 9]. In our experiments, reduction of the concentration of ionized calcium inhibited the development of superprecipitation but had no effect on its step-like character. Thus, it has been assumed that calcium had no direct effect on the step-like occurrence.

The effect of Mg^{2+} differs from that of Ca^{2+} because it activates actomyosin ATPase. Its effect correlates probably with the fact that Mg^{2+} exerts its effect through actin-activation in a form of Mg-ATP complex. The action of Mg^{2+} in changing the superprecipitation from step-like to a gradual type cannot be explained [8].

The step-like character of superprecipitation has recently been observed under circumstances similar to ours by Knyrim [6], Kakol et al. [4] and Kasman et al. [5]. Kakol assumes that one light chain of myosin (LC_2) is phosphorylated in the course of clearing, and this may be in correlation with the step-like character of superprecipitation. The duration of clearing is however also dependent on the concentration of ATP. An increase in ATP concentration causes a retardation of superprecipitation but does not change its step-like character.

In some other experiments we observed the special effect of ADP on the course of superprecipitation. We supposed that the presence of ADP had a direct or indirect effect on the ATPase activity of actomyosin. Experiments on this subject are to be published elsewhere [12].

Finally it may be assumed that the step-like character of superprecipitation is in correlation with the concentration or ratio of nucleotides, phasic changes of ATP concentration as a consequence of its regeneration from ADP (myokinase activity of actomyosin) and also with the phosphorylated state of LC_2.

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COMPOSITION OF PHOSPHOLIPIDS IN VARIOUS RAT TISSUES

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Total phospholipids and their various classes in erythrocytes, the blood plasma, liver, lung, myocardium, spleen and skeletal muscles were determined quantitatively by means of two-dimensional thin-layer chromatography.

Keywords: phospholipids, erythrocyte, blood plasma, liver, lung, myocardium, spleen, sceletal muscle, two-dimensional thin-layer chromatography.

In the literature [4, 7] the quantitative composition of phospholipids is given for only few rat tissues and there are no reports on the complex determination of total phospholipids and their various classes in different rat tissues. In view of this we have studied the phospholipids in the tissues of the rat.

Materials and methods

All the reagents were of highest analytical purity. The solvents were purified by redistillation. Kieselgel H (Merck, Germany) was purified by the Broekhuyse procedure [1]. Alkaline magnesium silicate ("for TLC" Woelm, Eschwege, Germany) was added to the silica gel powder prior to preparing the thin-layer plates.

Reference compounds

Sphingomyelin from bovine brain (Sigma). Phosphatidylethanolamine and lysolecithin prepared from eggs, L-α-lecithin from bovine brain (Koch-Light, England).

Animals

Ten-month-old Wistar strain male rats, weighing 450—500 g, were fed on standard LSM diet. They received only water twelve hours before blood and tissue samples were taken.

Preparation of lipid extracts

Under ether anaesthesia blood samples (10—12 ml) were taken from the abdominal aorta and mixed with EDTA (1 mg/ml). The blood plasma and erythrocyte lipids were extracted exactly according to the method described by Broekhuyse [2]. In tissue samples taken from the same rats the lipids were extracted by the method of Folch et al. [3].

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Identification of phospholipids

Lysolecithin, lecithin, sphingomyelin and phosphatidyl ethanolamine were identified by comparison with reference compounds, the R_f values of which corresponded to those obtained by Broekhuyse [2]. Other phospholipids were identified on the basis of their R_f value.

Quantitative two-dimensional thin-layer chromatography

The plates were prepared according to Broekhuyse [1]. Lipid extract (20 μg—30 μg of the plasma extract phosphorus, 60 μg—80 μg of the erythrocyte extract phosphorus and of the tissue extract phosphorus) were applied to the plates with micropipette. The chromatogram was developed in the first direction with cloroform–methanol — 7M ammonia (90 : 54 : 11, v/v) and in the second direction with chloroform — methanol — acetic acid — water (90 : 40 : 12 : 2, v/v). Between the two runs, the plates were dried in air current for 15 min or if the relative humidity exceeded 65%, they were placed in vacuum over concentrated H_2SO_4 for 1 hr. After chromatography, the spots were dyed with iodine vapour.

Determination of lipid phosphorus

The phospholipid spots and blanks were scraped with razor blades into Jena test tubes with ground-in stoppers and then digested. Lipid phosphorus was determined by the method of Martin and Dotty [6] as described by Jaroszewicz [5].

Results and discussion

A thin-layer chromatogram of lipids of the rat myocardium is presented in Fig. 1.

Tables I and II present the quantitative phospholipid composition of blood, and Table III shows that of the other tissues. In the blood plasma, five

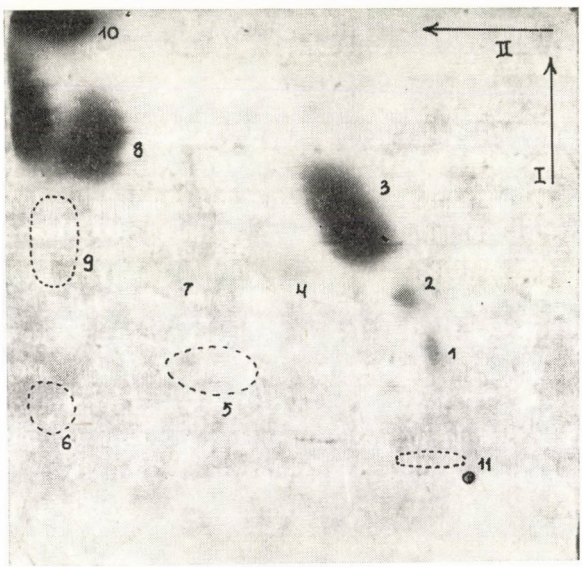

Fig. 1. Thin-layer chromatogram of rat myocardium lipid extract: ● — origin; 1 — lysolecithin; 2 — sphingomyelin; 3 — lecithin; 4 — unidentified; 5 — phosphatidyl serine; 6 — phosphatidic acid; 7 — phosphatidyl inositol; 8 — phosphatidyl ethanolamine; 9 — cardiolipin; 10 — cholesterol, cholesterol esters, triglycerides and other non-polar lipids; 11 — diphosphoinositides

classes of phospholipid were identified. Choline phospholipid constituted 85% of all the phospholipids. In the erythrocytes, 8 classes of phospholipid were found, expressed in mol per cent they were not comparable with the results published by De Gier and Van Deenen [4] who reported for lecithin, sphingomyelin + lysophosphatides and cephalins 56.0, 26.0 and 18.0 mol per cent,

Table I

The phospholipid composition of rat erythrocytes

Phospholipid	Composition
Lysolecithin	4.4±0.86
Sphingomyelin	10.5±1.63
Lecithin	47.4±2.50
Phosphatidyl inositol	2.9±0.55
Phosphatidyl serine	10.5±1.80
Phosphatidyl ethanolamine	23.1±1.64
Phosphatidic acid	0.9±0.20
Diphosphoinositide	0.4±0.20
Recovery of phosphorus after thin-layer chromatography, per cent	96.1±3.7
Total lipid phosphorus, μmol/ml packed cells	4.9±0.7

The phospholipids were extracted with chloroform–methanol (1 : 1 and 2 : 1 vol/vol) and separated by means of two-dimensional thin-layer chromatography

The values are means ± S.D. for ten rats each and are expressed in mol per 100 mol of phosphorus recovered

Table II

The phospholipid composition of rat blood plasma

Phospholipid	Composition
Lysolecithin	22.3±2.6
Sphingomyelin	8.5±1.5
Lecithin	62.6±3.7
Phosphatidyl inositol	4.5±0.8
Phosphatidyl ethanolamine	2.1±0.7
Recovery of phosphorus after thin-layer chromatography per cent	96.3±4.3
Total lipid phosphorus, μmol/ml plasma	1.2±0.2

The phospholipids were extracted with chloroform–methanol (2 : 1, vol/vol) and separated by two-dimensional thin-layer chromatography. The values are means ±S.D. for ten rats each and are expressed in mol per 100 mol of phosphorus recovered

Table III

Composition of phospholipids in myocardium, lung, liver, spleen, kidneys and skeletal muscle

Phospholipids	Myocardium	Lung	Liver	Spleen	Kidneys	Skeletal muscle
Lysolecithin	1.6±0.7	2.4±0.5	1.9±0.7	1.9±0.7	2.0±0.4	3.4±0.9
Sphingomyelin	3.2±0.4	11.8±1.7	4.8±0.9	6.9±1.1	11.7±3.2	4.6±0.7
Lecithin	45.1±4.9	49.8±3.8	53.6±6.1	47.3±3.2	35.6±2.7	52.6±5.9
Unidentified	0.5±0.2	1.1±0.2	0.2±0.2	lack	0.8±0.0	1.5±0.7
Phosphatidyl serine	2.4±0.4	8.0±0.9	4.5±1.8	9.2±1.1	6.3±1.1	3.4±0.7
Phosphatidic acid	0.7±0.2	1.1±0.2	0.4±0.2	4.3±0.6	0.9±0.2	0.5±0.2
Phosphatidyl inositol	4.7±0.8	3.3±0.4	8.3±1.4	8.5±1.9	5.5±1.0	7.9±1.8
Phosphatidyl ethanolamine	36.7±3.5	20.8±0.4	27.7±3.5	22.9±3.5	35.3±4.6	25.6±5.6
Cardiolipin	1.8±0.9	0.7±0.2	0.3±0.4	0.4±0.0	0.7±0.3	0.6±0.2
Diphosphoinosites	0.4±0.1	0.7±0.4	0.4±0.2	0.7±0.1	0.3±0.1	0.8±0.3
Total lipid phosphorus, μmol/1 g tissue	25.8—6.2	23.0—7.1	22.9—5.9	16.8—2.5	34.7—8.0	7.2—1.5

The phospholipids were extracted by the method of Folch et al. [3] and separated by two-dimensional thin-layer chromatography. The values are means ± S.D. for ten rats each and are expressed in mol per 100 mol of phosphorus recovered

respectively. In our experiments lecithin rised 47%, sphingomyelin + lysolecithin 15%, and cephalins 33% of all the phospholipids. The discrepancies were probably due to the fact that in our investigations the lipids were extracted directly from haemolyzed erythrocytes whereas De Gier and Van Deenen extracted them from ghost erythrocytes obtained by a lengthy procedure. Moreover, they applied one-dimensional chromatography which does not ensure a complete separation of the phospholipids.

The total amount of phospholipids (in μmol/g tissue wet weight), and the mol per cent values of the various phospholipid classes of the heart, lung, liver, spleen, kidney, and skeletal muscle are presented in Table III. The largest amount was found in the kidneys (34.7 μmol/g), the smallest in skeletal muscle and spleen (7.2 and 16.8 μmol/g, respectively). The amount in the liver, lung and myocardium were 22.9, 23.0, and 25.8 μmol/g, respectively.

In the tissues studied ten classes of phospholipid were found, one of which was unidentified. This unidentified phospholipid was lacking in the spleen.

Lecithin and phosphatidyl ethanolamine were the dominant phospholipids, constituting a total of 71% to 81% of all of the phospholipids. Lysolecithin, phosphatidic acid, cardiolipin, the unidentified phospholipid, and diphosphoinositides constituted the lowest percentage. The value of the last is somewhat questionable since a neutral mixture was used for extraction, while

for the quantitative extraction of diphosphoinositides a chloroform — methanol – HCl mixture is needed [8].

The quantitative composition of hepatic phospholipids was comparable to that reported by Tjiong et al. [7]. In the available literature we have not found data concerning the quantitative composition of all classes of phospholipids in other tissues. The agreement of our results concerning the composition of phospholipids in the liver with those reported by Tjiong et al. [7] indicates that the results obtained for the other tissues may be considered reliable.

The investigations presented here formed a basis for further studies on phospholipids under the influence of vanadium.

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Acta Physiologica Hungarica, Volume 62 (2), pp. 151–153 (1983)

INFLUENCE OF NEONATAL ENDOTOXIN TREATMENT AND HORMONAL IMPRINTING ON THYROTROPIN-INDUCED THYROXIN (T_4) PRODUCTION OF ADULT RATS

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A single subtoxic dose of endotoxin given to newborn rats by the intraperitoneal route decreased the T_4 level in adulthood. TSH administration 24 hours after the endotoxin treatment failed to abolish this effect but, in this case, the response to TSH provocation in adulthood exceeded even the control value.

Keywords: endotoxin, hormonal imprinting, thyroxin, thyrotropin, adult response.

Hormonal imprinting takes place in the critical perinatal period of maturation of receptors. At this time the receptor undergoes modification upon the influence of the adequate hormone, an event resulting in the development of the normal responsiveness characteristic of adulthood, and the hormone present in excess perinatally will amplify the receptor [2, 3]. On the other hand, when an inadequate hormone is bound to the receptors it will damage them; as a consequence also the response in adulthood will be smaller. The structures of gonadotropins and TSH are similar [7]; this may be the cause why a neonatal gonadotropin treatment with a single dose damages the TSH receptor to such an extent that, in adulthood, the thyroid response to TSH (thyroxin production) will become insignificantly small when compared to the control [4, 5].

Since the TSH receptors are membrane receptors it seemed worth to investigate whether a toxic agent (endotoxin, lipopolysaccharide, LPS 5–6) that causes a general membrane destruction was able to produce (when applied in the critical period of receptor maturation) such changes in the membrane of thyroid cells which would persist and could be demonstrated also in adulthood. In addition, it seemed worth to investigate whether TSH treatment in the peri-

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natal period, i.e. during the membrane perturbation [1] caused by endotoxin, would restore or influence in any way the responsiveness of the damaged membrane.

Materials and methods

Experiments were performed on 116 newborn CFY rats of both sexes. The newborn animals were treated with endotoxin within the first 24 hours of life. The endotoxin was prepared from E. coli 89 according to the method of Westphal and Jann [8]. On the basis of previous titration a subtoxic amount (1 μg/0.1 ml) of the toxin was administered intraperitoneally.

Certain groups of the animals treated with endotoxin were given also one I.U. of TSH (Ambinon, Organon, Oss, Holland) 24 hours after the endotoxin administration. The control animals were given only physiological saline solution when newborn.

At the age of 30 days the animals were separated from their mothers and at the age of 35 days 3 I.U. of TSH were given both the control and the endotoxin treated groups and also the animals treated with TSH neonatally. One hour later the animals treated or not treated with TSH in adulthood were bled and the serum level of T_4 was determined by the RIA method (Isotope Institute of the Hungarian Academy of Sciences, ^{125}I-T_4 RIA kit).

For statistical analysis Student's t test was used.

Results and discussion

The schedule and the results of the experiments are shown in Table I., and Fig. 1.

In animals untreated in newbornhood administration of TSH in adulthood significantly increased T_4 production. In animals treated with endotoxin in newbornhood the basal level of T_4 was decreased while the response to TSH in adulthood increased, although the value did not reach the level found in control animals treated with TSH only. From this one might conclude that endotoxin treatment in newbornhood had damaged the membrane receptors; therefore, the responsiveness to endogenous TSH is not the same as in the untreated controls. This might indicate that in the endotoxin-damaged membrane the endog-

Table I

Effect of endotoxin (LPS) and endotoxin+TSH given in newbornhood with or without subsequent treatment in the adulthood on thyroxin production of thyroid cells

Group	Treatment in newbornhood	Treatment in adulthood	No	T_4 μg/dl
1.	—	—	24	11.21±2.28
2.	—	TSH	19	13.85±2.41
3.	LPS	—	19	9.41±1.57
4.	LPS	TSH	20	12.12±1.29
5.	LPS+TSH	—	16	9.44±2.55
6.	LPS±TSH	TSH	19	16.38±1.92

$p < 0.001$: 1—2, 3—4, 5—6, 4—6, 1—5
$p < 0.01$: 1—3, 2—4, 2—6

enous imprinting was disturbed, an event perhaps accounting for the decrease in the basal level. However, the receptors were not damaged to such an extent as to become unable to respond to exogenous TSH. The degree of the response is similar to that of the controls but, in absolute values, the level is lower than that seen in normal rats.

When the neonatal endotoxin administration was followed by TSH treatment 24 hours later the basal level in adulthood did not change and remained as low as that of the animals treated with endotoxin only. The responsiveness of the receptors to exogenous TSH was, however, more marked and the T_4 level much higher than in the controls treated with TSH.

The identical basal levels and the dissimilar responses to TSH of animals treated in newbornhood with endotoxin only and with endotoxin + TSH suggest that the damage to the receptors impairs only the response to endogenous but not to exogenous TSH (cf. the group given endotoxin and also TSH). This can only be explained by assuming that the TSH, ovine and porcine in origin, is not exactly of the same structure as the rat TSH, given 24 hours after the endotoxin somehow made the receptors of the membrane under restitution to assume a shape more corresponding for the ligand.

These observations might be worthy of attention not only from a theoretical point of view as far as the hormonal imprinting is concerned; they might also lead to a better understanding of the background of the retardation of endotoxin-damaged subjects.

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INTRAVENOUS FEEDING IN THE RABBIT

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Experience with intravenous feeding in about forty rabbits forms the basis of this report. The results showed that low cost functional equipment and techniques for intravenous feeding and metabolic studies are possible in the rabbit. Restraining of the rabbit is necessary, but it hardly affects the results in short (up to three days) studies. Repeated at 24-hour intervals, heart punctures under anaesthesia allow to obtain large blood samples for microscopic, haematological and biochemical measurements during the same study.

Keywords: intravenous feeding, metabolic studies, rabbit, restraining.

The use of intravenous infusions has become general in clinical practice. Techniques for the total intravenous feeding of various laboratory animals have been described and mostly rats have been used in such studies [2, 3, 5—8, 10—12]. In view of the technical difficulties e.g. the limited amount of blood obtaiuable from the rat, an intravenous feeding system was developed for the rabbit. For laboratory use the rabbit has a low cost as compared with the dog and the volume infused is not so large as to require, an infusion pump. Experience with intravenous feeding in about forty rabbits formed the basis of this report.

Cannulation

It is possible to cannulate the rabbit under anaesthesia in the jugular or femoral vein after are skin incision. After a little training anaesthesia, incision and subcutaneous passage of the catheter could be avoided by using percutaneous ear vein puncture at the posterior edge and by insertion of an intravenous cannula (216/0.8 mm O.D. × 25 mm Venflon® Viggo AB, Helsingborg, Sweden). In some cases shaving was necessary for successful cannulation at the first puncture. The vein was usually made visible by using the left thumb and first finger proximally and the third and fourth fingers distally without tourniquet or assistant, and only rarely had alcohol to be applied to dilate the vein.

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The cannula was secured with hypoallergenic 25 mm wide Surgical Tape 3M (Micropore®, USA) and a slow infusion was started. To prevent dislodgement of the cannula by rapid ear movements the ears were tied together with Surgical Tape 3M.

Restraining the rabbit

Infusion with cannulas placed through incision or percutaneously did not succeed in unrestrained rabbits, as strong movements of the animal always caused displacement or breaking of the catheter. Therefore restraint of the rabbit was necessary. This was best achieved by at home-made adjustable lock around the neck. It never caused any excoriation or wound. A procedure like this is prone to cause fracture of the vertebral column due to strong twitching of the rabbit. This was prevented by a moveable lock so that the lower part of the rabbit's back could be forced against the rear of the metabolic cage.

No harmful effects from this restraining system were found and the rabbits were calm during the study. The heart rate (130 $\pm$ 11/min), frequency of respiration (57 $\pm$ 5/min), daily urinary excretion (100 $\pm$ 23 ml/kg body weight) and amount of faeces (16 $\pm$ 8 g/kg body weight) were normal (120—140/min, 50—60/min, 250 ml and 30—40 g respectively, for a rabbit weighing 2.5 kg (4). After laparatomy and the daily anaesthesia needed for heart punctures the physical activity of the rabbit was not intensive even without restraint.

Infusion solutions

The rabbits were infused with physiological NaCl solution or 10% glucose solution alone or with their combination (+51 mmol/l sodium and chloride) and 8% amino acid solution (Medipolar Pharmaceutical Co., Oulu, Finland). No food or fluids were given by any other means during the study. The amount of infused fluid (110 $\pm$ 10 ml/kg body weight) corresponded to the physiological needs of a male rabbit at 21 °C (104.1 $\pm$ 37.79 ml/kg body weight) [1] as did, despite the infusion of NaCl solution, the amount of energy given 250 kJ (60 kcal) for the first kg and + 84 kJ (20 kcal)/0.5 kg body weight over 2 kg [9].

The infusion was uncomplicated and no local changes were caused by it. The body weight of the rabbits was, in this short term study, dependent on the amount of fluid infused. Most of those rabbits who died during the infusion (four out of five) were from the group receiving physiological NaCl solution.

Metabolic cage

The metabolic cage was designed and constructed for these studies in our department. In a 25 cm high open steel box with a net made of 1.5 mm ∅ wire (11 $\times$ 11 mm holes) as a floor (35 $\times$ 40 cm) two parallel struts were attached 18

cm apart to fix the adjustable neck lock (7.5 to 3.5 cm hole), which in turn could firmly be fixed at any desired distance from the back of the box. The faeces and urine fell down a funnel which converged over a 21 cm distance to a tube 2.5 cm in diameter. The urine flowed to the surface of a glass oval (5.8×9.0 cm) suspended on a steel wire from the tube to a 300 ml flask with a mouth measuring in diameter less than that of the maximal horizontal diameter of the oval. The solid faecal material fell separately outside the urine flask. With this equipment and technique the daily urine and faecal outputs could be collected and measured.

Trauma and blood samples

After developing the infusion equipment and techniques in 16 rabbits a series of laparatomies followed by two days infusion was undertaken with four different regimens (physiological NaCl solution, 10% glucose (+51 mmol/l sodium and 51 mmol/l chloride) alone and 10% glucose together with two different 8% amino acid solutions) in four rabbits each. Healthy male rabbits (California) 15 weeks old were used kept before the experiment on the same stable diet and in the same environmental conditions. Intravenous sodium pentobarbital 30 mg/kg body weight was used as an anaesthetic. After shaving with sterile instruments and aseptic techniques, with the rabbit laying on his back, a mid-line laparatomy incision was made from the xiphoid to a five cm distance from the symphysis. The linea alba was closed by interrupted polyglycolic acid (4–0 Dexon®, Davis + Geck, Cyanamid of Great Britain Ltd., Hampshire) sutures and the skin by continuous sutures of polypropylene monofilament (4–0 Prolen®, Ethicon Inc., USA). Blood samples of 15 ml were taken by heart puncture (sterile disposable needle 0.90×38 mm, 20 g×1,5, sterile disposable 5 ml syringe, Pharma-Plast, Denmark) under anaesthesia preoperatively, 24 hours and 48 hours postoperatively. In sodium pentobarbital anaesthesia after a careful palpation the parasternal puncture succeeded in most cases at the first attempt. When several punctures had to be done to obtain blood, it caused cardiac tamponade and death in two rabbits. Tamponade was verified by rapid thoracolaparatomy. Three heart punctures repeated 24-hour apart were well tolerated. After blood sampling the rabbit was placed in antitrendelenburt (30 degrees) position under a warm bulb for 15 min and the lost volume was replaced by rapid infusion. The amount of blood taken daily allowed a series of measurements to be made e.g. blood counts, blood sugar. Astrup analyses, Smack® (e.g. serum sodium, potassium, chloride, proteins, albumin, urea, creatine, calcium, phosphorus, cholesterol, alkaline phosphatase and liver enzymes) and the concentration of serum amino acids. The fluid volume needed by a rabbit was so high that it was easy to adjust the infusion rate by gravity, it was controlled three times daily. Percutaneous puncture of the central ear

artery did not seem as practical as the heart puncture. From the ear rarely could three samples be taken and in spite of several punctures the sample was often insufficient or impaired by haemolysis or clotting.

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LACK OF PROTECTIVE EFFECT OF ADRENALINE TOLERANCE IN HAEMORRHAGIC SHOCK IN CONSCIOUS DOGS

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Dogs were made tolerant to lethal doses of adrenaline by treatment with increasing doses of the hormone up to 1 mg/kg. The conscious animals were then subjected to haemorrhagic shock with a hypovolaemic period of 3 hours. Survival was 8/17 in the pretreated group and 15/18 in the control group. 12 of 29 dogs died already during adrenaline treatment. Plasma catecholamine levels were higher in the treated group already before bleeding and also during hypovolaemia. Catecholamine induced myocardial lesions were found in the treated group. No evidence of a blunting of the sympathetic response or a protective effect in haemorrhagic shock was seen in adrenaline tolerance.

Keywords: Haemorrhagic shock, adrenaline tolerance, survival, catecholamines, myocardial lesions.

A protective effect of adrenaline tolerance in haemorrhagic, endotoxic and cardiogenic shock [14, 15, 13, 12, 8, 2] has been reported. Its mechanism has been attributed to a blunting of the hyperactive sympathetic response leading to excessive vasoconstriction in various vascular beds in the different forms of shock. Cardiac hypertrophy, enlargement of the adrenal glands, and possibly reticuloendothelial hyperplasia have been suggested as factors in the protection against cardiogenic shock [2]. Except in the latter type of shock [8, 2] a detailed description of the experimental protocol is not available.

We have made a study of the effect of adrenaline tolerance in experimental haemorrhagic shock in dogs. Tolerance to adrenaline was induced by repeated injections of the hormone in increasing doses during a 3-week period of treatment. Catecholamine levels were measured in the blood plasma as indicators of the degree of symphathetic activity. The studies were made in conscious (unanaesthetized) animals with chronically implanted vascular catheters to eliminate any possible influence of anaesthesia or sedation. A protective effect of adrenaline tolerance in haemorrhagic shock was not found.

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Materials and methods

Animal preparation

A total of 47 healthy mongrel dogs were used (average weight, 11.8 kg). They were instrumented with chronic indwelling carotid arterial and jugular venous Silastic catheters introduced by an aseptic surgical technique under intravenous sodium pentobarbital (30 mg/kg) anaesthesia. The catheters were passed into the aortic arch and superior vena cava and were led through subcutaneous tunnels and exteriorized at the dorsal part of the neck. The catheters were filled with a 3.3% sodium citrate solution at the time of operation and, from the second day, this was changed to heparin. Flushing and filling with fresh heparin was done daily. A bandage was applied to the neck to protect the catheters. The bandage was covered by adhesive tape and was changed daily. This procedure was well tolerated by the animals.

Production of adrenaline tolerance

Injections of adrenaline (Tonogen, Richter, Budapest) in increasing doses up to 1 mg/kg were done every second day using the jugular venous catheter. The volume of injections was 1 ml/kg. The diluent was saline. At the end of the injection the catheter (volume, 1 ml) was flushed with 5 ml saline. Adrenaline injections were begun on the 3rd day after operation (treatment day 1). The last injection was given on treatment day 19 and shock was elicited on the 21st day after treatment was begun. Two dosage schedules were used, the length of treatment period being identical. One group of animals was given 0.1 mg/kg as the first injection and then 0.2, 0.3, 0.4, 0.6, 0.8 and 1 mg/kg, the latter dose four times. The other group received 0.1, 0.3, 0.6, 0.9 and six times 1 mg/kg. Since the two groups were similar in the course and outcome of shock, in plasma catecholamine levels, blood pressure, heart rate, bleeding volumes, and haematocrit, they were pooled and evaluated together. The control group received 1 ml/kg saline injections, the frequency and duration of treatment was the same as in the treated group.

Elicitation of haemorrhagic shock

The dogs were placed into large plexiglass containers. These were open at the top thus permitting easy access. The animals were handled and petted very gently throughout the experiments, thus obviating the need for special restraint. The carotid cannula was used for bleeding. It was also used for blood pressure measurement through a T-connector. The tubing to the bleed-out reservoir was clamped during blood pressure measurement. Reservoir hydrostatic pressure could be measured by clamping the carotid cannula. Seven hundred and fifty units per kilogram heparin were given intravenously before bleeding. Bleeding was spontaneous into an elevated reservoir and was continued to a mean arterial pressure of 5.33 kPa (40 mmHg). Bleeding times to reach this level of pressure averaged 15 min. This pressure was maintained by further bleeding or reinfusion for one hour (= controlled hypotension). In the next two hours bleeding was stopped but no blood was reinfused (= compensation period). All shed blood except that taken for samples was reinfused intravenously. Sterile graduated glass reservoirs and interconnecting Silastic and Tygon tubings were used for the bleedings. The blood was passed through a 28 μ mesh size polypropylene filter before reinfusion.

Measurements

Mean arterial blood pressure was measured with Beckman transducers. Heart rate was measured using Beckman 9857 Cardiotachometer couplers. Blood pressure and heart rate were recorded on a Beckman R-611 Dynograph recorder. Haematocrit was measured by the microcapillary method. Noradrenaline and adrenaline levels in plasma were measured in 3 ml blood samples by the radioenzymatic method of DaPrada and Zürcher [7].

Statistical analysis

To avoid any assumptions on the distribution of the measured variables, non-parametric statistical methods were used. Stochastic significance [9] was estimated by the method of Wilcoxon [6]. The null hypothesis was rejected at P values less than 0.05. Mean ($\bar{x}$), median (m) and semi-interquartile range (sr) [10] values are given. Survival data were evaluated by the chi square method with Yates'correction [16].

Results

Survival

Eight out of 17 adrenaline treated and 15 out of 18 control dogs survived 48 hours after shock ($P > 0.05$). Adrenaline treatment itself caused an appreciable mortality with 12 of the 29 treated animals dying before the treatment was ended. There were no deaths in the saline treated control group before

Table I

Plasma catecholamine levels in shock (nmol/l)

		Adrenaline				
		1	2	3	4	5
Control	$\bar{x}$	2.41	18.08	38.56	15.66	4.61
Group	m	2.26	15.24	28.35	11.35	3.68
n = 18	sr	0.8	6.6	14.2	5.3	1.9
Stochastic significance*		$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	NS
Adrenaline pretreated	$\bar{x}$	7.65	82.95	69.15	38.68	8.38
Group	m	5.76	59.48	53.81	25.66	7.02
n = 17	sr	3.9	46.5	43.0	27.7	4.9

		Noradrenaline				
		1	2	3	4	5
Control	$\bar{x}$	2.15	6.46	15.28	9.33	4.25
Group	m	1.90	6.24	10.13	6.59	3.96
n = 18	sr	0.7	1.5	3.9	2.7	1.6
Stochastic significance*		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS
Adrenaline pretreated	$\bar{x}$	5.12	22.98	31.82	22.72	6.88
Group	m	3.90	13.27	24.39	17.16	6.17
n = 17	sr	2.3	6.4	12.9	10.3	3.1

Blood samples were taken at the following times:
1: prior to haemorrhage in the control period
2: when blood pressure reached the 5.33 kPa level
3: at the end of the controlled hypotension period
4: at the end of the compensation period
5: fifteen minutes after reinfusion

* = Wilcoxon rank sum test
$\bar{x}$ = mean
m = median
sr = semi-interquartile range

Table II

Haemodynamic

		Blood pressure kPa			Heart rate min^{-1}		
		1	4	5	1	2	3
Control	$\bar{x}$	13.8	7.7	14.5	90.5	99.1	131.6
Group	m	14.6	7.9	14.6	90.0	102.5	130
n = 18	sr	1.3	0.3	1.3	11	5	29
Stochastic significance*		NS	NS	NS	NS	NS	P<0.01
Adrenaline pretreated	$\bar{x}$	13.9	7.1	14.6	94.3	108.5	159.1
Group	m	13.3	7.3	13.3	100	120	155
n = 17	sr	1.5	2.2	2.2	12	23	21

Times of measurements listed (1, 2, 3, 4, 5) correspond to those given in Table I.
* = Wilcoxon rank sum test
$\bar{x}$ = mean

shock was elicited. Stochastic probability of the difference of survival rates of treated and control groups until day 21 ("treatment only") was $P < 0.01$. Taking all control and all treated animals into account irrespective whether they lived long enough to be subjected to shock, the overall ("treatment plus shock") mortality was significantly ($P < 0.001$) higher in the treated group than in the control group.

Catecholamine levels

Compared to the control group plasma adrenaline and noradrenaline concentrations were higher in the treated group even before haemorrhage. The difference persisted throughout the shock period and after reinfusion and was stochastically significant at most of the time points studied (Table I).

Blood pressure, heart rate, bleeding volumes, haematocrit

No major differences between the groups were detected in these parameters (Table II). Haematocrit had a tendency to be lower in the treated group during hypovolaemia.

Myocardial damage

An autopsy was performed on 28 animals subjected to shock (14 treated, 14 controls). Ventricular hypertrophy was present in the hearts of the adrenaline treated dogs (average heart weight, 8.3 g/kg; controls, 7.5 g/kg).

parameters in shock

Heart rate min^{-1}		Bleeding volume ml/kg		Haematocrit %				
4	5	2	3	1	2	3	4	5
160.9	95.0	40.6	54.0	35.7	37.3	35.7	36.1	37.1
150	92.5	39.4	53.8	36	36.5	35.5	36.5	36.5
13	5	5	7	3	4	4	4	3
NS	NS	NS	NS	NS	NS	$P<0.01$	NS	NS
164.4	111.0	40.4	53.4	34.8	32.7	28.4	31.5	37.2
180	100	41.4	54	34	33	28	33.5	35
46	8	8	9	3	5	6	4	2

m = median
sr = semi-interquartile range

Myocardial haemorrhages were observed in all autopsied treated dogs. These were of various localizations involving mostly the subendocardial region of the left ventricle, they occurred, however, in all four chambers of the heart, both subendocardially and subepicardially and were usually quite extensive. In contrast to this the hearts of the control dogs were normal, a slight haemorrhage being found in the left ventricle of one of the hearts.

Of the 12 animals which died during treatment in an average 6.5 days (range 2—14 days), 6 were autopsied. Three of these exhibited the typical intramyocardial haemorrhages seen in the treated group subjected to shock.

Discussion

We could not confirm earlier results on the protective effect of adrenaline tolerance in haemorrhagic shock [14, 15, 13, 12]. The reason for the different results is not clear. It is difficult to make comparisons between our experiments and previous work because of the scarcity of published information on the latter. No data are available on the exact dosage schedule and duration of adrenaline treatment, on the type of haemorrhagic shock model used and the presence or absence of anaesthesia during the experiments. A detailed description of an adrenaline treatment schedule is given only by Beckman et al. [2] in their paper on adrenaline tolerance in cardiogenic shock and our schedule is very similar to theirs. The highest single adrenaline dose was 1 mg/kg corresponding to that

used by Beckman et al. [2] and Dietzman et al. [8] in their cardiogenic shock experiments. In comparison, Vigran and Essex [19] found the minimal lethal dose (MLD) of adrenaline to be 0.1 mg/kg and the surely lethal dose (SLD) 0.8 mg/kg in dogs. These figures refer to first, single doses. Our highest single dose, given after a series of doses in increasing amounts is therefore above the surely lethal dose. It is only one half of the maximal single adrenaline dose used by Lillehei et al. [14, 15, 13, 12] yet we do not think that this difference in the maximal doses adequately explains the lack of a protective effect in our experiments. Lillehei et al. [14, 15, 13, 12] do not mention any adverse effects of adrenaline treatment at all, and Dietzman et al. [8] and Beckman et al. [2] mention only weight loss, excessive salivation, incontinence of urine and feces and rectal bleeding. While these effects were all present in our series, too, there was in addition an appreciable mortality in the treated group and extensive intramyocardial haemorrhages occurred. It is difficult to assume that all these adverse effects would not be further intensified by increasing the adrenaline dosage. The mortality of treatment itself was not unlike that observed by Vigran and Essex [19] in dogs treated with large doses of adrenaline.

These experiments do not support the hypothesis that adrenaline pretreatment would blunt the hyperactive sympathetic reaction in shock. On the contrary, an intensification of the response was found. Catecholamine levels in plasma are a good indicator of the degree of sympathetic activation [3, 5, 18]. The catecholamine concentrations had been elevated in the treated group already before shock was elicited and rose to higher levels during shock than in the controls. We have shown previously [1] that nonsurviving animals have larger increases in catecholamine levels than survivors during haemorrhagic shock. Majewski et al [17] have shown that noradrenaline release rate is increased by repeated injections of adrenaline by activating facilitatory presynaptic β-adrenoceptors. Berecek and Brody [4] provide evidence for the uptake of adrenaline by sympathetic nerve terminals innervating blood vessels. The catecholamine is then released from these nerves and acts like a classical neurotransmitter.

In cardiogenic shock the protective effect of pretreatment with adrenaline is documented [8, 2]. Remarkably, Beckman et al. [2] have abandoned the hypothesis of a blunting of the sympathetic response as the possible underlying mechanism.

Macroscopic examination of the hearts of our animals subjected to shock revealed myocardial lesions characteristic for high dose catecholamine administration. Such lesions which are similar in all species that have so far been studied including man [11] may also occur in severe shock with extremely high endogenous catecholamine concentrations. It is remarkable that only one such case was found (out of 14) in the control group indicating that endogenous catecholamine levels were not high enough to regularly produce the lesion in

this group. The uniform occurrence of the lesion in the treated group suggests that it was due to the high dose adrenaline treatment although a possible contributing role of the higher endogenous catecholamine concentration can not be excluded either.

In conclusion, we suggest that adrenaline pretreatment does not have a protective effect in haemorrhagic shock in dogs. It does not decrease the hyperactive sympathetic response and causes lesions of the myocardium. The treatment itself carries an appreciable mortality. It appears highly unlikely that increasing the adrenaline dose would confer protection in haemorrhagic shock.

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POSSIBLE ROLE OF AN ENDOGENOUS OPIOID IN THE ANTIHYPERTENSIVE ACTION OF PROPRANOLOL IN SPONTANEOUSLY HYPERTENSIVE RATS

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The effect on systolic blood pressure and heart rate of the acute and chronic intraperitoneal (i.p.) administration of *d*- and *dl*-propranolol was investigated on unanesthetised spontaneously hypertensive rats. The effect of naloxone on the propranolol induced hypotension was also studied to test the hypothesis that the antihypertensive effect of propranolol involves the release of an endogenous opiate. On i.p. administration, 3 mg/kg *d*-propranolol was inactive; 3 and 30 mg/kg *dl*-propranolol decreased blood pressure and heart rate in a dose-dependent manner. When the rats were pretreated with 2 mg/kg naloxone i.p., the effect of propranolol on the blood pressure was nearly completely abolished, while that on the heart rate was only partially blocked. Chronic administration of *dl*-propranolol (30 mg/kg b.i.d.) to spontaneously hypertensive rats from the age of 6 weeks (prehypertensive phase) for 29 days prevented the development of hypertension while the rats treated with physiological saline for 29 days (control group) developed hypertension. Naloxone (2 mg/kg i.p.) administered on the 29th day to chronically treated rats induced a reversal of the propranolol action on systolic blood pressure and heart rate, i.e., blood pressure and heart rate increased. Naloxone had no such effect in the control group. We suggest that the release of an endogenous opioid contributes to the acute and chronic antihypertensive action of i.p. propranolol in spontaneously hypertensive rats and that the secretion of endogenous opioids participating in the control of cardiovascular functions is influenced by adrenergic mechanisms.

Considerable efforts have been made to clarify the mechanism of the antihypertensive action of beta adrenergic blocking drugs. Several hypotheses have been forwarded, however the exact mechanism has remained controversial. By now it is clear that the blockade of beta adrenoceptors is the main mode of action, resulting in an inhibition of renin release from the kidney [18], a reduction of cardiac output [13], a reduction of the renal baroreflex threshold [5], a change in the elastic or autoregulatory modalities of carotid sinus smooth muscle thereby enhancing carotid sinus nerve activity [31], the presynaptic inhibition of noradrenaline release during cardiac nerve stimulation [36]. Antagonism of angiotensin II enhancement of sympathetic nerve transmission through the release of prostaglandins was also suggested for the explanation of

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the antihypertensive action of propranolol, an effect probably not related to beta adrenoceptor blockade [11].

The effect of beta adrenoceptor antagonists on the central nervous system (CNS) is widely accepted as one of the major factors in the antihypertensive effect of propranolol [8, 10, 12, 19, 24, 30, 33]. Decrease in central sympathetic tone [8, 27, 37], increase in parasympathetic tone [8] is the result of this action. The sedative and anxiolytic effect of beta-blockers is thought to be the consequence of the blockade of symptoms of sympathetic hyperactivity at the periphery (heart, blood vessels) as well as inhibition of central sympathetic tone [30].

The action of beta-blockers and symptoms of their withdrawal resemble those of the opiates [4, 9, 16, 20, 28, 34]. Furthermore, the antihypertensive effect of centrally acting alpha adrenoceptor agonists, clonidine and alpha methyldopa, is blocked by opiate antagonists, naloxone and naltrexone [6]. These data led us to form a hypothesis that the activation of opioidergic mechanisms might also contribute to the CNS effects of propranolol. We tested this possibility by studying the effect of opiate antagonists on the antihypertensive action of propranolol, and found that the blood pressure-decreasing effect of intracerebroventricularly (icv.) or orally administered propranolol was inhibited by naloxone or naltrexone in spontaneously hypertensive rats (SHR) [23].

We report here that the acute and chronic antihypertensive effects of intraperitoneally (i.p.) administered propranolol can also be blocked by naloxone.

Materials and methods

Male spontaneously hypertensive rats of the Okamoto-Aoki strain (Canadian Breeding Farms Ltd, Montreal, Canada) were used for the experiments. Animals were kept at a constant temperature, lighting conditions and diet with tap water ad libitum. Systolic blood pressure of unanesthetised rats was measured by the tail-cuff method as described in detail elsewhere [14]. Heart rate was calculated from the pulse curve. Baseline systolic blood pressure and heart rate values were obtained as a mean of 10—12 measurements. The effect of different drugs on these parameters was assessed from readings (mean of three) done at 5 to 10-min intervals for a period up to 60 minutes.

Acute experiments

dl- or *d*—Propranolol hydrochloride (ICI, USA) was administered i.p. in a volume of 0.20 ml, five minutes after the i.p. injection of physiological saline (0.6 ml) as well as after 2 mg/kg naloxone hydrochloride (Endo Labs. USA), also given in 0.6 ml. The dose of *d*-propranolol was 3 mg/kg, and that of *dl*-propranolol was 3 or 30 mg/kg. On any one day, only a single dose of propranolol with or without the prior administration of naloxone was tested in the same animal. Six rats were used in this series of experiment.

Chronic experiments

A group of six young (6 weeks old) SHR were chronically treated with *dl*-propranolol (30 mg/kg b.i.d., i.p.)for 29 days. The blood pressure and heart rate were 144 $\pm$ 7 mm Hg and 422 $\pm$ 7 beats/min, respectively, before the treatment. Another group (control group) of five SHR of the same age was also treated for 29 days with physiological saline (0.6 ml b.i.d. i.p.),

whose blood pressure (148 ± 2 mm Hg) and heart rate (403 ± 9 beats/min) were not statistically different from the values in the propranolol-treated group before the treatment. On the 29th day of propranolol or saline therapy the effect of 2 mg/kg i.p. naloxone on blood pressure and heart rate were measured in both groups.

For statistical analysis paired or unpaired *t* tests were used as appropriate.

Results

Acute experiments

dl-Propranolol, in a dose of 3 mg/kg, decreased systolic blood pressure from 196 ± 3 to 148 ± 5 mm Hg, and heart rate from 353 ± 5 to 294 ± 16 beats/min. The onset of the effect manifested five minutes after the administration, peaking at 15 min. The hypotensive action lasted for about 30 min and the bradycardiac effect for longer than 60 min (Fig. 1). When the rats were pretreated with naloxone (2 mg/kg i.p.) the same dose of propranolol decreased blood pressure from 198 ± 2 to 183 ± 3 mm Hg, and heart rate from 357 ± 3 to 314 ± 6 beats/min. This hypotensive action of propranolol could be detected only 5 min after the application, while 10 min after the administration of pro-

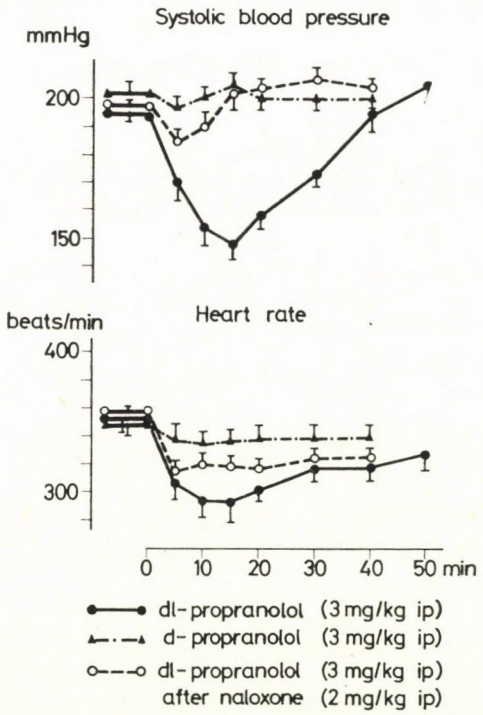

Fig. 1. Acute effects of 3 mg/kg i.p. propranolol. *dl*-Propranolol decreased systolic blood pressure and heart rate while *d*-propranolol was not effective. Naloxone blocked the blood pressure response and inhibited the heart rate response to *dl*-propranolol

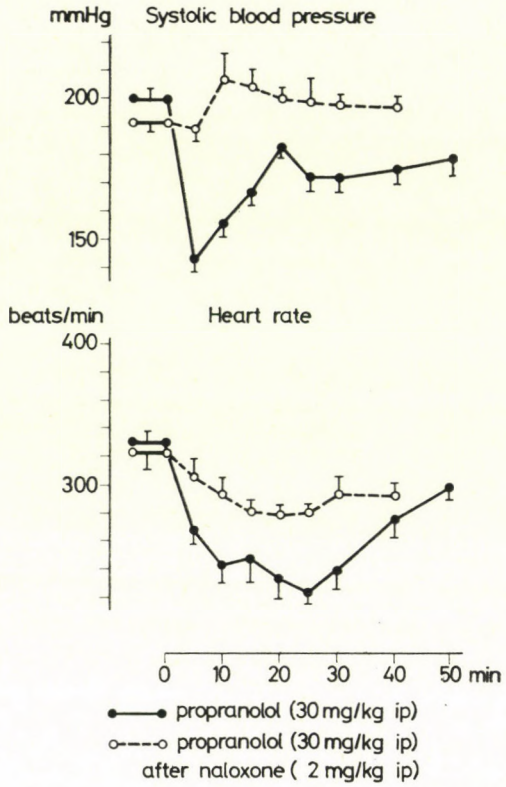

Fig. 2. Acute effects of 30 mg/kg i.p. propranolol. *dl*-Propranolol decreased systolic blood pressure and heart rate. Naloxone blocked the hypotensive response and inhibited the heart rate response to propranolol. There was a slight increase in systolic blood pressure after propranolol when naloxone pretreatment was applied

pranolol blood pressure was not different from the baseline level. Heart rate response to propranolol was only partially inhibited by naloxone (Fig. 1).

d-Propranolol, in the dose of 3 mg/kg i.p., did not affect blood pressure and heart rate (Fig. 1).

dl-Propranolol, in the dose of 30 mg/kg i.p., decreased systolic blood pressure from 200 $\pm$ 2 to 142 $\pm$ 3 mm Hg, and heart rate from 350 $\pm$ 8 to 244 $\pm$ 4 beats/min. The maximal effect of propranolol on blood pressure was detected 5 min, on heart rate 25 min, after the i.p. injection. Both the hypotensive and bradycardiac action lasted longer than 60 min (Fig. 2). When the rats were pretreated with naloxone (2 mg/kg i.p.), propranolol, in the same dose, decreased blood pressure from 192 $\pm$ 3 to 189 $\pm$ 4 mm Hg (not significant) 5 min after the injection, while causing a significant increase to 207 $\pm$ 11 mm Hg at the 10th minute with a gradual decrease to the baseline level afterwards. Heart rate was decreased by propranolol from 341 $\pm$ 11 to 300 $\pm$ 4 beats/min, the maximal effect being recorded 20 min after the application of propranolol (Fig. 2).

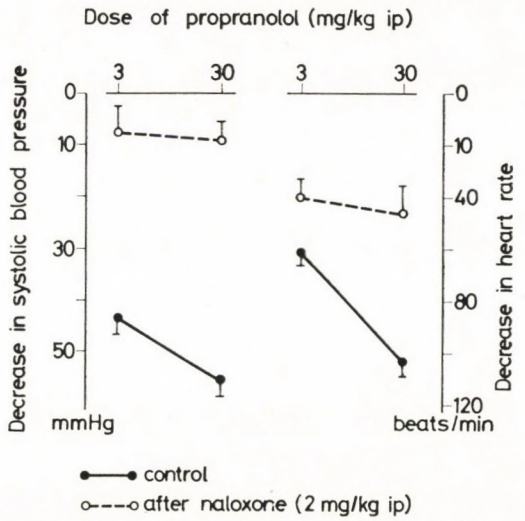

Fig. 3. Relationship between the dose and effect of propranolol. Higher doses of propranolol caused a more pronounced effect on blood pressure and on heart rate. After naloxone this dose-response relationship disappeared

The dose — effect relationship with and without the naloxone pretreatment is presented in Fig. 3.

Chronic experiments

Young, 6 weeks old, SHRs were treated with propranolol (30 mg/kg b.i.d., i.p.) or saline (0.6 ml b.i.d., i.p.). The means of blood pressure and heart rate in the two groups were not different. On the 29th day of treatment blood pressure of the control group was 170 $\pm$ 8 mm Hg while that of the propranolol-treated group was 147 $\pm$ 5 mm Hg, with heart rates 385 $\pm$ 20 and 302 $\pm$ 10 beats/min, respectively.

Naloxone, 2 mg/kg i.p., significantly increased both systolic blood pressure and heart rate of the propranolol-treated group to 162 $\pm$ 5 mm Hg and 348 $\pm$ 5 beats/min, respectively. The effect of naloxone on blood pressure lasted for about 60 min, and on the heart rate for longer than 60 min (Fig. 4), peaking between 5 and 15 min after the administration.

In contrast, naloxone, in the same dose, caused a slight but significant decrease in blood pressure of the control group (to 164 $\pm$ 4 mm Hg) and an increase in heart rate (to 408 $\pm$ 3 beats/min). These changes lasted only for about 10 min (Fig. 4).

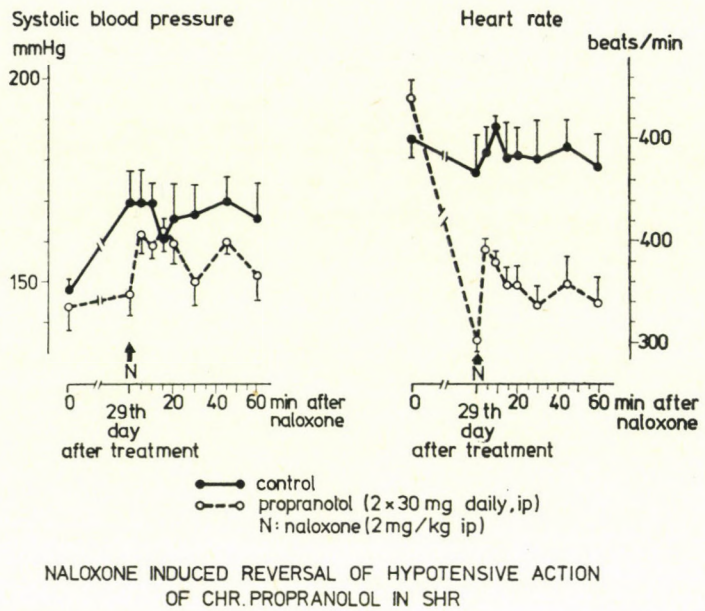

Fig. 4. Chronic effects of propranolol and its reversal by naloxone. 2×30 mg/kg i.p. daily dose of propranolol for 29 days inhibited the development of hypertension in young SHR. Naloxone, given on the 29th day of propranolol treatment, increased systolic blood pressure and heart rate. In the control, saline-treated group naloxone slightly decreased systolic blood pressure and increased heart rate to a much lesser extent than in the propranolol-treated group

Discussion

Data in the literature concerning the *acute* antihypertensive action of propranolol are controversial. On intravenous administration of 1—2 mg/kg *dl*-propranolol to SHR an increase in blood pressure and a decrease in heart rate occurred [37], or there was no change in these parameters [24]. Others reported a decrease in both blood pressure and heart rate on intraperitoneal or oral administration of higher (10 to 60 mg/kg) doses to SHR [5, 13, 17, 21, 25]. By application of propranolol into the CNS a decrease in blood pressure and heart rate was noted in SHR [23, 37], in cat [8], in dog [8, 19] and in the rabbit [24]. The decrease in blood pressure and heart rate was associated with an early decrease in cardiac output followed by a reduction of total peripheral resistance [17] and with suppressed nerve activity of centres involved in the cardiovascular regulation [8].

In our experiments a dose-related decrease in both blood pressure and heart rate was found after i.p. administration of *dl*-propranolol, while *d*-propranolol was not effective. This finding indicates that the action of the drug is probably related to its beta adrenoceptor blocking activity. Application to the CNS of *d*-propranolol in higher concentration also affected blood pressure pre-

sumably because of the local anesthetic activity of the substance [24, 37]. When propranolol was administered 5 min after naloxone in our experiments, the blood pressure response was nearly abolished, while the heart rate effect was only partially blocked. Our results might point to the role of the activation of opiate receptors in the antihypertensive action of *dl*-propranolol, if this inhibitory action of naloxone is the consequence of the blockade of opiate receptors. Naloxone is thought to be a "pure" opiate antagonists, although a weak antagonist activity and antagonism of other substances than opiates (nitrous oxyde, substance-P, acetylcholine, haloperidol, barbiturate, ethanol, amphetamine, diazepam, adenosine ect.) was also reported, although the effect of some of these substances may also be related to the release of endogenous opiates [26]. However, naloxone and naltrexone readily antagonized also the hypotensive effect of icv. administered propranolol indicating an activation of *central* opiate receptors in the action of propranolol [23].

Chronic administration of propranolol decreased blood pressure of SHRs and of rats with isolation-induced hypertension [1, 2]. It also prevented the development of isolation-induced hypertension in rats [1]. Others found propranolol, even during chronic administration, ineffective on SHRs with definitive hypertension, but it prevented the development of hypertension when the treatment had been started in the young, prehypertensive phase [35].

In agreement with these data propranolol inhibited the development of hypertension for 29 days in our experiments. In the propranolol-treated group of SHR naloxone induced a reversal of propranolol hypotension: it increased systolic blood pressure and heart rate.

The partial blockade of the bradycardiac action of propranolol by naloxone in both the acute and chronic series of our experiments is supposed to be related to the direct cardiac effect of propranolol.

If our presumption, that the effect of naloxone is the consequence of the blockade of opiate receptors, is valid, then our results indicate the stimulation of opioidergic mechanisms not only by acute but also by chronic administration of propranolol. The mechanism(s) of this stimulation is not clarified yet.

The binding of ^{3}H-naloxone to the brain tissue was displaced by propranolol [3] showing the possibility of the direct stimulation of CNS opiate receptors by beta adrenoceptor antagonist, propranolol. However, the concentration in the CNS of propranolol used in our experiments is estimated to be at least two degrees of magnitude lower [7] than that in the cited binding study [3]. Moreover, in our study propranolol (10^{-8}—10^{-6} M/l) did not suppress ^{3}H-naloxone binding to rat brain membranes (unpublished observations). The direct interaction of propranolol and naloxone at the same opiate receptors is unlikely to explain the suggested antagonism also in view of the finding that in the chronically (orally) treated SHR propranolol was practically completely eliminated by the time of the test with the opiate antagonist [14, 23].

The analogy between the above described or previous [23] findings and the reported reversal of the antihypertensive effects of CNS alpha-2 adrenoceptor agonists by opiate antagonists [6] indirectly supports the concept that the central hypotensive action of propranolol is, at least partially, mediated by the release of noradrenaline stimulating alpha-2 adrenergic receptors inhibitory to the sympathetic tone [29]. Blockade of beta adrenoceptors can also elicit similar effects as stimulation of alpha adrenergic receptors by unmasking tonic alpha adrenoceptor activity [5].

In a previous series of experiments we found that stimulation of alpha-2 adrenoceptors by clonidine or *l*-alpha-methylnoradrenaline increased the release of a substance with *beta endorphin*-like immunoreactivity from the brain stem + hypothalamus slices of SHR, in vitro [15]. Adrenergic control of beta endorphin secretion from rat pituitary gland has also been reported [22, 32]. Beta endorphin has a potent hypotensive action of its own [16]. Thus, we suggest that the release of an endogenous opioid (probably beta endorphin) contributes to the antihypertensive action of propranolol in SHR.

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Book Reviews

MOSHE ABELES (Ed.)

Local Cortical Circuits

(An electrophysiological study) Studies of Brain Function, Vol. 6. Springer Verlag, Berlin—Heidelberg—New York 1982

Moshe Abeles' book is a bold, straightforward theory about the function of the cerebral cortex. It is based on meticulous experimental work performed in the last ten years, developing methods apt to clarify the nature of cortical connections. The methods included the exploration of multiunit activity with the help of microelectrodes and a computer-based separation of spikes belonging to neighbouring neurons. Recording of spikes from two neurons revealed only that relations between the units change with the changing situations, suggesting that the cortex is not merely a simple relay station along the sensory pathway. Separation of three units, however, clearly showed that cortical neuronal activity is organized along chains of synchronously firing groups of neurons. These groups emerge occasionally from the multitude of cortical neurons. Thus, the author presents the concept of synfiring neuronal chains in the cortex. Anatomical, physiological and a vast amount of theoretical evidence is provided to support this hypothesis.

The new concept of cortical function created by an excellent scientist is a promising theory and fascinating reading at the same time. It is recommended to neurophysiologists, cybernetics and for all who are interested in the function of the cerebral cortex.

F. OBÁL

J. ALTMAN, S. A. BAYER

Development of the Cranial Nerve Ganglia and Related Nuclei in the Rat

Advances in Anatomy, Embryology and Cell Biology, Vol. 74, Springer Verlag, Berlin—Heidelberg—New York 1982, 90 pages, with 54 figures, price DM 54,—; approx US $ 24,—

This monograph summarizes observations on three aspects of the morphogenetic development of the cranial nerve system of the rat: time of origin of neurons of the trigeminal, facial, vestibular, glossopharyngeal and vagal ganglia: development of these ganglia from the earliest stages until birth: how the development of the cranial nerve ganglia and nerves is related to the development of the sensory and motor nuclei of the medulla that are directly associated with these cranial nerves. The description of the development of the ganglia in relation to the respective brain stem nuclei is followed by a general discussion in which important questions such as sequence of production of motor neurons, ganglion cells and sensory neurons of the cranial nerves, placodal origin of ganglion cells, sites of neuron production, routes of migration, are discussed. The material is presented clearly and is well documented. The monograph provides a description of the major early events in the embryonic development of cranial nerve ganglia and related brain stem nuclei in the rat and offers insights into the mechanisms that regulate this growth.

B. HALÁSZ

I. C. BROWN

Gastric Inhibitory Polypeptide

Monographs on Endocrinology, Volume 24. Springer Verlag, Berlin—Heidelberg—New York 1982. 88 pages with 32 figures and 1 table, price DM 68,— approx. US $ 30.20

The author while purifying CCK-preparations with Professors Mutt and Jorpes established that in the extracts there exists an inhibitory material for acid secretion not identical with the CCK molecula. Purification of the inhibitory factor was done rapidly and the structure and amino acid sequence of the newly discovered peptide was described by Brown in 1970. He called it gastric inhibitory peptide (GIP).

In the small monograph the author gives a comprehensive overview of present knowledge about GIP. The first studies were based on the "enterogastrone" line, and GIP was shown to be a powerful inhibitor of acid secretion in dogs with denervated gastric pouches. It was concluded that the inhibitory effect of GIP on the parietal cell is not a direct one: GIP exerts its acid inhibitory effect most probably via somatostatin release. In the physiological situations where GIP is released, numerous interacting mechanisms are activated, which explains the poor effect of exogenous GIP in the presence of parasympathetic innervation.

GIP proved to be also a *g*lucose-dependent *i*nsulin-releasing *p*eptide and later a series of experiments was conducted on the "incretin" line. Insulin release has been induced with a dose of GIP which elevated the serum immunoreactive GIP level by approximately 1.0 ng/ml.

In the booklet the reader can find informations about the structure, the radioimmunoassay procedure and cellular localization of GIP. All data are critically evaluated concerning the hormonal interactions and the role of the peptide in various pathophysiological situations.

The reviewer was unhappy to find in the first part of the book some pages that have lost their way.

V. VARRÓ

D. DENTON

The Hunger for Salt

Springer Verlag, Berlin—Heidelberg—New York 1982. 650 pages with 346 figures, price DM 360,—; approx US $ 150.20

The "Hunger for Salt" is a monograph of exceptional qualities, as perhaps indicated even by its title. The importance of sodium balance in the maintenance of constancy of the *milieu intérieur* has been well known since the pioneering work of Claude Bernard. Sodium balance is influenced both by intake and excretion and we attribute the final control of intake to sodium appetite, more commonly called salt appetite. There is some romanticism in the title "Hunger for Salt" and this romanticism is perhaps a reflection of the author's exceptionally wide interest and education. Dr. Denton has been a well known expert of adrenal physiology since the late fifties. At the same time he has been involved in the problems of salt appetite. This way, as reflected by this monograph, he has a perfect survey over all the aspects of the control of salt–water balance including theoretical questions and methodological problems. Accordingly, the 27 chapters of the monograph discuss not only the central control of sodium appetite but all the factors influencing it (salt–water balance, hormonal status, taste, etc.) in cluding the clinical aspects and also give a short but up-to-date review on other important aspects of salt–water balance. Thanks to the aforementioned wide interest and education of the author we can also read about the phylogenesis of sodium deficiency and salt appetite, with special attention to hominoid evolution, about cannibalism or the symbolic and social aspects of salt in history. It is perhaps surprising to find details from the diary of Captain Cook in a strictly physiological monograph but these details make the book not only informative but also an often exciting reading.

A. SPÄT

DONALD S. FARNER, KARL LEDERIS (Eds)

Neurosecretion: Molecules, Cells, Systems

Proceedings of the Eight International Symposium on Neurosecretion held Sept. 4—10 1980, in Friday Harbor Washington. Plenum Press, New York and London 1981. 531 pages, price US $ 59.50

This volume deals with neurosecretory function in its broadest sense, focussing on the relationship of neurosecretion, neurosecretory cells and neurosecretory systems to other selected aspects of neurobiology. It describes among others the relationship of neurosecretory neurons to the molecular aspects of their functions, vertebrate and invertebrate neurosecretory phenomena and neurosecretory cells whose products may function as modulators within the central nervous system.

The papers of the invited speakers are grouped into five section.

I. In this Section paraneurons, the APUD concept, the classical neurosecretory system, distribution of some hypophysiotrophic neurohormones, endorphinergic and enkephalinergic pathways, monoamine-containing neurons, interactions of peptidergic and monoaminergic structures are discussed.

II. The Section entitled "Synthesis, transport and release of neurosecretory substances" contains 10 papers dealing with questions such as secretory activity of peptidergic and aminergic neurons, monoaminergic and peptidergic control of anterior pituitary secretion, regulation of secretion from the neurohypophysis, retrograde transport of neuropeptides as a pituitary-brain communication system, fine structural aspects of release of the peptide hormones, timing of synthesis, transport and release of neurosecretion in the insect brain.

Section III is concerned with the electrophysiological aspects of neurosecretion. Papers describe electrical activity in relation to hormone secretion in the × organ, sinus-gland system of the crab, an opiate mediated inhibition of peptide release from neurosecretory terminals, an in vitro technique for electrophysiological studies of the hypothalamic magnocellular endocrine neurons, receptor functions affecting neuronal excitability and nonsynaptic peptidergic neurotransmission in the abdominal ganglion of Aplysia.

IV. Papers deal with the role of neuropeptides in osmoregulation of various organisms and in regulation of body weight.

In Section V some hormones and their functions in insects, in hydra, in the common sucker and the carp are discussed.

In a closing and summarizing paper B. T. Pickering discusses neurosecretion in the light of findings of the recent years.

47 short communications ranging on a broad scale were presented posters at the symposium they are also included in this volume. A subject index is attached.

This book will be of interest to researchers and teachers in many fields of neurobiology, vertebrate and invertebrate zoology.

B. HALÁSZ

Y. HOUDIS, E. F. J. RING

Human Body Temperature. Its Measurement and Regulation

Plenum Press, New York and London, 1982. pp XI + 238. 64 Figures, 15 Tables, price US $ 32.50

The subjects dealt with can be roughly indicated by the titles of the chapters and the space allotted to them: 1) Terminology used in thermal physiology (pp. 8), 2) Principles of heat transfer (pp. 24), 3) Temperature and humidity measurements (pp. 24), 4) Man and his environment (pp. 24), 5) Temperature distribution (pp. 23), 6) Temperature regulation (pp. 37), 7) Heat loss and conservation (pp. 36), 8) Acclimatization to heat and cold (pp. 9), 9) Disorders of thermoregulation (pp 19), Appendices A—F: enumeration of possible applications of thermography (pp. 11), Bibliography and references (pp. 11), Subject index (pp. 4).

According to the preface, the authors intended this volume "to assist the coordination of the many disciplines with an interest in human body temperature". These disciplines will interest, as suggested by a preceding sentence, engineers, physicists, technicians, and clinicians. Accordingly, the text is, in general, elementary for those who are well versed in the subjects of

some chapters, the same may, however, suit or even exceed the needs of those not familiar with the subject, e.g. the first chapters may be useful to clinicians, and some others to physicists, engineers, and technicians. Considering the wide range of readers for whom this volume was intended, apodictic treatment of many subjects could hardly be avoided. Beside physical aspects, thermography is the pet subject of the authors, a feature which has been evidently appreciated by manufacturers of thermographic equipment. Revision of the text and proofreading appears to have been rather perfunctory, for example on p. 163: "In a newborn baby sweat glands do not develop. Maximal secretion is only one third or one half that of an adult. Maximal sweat production increases rapidly with increasing postnatal age or on p 167: "If $\dot{E}_{req} \ll \dot{E}_{max}$" and as an antithesis "Conversely, where $\dot{E}_{req} \ll \dot{E}_{max}$" or on p. 157: " . . . an increase in heat loss can be effected by raising the *insensible* heat loss, that is evaporative heat loss. In humans this is achieved by the secretion and *evaporation of sweat*" (reviewer's italics).

Sz. DONHOFFER

SHIRO KAKIUCHI, HIROYOSHI HIDAKA, ANTHONY R. MEANS (Eds)

Calmodulin and Intracellular Ca^{++} Receptors

Proceedings of a satellite symposium of the Eighth International Congress of Pharmacology, held July 25—27 1981 in Kyoto, Japan. Plenum Press New York and London 1982. 464 pages plus indexes, price US $ 49.50

Since 1970 the year when it was discovered, calmodulin was shown to be an extremely, and generally important intracellular regulator substance and it is no wonder that it was chosen as the topic of a satellite symposium of the International Congress of Pharmacology in 1981.

I went to begin with the last chapter of the volume, that on "Calcium ion and calcium binding proteins" by S. Ebashi. This short paper of 4 pages is a beautiful and fascinating reading to anybody who is interested in the evolution of ideas and discovery of facts. Beginning with Sidney Ringer's work in the last century Ebashi describes in an admirable manner how limited knowledge interfered with the acquisition of more information and how the observation of minor and seemingly unimportant effects finally led to important discoveries. The first section, entitled "Molecular basis of calmodulin function" contains 14 papers. It begins with a paper by F. F. Vincenzi on the pharmacology of calmodulin antagonism and serves as a useful introduction to the problems. There are further papers on calmodulin antagonists, on binding of Ca^{2+} to the protein and conformational changes caused by the metal binding and several papers concerning regulatory phenomena in smooth muscle. A few papers in this section deal with interdependence of the Ca^{2+}-calmodulin and cyclic nucleotide systems.

The next section is entitled as "Ca^{++} and protein phosphorylation". It contains 8 papers and deals with myosin and protein kinase phosphorylations. The third section is on "Ca^{2+} regulation of microfilament system": it contains 4 papers on the subject.

The papers published are generally of high quality. Some are published first in the present volume and in the data in some are taken from very recent publications. In addition to the papers printed in extens, abstracts of the posters presented at the symposium are also included

I should like to stress the even high level of reproduction of the manuscripts including half-tone figures.

A. FONYÓ

C. KING, H. AKAI (Eds)

Insect Ultrastructure

Plenum Press, New York and London 1982. 485 pages Price US $ 55.00

As the class of insects is the most populous one in the animal kingdom, it would be difficult to write separately on the ultrastructure of every single member of the class. In this volume which is the first part of a two-volume book, the ultrastructure of such insects is described which are interesting from the developmental, biological or genetical point of view as model animals. Mostly the development of those insects and their cell organelles involved in development are analysed. The development of germ cells occupies about 200, the morphogenesis about

60 and organogennesis about 200 pages in the book, and this last part contains the organ ultrastructures, too. In these chapters the junctional complexes and gap junction, have an outstanding importance. As the editors note, the last similar book was published 14 years ago and since that time many techniques have been introduced and improved in electron microscopy (e.g. chromosomal spreading technique, freeze-fracture, scanning electron microscopy, electron microscopic cytochemistry, etc.) and these techniques have resulted in new discoveries. The primary aim of the book is to acquaint the reades with the recent results and to compare them with early ones. For that very reason the volume is not a detailed insect electron microscopy, although it satisfies the requirements of experimental biologists. The authors of the chapters are prominent representatives of different fields and this is mirrored by the reports of their own results, by the abudance of their own and foreign literature, and the very fine transmission and scanning electron microscopic pictures. These are supplemented by didactic schematic drawings.

As many conclusions drawn from experiments on insects apply to general biology as well (e.g. observations on meiosis, gap junction, etc.) the book is useful for every biologist and especially for developmental biologists, geneticists, entomologists and cell biologists.

G. CSABA

P. KUGLER

On Angiotensin-Degrading Aminopeptidases in the Rat Kidney

Advances in Anatomy, Embryology and Cell Biology Vol. 76. 86 pages, with 88 figures. Springer Verlag, Berlin—Heidelberg—New York 1982, price DM 48,—; approx. US $ 21.4

The octapeptide angiotensin II plays an essential role in the hormonal control of salt–water balance. Beside stimulating aldosterone secretion by the adrenal cortex, angiotensin II produced in the kidney may modify the glomerular filtration rate and tubular sodium reabsorption. Therefore, angiotensin degrading enzymes may exert an important effect on renal function. Considering that angiotensin II inhibits renin release by the juxtaglomerular system and thus exerts a feedback control on its own production, the activity of these enzymes may also modify plasma renin activity and the concentration of circulating angiotensin II.

Dr. Kugler summarizes his morphological and biochemical studies on renal angiotensin-degrading aminopeptidases in this monograph. The reader may find a detailed description of the histochemical, ultracytochemical and biochemical fluorometric methods applied for the examination of aminopeptidases. It is demonstrated that aminopeptidase A is identical with angiotensinase A and that both aminopeptidase A and M take part in the degradation of angiotensin II is said to be. Aminopeptidase A localized in the glomeruli and the brush border of the proximal tubuli and in the juxtaglomerular apparatus, and aminopeptidase M in the brush border of the proximal tubuli. Experimental results have been obtained concerning the control of enzyme activity with special reference to changes in salt–water balance and adrenocortical activity. Interesting physiological conclusions have been drawn from these data, shedding new light on the role of these enzymes in the control of glomerular filtration rate and renin release. The list of references contains nearly 200 items.

A. SPÄT

J. MICHEL, H. C. REISSMANN, H. SCHUMANN (Eds)

Automatisierte Erfassung und Verarbeitung von Biosignalen in Forschung und Praxis

Ergebnisse der Experimentellen Medizin, Vol. 40. Verlag Volk und Gesundheit, Berlin 1982. 392 pages Price DM 43,—

The title promises an up-to-date content of interest which is outlined in more detail in the Foreword by the editors. The book gives a survey of the subjects investigated in the German Democratic Republic and of the results obtained in the automatic processing of biological signals.

The papers are grouped in three chapters. One of them deals with general fundamentals, one with the results of research and the third part with clinical applications, there are, however, no strict boundaries separating them. Further, it becomes obvious at first sight that three aspects are dominant:

— mathematical basis of signal processing,
— the technical apparatus used in processing, and
— the medical (scientific) problems and evaluation.

In the solution of a given problem nowadays the applicable technical methods play a decisive role. Still, the reader with some experience in signal processing will not be much impressed either by the block diagram level description of equipment or by the "philosophy" of apparatus construction. At most we may be glad that our German colleagues use numerous Hungarian-made equipments and hope they are satisfied with them.

It is obvious from the papers that the researchers know and apply the most recent mathematical methods.

The theoretical papers are well constructed and give much information. Their main topics summarized in keywords are:

— linear transformations (especially Walsh and Slant transformations),
— the autoregressive method,
— HJORTH-algorithms,
— the method of sychronous averaging.

The methods usually illustrated by practical examples serve a double aim: on the one part they increase the signal to noise ratio, and on the other they facilitate the classification of various signals (mainly EEG, EMG, ECG signals). This is important not only in the quick evaluation of a great number of measurements but in the detection of so small changes in a stochastic signal (mainly in EEG) which are unobservable by our senses.

The second part deals with topics of research. The five contributions study physiological changes during psychic and physical loading, mainly in the functions of the brain and the heart. Detailed analysis of the construction of combined programme controlled measuring systems is given, but here already the medical result is predominant. A striking feature on the other hand is that we meet not only the new methods described previously in the processing of the results but also traditional procedures such as the averaging of evoked potentials, interval histograms and the better known methods of statistical analysis.

The third chapter, occupying half of the volume discusses the clinical applications. The great variety of signal types presented by the authors shows the diagnostic and therapeutic possibilities. A long summary is given e.g. on the methods and problems of computerized ECG signal processing, on the automatized processing of impedance plethysmography signals, EEG signals, evoked potentials and the electromyogram, on the analysis of respiratory arrhythmias and on objective audiometry. It can be seen from this chapter that our colleagues are strongly interested in the method of evoked potentials. From the analysis of averaged signals the reader becomes acquainted with further applications of the up-to-date mathematical methods. Finally, two papers deal with the evergreen subject of monitoring systems in intensive therapy. The last paper discusses in connection with perinatal monitoring the problem of monitoring considered most important, though solved only partly, the trend analysis.

The authors of the 25 papers collected in the volume keep to the rules applied generally in scientific journals. This helps the reader to follow the aims and conclusions of the works. The book is useful for those who already know the fundamentals of computer-supported data collection and procession and wish to be informed on the possibilities of application.

P. HORVÁTH

GÜNTHER PALM

Neural Assemblies

An Alternative Approach to Artificial Intelligence Studies of Brain Functions, Vol. 7. Springer Verlag, Berlin—Heidelberg—New York. 1982. 240 pages, 147 Figures, 322 references, Price DM 88,—; approx. US $ 39.10

A new attractive volume of the Brain Function Series of Springer is the work of a young German mathematician who sacrified himself to understand how the human brain works. Among this kind of specialists the author belongs to the class whose writing is a pleasant synthesis of mathematical ideas and neurobiological knowledge. Concerning its character his book is a selective collection of certain current trends of the field both with didactically well written introductory parts and original results. The text is lucid and expressing the author's enjoyment when writing; most probably the reader will follow him. The style and reasoning is productively fertilized by the spirit of Braitenberg's shool of neurobiology which he is belonging to.

The volume includes 15 chapters, supplements and appendices. Chapter 1 gives basic data about numbers of neurons, connections and parameters of information flow with respect of the human brain. Reading Chapter 2 we can taste some traditional topics of Artificial Intelligence (paradoxes, Turing test, Gödel). Chapter 3 touches knowledges about formal neurons and their networks. In Chapter 4 concepts like organization and cooperativity appear. A principal part including original ideas of the author chapters 5 through 7 is dealing with the so-called matchbox algorithm with improvements by associative matrix memory and minimax look-ahead algorithm. Because of described reasons he calls this as a survival algorithm of which detailed specifications are presented. The 2nd part of the volume starts with an instructive introduction to the anatomy of cortical connectivity ended with mentioning concepts of the Tübingen school. Essentially 2 chapters are devoted to visual processes (9, 10) including plastic changes and deprivation experiments. Again two important chapters follow dealing with neural dynamics, definitions of cell assemblies and associative processing of information including numerous original statements. The closing chapters are sacrified to agreable speculations and comparisons of men, monkeys and machines. It is filled with non-artificially but naturally intelligent questions and open problems. The author's commentary to the wild discussion around reductionism is ironically condensed into an almost nicequantum-poem originating from an anonymous German poet (the author?).

In the Supplements the following topics are included to help the reader in digestion: electrical neural signals, basic information theory, sets and mappings, local synaptic rules and a flow diogram of Survival Algorithm.

Appendix 1 is an interesting recent lecture of the author on the storage capacity of associative memories. Appendix 2 summarizes the author's views of neural modelling with good quality diagrams and tables. The two last appendices contain concepts of cell assemblies put together and graph theoretical representations of wiring in cell assemblies. Here numerous definitions, small propositions and a short theorem can be found.

The book is devoted both to beginners and advanced specialists speculating on the border-lines of sciences about the performance of human brains.

E. LÁBOS

J. SALÁNKI (Ed.)

Physiology of Non-excitable Cells

Advances in Physiological Sciences Vol. 3. Proceedings of the 28th International Congress of Physiological Sciences, Budapest 1980. Pergamon Press and Akadémiai Kiadó 1981. 323 pp. plus index, ISBN 963 05 2729 4.

The 1980 Congress of the IUPS had a Section on General Cell Physiology composed of 5 Symposia. The present volume contains part of the text of the papers presented at the Symposia as well as the complete text of two of the Plenary Lectures. Due to differences in the willingness of the contributors to cooperate, the various Symposia are represented at different levels, some being complete while others are much less so.

I cannot tell how useful the texts of the plenary lectures are for the specialists. For the reviewer who is not an expert in either field, the reviews of both Minakami and Takeshige on "Oxidative Metabolism of Phagocytosing Leukocytes" and of Sömjen on "Physiology of glial cells" were quite interesting, both being highly informative on the "State of the Art 1980". Both reviews are supplemented with a list of references with titles included what makes them even more useful. I wish the reproduction of the electron micrograph published were of better quality.

Of the 5 Symposia, that on epithelial transport is the one represented best. The introduction and the concluding remarks were given by H. H. Ussing. The frog skin, the turtle and the toad bladder are apparently still best suited for the investigation, as 7 out of 8 contributions used these as experimental objects. Computer analysis of current-voltage and current-time curves as well as extensive biophysical and biochemical analysis of various transporting parameters are found in the single contributions. I also got a reasonable quantity of information in the Symposium on "Models of iso-osmotic transport". The Symposium on "Relationship of cell transport and metabolism" is somewhat more heterogeneous. "Cell to Cell Communication" is represented by 3 contributions. "Time in Cell Physiology" is an interesting topic but I have the feeling that it is somewhat loosely coupled to the other topics already listed. On the other hand

it is perfectly clear that in the publication of a large international meeting as the 28th Congress was there is no perfect solution and certain compromises are inevitable.

On the balance, this is a well organized volume in which there are excellent parts and for these one is willing to pay also for the rest.

A. Fonyó

Joachim Ude und Michael Koch (Grafik)

Die Zelle. Atlas der Ultrastruktur

VEB Gustav Fischer Verlag, Jena, 1982, pp. 260, Price M 55,0

The book represents an electron microscopic atlas of the cell containing 196 micrographs and 39 schematic figures in colour.

As an introduction the principle and structure of the electron microscope is detailed and compared to the light microscope. Special requirements of sample preparation and fixation are mentioned as well.

Part II entitled "The cell and its organelles" begins with a short comparison of the procaryotic and eucaryotic cells and of animal and plant cells. Following this, the typical constituents of eucaryotic cells are dealt with systematically: the nucleus and nucleolus, the endoplasmic reticulum and ribosomes, the mitochondria, the chloroplasts, the Golgi apparatus, the lysosomes and the microtubuli are detailed in individual chapters. Besides the typical morphological pictures the structure of the different organelles is explained in simplified diagrams — like that of the microtubuli, chloroplasts, relation of endoplasmic reticulum and ribosomes, etc. The special merit of the atlas is the emphasis of the connection between structure and function: it gives a general overview on the role of each organelle. The details of these processes can then be studied in physiological or biochemical textbooks, but the basic ideas of intracellular localization are fixed in line with the morphological studies.

It should be mentioned that the functional aspects are rather up-to-date and supplemented with adequate references of literature. The last four chapters of Part II deal with the cell membrane (explaining the fluid-mosaic model, too), types of intercellular connections and special formations on the cell surface — like microvilli, endocytotic vesicles and cilia.

The last part describes the characteristic properties of some cells of specialized function — like secreting cells of glands, absorptive cells of the kidney, sensory cells of the retina, neurons and synapses and muscle cells. Again, the connection of the morphological appearance with the specialized function is stressed.

The atlas is a good start for getting acquainted with the structure and general function of the cell, and it can be recommended both to students and to graduates beginning their research work in biology.

Erzsébet Ligeti

D. G. Weiss (Ed.)

Axoplasmic Transport

Springer Verlag, Berlin—Heidelberg—New York, 1982, pp. 477, with 181 Figures

This book is a collection of lectures held on the Workshop on Axoplasmic Transport in 1981. Volumes of this type have usually the advantage over monographs that they yield unselected information concerning the most recent ideas and developments as viewed from different angles including even controversial issues. On the other hand they suffer from the drawback of requiring from the readers some experience in the field. This book is no exception, however, its requirement of expertise in axoplasmic transport research is compensated by the novelty of data contained: it was published within less than a year after the Workshop was held. This fact and the contribution of almost all competent scientists known from the literature of this field makes this Volume a most interesting and useful reading for the specialist.

Lectures are divided into 6 Sections the first of which deals with non-neural intracellular motility. It is aimed at giving an introduction to the field but in spite of the editor's foreword one feels two of the three papers (the second and third) a bit irrelevant. It is Section 2 that gets down to the topic by covering Molecular and Structural Components of the Axon Boundary Conditions for Transport. Among many interesting papers an account of nerve cell contractile

proteins by D. J. Goldberg is found. Section 3 appears to be more heterogenous in scope as it may also be judged from its title: General Characterization of Axoplasmic Transport Materials and Properties. The reader may find papers on isolation, subcellular distribution and nature of transported proteins, peptides, organelles, on transport in different organs and species, etc. The paper by the group of B. Droz, one of the pioneers in axoplasmic transport research, exposes some new aspects of phospholipid transport and the contribution of glial elements. Section 4 is devoted to Transport Mechanism Prerequisites with an interesting contribution of the Kreutzberg-group on neuroplasmic actin, while Section 5 comprises current theoretical models for axonal transport. It is difficult to assess at present the value of the hypotheses presented but it is good to see how people speculate today about possible mechanisms, something what they cannot freely afford when writing research communications to Journals. Section 6 is useful from the methodological point of view as it considers Experimental Techniques to Study Axoplasmic Transport.

Each paper is concluded by a comprehensive list of references. All papers are written in a clear and concise English, illustrations are of adequate number and quality. Printwork is excellent.

In conclusion, the wealth of up-to-date information contained makes this book most valuable for the expert investigator of axoplasmic transport either for general orientation about current status of the field or for quick reference.

F. HAJÓS

ACTA MORPHOLOGICA

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EDITOR-IN-CHIEF
E. SOMOGYI

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CONTENTS

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CONTENTS

PHYSIOLOGY — PATHOPHYSIOLOGY

ABSTRACTS OF THE LECTURES AND POSTERS PRESENTED AT THE MEMBRANE SYMPOSIUM, SÜMEG (HUNGARY), MAY 10–14, 1982

LECTURES

POSTERS

BOOK REVIEWS

Acta Physiologica Hungarica, Volume 62 (3—4), pp. 187—190 (1983)

EFFECT OF THYROTROPIN (TSH) TREATMENT ON THE RESPONSIVENESS OF THE THYROID GLAND DURING ENDOTOXIN SHOCK IN ADULT RATS

Susanna U. NAGY, G. CSABA, L. BERTÓK*
DEPARTMENT OF BIOLOGY, SEMMELWEIS UNIVERSITY MEDICAL SCHOOL AND *FREDERIC JOLIOT CURIE NATIONAL RESEARCH INSTITUTE OF RADIOBIOLOGY AND RADIOHYGIENE, BUDAPEST, HUNGARY

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The shock-inducing dose (1.0 mg/200 g i.v.) of bacterial endotoxin (*E coli* 089) significantly decreased the serum levels of T_4 and inhibited the T_4 increasing action of thyrotropin in rats. It is suggested that the changes observed were due to the membrane damaging effect of endotoxin.

Keywords: endotoxin, TSH, thyroxine production, endotoxin shock

It is well-known that thyroid hormone production is controlled by thyrotropin (TSH) synthesized in the pituitary gland which in turn is regulated by the thyrotropin releasing hormone (TRH) produced in the hypothalamus and other areas of the brain. For the TSH action specific membrane receptors are needed which are known to develop in rats after birth during the maturation period [8, 9].

It has long been known that the cell wall of Gram negative bacteria (*E. coli*, *Proteus vulgaris*, etc.) constantly present in the intestinal flora of humans and animals contain a lipopolysacharid-like substance, the endotoxin, which under physiological conditions is cleaved to non-toxic fragments under the detergent action of bile. The toxin can only be absorbed and cause enteroendotoxinaemia if no bile is present for some reason [2, 3].

The endotoxin is a biologically highly active macromolecule. Its membrane damaging, capillary permeability increasing, pyrogenic, etc., effects are well known [12]. The membrane damaging effect is probably due to the fact that the endotoxin macromolecule (lipopolysacharide) binds to the lipid bilayer of the membrane and causes its perturbation [13]. This is reflected by the enhanced release of lysosomal enzymes, the depression of the mitochondrial oxigenase system and the augmentation of the blood lipoprotein level [6].

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In view of these facts and on the basis of data in the literature it appears that in endotoxin shock the hormonal reception might be disturbed. Hadjikostova et al. [11] have demonstrated that in endotoxin shock the plasma adrenocorticotropin (ACTH) and somatotropin (STH) levels are increased which those of TSH, prolactin, triiodothyronine (T_3) and thyroxine (T_4) decreased. They attributed the changes to the reduced blood flow of the brain. It is, however, possible, that the reduction of TSH, T_3 and of T_4 is due to the damage of the specific hormone receptors in the membrane of corresponding cells. It was therefore interesting to study how the thyroid response to TSH is altered in adult rats during endotoxin shock.

Materials and methods

CFY albino rats of both sexes weighing 180–220 g were used. The endotoxin was produced from *E. coli* 089 fermentor culture by the warm phenol-water method of Westphal et al [16]. Thyrotropin (Ambinon, Organon-Oss) was given at a dose of 3 IU/animal four hours after endotoxin treatment (1 mg/animal i.v.) at the time when the endotoxin shock had developed. One hour later the animals were bled and the serum T_4 level was estimated using ^{125}I-T_4 RIA kit (produced by the Isotope Institute of theHungarian Academy of Sciences). Data were analysed for statistical significance by Student's *t* test.

Results and discussion

Groups and results are shown in Table I. Endotoxin shock with its characteristic symptoms (diarrhoea, hyperventilation, tachycardia, adynamia, etc.) was observed in the animals.

After TSH administration the plasma T_4 concentration was considerably increased (13.5 ± 1.06 μg/dl) as compared to the control value (9.24 ± 1.32 μg/dl). In response to the endotoxin, the plasma T_4 level decreased appreciably

Table I

Effect of endotoxin on T_4 production of the thyroid gland

Group №	Treatment	No. of rats	T_4 μg/dl
1	—	13	9.24 ± 1.32
2	3.I.U. of TSH i.p. Animals were bled 1 h later	10	13.54 ± 1.06
3	Endotoxin 1 mg/animal i.v. Animals were bled 4 h later	20	5.6 ± 0.84
4	Endotoxin 1 mg/animal i.v. 4 h later 3.I.U. of TSH i.p. Animals were bled 1 h later	20	5.58 ± 0.98

Values of Group 3 were not significant as compared to those in Group 4
Significance (Groups 1 vs 2, 1 vs 3, 1 vs 4, 2 vs 3, 2 vs 4) $P < 0.01$

(5.6 ± 0.84 μg/dl) and the fall was not compensated by TSH (5.58 ± 0.98 μg/dl). This appears to indicate that in response to the shock-inducing dose of endotoxin the T_4 production of the follicular cells of the thyroid was potently suppressed.

We suppose that the endotoxin macromolecule causes membrane damage which results in an apparent reduction of TSH receptor sites or reduces the binding affinity. One cannot rule out the possibility that the cerebral blood flow reduced by the endotoxin treatment may have appreciably contributed to the alterations of hormone concentrations as suggested by Bryan and Emerson [7, 14]. Nevertheless, the increment in the concentrations of some hormones (such as ACTH, STH) and the decreases of others (TSH, T_3) appear to contradict this view. It is also possible that owing to the metabolic disturbances in endotoxin shock the peripheral utilization of T_4 was enhanced. This, however, does not seem probable since the T_4 concentration was not elevated or even maintained after TSH administration. In the knowledge of the general membrane damaging effect of endotoxins [5, 13], and their interactions with other hormone receptors [1, 15] our assumption appears to be more acceptable. Further studies are of course required to settle the problem. It is expected that our recent examinations with irradiated (radio-detoxified) endotoxin — which has practically no membrane damaging effect [4] — will furnish further data in support of our view.

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EFFECT OF AUTONOMIC BLOCKING AGENTS ON LOCAL CEREBRAL BLOOD FLOW IN THE RAT

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The local effect of autonomic blocking agents on the resting cerebral blood flow of anaesthetized rats was investigated in 78 anatomical structures. The existence of an α-adrenergic vascular tone was indicated by the average flow increase of 28 ± 3% and 17 ± 3% after the administration of the α-receptor blocking agents phenoxybenzamine (5 mg/kg i.p.) and phentolamine (1 mg/kg i.v.), respectively. The injection of atropine (1 mg/kg i.v.) increased the average blood flow by 19 ± 2%, whereas the overall effect of the β-blocking agent propranolol (1 mg/kg i.v.) was statistically not significant. The magnitude of local blood flow changes in specific cerebral areas exhibited considerable variation in all experimental groups. The comparison of individual and overall flow changes suggests that some of the discrepancies among different investigations could have resulted from the intraindividual heterogeneity of evoked flow changes.

Keywords: cerebral blood flow, phenoxybenzamine, phentolamine, propranolol, atropine

In spite of a great number of studies dealing with the neurogenic regulation of the cerebral circulation there is still no unified attitude regarding the importance of the observed phenomena under physiological conditions. One factor responsible for the conflicting results might have been the heterogeneous reactivity of different cerebral areas to neural and neurohumoral stimuli. However, no systematic investigations concerning the nature and extent of this heterogeneity have been carried out although the recent perfection of autoradiographic techniques employing diffusible indicators has made it possible to study haemodynamic changes in practically any part of the brain.

The histological evidence for differences in the innervation density of cerebral areas constitutes the basic hypothesis for the explanation of regional heterogeneity. Unfortunately, the stimulation of the corresponding receptors by neurotransmitters or their agonists is very difficult since most of them can pass the blood-brain barrier only to a limited extent. While the opening of the blood-brain barrier with hyperosmotic solutions can increase the passage of neurohumoral substances accross the barrier, it probably also imposes another

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source of inhomogeneity and might itself alter the normal metabolic/haemodynamic pattern of the brain.

On the other hand, some adrenergic and cholinergic antagonists are able to reach the cerebral tissue from the circulation in substantial amounts. This enables the determination, without major disturbance of the physiological condition, of the associated vascular tone in the brain, whose existence is still controversial. Since the vascular tone depends on the activation of the corresponding receptors, it is likely to exhibit the same regional distribution as the receptors themselves. In order to gain quantitative information about the basic heterogeneity of neurohumoral effects in the brain, we investigated the effect of different autonomic blocking agents on the local cerebral circulation of the rat in 78 anatomical structures.

Materials and methods

Preparation of experimental animals

The experiments were carried out on male Wistar rats anaesthetized with 50 mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories) i.p. Both femoral arteries and both femoral veins were cannulated with polyethylene catheters (PE-50) for the continuous recording of arterial blood pressure, withdrawal of blood samples and infusion of different substances. The electroencephalogram (EEG) was recorded from two brass screws inserted biparietally into the skull.

After tracheotomy the animals were connected to a rodent ventilator (Harvard Apparatus) delivering 30% oxygen and 70% nitrogen. Immobilization was accomplished with 30 mg/kg gallamine triethiodide (Flaxedil, Specia) i.v. The frequency of ventilation was adjusted continually, in order to maintain the arterial CO_2 tension (PCO_2) between 35 and 40 mmHg. The CO_2 content of the expired air was monitored with an infrared analyzer (Beckman Instruments), blood gases and pH were checked just before the determination of cerebral blood flow with a microanalyzer (Radiometer). With the aid of a temperature controller (Yellow Springs Instrument Co), the rectal temperature of the animals was prevented from dropping below 37 °C.

Experimental groups

The cerebral blood flow in different areas of the brain was determined in the following experimental groups:

(a) control,
(b) 5 mg/kg phenoxybenzamine (Dibenzyline, Smith Kline and French),
(c) 1 mg/kg phentolamine (Regitine, CIBA-GEIGY),
(d) 1 mg/kg propranolol (Inderal, Ayerst Laboratories),
(e) 1 mg/kg atropine (Atropine sulphate, EGYT).

The irreversible α-adrenergic antagonist phenoxybenzamine was administered intraperitoneally 24 hours before the experiment, in order to prevent the systemic hypotension characteristic of the acute use of this drug. The effectiveness of the α-adrenergic blockade was demonstrated by the virtual absence of the pressor response to the intravenous infusion of 20 μg/(kg · min) norepinephrine in earlier studies [56]. All other agents were given in a slow intravenous infusion that lasted for 5 minutes, and the blood flow measurements were carried out 15 minutes later. The completeness of cholinergic blockade established by 1 mg/kg atropine was corroborated in each experiment by the lack of systemic blood pressure response to 0.1 μg/kg acetylcholine. The doses of phentolamine and propranolol were chosen in accordance with the data of other investigators [17].

Determination of local cerebral blood flow

The blood flow in different areas of the rat brain was measured with the chemically inert diffusible tracer iodoantipyrine (4-[N-methyl-^{14}C]-iodoantipyrine, New England Nuclear). At the time of the flow measurement approximately 100 μCi/kg of the tracer, dissolved in 1 ml physiological saline, was infused into the femoral vein over a 1-minute period. The amount of indicator accumulated in any given site of the tissue during tracer infusion can be calculated according to the equation [47]

$$C(T) = \lambda \cdot K \int_0^T \{C_a(t) \cdot \exp[-K(T - t)]\}\, dt$$

where C(T) is the concentration of tracer in a given anatomical structure at time T, $C_a(t)$ is the time course of the arterial tracer concentration, and λ is the brain : blood partition coefficient, which was shown to be 0.8 for iodoantipyrine [50]. For a tracer that is not diffusion limited, the factor K is defined as the blood flow (F) per unit mass of tissue (W) divided by λ, that is,

$$K = F/(W \cdot \lambda) = 1CBF/\lambda$$

where 1CBF is the local cerebral blood flow, usually given in ml/(min · g).

At the 60th second of the iodoantipyrine infusion the animal was decapitated in order to instantaneously cut off the blood supply to the cerebral structures. The brain was removed from the skull in approximately 2 minutes and frozen in liquid Freon 12. Until sectioning, which was done within 48 hours, the specimen was stored in an airtight plastic bag on dry ice.

For the reconstruction of the arterial iodoantipyrine concentration during the infusion, 20 μl samples were collected from the femoral artery approximately every 3 seconds. Since the total length of the catheter used for sampling was less than 30 mm, the effect of catheter smearing was considered to be negligible. Blood coagulation was prevented by 1000 IU/kg heparin (Panheprin, Abbott Laboratories) administered intravenously just prior to sampling. After the infusion the samples were transferred to chromatography paper. When dry, the blood spots were cut out and placed in scintillation vials containing 1 ml of distilled water and 10 ml of a dioxane-based scintillator (Bray's solution, New England Nuclear). The determination of the ^{14}C-concentration was performed in a liquid scintillation counter (Packard Instrument Co), using external standard counting for quench correction.

The frozen brain was cut frontally into 20 μm sections with a microtome (American Optical Corp) at -18 °C. Every 15th section was picked up with a cover glass and dried on a hot plate at 60 °C. The brain slices and a set of carbon-14 standards for autoradiography (Poly-[^{14}C]-methyl methacrylate, Amersham) were placed on X-ray film (SB-5, Eastman Kodak Co) for 10 days. The plastic radiation sources had been previously calibrated for brain tissue of 20 μm thickness, so that differences in absorptance could be taken into account.

The optical density of the autoradiographs was measured with a microdensitometer (Gamma Scientific) connected to a PDP-11 computer (Digital Equipment Corp). From the arterial iodoantipyrine concentrations and the ^{14}C-concentration vs. optical density curve, which was determined individually for each film, the computer program calculated the local blood flow value corresponding to the film darkness in a specific point of the autoradiograph. The area of analysis had a diameter of 200 μm. Smaller structures were scanned manually, larger structures, like the cortical areas, were represented by measurements at approximately the same site in each experiment. Symmetrical structures were analysed at random on either side. The blood flow of each structure was determined in at least two different brain slices of the same animal, and the mean values were included into the final analysis. The anatomical identification of the cerebral structures was made with the aid of a stereotaxic atlas of the rat brain [26].

Statistical evaluation of data

One-way analysis of variance was performed on the general physiological variables listed in Table I. In order to test the significance of differences in average blood flow and vascular resistance, the mean values of individual cerebral structures were arranged according to the anatomical localization and the two-way analysis of variance was applied (Table II). When the analysis of variance indicated significant differences, multiple comparisons between control and each experimental group were carried out with modified t-statistic. The critical values were calculated by the method of Bonferroni [57].

Table I

General physiological variables, cerebral blood flow and vascular resistance after the administration of autonomic blocking agents[a]

	Control	5 mg/kg PBZ	1 mg/kg PHA	1 mg/kg PPL	1 mg/kg ATR
Number of experiments	11	8	8	13	10
Arterial CO_2 tension, mm Hg	35.9±0.7	36.4±0.7	37.3±0.4	37.6±0.4	37.1±0.5
Arterial O_2 tension, mm Hg	112±5	111±4	92±6*	114±5	104±4
Arterial pH	7.45±0.01	7.41±0.01	7.39±0.01***	7.42±0.01	7.40±0.01**
Mean arterial blood pressure, mm Hg	114±7	120±4	103±4	113±3	129±4
CBF[b], ml/(min · g)	0.82±0.03	1.07±0.06***	0.93±0.03**	0.83±0.04	0.97±0.04***
CVR[c], mm Hg · min · g/ml	1.66±0.06	1.44±0.05***	1.35±0.04***	1.75±0.07	1.85±0.07***

[a] All values reported are mean ± SEM
(PBZ: phenoxybenzamine, PHA: phentolamine, PPL: propranolol, ATR: atropine
*, **, ***: Significantly different from control with $P < 0.05$, $P < 0.01$, or $P < 0.001$)
[b] Average cerebral blood flow (see text)
[c] Average cerebral vascular resistance (see text)

Table II

Two-way analysis of variance of blood flow data classified according to treatments and cerebral structures

	Degree of freedom	Sum of squares	Mean square	F
Treatments	4	32575	8143	24.38***
Structures	77	428961	5570	16.68***
Residuals	308	102919	334	
Total	389	564455		

*** Significant at $P < 0.001$ level

Multiple comparison of treatments

	Modified t-statistic	Critical value
Control vs. phenoxybenzamine	8.40	3.74 ($P < 0.001$)
Control vs. phentolamine	3.61	3.04 ($P < 0.01$)
Control vs. propranolol	0.23	2.51 (NS)
Control vs. atropine	4.84	3.74 ($P < 0.001$)

Since the local blood flow data did not always follow the normal distribution, statistical significances presented in Table III were calculated by distribution-free multiple comparisons based on Kruskal-Wallis rank rums with large sample approximation according to Dunn [23].

Results

Table I summarizes the main physiological parameters measured just before the determination of cerebral blood flow. Significant differences as compared to control existed only with regard to the partial pressure of oxygen in the arterial blood after phentolamine, and with regard to the arterial pH in both the phentolamine and the atropine treated groups. The absolute differences were, however, small and unlikely to have influenced the blood flow values substantially. Based on qualitative analysis, the applied agents had no effect on the EEG of the experimental animals.

The local cerebral blood flow (1CBF) was determined in several anatomical structures that could be identified on the basis of the autoradiographs. Eventually, 78 different areas were included into the analysis.

For the quantitative characterization of the cerebral blood supply the average blood flow of each experimental group was calculated by the formula

$$CBF = \left(\sum_{i=1}^{78} 1CBF_i\right) \Big/ 78$$

with

$$1CBF_i = \left(\sum_{j=1}^{n} 1CBF_{i,j}\right) \Big/ n$$

where $1CBF_{i,j}$ is the local cerebral blood flow of structure i in the experiment j from a total of n experiments that compose the series in question. The average vascular resistance was calculated analogously for each experimental group after the flow values had been converted to resistances according to the formula

$$1CVR_{i,j} = MABP_j / 1CBF_{i,j}$$

where $MABP_j$ equals the mean arterial blood pressure in the experiment j at the time of the blood flow measurement. These values are also contained in Table I.

Phenoxybenzamine, phentolamine and atropine all increased the average blood flow significantly. The increase amounted to $28 \pm 3\%$, $17 \pm 3\%$ and $19 \pm 2\%$, respectively. The α-blocking agents decreased the calculated vascular resistances by an average of $13 \pm 2\%$ and $16 \pm 2\%$, while atropine increased them by $13 \pm 2\%$. The β-blocking agent propranolol had no significant effect on these parameters. The details of the statistical analysis are shown in Table II.

Table III

Local blood flow of cerebral areas affected significantly by autonomic blocking agents[a]

	Control	5 mg/kg phenoxybenzamine	1 mg/kg phentolamine	1 mg/kg propranolol	1 mg/kg atropine
Commissura anterior	0.64±0.06 (11)	0.57±0.04 (7)	0.84±0.10 (8)	0.49±0.04 (11)	0.79±0.12 (10)
Cingulum	0.44±0.04 (11)	0.59±0.06 (8)	1.02±0.15 (8)***	0.78±0.07 (11)**	0.84±0.04 (8)***
Genu corporis callosi	0.43±0.03 (11)	0.57±0.06 (8)	0.52±0.07 (8)	0.33±0.03 (11)	0.43±0.04 (10)
Truncus corporis callosi	0.49±0.05 (11)	0.49±0.04 (8)	0.53±0.06 (8)	0.35±0.03 (11)	0.57±0.08 (10)
Globus pallidus	0.73±0.06 (11)	1.18±0.18 (8)*	1.22±0.15 (8)**	0.87±0.08 (12)	1.17±0.12 (10)**
Capsula interna	0.43±0.04 (11)	0.60±0.07 (8)*	0.54±0.07 (8)	0.38±0.02 (12)	0.57±0.05 (9)*
Stria medullaris thalami	0.77±0.06 (10)	0.65±0.07 (8)	1.03±0.13 (8)	0.60±0.04 (13)	0.71±0.07 (9)
Tractus opticus	0.63±0.03 (9)	0.55±0.03 (8)	0.62±0.05 (8)	0.48±0.04 (12)*	0.72±0.06 (9)
Colliculus superior	1.06±0.09 (11)	2.48±0.49 (8)**	1.63±0.16 (8)*	1.02±0.12 (12)	1.25±0.16 (10)
Pedunculus cerebellaris med.	0.87±0.11 (11)	1.26±0.19 (8)	0.77±0.13 (8)	0.57±0.05 (11)	1.09±0.19 (8)
Substantia alba cerebelli	0.41±0.03 (9)	0.62±0.07 (8)*	0.63±0.08 (8)*	0.46±0.04 (13)	0.48±0.07 (10)
Tractus corticospinalis	0.46±0.03 (11)	0.65±0.06 (8)*	0.59±0.04 (8)	0.49±0.05 (13)	0.46±0.06 (9)
Nucleus olivaris superior	2.09±0.34 (11)	3.37±0.18 (7)*	1.49±0.15 (7)	1.95±0.26 (13)	1.87±0.39 (10)
Flocculus	0.90±0.07 (9)	0.95±0.09 (8)	1.00±0.11 (8)	0.62±0.05 (9)	0.80±0.11 (9)
Pedunculus cerebellaris sup.	1.77±0.27 (11)	2.41±0.26 (8)	0.97±0.12 (7)	1.65±0.21 (13)	1.65±0.33 (9)
Pedunculus cerebellaris inf.	1.47±0.13 (10)	2.83±0.26 (8)*	1.02±0.15 (7)	1.37±0.14 (10)	1.82±0.36 (10)
Tractus spinocerebellaris vent.	0.73±0.05 (11)	0.80±0.06 (8)	0.68±0.12 (8)	0.49±0.05 (12)*	0.52±0.06 (8)
Nucleus principalis vestibul.	1.41±0.13 (11)	2.24±0.16 (8)*	1.38±0.19 (7)	1.85±0.32 (11)	1.67±0.34 (8)
Tractus solitarius	0.73±0.07 (10)	1.03±0.06 (7)*	0.82±0.12 (8)	0.64±0.05 (10)	0.89±0.13 (10)

[a] All values reported are mean ± SEM in ml/(min · g)
Figures in parentheses represent the number of experiments in which the blood flow of the particular structure was determined
(*, **, ***: Significantly different from control with $P < 0.05$, $P < 0.01$, or $P < 0.001$ after multiple comparisons)

The average flow and resistance values as given in Table I do not reflect the diversity of individual responses adequately since opposing effects in different structures can effectively cancel each other without changing the mean.

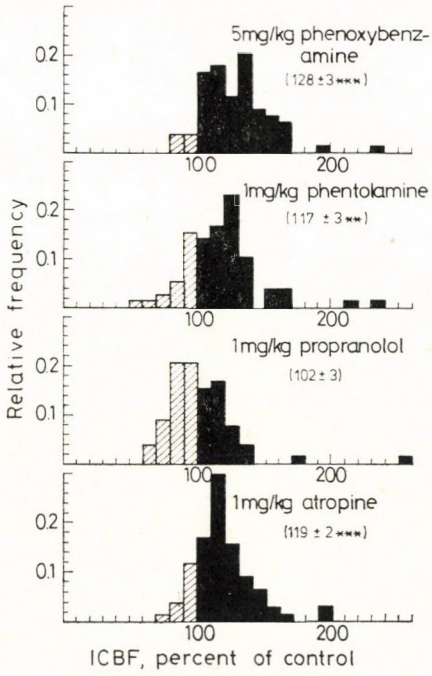

Fig. 1. Histogram of mean local cerebral blood flow (1CBF) values measured in 78 structures of the rat brain after the administration of different blocking agents. Values in parentheses stand for the average flow in percent of control. Statistical significance was calculated by the modified t-test according to Bonferroni after two-way analysis of variance (** $P < 0.01$, *** $P < 0.001$)

Figure 1 shows the actual variability of the evoked changes by displaying the frequency histogram of percent flow changes in each experimental group. The relative data for the histograms were calculated by the equation

$$1CBF\%_{0i} = 100 \cdot 1CBF_i/1CBF_{ic}$$

where $1CBF_{ic}$ is the mean flow of structure i in the control group. The percent changes in local vascular resistances are presented in the same way in Fig. 2.

The histograms clearly demonstrate that there is a large scatter of responses behind the mean values. The observed changes in local blood flow ranged from —14% (stria medullaris thalami) to +133% (colliculus superior) after phenoxybenzamine, from —44% (pedunculus cerebellaris superior) to

+134% (cingulum) after phentolamine, from —34% (pedunculus cerebellaris medius) to +153% (chiasma optici) after propranolol, and from —27% (tractus spinocerebellaris) to +93% (cingulum). However, structure by structure com-

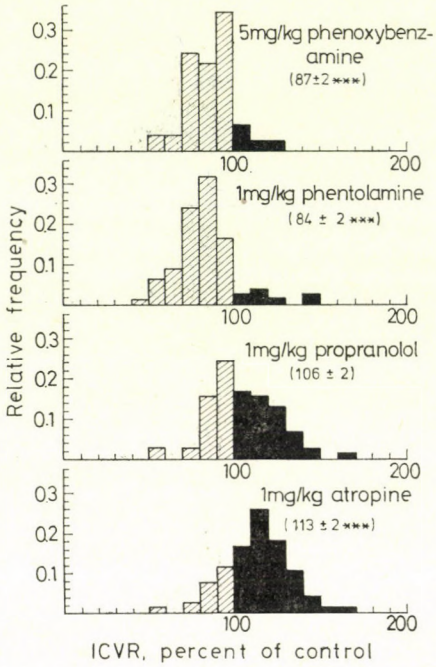

Fig. 2. Effect of different blocking agents on calculated cerebrovascular resistance (ICVR) of 78 anatomical structures. Values in parentheses represent the mean resistance expressed in percent of control. Statistical significance was determined as for the data in Fig. 1 (*** $P<0.001$)

parison of the drug effects revealed statistically significant differences only for the areas with the highest reactivity to the stimulus in question. Table III presents the blood flow data of all investigated areas where the null hypothesis of no treatment effects was discarded. Since total cerebral blood flow is determined largely by the blood supply of the brain cortex, it is worth mentioning that under the described experimental conditions no cerebrocortical region was influenced significantly by any of the drugs investigated.

Discussion

The morphological evidence for the adrenergic and cholinergic innervation of cerebral arteries has been known for several years [43], however, the discussion concerning the physiological importance of these findings is far from being

settled [20, 27, 45]. The conflicting results regarding certain aspects of neurogenic regulation make it very difficult to synthetize the findings, thus reviewers are sometimes constrained to the listing of contradictory data [12, 29]. The thorough analysis of quantitative cerebral blood flow data in the literature [31] suggests that neither the methodological differences nor the varying experimental conditions can account for all the deviations observed even in baseline measurements. The situation becomes even more complicated if the effects of different physiological stimuli are investigated since the reactivity of the cerebral circulation to many interventions depends also on the site of the actual measurement [4, 52].

Most studies in which measurements are made in multiple areas deal with cerebral structures that are either easily accessible or thought to be important for physiological phenomena. Our experiments were aimed at the evaluation of flow changes in as many as possible areas on the basis of autoradiographs, while no effort was undertaken to weigh the individual structures according to their actual size. For this reason the average flow and resistance values as referred to in this paper are to be used for comparison of the individual experimental groups only and should not be considered as physiological parameters. On the other hand, the average of per cent changes is a good estimate of the relative strength of the applied stimuli.

Cerebrovascular receptor blockade was chosen as the stimulus since it can be carried out without major surgery or interference with other factors known to affect the cerebral circulation. The need for simultaneous flow measurements in different areas with high spatial resolution made the application of a diffusible tracer technique combined with autoradiography obvious.

Blockade of α-receptors

The possible existence of an α-adrenergic vascular tone was investigated by the administration of two different blocking agents. Phenoxybenzamine and phentolamine are widely used α-blockers that inhibit norepinephrine induced blood flow changes both in vivo and in vitro [13, 17, 28]. The effect of phenoxybenzamine lasts over several days whereas phentolamine is dissociated from the receptors in much shorter time. Phenoxybenzamine passes the blood-brain barrier easily [37], and if it is administered as a pretreatment 24 hours before the experiment, its acute blood pressure reducing effect can be eliminated. Even though phentolamine has been employed in many intravascular applications, its passage accross the blood-brain interface may be limited [2].

Phenoxybenzamine was reported to increase [24, 39] or to decrease [10] the cerebral blood supply, or to have no effect [19, 48]. The administration of phentolamine increased the resting cerebral blood flow in some studies [1, 11, 34, 46] and had no significant effect in others [8, 28, 32, 53]. All experiments were performed in species other than rat.

The average lCBF increase in our studies lies in the range of the above mentioned investigations. Since both agents block the α-receptors competitively, the quantitative difference between their effect is probably related to the completeness of the blockade.

The tight coupling between cerebral blood flow and metabolism [54] raises the possibility that the observed changes are not mediated by vascular receptors, but reflect increased metabolism. Savaki et al. [51] investigated the effect of phenoxybenzamine and phentolamine on local cerebral glucose utilization. They found reduced metabolism in most structures, however, these changes might have been the consequence of the reportedly very low arterial pressure, decreased body temperature, and reduced arterial PCO_2, which were well controlled in our experiments. In some areas increased glucose consumption was observed, but only after the administration of rather high doses (21 and 42 mg/kg phenoxybenzamine, 0.5×85 and 0.85×85 mg/kg phentolamine). Since we used much lower doses in our studies, the flow increase was probably not caused by an underlying increase in metabolism.

Blockade of β-receptors

Propranolol is also able to penetrate from blood into the parenchyma [42], but the speed of passage may be regionally different [33, 36]. Some investigations found a decrease of cerebral blood flow due to propranolol [1, 3, 10, 42], others could not demonstrate any effect [7, 11, 32, 35, 39, 41, 48, 55]. In rat studies, cerebral blood flow did not change after intravenous propranolol administration [5, 6] except if it concurrently reduced the oxygen consumption of the brain [22]. In our studies, neither the average blood flow nor the vascular resistance differed significantly from control. This is in good agreement with the finding that the cerebral vasculature of rats is devoid of β-receptors [38].

Effect of intravenous atropine injection

Experiments with parasympathomimetic agents indicated that the muscarinic receptors of the cerebrovascular wall mediate vasodilatation [18, 30, 44]. Accordingly, the inhibition of the cholinergic tone, if there is any, should result in vasoconstriction. The flow increase observed in our experiments is therefore probably not the result of muscarinic receptor blockade. In one study [30], unspecific dilatation of pial arteries was observed after perivascular application of relatively high atropine concentrations, but the dose used in our experiments seems not to be excessive. It was also demonstrated that higher acetylcholine concentrations can induce contraction in isolated cerebral arteries and this effect could be blocked by atropine [43], but there is no evidence that comparable situation may occur under in vivo conditions. It is a possibility that the well

documented axo-axonal relationship between the cholinergic and adrenergic system [15, 40] is responsible for the observed phenomenon. However, since no previous study was able to demonstrate any change in cerebral circulation after intravascularly applied atropine [9, 11, 21, 25, 32, 49], our observations concerning this drug require further investigation.

The cerebral effect of atropine in our experiments is more complicated than simple receptor blockade since both the average flow and the average resistance were increased by this drug even though the increase in mean arterial blood pressure was found to be not significantly different from control. This apparent contradiction is due to the fact that the flow and resistance changes are based on much more values than the blood pressure data (78 versus 1 per experiment) and they are therefore not directly comparable. It must be emphasized in this context that the calculated vascular resistances represent only a rough estimate since the arterial pressure cannot properly reflect the cerebral perfusion pressure at the local level.

Reactivity of the circulation to neurohumoral stimuli

As it is evident from the histograms in Figs 1 and 2, the variation of responses to all investigated agents is very large. Even if the outliers are not considered, the range of evoked changes extends to approximately $\pm 40\%$ from the mean. It is likely that there is more than one factor that contributes to this heterogeneity, but it is difficult to estimate their individual participation in producing the observed variability.

The basic question concerns the accuracy and reproducibility of the method employed for the determination of local blood flow. According to the extensive comparison of quantitative tracer techniques [31], the tissue saturation method falls into the group of techniques with the highest accuracy. On the other hand, the sensitivity of the method to detect changes must be below average since only one measurement is possible in each experimental animal. Apart from the difficulty of identifying the same anatomical structure on different autoradiographs, animal to animal variations cannot be eliminated, and it is difficult to statistically substantiate small flow changes. Our results clearly reflect this problem since the highly significant changes over all investigated cerebral structures (Table II) could not be demonstrated in many individual areas compared with tests for independent samples (Table III). Similar experience was reported concerning the effect of intravenous carbacholine infusion measured with the ^{14}C-ethanol technique [44]. The authors found that the average flow increase of 16% in different areas of the cerebral cortex was significant according to the paired t-test, but the increase in the respective brain areas was statistically meaningless.

It must be noted that the above mentioned interindividual variability was significantly smaller in our control experiments than in any other group.

This strongly suggests that the effect of neurohumoral stimuli depends on factors that are not controlled under the usual experimental conditions.

The regional heterogeneity of the cerebral circulation as a physiological fact is well known. It has also been established that its reactivity varies from structure to structure. Our studies demonstrate that the differences can be both qualitative and quantitative. Obviously, if the effect is strong enough, only the quantitative differences will remain. In the case of neurohumoral studies, the inhomogeneities of barrier permeability (cf. [16]), innervation density [14] or receptor distribution could all account for some variance. However, the fact that the same stimulus can elicit opposing effects in different regions of the brain indicates a much more complex situation.

In conclusion, our data suggest that some of the contradictory findings concerning the neural regulation of the cerebral circulation might have been the result of regional differences whose mechanism needs further investigation.

Acknowledgements

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THE EFFECT OF LINEAR SOMATOSTATIN ON THE ACTIVE AVOIDANCE BEHAVIOUR AND OPEN-FIELD ACTIVITY ON HALOPERIDOL, PHENOXYBENZAMINE AND ATROPINE PRETREATED RATS

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Somatostatin administered intracerebroventricularly inhibited the extinction of active avoidance behaviour. The dopamine receptor blocking agent haloperidol, the α_1-receptor blocker phenoxybenzamine and the muscarinic anticholinergic agent atropine inhibited the behavioural effect of the peptide. Furthermore, somatostatin increased the locomotor activity of the animals. Neither of these drugs influenced the effect of the peptide exerted on locomotor activity. The peptide was ineffective on other parameters of the open-field test while phenoxybenzamine decreased the defecation rate of the animals and this effect was not influenced by somatostatin. The results suggest that the catecholaminergic and the cholinergic system play an important role in the inhibition of extinction produced by somatostatin but these mechanisms do not have a role in the locomotor activity induced by the peptide.

Keywords: linear somatostatin, active avoidance behaviour, haloperidol, phenoxybenzamine, atropine, locomotor activity.

The presence of the growth hormone release-inhibiting factor somatostatin in hypothalamic tissue was first observed by Krulich et al. [15] and a substance which suppresses growth hormone release was isolated by Brazeu et al. [4]. The distribution of this peptide in the central nervous system is well documented [6, 10] but the function of somatostatin in the organization of behaviour is less known [3, 19, 20].

Somatostatin is able to influence the locomotor activity (20, 26), the pentobarbital sleeping time and the strychnine induced convulsion [5, 7] while it induces scratching behaviour [11] and modifies self-stimulation behaviour [25].

Furthermore, somatostatin inhibited the extinction of active avoidance behaviour and increased the open-field activity in a dose related fashion [26]. The central dopaminergic, noradrenergic and cholinergic systems are known to play an important role in the organization of memory [9, 12, 14, 17] and motor behaviour [16].

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In the present study the role of the dopaminergic, noradrenergic and cholinergic systems was investigated in the effect of somatostatin on the extinction of active avoidance behaviour and open-field activity caused by the drugs haloperidol, a relatively specific dopamine receptor blocker [1], the α_1-receptor blocker phenoxybenzamine, [2] and the muscarinic anticholinergic agent, atropine [23] in the rat.

Methods

Animals

The experiments were performed on male CFY rats weighing 150–170 g. The animals were kept at constant temperature in artificial light, with 12 h light (started at 6 a.m.) and 12 h dark periods. A standard diet and water were given ad libitum.

Surgery

The animals were anaesthetized with pentobarbital-Na (Nembutal 35 mg/kg intraperitoneally i.p.) and a cannula was placed into the lateral cerebroventricle and fixed to the skull with dental cement. The rats were used after a recovery period of 7 days. Correct positioning of the cannula was checked by dissection of the brain [22, 24].

Behavioural methods

Active avoidance behaviour was investigated in a platform jumping apparatus described earlier [21, 22, 24, 26]. The rats were trained to avoid an electric shock to the paw by jumping onto a platform during 10 s of a conditional stimulus, the light was a 45 V bulb. Lack of performance during this time was associated with an electric shock of 0.2 mA. 10 trials were given daily, in a fixed intertrail interval of 55 s (range 50–60 s). On the fourth day, extinction trials were run and the unconditional stimulus was no longer applied. Animals which produced at least 8 conditional avoidance responses in 10 trials in the first extinction session were used for further experiments. These animals were allocated to different treatment groups and treated immediately after the session. In the first experiment haloperidol (G. Richter, Budapest) (20 μg/kg i.p.) or as a control saline was injected (Fig. 1, first arrow(I)) immediately after the extinction session. This was followed 30 min later by the intracerebroventricular (i.c.v.) administration of linear somatostatin (Serono, Italy) (1 μg/2 μl) or saline (2 μl) (Fig. 1, second arrow(II)). In the second experiment the phenoxybenzamine (Smith Kline French, Herts) (2 mg/kg i.p.) or saline administration (Fig. 2, I) was followed by the i.c.v. administration of somatostatin (Fig. 2, II). In the third session atropine (EGYT, Budapest) was administered (2 mg/kg i.p.) (Fig. 3, I) and this was followed 30 min later by the administration of somatostatin (Fig. 3, II).

Exploratory activity

The exploratory activity of the animals was measured by an open-field method. The animals were placed in an open-field box with 36 squares measuring 10×10 cm each. The activity was characterized by the total number of squares explored and the total number of rearings and groomings during the 3 min session. Somatostatin 1 μg in a volume of 2 μl was injected i.c.v. and the animals were tested 30 min later. In the first experiment haloperidol (Fig. 4), in the second phenoxybenzamine (Fig. 5) and in the third atropine (Fig. 6) was administered ip. in the same dose as in the active avoidance behaviour test 30 min before injecting the somatostatin.

Statistical analysis

Statistical evaluation of the active avoidance data was performed by the test of Kruskal-Wallis and the U-test of Mann-Whitney. In open-field activity the analysis of variance was used.

Results

Somatostatin delayed the extinction of the active avoidance behaviour 6 h ($p < 0.05$, Mann-Whitney; $p < 0.01$, Kruskal-Wallis' test) and 24 h ($p < 0.05$, Mann-Whitney; $p < 0.05$, Kruskal-Wallis' test) after treatment. Haloperidol had no effect on the extinction but blocked the inhibitory effect of the peptide (Fig. 1). Phenoxybenzamine which itself had no effect on the extinction of active avoidance behaviour also blocked the somatostatin induced inhibition of extinction at 6 h ($p < 0.05$, Mann-Whitney; $p < 0.01$, Kruskal-Wallis' test) after the test (Fig. 2).

Atropine in itself had no action on this behaviour, but at 6 h after treatment it blocked significantly the somatostatin induced effect ($p < 0.05$, Mann-Whitney; $p < 0.05$, Kruskal-Wallis' test) (Fig. 3).

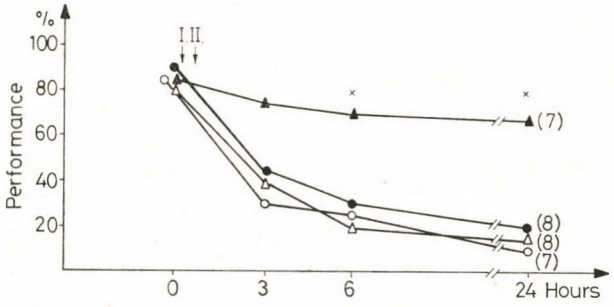

Fig. 1. Effect of somatostatin on extinction of the active avoidance response of haloperidol pretreated rats. △-------△ control, ▲-------▲ somatostatin (1 μg/2μl icv.), ○-------○ haloperidol (20 μg/kg ip.), ●-------● haloperidol + somatostatin. The first arrow indicates the administration of haloperidol the second the administration of somatostatin. Numbers in brackets show the number of animals used. Asterisk represents a significant difference

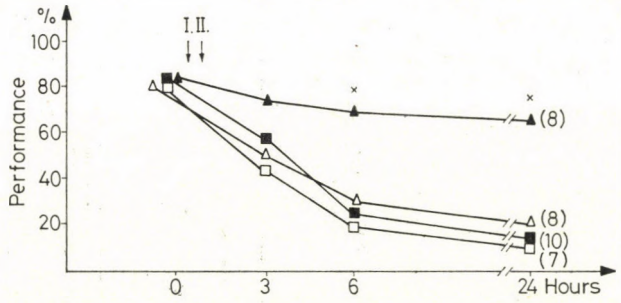

Fig. 2. Effect of somatostatin on extinction of active avoidance behaviour of phenoxybenzamine pretreated rats. △-------△ control, ▲-------▲ somatostatin (1 μg/2 μl), □-------□ phenoxybenzamine (2 mg/kg ip.), ■-------■ phenoxybenzamine + somatostatin. The first arrow indicates the administration of phenoxybenzamine, the second arrow the administration of somatostatin. Numbers in brackets show the number of animals used. Asterisk represents a significant difference

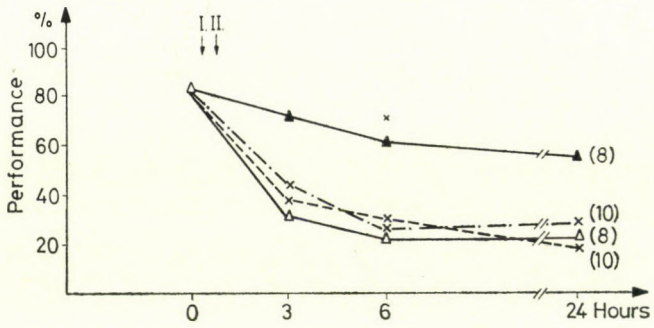

Fig. 3. Effect of somatostatin on extinction of active avoidance behaviour of atropine pretreated rats. △------△ control, ▲------▲ somatostatin (1 μg/2 μl), x------x atropine (2 mg/kg ip.), x—.—x atropine + somatostatin. The first arrow indicates the administration of atropine, the second the administration of somatostatin. Numbers in brackets show the number of animals used. Asterisk represents a significant difference

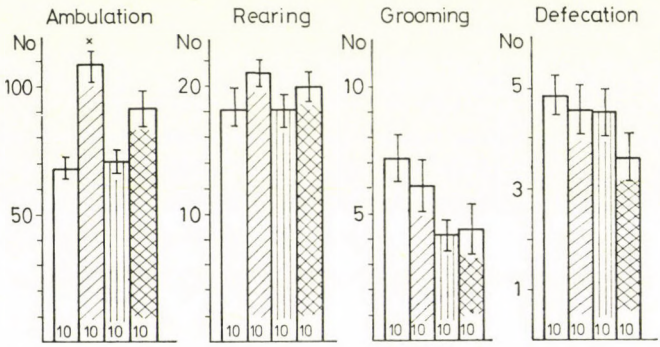

Fig. 4. Effect of somatostatin on open-field activity of haloperidol pretreated rats. □ control, /// somatostatin (1 μg/2 μl), ||| haloperidol (20 μg/kg ip.), ▨ haloperidol + somatostatin, x = $p < 0.05$ versus control (ANOVA). Numbers in brackets show the number of animals used

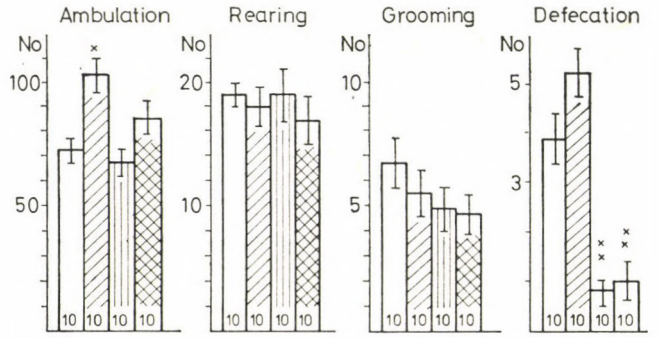

Fig. 5. Effect of somatostatin on open-field behaviour of phenoxybenzamine pretreated rats. □ control, /// somatostatin (1 μg/2 μl), ||| phenoxybenzamine (2 mg/kg ip.), ▨ phenoxybenzamine + somatostatin, x = $p < 0.05$ versus control (ANOVA), xx = $p < 0.01$ versus control (ANOVA). Numbers in brackets show the number of animals used

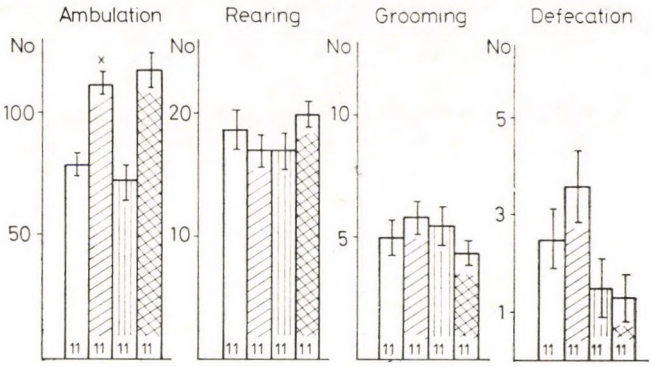

Fig. 6. Effect of somatostatin on open-field behaviour of atropine pretreated rats. □ control, /// somatostatin (1 μg/2 μl), ▥ atropine (2 mg/kg ip.), ▩ atropine + somatostatin, x = $p<0.05$ versus control (ANOVA). Numbers in brackets show the number of animals used

In open-field behaviour somatostatin increased the ambulation ($F/3.28/ = 3.12$, $p < 0.05$) but haloperidol pretreatment slightly but not significantly attenuated this effect (Fig. 4). Somatostatin also increased ($F/3.28/ = 3.42$, $p < 0.05$) the ambulation rate of the rats but phenoxybenzamine attenuated this effect slightly but not significantly and markedly decreased the defecation in somatostatin treated animals also ($F/3.28/ = 4.62$, $p < 0.01$) (Fig. 5). Atropine was ineffective on the activation of ambulation induced by somatostatin ($F/3.29/ = 3.35$, $p < 0.05$). After treatment with atropine the rate of defecation decreased but the change was not significant compared with the controls (Fig. 6).

Discussion

The role of extrahypothalamic somatostatin in the organization of behaviour is a controversial issue [3]. In the present study it inhibited the extinction of active avoidance behaviour.

The effect of haloperidol on a conditional avoidance response was investigated by Monti and Ruiz [17]. Haloperidol effected in a dose-related manner the avoidance behaviour, which suggested the importance of the dopaminergic system. Other theories [9, 14] postulate that the noradrenergic projection from the nucleus locus coeruleus to the hippocampus and cortex is fundamentally involved in learning and in memory consolidation. Furthermore, in most studies scopolamine and other cholinergic blocking agents were found to affect the performance of tasks involving discrimination, active avoidance response, passive avoidance response, and learning of fear (conditioned suppression) responses, whereas physostigmine generally improved learning [12].

In the present experiments haloperidol [1], phenoxybenzamine [2] and atropine [23] all antagonized the somatostatin induced inhibition of extinction, but the effect of atropine reached significance only 6 hours after its application. These results are in good agreement with the findings of Garcia-Sevilla et al. [13] who reported that somatostatin stimulated both the synthesis and utilization of dopamine and noradrenaline, suggesting an increased impulse flow in the neurons.

Concerning the cholinergic system Nemeth and Cooper [18] reported that somatostatin induced a release of acetylcholine from hippocampal synaptosomes, although this effect may not have been physiological. In behavioural experiments Cohn and Cohn [8] reported that somatostatin administered in high doses induced barrel-rotation. This behaviour was inhibited by atropine and this suggested that somatostatin may be acting, at least partly, through cholinergic mechanisms. In addition, atropine partly antagonized the effect of somatostatin on self-stimulation. These behavioural experiments suggested the important role of the cholinergic system in the behavioural effect of somatostatin.

Somatostatin facilitated the locomotor activity [20, 26] and haloperidol, phenoxybenzamine and atropine did not influence this effect. Phenoxybenzamine decreased the rate of defecation; this effect might be connected with the altered peristaltic activity [27].

The present results suggest that the somatostatin induced delayed extinction of active avoidance behaviour is mediated by dopaminergic, noradrenergic and cholinergic mechanisms. The same dose of the drug which was able to block the delayed extinction induced by somatostatin was not able to influence the locomotor activation induced by somatostatin. Still, higher doses of the receptor blockers than these applied by us might produce a complete block of the effects of somatostatin.

The difference between the two actions exerted on extinction and open-field activity suggests that different mechanisms are involved in the organization of somatostatin induced behaviour.

Acknowledgement

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MODIFICATION OF BURSTING IN A *HELIX* NEURON BY DRUGS INFLUENCING INTRACELLULAR REGULATION OF CALCIUM LEVEL

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The effect of ruthenium red, caffein and EGTA (ethyleneglycol tetraacetic acid) influencing intracellular Ca^{2+} level as well as that of pH-lowering was investigated on identified RPal neuron of *Helix pomatia* characterized by bimodal pacemaker (bursting) activity. Drugs were applied both extracellularly and intracellularly. Intracellular injection was performed from micropipettes by pressure.

It was found that intracellular injection of ruthenium red, caffein, EGTA and pH-lowering caused immediate short hyperpolarization and suspension of bursting. The effect of caffein and lowering of pH was biphasic, hyperpolarization was followed by an increase of spiking. Following EGTA injection the amplitudes of interburst hyperpolarizing waves decreased, and prolongation of spikes occurred.

Extracellular application of ruthenium red caused slight depolarization, while caffein produced mainly effects that were similar to those of the intracellular injection. Adding EGTA into the bath resulted in cessation of bursting, and later on also spike generation was blocked. All these effects could be eliminated by washing.

It is concluded that Ca-influx during spiking cannot be considered as a single factor in maintaining bursting activity, nevertheless, intracellular binding and liberation of Ca depending on the cell metabolism should also be taken into consideration as a possible mechanism of burst regulation.

Keywords: Helix, bimodal pacemaker, bursting activity, intracellular calcium, ruthenium red, caffein, EGTA.

According to a generally accepted idea the fluctuation of intracellular Ca^{2+} level is responsible for the slow oscillation of the membrane potential in bimodal pacemaker neurons of gastropods [14, 5]. This theory is based on the fact that in burst generating neurons potassium conductance shows Ca-dependent cyclic variations [9], elevated intracellular free calcium concentration $[Ca^{2+}]_i$ increases K-permeability of the membrane resulting in hyperpolarization, while lovering of $[Ca^{2+}]_i$ evokes an opposite effect. Influx of Ca^{2+} during spiking was demonstrated by direct and indirect methods [10, 28] and it was also shown that intracellular injection of Ca^{2+} into the neuron increases K-permeability and evokes hyperpolarization [16, 17]. Free Ca^{2+} concentration of the neuron which is elevated during spiking decreases comparatively rapidly due to binding to intracellular particles and extrusion from the cell and, as a

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result, K-permeability of the membrane returns to the previous level. When hyperpolarization is eliminated the cell starts to fire again and the cycle of the oscillation of membrane potential is initiating again.

Two basic conclusions follow from this theory: (1) Waving of the membrane potential occures only if the neuron is spiking; (2) When neuron is spiking, hyperpolarization should occasionally brake the activity. However, in earlier investigations carried out on the bimodal pacemaker neuron of Helix pomatia (RPal cell, 23) we found that after some pharmacological treatment (TMA) the cell produced membrane oscillation without spiking [32]. The same was observed after application of methyonine enkephalin to the soma [27]. On the other hand, in the RPal neuron kept at 5–8 °C, activity is present without appearance of hyperpolarizing waves and also long synaptic activation of the neuron does not cause long hyperpolarization [25], what would be expected as a result of Ca^{2+} increase inside the cell. For this reason the idea may be considered that the cyclic change of membrane permeability is not coupled entirely to Ca-influx during spiking but, in the fluctuation of intracellular Ca-level significant role should be ascribed to the intracellular metabolism regulating Ca release and binding. Such a possibility is supported by the fact that increasing the level of intracellular cyclic nucleotids both in Aplysia R15 neuron and in Helix bimodal pacemaker cell hyperpolarization can be evoked [31] supposedly through an intracellular mechanism other than Ca-influx.

In the present investigations we wanted to obtain data whether drugs affecting intracellular Ca^{2+} level can influence the course of the bimodal pacemaker activity, what could be an indirect evidence to the understanding of the mechanism of slow waving in bimodal pacemaker neurons.

Materials and methods

Suboesophageal ganglionic ring of *Helix pomatia* L. was isolated and pinned in perfusion chamber containing 1.5 ml physiological saline. Removing the connective tissue of the right parietal ganglion the RPal neuron became well visible. Microelectrodes filled with 2.5 mol/l KCl (5–10 MOhm resistance) were used for recording membrane and action potentials. Electrical signals were fed into a negative capacitance (FET) amplifier [35], they were visualized on a Tektronix oscilloscope and recorded on a MFE 3M3V recorder.

Intracellular injection

For intracellular application of drugs micropipettes with about 2 μm tip diameter were used, and injection was carried out by pressure. To inject a controlled amount of solution into the cell we developed an appropriate system which was calibrated within 1–1000 picolitre (pl) range using a large number of micropipettes with the same diameter. The working pressure of the N_2 balloon was 10 bar, which could be decreased to 1–10 bar with a second pressure-regulator (Festo, typ: FRC-1/8). The duration of the pressure within the micropipette was regulated through an electronically commanded pressure valve (MECMAN, Typ: 4432-10-434), which could be timed in two steps (10–100 ms or 100–1000 ms). The circuit is shown on Fig. 1. The system was supplied by +24 V battery for the pressure valve and +5 V battery for the timing circuit.

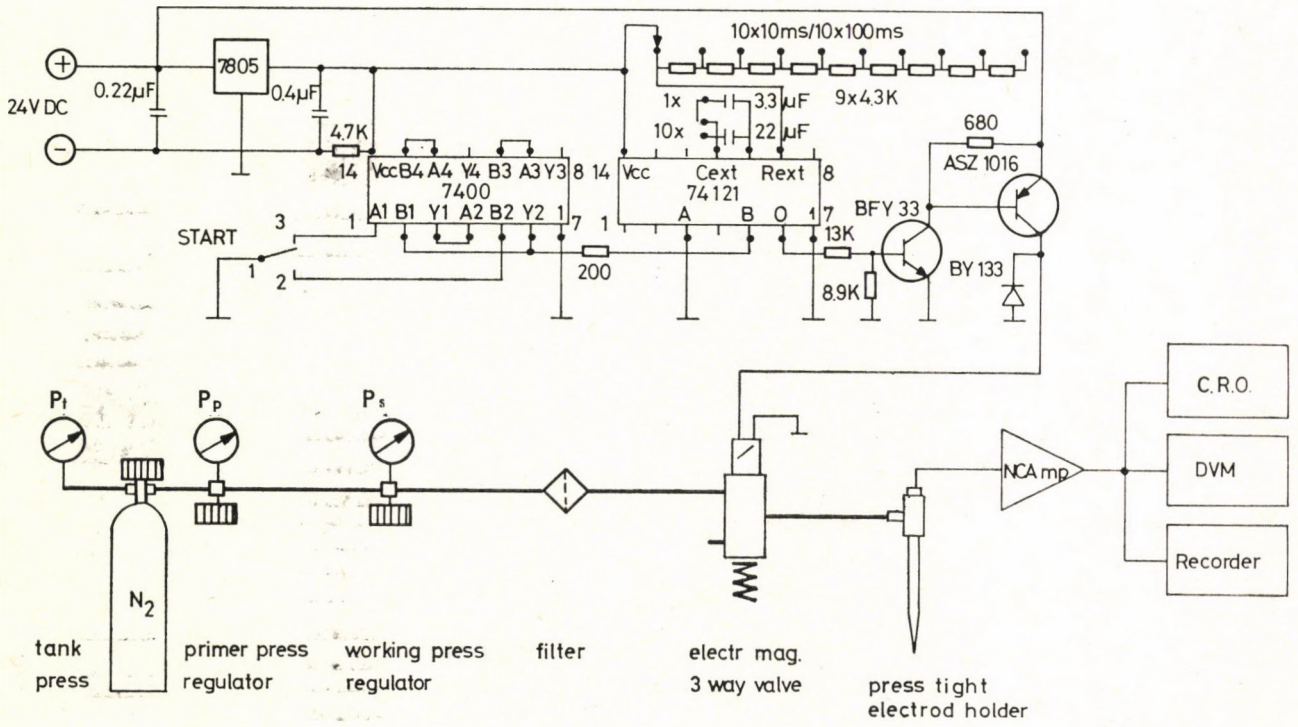

Fig. 1. Design of electrical circuit constructed for controlled injection of substances and scheme of the system. N_2 = nitrogen tank; P_t = tank pressure; P_p = primary pressure; P_s = secondary pressure; NC.Amp = negative capacitance amplifier; CRO = cathode ray oscilloscope; DVM = digital voltmeter

A special holder was constructed to keep the micropipette stable during pressure, and at the same time to assure fast change of the micropipette when necessary. With the system also electric signals could be recorded assuring the control of penetration of the micropipette into the cell.

Solutions

The composition of the physiological solution used in the bath was (in mmol/l): NaCl, 60; KCl, 4; $MgCl_2$, 12; $CaCl_2$, 10; Tris-HCl, 5; pH was adjusted to 7.6. In Ca-free solution Tris was replaced for Ca.

In extracellular application we used the following solutions: 2.5 mmol/1 EGTA in Ca-free physiological solution; 0.5 mmol/l ruthenium red and 3 mmol/l caffein solved in physiological saline.

Substances for intracellular injection: 0.1 mol/l $CaCl_2$; 0.1 mol/l K-EGTA (0.1 mol/l EGTA, 0.3 mol/l KOH adjusted with HCl to pH 7.2); 0.5 mmol/l ruthenium red; 0.05 mol/l caffein. For lowering intracellular pH we injected 10 μM/l HCl complemented with 0.05 mol/l KCl for assuring recording of voltage changes.

Experiments were carried out at room-temperature (20–24 °C).

Results

Effect of direct change of intracellular calcium concentration

Intracellular Ca^{2+} concentration was increased by about one magnitude with microinjection of 0.1 mol/l $CaCl_2$ into the cell. The injected amount was calculated according to the volume of the neuron ($\sim$ 4000 picolitre). Injection of 0.4 pl $CaCl_2$ caused 40–50 s long clamping of the membrane potential at a comparatively high level (Fig. 2).

When K-EGTA, a Ca-binding compound was injected into the neuron, an initial hyperpolarization occurred than the amplitude of the subsequent interburst hyperpolarizing phases decreased to about a half (Fig. 3). At the same time there was also a change in the spike generation: the last action potential of the burst became characterised by a long plateau referring to the damage

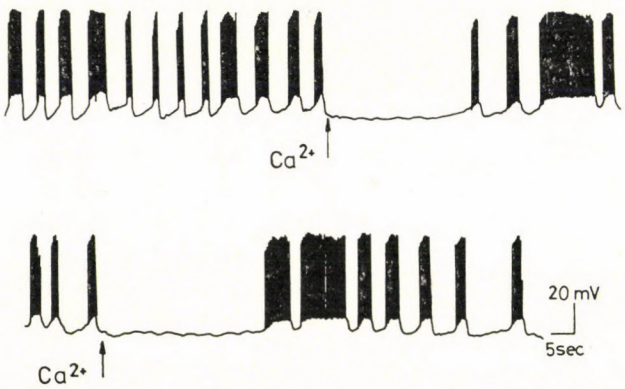

Fig. 2. Effect of intracellular injection of $CaCl_2$ on the bursting activity. Repetition of injection after 90 s

of the mechanism of repolarization. The number and amplitude of spikes within the burst did not change.

In contrast to this, in Ca-free bath supplied with K-EGTA in order to bind even Ca^{2+} leaving the cell, together with eliminating slow waving of the membrane potential, also the generation of action potentials was influenced markedly (Fig. 4). First the overshoot was eliminated, but after about 25 min the generation of action potentials was totally blocked. The effect was reversible by washing: first frequent spiking occurred, and in a few minutes also the bursting of the neuron was restored. The effect of extracellular EGTA on the RPal neuron was reported previously [32].

Effect of indirect change of Ca-concentration

a. Ruthenium red. Mitochondria are storage places for Ca also in nerve cells and carrier mechanisms of internal mitochondrial membrane control the calcium distribution between the cytosol and the mitochondrial matrix [22]. Ruthenium red inhibits uptake of Ca by isolated mitochondria without effecting release of Ca [34, 20].

To test whether influencing mitochondrial kinetic control of intracellular calcium has an effect on the membrane potential or not, we injected 80 p

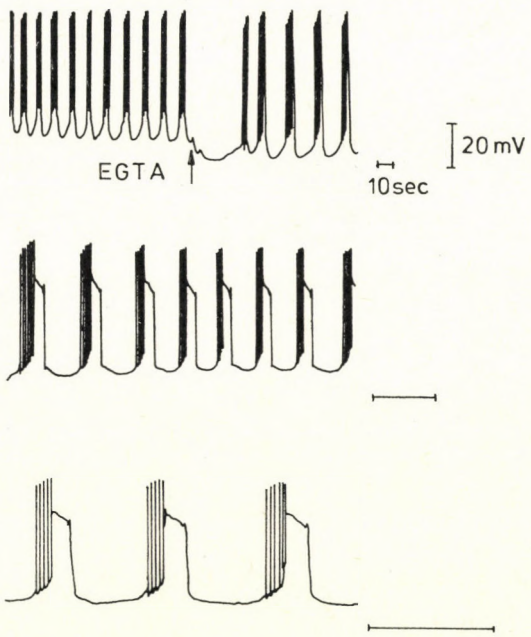

Fig. 3. Intracellular injection of EGTA into the bursting neuron. Upper, middle and lower records are immediately continuous, only the speed of the beam was changed, as shown on the time scale

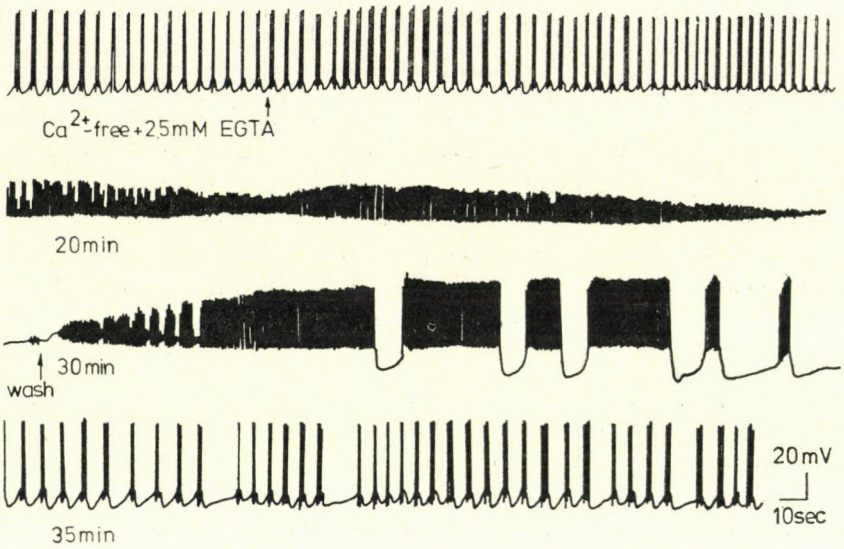

Fig. 4. Effect of extracellular application of EGTA added to Ca-free saline on the bursting activity. Minutes mark the time elapsed from EGTA application

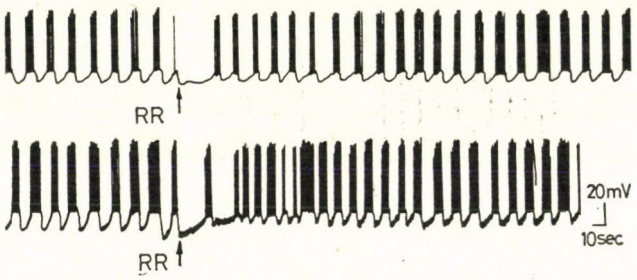

Fig. 5. Effect of intracellular injection of ruthenium red into the bursting neuron, in two preparations

ruthenium red into the neuron. We found that even about 10 μmol/l intracellular ruthenium red concentration caused only a short hyperpolarization and a transient inhibition in the potential generation (Fig. 5).

At the same time, if ruthenium red was applied extracellularly into the bath (0.5 mmol/l), the threshold of bursting was shifted by about 15 mV to the positive side, and as a result the frequency of bursting was decreased significantly (Fig. 6). This effect proved to be irreversible.

b. Caffein. One of the main physiological effects of caffein is the release of Ca from the intracellular storage structures [36, 13], the other being the activation of adenyl cyclase resulting in the increase of cyclic nucleotid level within the cell [8].

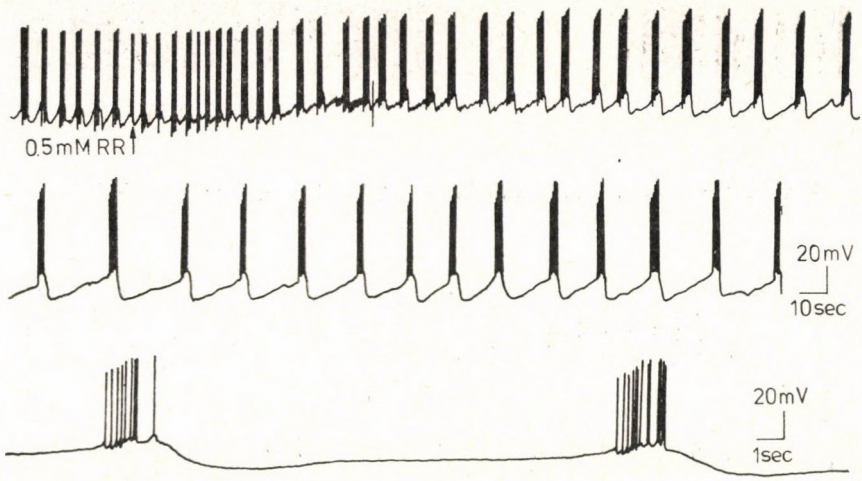

Fig. 6. Effect of ruthenium red added to the bath on the activity of the bursting neuron. The middle record is the immediate continuation of the upper one; lower: after 10 min, with 10 times higher speed

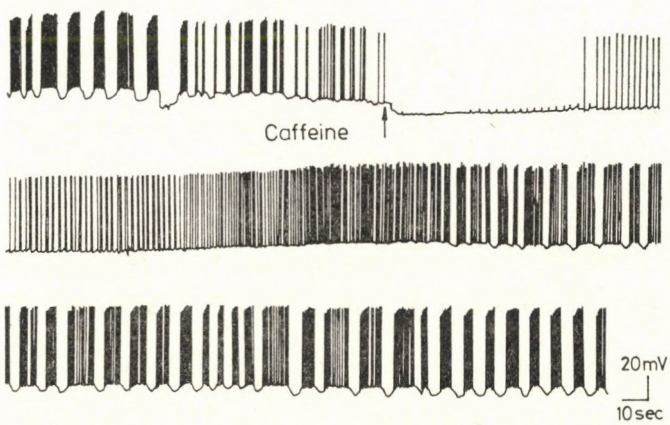

Fig. 7. Effect of caffein injected into the bursting neuron. Continuous recording

In the experiments we injected varying amounts of 0.05 mol/l caffein into the neuron. As a result a biphasic effect could be observed (Fig. 7): first a hyperpolarization occurred lasting for about 80 s, when spiking was blocked. In the second phase inhibition was gradually eliminated (within 5 min), generation of spikes was restored and, reaching the original level of membrane potential, slow oscillation and bursting started again.

When applied extracellularly, caffein (3 mmol/l) changed very potently the activity of the bursting neuron (Fig. 8). Following a short hyperpolarization there was a 15 mV depolarization of the membrane and the interburst hyper-

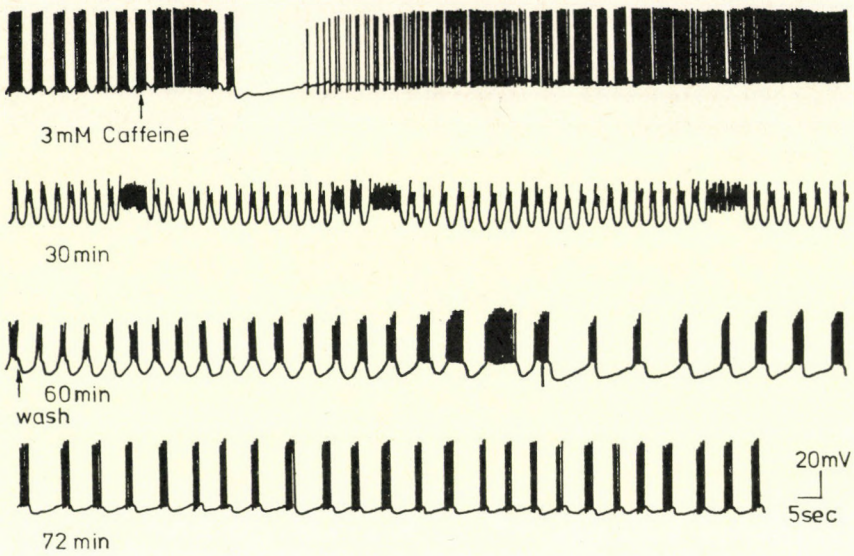

Fig. 8. Effect of caffein added to the bath on bursting activity. Minutes mark the time elapsed from caffein application

polarizing waves disappeared. After about 15—25 min a membrane potential oscillation occurred, which corresponded to the original bimodal pacemaker rhythm, but its amplitude was about three times higher, and no real spikes were present. Also the generation of action potentials changed characteristically, bursts were composed of 3–4 abortive spikes with decreased amplitudes. This effect was reversible, it could be eliminated by washing in a few minutes.

Effect of intracellular pH-lowering

Injection of Ca and other divalent cations and also of EGTA into invertebrate neurons causes, besides an increase of potassium permeability, also a fast, transient lowering of pH [18, 1]. According to our results shown on Fig. 9, injection of 10 μM/l HCl was able to hyperpolarize the membrane. The effect was biphasic: first depolarization lasting for about 15 s occurred, which was

Fig. 9. Effect of HCl injection into the bursting neuron. Continuous recording

followed by hyperpolarization for about 80–90 s. The control activity of the cell returned in about 5 min. Injection of the same amount of KCl at pH 7.4 did not cause similar change in the activity of the neuron.

Discussion

It has been demonstrated earlier that extracellular calcium plays an important role in the spike generation in a number of molluscan neurons [21, 3, 10, 28, 24]. It is also generally accepted that in bimodal pacemaker cells intracellular free calcium takes part in the regulation of the slow membrane oscillation through regulating potassium permeability of the membrane [9, 7, 14, 15, 11]. Our results are in accordance with these data since the bursting activity of the bimodal pacemaker neuron of *Helix pomatia* (RPal cell) was significantly modified by influencing directly or indirectly the intracellular Ca-level. Direct Ca-injection evoked hyperpolarizing wave, due probably to activation of a Ca-dependent K-current [17, 4]. Injection of EGTA decreased the amplitude of the interburst hyperpolarization what can be interpreted as the result of reduction of $[Ca^{2+}]_i$ and Ca-dependent potassium current as a consequence of Ca-binding. The protracted plateau appearing on the last spike of the bursts refers to the damage of the activating mechanism of the fast potassium current. These results are in agreement with the data of Meech obtained on *Helix aspersa* [14] and *Aplysia* [15].

Besides Ca^{2+} entering the cell during spikes intracellular Ca-binding mechanisms play an important role in the regulation of the intracellular Ca-level. By altering Ca-binding the increased potassium permeability activated by calcium ions could be prolonged [19]. Therefore it cannot be excluded that in the regulation of potassium permeability intracellular Ca-release is also involved. For this reason the conclusion that only Ca-influx occurring during spikes is responsible for the regulation of the bimodal pacemaker mechanism [5] should be accepted with reservation. The cycle of Ca-influx → increase of $[Ca^{2+}]_i$ → increase of K-permeability → Ca-binding → decrease of $[Ca^{2+}]_i$ → decrease of K-permeability → spiking, seems to be problematic as a single mechanism because it cannot explain the fact that a slow oscillation of the membrane potential may occur even in case when no spiking is present [29, 32, 27], consequently no Ca-influx exists. We suggest that one should consider also an additional mechanism which contributes to the oscillation of the membrane potential through releasing and binding of the intracellularly available Ca.

Our earlier data [25, 33] showing that intracellular injection of DNP, $CdCl_2$ or $HgCl_2$ can depress slow waving without stopping cell activity support the existence of such an intracellular oscillatory mechanism. Hyperpolarization evoked by injection of caffein and by lowering intracellular pH as well as the

slight effect of intracellular ruthenium red reflect intracellular processes which are certainly independent of Ca-influx and may serve as arguments for suggesting an intracellular cyclic mechanism. Caffein is capable of releasing Ca^{2+} from storage places as it was described for sympathetic ganglion cells [12, 13], while ruthenium red may prevent binding of Ca^{2+} to mitochondria [34], not only of that portion which enters the cell during spiking [19] but also of that is liberated inside the neuron.

The role of intracellular metabolic processes in the oscillation of bimodal pacemakers is supported also by the investigations of Treistman and Levitan [31] showing that increase of cyclic nucleotid level within the cell either through activating adenylate cyclase or as a result of direct injection of c-AMP causes hyperpolarization of R15 neuron of Aplysia. Taking this in consideration it may be suggested that caffein has a biphasic effect, the fast effect being the result of intracellular Ca-release, while the late one is caused by increase of c-AMP level since caffein activates adenylate cyclase [8]. However, in our study this latter effect was not an unambiguous hyperpolarization, only depression of slow oscillation occurring for a few minutes without affecting spiking. In contrary to that caffein added to the bath and so having a long time effect caused increase of the frequency of oscillation and alteration of spiking, an effect which was reversible.

The theory of bimodal pacemaker regulation built on Ca-influx can be subjected to criticism also because it has recently been shown that Ca-influx during spikes may evoke various permeability changes on the membrane. Standen [26] has found that increase of Ca^{2+}_i inactivates Ca-channels for a short time which, besides increasing K-permeability, can contribute directly to hyperpolarization. The multifunctional role of Ca is emphasized also by the results of Hofmeyer and Lux [6], showing that there is an initial inward current following intracellular injection of Ca. They have made the conclusion that the change of K-permeability caused by Ca-injection is not a direct effect of Ca on the membrane, but it realizes itself through the cytosol, by influencing some intracellular processes. Nevertheless, it should be noted that the effect of Ca^{2+} on the internal surface of the membrane causing increase of K-permeability was proved on isolated, intracellularly perfused neuronal membranes [11].

Probably the effect of divalent cations on the pH of the neurons [19] cannot be neglected as well, although usually it is believed to be insignificant due to the high puffer-capacity of cytosol [30]. It is probable that the decrease of pH originates from the functioning of mitochondria, since uptake of cations results in release of protons [22, 20]. Injection of EGTA causes also lovering of pH [1]. Hyperpolarization evoked by injection of HCl (Fig. 8) can be a result of increased $Ca^{2+}{}_i$ [1], but also of alterations of other intracellular processes.

Our aim was to obtain data on the direct effect of substances influencing intracellular metabolism in cells exhibiting continuous bimodal activity and on the basis of the present results no direct conclusions can be made about specific changes of membrane permeability or ionic currents. However, both our data and a number of other investigations show that one should consider the possibility that the slow oscillation of the membrane potential in gastropoda neurons is regulated not by the cyclic character of the Ca-influx occurring during burst, but it is rather determined by an intracellular metabolic cycle. Ca-influx and the cyclic change of Ca-dependent potassium permeability is superimposed to this oscillation, increasing its amplitude. Such an idea is supported also by the data of Drake and Treistman [2] showing that usually non-bursting neurons can be transformed to bursting ones by increasing intracellular cyclic nucleotide level, what means that the properties of the underlying processes resulting in a bimodal pacemaker activity are not inherent for the membrane itself.

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EFFECT OF WEIGHTLESSNESS ON THE FUNCTION OF RAT SKELETAL MUSCLES ON THE BIOSATELLITE "COSMOS-1129"

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Isometric tension of glycerinated fibrils of different skeletal muscles was studied in white rats carried in the biosatellite "Cosmos-1129" for 18.5 days. Expression and trends of the changes in contractile properties of muscle preparations depend on their functional specialization. The most sensitive reaction was displayed by the soleus muscle which consists predominantly of slow fibres.

Keywords: Cosmos-1129, weightlessness, muscle, contractility

In experiments performed aboard the biosatellites Cosmos-605 and 936, Oganov et al. [1, 2, 3] observed a decrease in muscle function and changes in the metabolism and structure of muscles [5]. In order to study muscular atrophy, changes in the contractile properties of muscles and structural changes in myofibrillar proteins in the state of weightlessness, a new biosatellite experiment was performed.

Materials and methods

Male Wistar rats weighing about 300 g were exposed to weightlessness for 18.5 days during a space journey in the biosatellite Cosmos-1129. As in previous experiments, the soleus, extensor digitorum longus (EDL), brachialis and triceps (medial head) muscles were studied.

As earlier, the soleus muscle was studied as a representative of a slow muscle, the EDL as one consisting mainly of fast fibres, and the brachialis and triceps (medial head) muscles as ones with mixed properties.

The rats that participated in the space flight were called the flight (F) group. In addition there were two control groups: one made up of animals living under terrestrial conditions (air pressure, temperature, O_2-content etc.) identical to those on the biosatellite (S) and another group, containing rats living free in the vivarium (V).

The contractile properties of muscles were studied using glycerinated muscle fibres. Glycerol treatment destroys the sarcolemma, the sarcoplasmic reticular system, the mitochondria and other cell organelles. The structure of myofibrils is identical with the living muscle, thus the glycerinated fibre can be regarded as an organised actin-myosin-tropomyosin-troponin system permeable to ATP, cations and anions [6]. Details of the experimental procedures have been described elsewhere [10]. Care was taken to maintain the original muscle length during

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the study first by means of 50% glycerol (containing 0.006 67 M phosphate buffer pH 0.7; temperature 0 °C) then after 30 minutes with slightly hypotonic salt solution (0.1 M KCl, 0.001 M $MgCl_2$, 0.006 67 M phosphate buffer pH 7.0; temperature 0 °C). The procedure was repeated two more times, then the muscle was stored in 50% glycerol at 0 °C for 24 hours and then at −20 °C until required.

The results of this treatment was that the preparations could be used for experiments as early as after 48 hours, furthermore that they have kept their contractile properties for 2–3 months or more.

Isometric contractions were registered using a differential capacitance transducer which gave a linear response within the range of 0–2.5 g. The solutions used contained 0.1 M KCl, 0.005 M $MgCl_2$ and 0.005 M histidine buffer pH 7.0. The relaxating solution contained in addition 0.004 M EGTA and 0.005 M ATP, while the contracting solution contained 0.00015 M EGTA, 0.00015 M $CaCl_2$ and 0.005 M ATP.

The contractile parameters were measured in a series of experiments and evaluated statistically by Student's t test; the level of significance was set at $p < 0.05$.

Results and discussion

The muscles of the rats in the flight group showed a considerable loss of weight. Compared to the animals in group V a significant loss of weight was observed in all muscles of the S-group rats. In the rats which participated in the spaceflight (F) the weight loss of the muscles was highly significant as compared to both control groups. The most pronounced difference was found with the soleus muscle.

Results for the tension of muscles are summarized in Fig. 1. The muscular contractions of the animals in group V were taken as 100%, and the values for groups F and S were expressed in percents of the former. Maximum tension of the soleus muscle decreased in group S too, but in group F the decrease was much greater — and highly significant in comparison to group S — after the 18.5 days weightlessness. The maximum tension of the triceps (caput mediale) and brachial muscles also decreased in both groups S and F and here the decrease was again much greater in the muscles of group F animals. In contrast maximum tension of the EDL muscle, in spite of slight differences did not significantly differ in groups V, S and F.

To evaluate the results for the speed of contraction the values obtained for group V were taken as 100% and the data found in groups S and F were

Table I

Effect of weightlessness on the weight of the soleus, EDL, brachialis, triceps muscles

	Muscle weight, mg			
	m. soleus	m. EDL	m. brachialis	m. triceps (caput mediale)
Vivarium (V)	184.29±6.73	173.00±7.91	166.43±12.80	137.43±11.27
Synchron (S)	134.29±12.05^{+++}	153.43±4.12^{++}	143.43±7.27^{+++}	105.00±5.32^{++}
Flight (F)	80.57±8.30^{+++}	108.86±11.87^{+++}	112.29±11.87^{+++}	79.29±8.83^{+++}

$^{++}$ $p < 0.01$
$^{+++}$ $p < 0.001$

related to these (Fig. 2). The brachial and triceps muscles of the rats participating in the spaceflight (F) showed a slight but significantly lower velocity than the corresponding muscles of groups S. In contrast there was no change in the speed of contraction of the EDL muscle, whereas that of the soleus muscle rather increased.

Readaptation of the muscles after the spaceflight was also studied. It was found that as early as on the 6th day weight of the EDL and brachial muscles returned to normal. It took, however, about four weeks before all the muscles had completely regenerated.

Under spaceflight conditions developed all the muscles underwent some degree of atrophy. The most pronounced change was observed in the soleus muscle. A smaller degree of atrophy was also found in animals belonging to

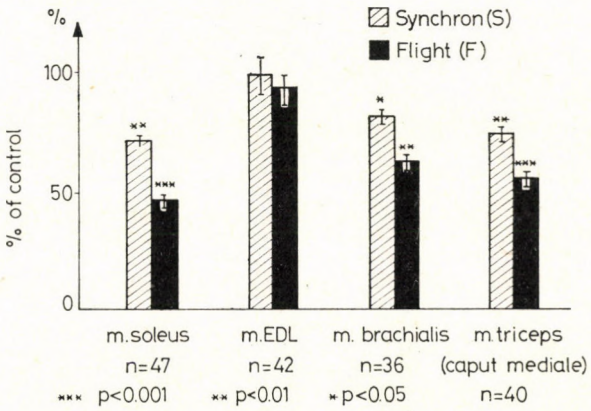

Fig. 1. Effect of weightlessness on the ATP-Ca^{2+}-induced maximum tension of glycerol-treated rat skeletal muscle preparations

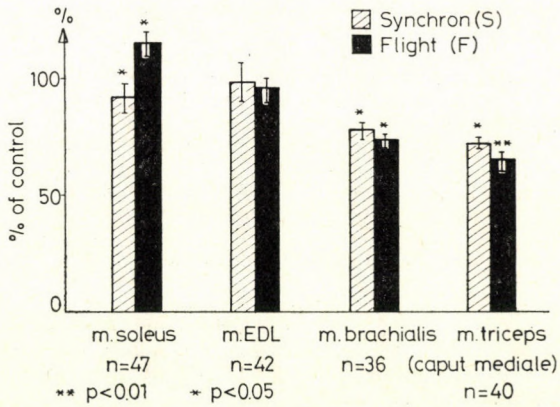

Fig. 2. Effect of weightlessness on the rate of ATP-Ca^{2+}-induced enhancement of tension of glycerinated rat skeletal muscle preparations

group S. These animals were restricted in their movements as those in group F and in addition several conditions including O_2 supply and feeding possibilities were also less favourable for them than for animals living in the vivarium. The present results were in good agreement with those of our earlier model experiments [3, 4, 7, 8, 9, 11]. In the immobilization experiments the severest atrophy was found in the soleus muscle. This might be indicative of a correlation between atrophy and the functional and dynamic specialization of the muscles.

In the soleus of group F PAG electrophoresis showed that the amount of LC_3 light chain increased in correlation with the degree of atrophy, indicating a transformation of type I fibres to type II fibres [12]. Evaluating the changes of the contractile parameters of the soleus muscle in groups F and S, it could be established that weightlessness alters the slow antigravitational function to a fast one. Such a change cannot be found in animals whose movements were restricted without being exposed to weightlessness.

We do not know what kind of role is played in the phenomenon by the genetic apparatus regulating the synthesis of myofibrillar proteins or by the mechanisms regulating muscle functions.

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EFFECT OF WEIGHTLESSNESS ON MYOFIBRILLAR PROTEINS OF RAT SKELETAL MUSCLES WITH DIFFERENT FUNCTIONS IN EXPERIMENT OF BIOSATELLITE "COSMOS-1129"

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The composition of contractile and regulatory proteins was studied in rat muscles with different functions. The rats were exposed to weightlessness for 18.5 days during a space journey in biosatellite Cosmos-1129. Under the effect of weightlessness the myosin light chain composition changed, the quantity of myosin LC-3 subunits increased in the soleus and extensor digitorum longus (EDL) while it decreased in the triceps and brachialis muscles. The experiments showed changes in the subunit composition of the TN-TM complex, too. The results obtained are in favour of a possible adequate transformation of fibril phenotypes of some (antigravitational) muscles under the effect of spaceflight.

Keywords: weightlessness, muscle proteins, myosin LC subunit, TN-TM complex

Previous investigations on muscles of rats exposed to weightlessness demonstrated signs of functional inadequacy of the skeletal muscles, being most distinct in the soleus [9, 13]. According to Gazenko et al. [3] metabolic and hormonal changes as well as atrophy of skeletal muscles are attributable to the space environment i.e. the lack of muscle activity. It was postulated [15] that atrophy of muscles and adaptive transformation of their contractile properties following 20—22 days' spaceflight may reflect changes in the protein composition of myofibrils. The purpose of the present experiments was to verify this concept in experiments on skeletal muscles of animals flown for 18.5 days aboard the biological satellite "Cosmos-1129".

Materials and methods

In the biosatellite "Cosmos-1129" male rats of 300–360 g participated in a spaceflight of 18.5 days (group F). In addition there were two control groups, one of animals living free in the vivarium (V), and another containing rats living in the laboratory under conditions, as described by Gazenko [4], synchronous on the biosatellite. The F and control (V, S) groups

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were sacrificed at the same time and the soleus, triceps (caput mediale), brachialis, and extensor digitorum longus (EDL) muscles were excised weighed and frozen in liquid N_2. For biochemical experiments the samples were divided into three parts: about 5–8 mg for the determination of total protein; 20–25 mg for the preparation of myofibrils, and the remaining part for the preparation of soluble proteins [14, 18]. The protein composition of myofibrils was studied from 20–25 mg preparations dissolved in 1% SDS, 1% mercaptoethanol using 7.5% SDS containing polyacrylamide gel according to the method of Weber and Osborne [19]. The subunit composition of troponin-tropomyosin (TN-TM complex) was examined by the method of Greaser and Gergely [5].

Results and discussion

Results of the analysis of the composition of myofibrils is seen in Table I. To illustrate the changes, only the relative quantities of myosin light chains will be detailed. Under the effect of weightlessness the quantity of LC-3 fast myosin subunit increased in the soleus and EDL, while it decreased

Table I

Percentual distribution of myosin subunits in the muscles investigated, taking the sum of light chains as 100% (n = 8)

	Muscles					
	soleus			EDL		
	V	S	F	V	S	F
LC-1F	11.7	14.9	12.1	44.0	34.3	32.6
LC-2F	4.4	2.7	6.9	39.3	29.2	32.8
LC-3F	1.9	3.7	8.2	3.5	9.4	6.2
LC-1S	50.4	50.8	37.4	5.3	1.4	1.2
LC-2S	31.5	27.9	35.2	7.8	25.5	27.0
F%	18.1	21.2	27.3	86.8	72.9	71.6
S%	81.9	78.8	72.6	13.1	27.0	28.3

	Muscles					
	brachialis			triceps (caput mediale)		
	V	S	F	V	S	F
LC-1F	30.9	30.8	32.2	29.8	24.2	27.1
LC-2F	43.5	31.5	31.5	34.3	22.9	18.8
LC-3F	10.6	10.0	7.1	10.6	9.2	8.2
LC-1S	1.8	1.9	5.4	8.6	17.8	8.2
LC-2S	13.1	25.7	23.7	16.7	28.5	37.7
F%	85.0	72.3	70.8	74.7	56.3	54.1
S%	14.5	27.7	29.1	25.3	44.2	45.9

in the triceps and brachialis muscles. The changes in the LC-1 fast subunit were not parallel with those in the LC-3 subunit. This means that the synthesis of the homodimer LC-3 myosin population was more pronounced under the experimental conditions. The EDL, triceps (caput mediale) and brachialis muscles are of fast-twitch, glycolytic or mixed type. The quantity of fast light chains in these muscles represents 70–80% of the total light chain peptides while the slow myosin light chains represent about 20–30%. In the fast muscles exposed to weightlessness the relative quantity of slow light chains inoreased. In the EDL and triceps only the LC-2S showed an about three-fold increase (from 7.8% to 27.0%), but in the brachialis both LC-1S and LC-2S increased. This means that in the fast muscles the slow myosin population accumulated to twofold during flight.

Similar changes occurred in the muscles of synchronous groups. These data are in good agreement with our previous experiments on rabbits [16] and rats [17] confirming that the effects of weightlessness on muscles can be modelled under terrestrial conditions, e.g. immobilization.

Our studies indicate that the type of reaction of different skeletal muscles to the space environment strongly depends on their functional specialization and biomechanism. The pattern of changes in muscle weight [13] lends support to the previously demonstrated relationship between the level of changes and the degree of involvement of various muscles in the antigravity function on Earth (Cosmos-605- [9, 10] and 936 [11]). These data seem to illustrate the pattern of experimentally induced transformation of the phenotype of soleus fibres that conforms to the physiological parameters and submolecular composition of contractile and regulatory proteins. This pattern confirms the notion of the principial lability of the phenotype of skeletal muscle fibres [6], and is consistent with the evidence of important structural changes [7], rearrangements of the isoenzyme pattern of lactate dehydrogenase of the soleus yielding an increased activity of M subunits [2, 12] and possible changes in the composition of TN-TM complex [8] obtained in previous biosatellite experiments. Our studies showed reversible changes in the subunit composition of the TN-TM complex, which had no specificity in the muscles with different function (Fig. 1).

In agreement with the present hypothesis [1] this finding gives evidence of changes taking place in the regulation of actin and myosin interaction.

The results showed that in spaceflight the skeletal muscles of rats develop (or at least start) the process of transformation of the phenotype of muscle fibres. It is assumed that in all likelihood changes in the pattern of physical load particularly of the antigravity muscles, activate mechanisms controlling the synthesis of muscle proteins, Ca-dependent systems of regulation of actomyosin formation, and post-translational mechanisms controlling the myosin structure based on the life-time of their molecules.

It should be, however, noted that the changes in the contractile properties and composition of contractile and regulatory proteins observed in the present study have similar trends in both synchronous and flight animals, being never-

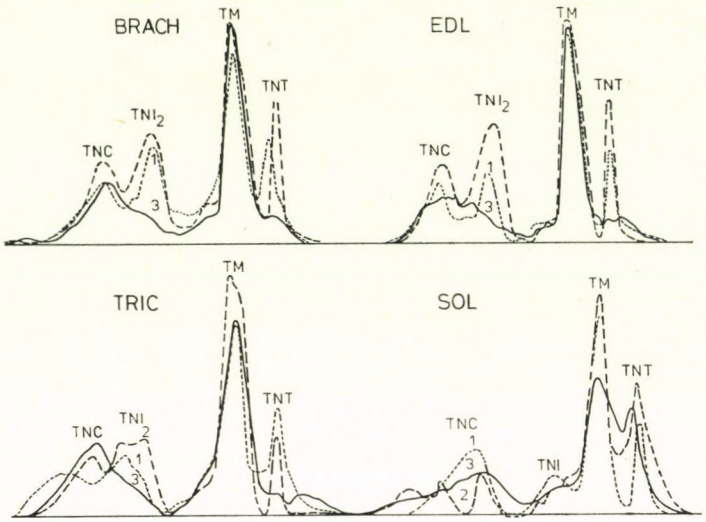

Fig. 1. Densitograms of the isolated tropomyosin-troponin preparations in postflight rats, "Cosmos-1129". Symbols: TNC = Ca-binding subunit, TNI = inhibitor subunit, TNT = regulatory subunit of troponin; TM = Tropomyosin; 1 = vivarium, 2 = synchron, 3 = flight animals. Brach. = M. brachialis, EDL = M. extensor digitorum longus, Tric. = M. triceps, Sol. = M. soleus

theless more pronounced in the latter. It is postulated that these changes develop as a result of combined effects of spaceflight factors and animal housing but manifest in a different manner depending on the functional specialization of muscles.

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LITHIUM INHIBITION OF RENAL TUBULAR *P*-AMINOHIPPURATE TRANSPORT IN THE RAT: RELATION TO PLASMA 2-OXOGLUTARATE

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Effects of lithium on renal haemodynamics and renal handling of *p*-aminohippurate (PAH) and 2-oxoglutarate (2-OG) were studied in anaesthetized rats. LiCl given as a single intra-arterial injection of 5 mmol/kg body wt followed by an infusion at 0.01 mmole/min · kg did not affect glomerular filtration rate (C_{in}) or renal plasma flow (C_{PAH}/E_{PAH} and C_{in}/E_{in}). The maximal tubular transport capacity for PAH (Tm_{PAH}) was reduced from about 240 to 160 μg/min. Simultaneously, lithium significantly raised plasma concentration of 2-OG: its renal clearance increased more than 20 times and net tubular secretion was demonstrated. The inhibition of PAH transport strongly correlated with plasma 2-OG level. The data suggest that lithium inhibits tubular PAH secretion indirectly, by raising plasma 2-OG; the two substances could compete for a common tubular transport mechanism.

Keywords: renal tubular *p*-aminohippurate transport lithium inhibition, 2-oxoglutarate, GFR, renal plasma flow, Tm-PAH

As may other organic anions, *p*-aminohippurate (PAH) is excreted by the kidney by way of tubular secretion [12]. Despite introduction of direct techniques for measurement of the renal blood flow, PAH clearance, alone or factored by the extraction ratio (C_{PAH}/E_{PAH}), as well as the tubular maximal transport capacity of this substance (Tm_{PAH}) remain valuable indices for noninvasive functional exploration of the kidney.

Our recent studies on the effect of lithium on renal function disclosed a lithium-dependent inhibition of PAH secretion in the rat, which was associated with increased plasma and renal tissue concentration of 2-oxoglutarate and increased excretion of this metabolite in the urine [1, 2]. Since oxoglutarate is a potent inhibitor of PAH transport in the proximal tubule [3, 4], in the present work we attempted to evaluate its possible role in alterations of the renal handling of PAH observed in lithium-treated rats.

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Materials and methods

Male Wistar rats weighing 180–220 g were used for the experiments. They were fed a standard pellet diet and given water ad libitum. Anaesthesia was induced with intraperitoneal sodium pentobarbital, 40 mg/kg body wt.

Standard clearance technique was used; the maintenance infusion contained (per litre) NaCl, 140 mmol; KCl, 3.5 mmol; mannitol, 50 g; inulin, 10 g (pH 7.5), and was infused at a rate of 0.1 ml/min. In some experimental groups *p*-aminohippurate (PAH) was infused at 0.5 or 2.0 mg/min · kg body wt. Urine was collected through a bladder catheter. After a 90 min equilibration period three 10 min control urine collections were made; arterial blood samples were withdrawn from the femoral artery in the middle of the first and third period. Subsequently, LiCl was injected i.a. and different experimental protocols in different groups of rats were used.

Group 1 ($n = 5$)

Effect of lithium on renal handling of PAH and 2-oxoglutarate at high plasma levels of PAH (9–15 mg/100 ml). After control periods an intraarterial injection of 5 mmol LiCl per kg body wt was given, followed by a sustaining infusion at a rate of 0.01 mmol/min · kg body wt. This is referred to below as standard lithium dosage. Four experimental (lithium) periods were made.

Group 2 ($n = 10$)

Effect of lithium on PAH extraction ratio (E_{PAH}) at high plasma PAH levels (9–11 mg/100 ml). All animals were infused with PAH at 2 mg/min · kg body wt. After equilibration five rats received LiCl at standard dosage while five others were given isotonic saline. Thirty min after the start of LiCl or NaCl infusion, femoral artery and renal vein blood samples were withdrawn and analyzed for PAH.

Group 3 ($n = 19$)

Effect of lithium on renal plasma flow measured as C_{PAH}/E_{PAH} or C_{in}/E_{in}. All animals received maintenance infusion delivering PAH at a rate of 0.5 mg/min · kg body wt, which produced arterial plasma PAH levels below 5 mg/100 ml. After equilibration 10 rats received LiCl at standard dosage while 5 others were given isotonic saline. Thirty min after the start of LiCl or NaCl infusion femoral artery and renal vein blood samples were withdrawn and analyzed for PAH and inulin.

Group 4 ($n = 30$)

Effect of lithium on Tm_{PAH}. Three subgroups of rats, 5 animals in each, received PAH at a rate of 0.5, 1.0, or 2.0 mg/min · kg body wt. Three control subgroups (3×5 rats) received isotonic saline instead of LiCl. Urine was collected between the 20th and 30th min of LiCl or saline infusion and arterial blood samples were withdrawn at the end of this collection.

Group 5 ($n = 30$)

Effect of different lithium doses on PAH secretion. All rats were infused with PAH at a rate of 2 mg/min · kg body wt. Six subgroups (6×5 animals) received LiCl as single injections of 0.1, 0.25, 0.5, 1.0, 2.5 or 5.0 mmoles/kg body wt, followed by sustaining infusion at 0.2, 0.5, 2, 5, or 10 μmol/min · kg body wt. respectively. Urine and blood were sampled as described for group 4.

Group 6 ($n = 9$)

Renal transport of 2-oxoglutarate (2-OG) in lithium-treated rats. All animals received standard maintenance infusion without PAH. Five rats received LiCl at standard dosage and four control rats were given saline. Urine was collected between the 20th and 30th min of LiCl or saline infusion and arterial and renal vein blood was sampled at the end of this collection.

Group 7 (n = 5)

Effect of PAH on renal transport of 2-OG in lithium-treated rats. The animals received PAH infusion at 2 mg/min · kg body wt and after control periods LiCl was administered at standard dosage. Urine and blood were sampled as in group 6.

Analytical techniques and calculations

PAH was determined by the method of Bratton and Marshall [7] and inulin by the method of Heyrowsky [11], using 3-indoloacetic acid as indicator. 2-OG was determined using glutamate dehydrogenase [6].

The renal clearance of any substance X (C_x) was calculated from the standard formula. The fractional excretion of x was defined as C_x/C_{in} (%). The renal extraction ratio (E_x) was calculated as $(A_x - RV_x)/A_x$, where A_x and RV_x denote concentrations in arterial and renal vein plasma, respectively. Renal plasma flow was calculated as C_{PAH}/E_{PAH}. However, since lithium affected renal handling of PAH, the data were compared with RPF calculated as C_{in}/E_{in}; to increase the accuracy in estimating (A — RV) inulin concentration difference, six parallel determinations were made on each arterial and renal vein plasma sample.

Statistical significance of differences between mean values was estimated using Student *t* test for paired or unpaired samples, as appropriate. Throughout the paper the standard error of mean (SEM) is used as an index of data dispersion.

Results

The preliminary data describing effects of lithium on renal handling of PAH and 2-OG (group 1) are summarized in Table I. It shows that LiCl did not significantly affect GFR (C_{in}) but markedly decreased C_{PAH} (measured at high plasma PAH) and increased arterial plasma PAH above control values. Consequently, the calculated absolute PAH secretion rate (T_{PAH}) was for all lithium periods significantly lower than control. This finding was in agreement with the E_{PAH} value (also measured at high plasma PAH level) for lithium-treated rats of 0.44 ± 0.06, i.e. significantly lower than the value of 0.87 ± 0.05 found in the control animals (Group 2).

Simultaneously with PAH changes, after LiCl treatment plasma 2-OG concentration doubled, its renal clearance increased dramatically and its fractional excretion rose to values exceeding 100%, indicating net 2-OG secretion.

Since renal blood flow (RBF) was not measured directly and the data of Table I show a tendency of the glomerular filtration rate (C_{in}) to decrease, it could be suspected that the decrease in T_{PAH} might have been due to a fall in RBF. However, combining C_{PAH} data of Table I with E_{PAH} values quoted above yielded renal plasma flow (C_{PAH}/E_{PAH}) value of about 4.4 ml/min · kg body wt in control periods and 5.0 ml/min · kg body wt after lithium treatment.

Although this rough estimation speaks against a lithium-dependent decrease in RBF, one has to admit that at the high arterial plasma PAH levels observed in Groups 1 and 2 (9–15 mg/100 ml), C_{PAH}/E_{PAH} might not be a reliable index of renal plasma flow. Moreover, it can be argued that since lithium presumably affects tubular PAH transport, in the presence of LiCl, PAH is unsuitable as a test substance for measurement of renal hemodynamics.

Table I

Effect of lithium on renal handling of p-aminohippurate and 2-oxoglutarate (preliminary data)

Urine collections min	V µl/min	C_{in} ml/min	A_{PAH} mg/100 ml	C_{PAH} ml/min	T_{PAH} µg/min	A_{OG} µmol/l	C_{OG} ml/min	$\frac{C_{OG}}{C_{in}}$ %
PAH infusion, 2 mg/min · kg body wt								
Control periods								
0—10	110±5	1.21±0.10	9.1±1.2	3.8±0.2	231±23			
10—20	95±3	1.33±0.12		3.9±0.2	260±25			
20—30	100±4	1.33±0.11	9.7±1.7	3.7±0.3	242±28	67±7	0.07±0.03	5±2
LiCl, 5 mmol/kg body weight i.v., followed by 0.01 mmol/min · kg body wt								
30—40	125±7	1.13±0.06		1.6±0.2[a]	59±32[a]			
40—50	120±5	1.08±0.17	13.6±1.2[a]	2.2±0.2[a]	147±13[a]	125±14[a]	1.69±0.29[a]	153±27[a]
50—60	100±3	1.25±0.17		2.3±0.3[a]	168±14[a]			
60—70	105±3	1.22±0.21	15.1±0.5[a]	2.1±0.2[a]	138±13[a]	135±10[a]	1.69±0.15[a]	139±13[a]

V, urine flow; C_{in}, inulin clearance; A_{PAH}, arterial plasma PAH concentration; C_{PAH}, PAH clearance; T_{PAH}, PAH secretion; A_{OG}, arterial plasma 2-oxoglutarate concentration; C_{OG}, 2-oxoglutarate clearance; $\frac{C_{OG}}{C_{in}}$, fractional 2-oxoglutarate excretion. Each value represents mean ± SEM of five experiments.

[a] significantly different from mean control value at $p < 0.05$.

Therefore in rats of Group 3 we determined RPF as C_{PAH}/E_{PAH} at plasma PAH concentration below 5 mg/100 ml and also on the basis of the inulin clearance and the extraction ratio (C_{in}/E_{ni}). The data presented in Table II disclosed no difference in RPF between lithium-treated and control rats,

Table II

Renal plasma flow (RPF) measured as C_{PAH}/E_{PAH} or C_{in}/E_{in} in lithium-treated and control rats

Group	C_{PAH} ml/min	E_{PAH}	C_{PAN}/E_{PAH} ml/min	C_{in} ml/min	E_{in}	C_{in}/E_{in} ml/min
LiCl n = 10	4.0±0.3	0.87±0.04	4.7±0.4	1.4±0.1	0.28±0.02	4.9±0.4
NaCl n = 9	3.9±0.2	0.88±0.04	4.5±0.3	1.4±0.1	0.27±0.02	5.2±0.5

E_{PAH}, E_{in}, renal extraction ratios of PAH and inulin.
No significant differences between LiCl and NaCl groups were found.

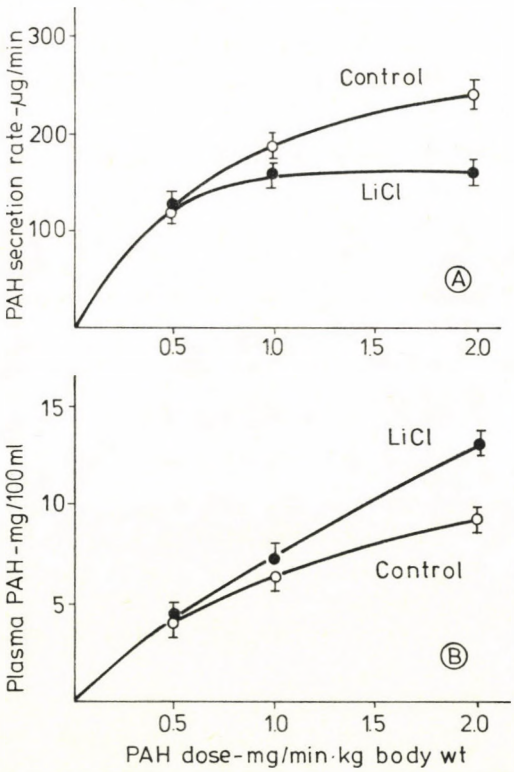

Fig. 1. Effect of lithium on Tm_{PAH}. PAH secretion rate and plasma [PAH] as related to infusion rate were determined 30 min after start of LiCl infusion or isotonic saline infusion. Each point represents mean ± SEM for 5 experiments

regardless of the test substance used. Also C_{PAH} and E_{PAH} values were similar in control and lithium-treated rats, in contrast to data obtained previously with high plasma PAH concentrations.

If lithium specifically inhibits tubular PAH transport, it should decrease the Tm_{PAH} value. This was tested in animals of Group 4 and the relevant

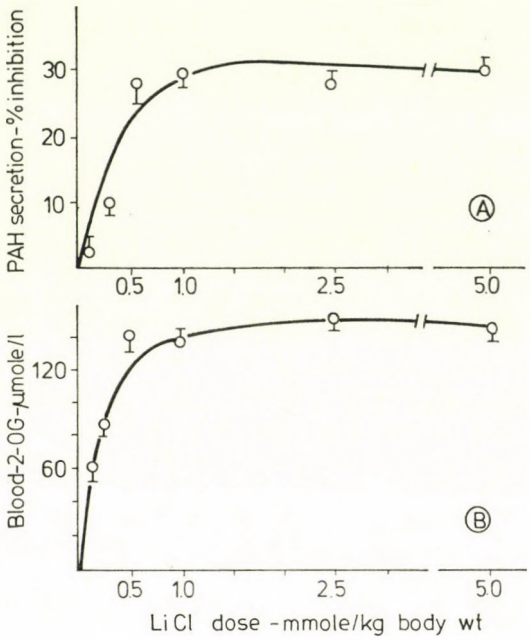

Fig. 2. Inhibition of tubular PAH secretion (A) and plasma 2-oxoglutarate (B) as related to LiCl dose. Per cent inhibition of PAH secretion and plasma 2-OG concentration were determined 30 min after i.v. injection of different lithium doses. Each point represents mean ± SEM for 5 experiments

data are presented in Fig. 1. It shows that in control rats Tm value was approached at a secretion rate (T_{PAH}) of about 240 μg/min (infusion rate, 2 mg/min · kg body weight) while in lithium-treated animals it was attained at 160 μg/min (infusion rate, 1 mg/min · kg body wt). The effect on PAH transport was also reflected by higher plasma PAH levels that were obtained in lithium-treated compared to control rats, both grups being infused at the same rate (Fig. 1B).

Inhibition of PAH secretion as related to the dose of LiCl was studied in animals of Group 5. Figure 2A shows that a marked effect on T_{PAH} was already seen with a single dose of 0.25 mmol/kg body wt followed by an infusion at 0.5 μmol/min · kg body wt. Maximal inhibition (28%) was observed with the injection of 0.5 mmol/kg followed by an infusion at 1 μmol/min · kg higher

doses did not produce any further effect. The pattern of the increase in plasma 2-OG depending on LiCl dose (Fig. 2B) was strikingly similar: a significant increase was observed with the injection of 0.25 mmol/kg whereas maximal effect was obtained with 0.5 mmol/kg. Quite remarkably, per cent inhibition of T_{PAH} was clearly related to plasma 2-OG concentration (Fig. 3).

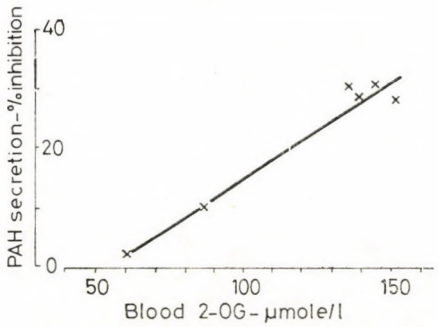

Fig. 3. Inhibition of PAH secretion as related to plasma 2-oxoglutarate concentration in lithium-treated rats. Each point represents a mean for five experiments

The data of Groups 6 and 7 were used to evaluate the action of lithium on renal handling of 2-oxoglutarate in the presence and absence of PAH. In control animals most of the filtered 2-OG was reabsorbed and only 10% was excreted in the urine. Due to an increase in plasma level, the filtered load of 2-OG (F_{OG}) in lithium-treated rats was definitely higher than in control animals. Since urinary 2-OG excretion was even higher than F_{OG}, net tubular 2-oxoglutarate secretion was observed. It is noteworthy that the secretion rate was slightly but significantly lower in the presence of PAH.

Discussion

The preliminary data of the present experiments showing a concurrent increase in renal clearances of p-aminohippurate and 2-oxoglutarate in response to lithium treatment were not readily interpretable. We have shown before that lithium increases plasma 2-OG level and its urinary excretion [1, 2]. However, the mechanism of the decrease in C_{PAH} observed here and the relationship, if any, of PAH and 2-OG changes were not clear. The depression of tubular secretion of PAH (T_{PAH}) could have been due either to a defect of tubular transport or to reduced delivery of PAH to the tubules, depending on a decrease in renal blood flow. The latter possibility had to be examined very carefully since the glomerular filtration rate (GFR) tended to decrease after lithium (Table I) and it had been claimed that in the rat filtration pressure

equilibrium is obtained at the end of the glomerular capillary and, consequently, GFR changes are strikingly plasma flow-dependent [8].

In further studies we were able to prove that renal plasma flow (RPF) measured as C_{PAH}/E_{PAH} did not decrease after lithium treatment. Since a defect of tubular transport was suspected, we thought that the best marker substance for measurement of RPF using Fick is principle would be one that is not transported by the tubules and is extracted by the kidney solely by glomerular filtration. Therefore RPF was determined also as clearance to extraction ratio of inulin (C_{in}/E_{in}) and it was found, again, that total renal plasma flow remained stable after lithium treatment.

A major increase in renal medullary blood flow, balanced by a decrease in cortical blood flow to keep total renal blood flow constant, could reduce T_{PAH} in the absence of any defect of tubular secretion. However, since LiCl treatment did not change E_{PAH} or C_{PAH} measured at plasma PAH levels well below that corresponding to Tm_{PAH}, this seems a rather remote possibility.

As a whole, these data exclude with a reasonable certainty that the decrease in T_{PAH} observed in lithium-treated rats should be due to changes in renal hemodynamics. They also confirm that, when in the presence of lithium plasma PAH concentration is kept sufficiently low, i.e. below 5 mg/100 ml in the rat, C_{PAH}/E_{PAH} or even C_{PAH} alone may still be regarded as valid indices of total or cortical ("effective") renal plasma flow, respectively.

The defect of tubular PAH transport related to lithium treatment was ultimately documented by tubular titration studies which showed an approximately 30% reduction in Tm_{PAH} (Fig. 1). Up to the dose of 0.5 mmol/kg followed by an infusion at the rate of 2 μmol/min · kg body wt, the per cent inhibition of T_{PAH} was correlated to lithium dose, higher doses did not cause

Table III

Effect of lithium in absence and in presence of PAH on the renal handling of 2-oxoglutarate. Mean values ± SEM, nmol/min

Group	$E_{OG} = A_{OG} - RV_{OG}/A_{OG}$	$F_{OG} = A_{OG} \cdot C_{in}$	$U_{OG} \cdot V$	$T_{OG} = U_{OG} V - F_{OG}$
NaCl (control)	0.05±0.02	116±4	12±2	−104±17[a]
n = 4				
LiCl	0.43±0.03[b]	192±6[b]	248±26[b]	56±13[b]
n = 5				
LiCl + PAH	0.36±0.03[b]	208±12[b]	220±36[b]	12±12[b]
n = 5				

E_{OG}, extraction ratio of 2-oxoglutarate (dimensionless); A_{OG}, RV_{OG}, arterial and renal vein plasma 2-oxoglutarate concentration; F_{OG}, filtered load of 2-oxoglutarate; C_{in}, inulin clearance; $U_{OG}V$, urinary 2 oxoglutarate excretion; T_{OG}, tubular 2-oxoglutarate secretion. [a] reabsorbed, [b] significantly different from the value for NaCl group at $p < 0.05$.

any further change in PAH transport (Fig. 2A). This pattern was remarkably similar to the relation between lithium dosage and plasma 2-oxoglutarate concentration (Fig. 2B), indeed, the inhibition of PAH secretion appeared to be a linear function of plasma 2-OG concentration (Fig. 3).

Earlier studies of renal cortical slices [9] and clearance experiments [3, 4] have shown that some substances, including 2-oxoglutarate, inhibit tubular secretion of PAH by competing for a common transport mechanism. Accordingly, the present experiments suggest that the inhibitory action of lithium on PAH transport is mediated by increased delivery of a competitive substance (2-OG) to the tubules. The data of Table III show that, indeed, some fraction of 2-oxoglutarate is secreted by tubular cells into the urine, similarly as is the case with PAH. The tubular secretion of 2-OG in lithium treated rats was slightly but significantly lower in the presence of PAH, which is also compatible with a competition of the two substances for a common secretory mechanism.

The present studies cannot exclude a possibility of a direct action of lithium on the transport system for PAH. This alternative explanation should be given a consideration since it has been shown that lithium can be transported into the human erythrocyte in form of $LiCo_3$-. If lithium were transported as an anion across the antiluminal membrane of tubular cells, its interference with PAH transport at the same site is conceivable.

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ABSTRACTS

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Lectures

INTERACTION OF PLASMA LIPOPROTEINS AND CELL MEMBRANES

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Lipoproteins are macromolecular protein-lipid complexes, their main biological functions are the solubilization and transport of water-insoluble lipids. Lipids important both in energy homeostasis (e. g. triglycerides) and as structural elements (e. g., cholesterol) are transported in plasma lipoproteins from their sites of absorption or synthesis to cells, where they are utilized or stored. The structure of plasma lipoproteins, the nature of protein-lipid association resembles the modern concept of membrane structure. The three important areas of the lipoprotein-membrane interaction are the following.

(1) Cell surface receptor mediated endocytosis of cholesterol and its most common pathological disturbance: atherosclerosis

Cholesterol is moved into the cells by means of absorptive endocytosis, after internalization, the liberated free cholesterol suppresses endogenous cholesterol synthesis. Another possibility for cholesterol uptake is an internalization through the "scavenger" receptor, without interfering with intracellular cholesterol synthesis. The ratio of regular and scavenger receptors is 2:1. During the development of atherosclerosis the suspected cause of intracellular cholesterol deposition is the increase of C uptake through the scavenger receptor system. This increase is evoked either by high levels of plasma and/or intraintimal LDL or by "abnormal" LDL. The structurally abnormal LDL is formed in the blood, in the intima, and platelet interaction may also play a role in its development. Thus this cellular theory of atherosclerosis appears to cover all the important factors recently implicated in the pathogenesis of this disease.

(2) TG uptake of cells

Lipoproteins can enter the cells either in toto or as fatty acids and glycerol, after hydrolysis by the lipoprotein lipase enzyme system. The in toto uptake of VLDL is mediated by specific receptors, but the details of regulation

are unknown, as well its role in certain pathological disturbances in which an increased cellular TG accumulation can be observed (e.g. obesity, fatty infiltration of the liver and heart, etc.).

(2) Immunoregulatory lipoproteins

Plasma lipoproteins containing apo B and apo E apoproteins suppress mitogen-induced lymphocyte proliferation. The lipoproteins inhibit the mitogen enhanced Ca^{2+} accumulation and subsequently the DNA synthesis. The inhibitory lipoproteins interact with cell surface receptors, however, the LDL and immunoregulatory receptors are different, since internalization of lipoproteins is not required for the immunosuppression.

The physiological and possible pathological significance of the regulatory receptor has not yet been elucidated.

The data suggest new directions for investigators studying the interaction of plasma lipoproteins and cell membrane and the role of lipoproteins in cell biology.

DIFFERENTIAL SCANNING CALORIMETRY (DSC) AND ITS APPLICATION IN MEMBRANE RESEARCH

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Differential scanning calorimetry is a thermoanalytical method to measure the temperature and enthalpy of endothermic and exothermic processes. The last two decades in the history of DSC brought about a distinct improvement in sensitivity, accuracy and stability of equipments, resulting in greater appreciation and wider application of the method. Recently, it has been recognized that basic thermodynamic data yielded by DSC are acceptable and sufficiently precise for physical, chemical and biological systems too.

In the first part of this lecture the principle of the method, the technical problems of the measurements, the evaluation of the thermograms and the accuracy of the obtained data will be summed up. A short survey about the possibilities and limits in the application will be offered.

In the second part, examples will be given about the results in the membrane research achieved by the help of the DSC method. In the early studies of model membranes containing only one sort of lipids the interpretation of DSC-thermograms was relatively simple. More difficulties emerged later

from the interpetation of the thermograms of model membranes containing two or more different kinds of lipids and even of model membranes modified with various materials (e.g. ions, alcohols, surfactants, anaesthetics, antibiotics, proteins, enzymes, etc.).

Meanwhile DSC gained a higher role in the investigation of real biological membranes too.

The development in the application of DSC method for membrane research will be illustrated with results taken from the literature and from our work.

RELATION BETWEEN THE REQUIREMENTS OF THE PRACTICAL PLANT PRODUCTION AND THE RESEARCH IN NUTRIENT UPTAKE

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According to the actual fertilizer practice, by introducing fertilizer in one or more charges, the nutrient level will be high enough to meat the plant's requirements of rerying levels in various growing seasons which can be characterized also by nutrient intake ratio. Nutrient intake implying great differences in concentration does not mean optimal conditions for the plants and — on the other hand — results in considerable loss in nutrients (washing out, decomposition).

For the next decade our main goal is to develop a new system feasible also in farm production, which can warrant optimal nutrient quantities during the growing seasons, as far as territory, season and ratio are concerned.

During our present research work we approach our goals simultaneously from both practical and theoretical sides.

In the first phase we intend to find a solution for the harmonic plant nutrition, and later on for the active regulation of nutrient intake process in farm production.

In our present lecture we try to give a full picture on our nutrient intake analysis carried out on 10–12 most important field cultures by the Plant Protection and Agrochemistry Centres of the Ministry of Agriculture and Food (MÉM), and on our plant nutrition program.

On the basis of our practical experiences we would like to outline the focussed task of the research work.

EFFECTS OF STRESS FACTORS ON POTASSIUM TRANSPORT OF THE TERMOPHILIC PLANTS

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Stress effects of plants directly or indirectly cause injuries which can lead to the damage of the metabolic processes and to the disturbance of transport. If the effect of the environmental factors is slow enough, the transport processes connected to the metabolism of plants are damaged to a lesser extent than in the case of sudden changes.

One of the most common stress factors, which is also important from the point of view of practice, is the low temperature. The strong acidification of soils experienced recently directs our attention of the damaging effect of the low pH (H^+-stress). These facts justify further investigations concerning the K^+ uptake of two important plants: the thermophilic rice and the non-thermophilic winter wheat. Measuring (i) the K^+ influxes of these plants after a sudden fall of the temperature and pH, and (ii) the changes of the structure of the root plasmalemma of these plants after the same stress, and comparing the results obtained with rice and wheat, we can summarize our results as follows:

(1) The roots of the thermophilic plants showed and anomaly in their K^+ uptake after sudden changes (decrease) in temperature and/or under the effect of H^+-stress. As a result of them, the K^+ uptakes at 0 °C and/or at pH 3–4 were larger than expected. The anomalous K^+ uptake decreased remarkably when roots were gradually exposed to the low temperature effect.

(2) The addition of an "uncoupler" (2,4-DNP) to the absorption solution practically did-not influence the temperature induced K^+ uptake anomaly of roots, but addition of Ca^{2+} eliminated this effect.

(3) The roots of the thermophilic (stress-sensitive?) plants responded to the stress factors with an increased K^+ influx and a decreased K^+ content, suggesting and increased exchange between the cytoplasmic K^+ pool and the external medium.

(4) From the facts mentioned above it can be concluded that the K^+ uptake anomaly is a phenomenon connected primarily to the plasmalemma of roots, i.e. there must be an essential difference between the thermophilic and non-thermophilic plant root cells in the composition and/or structure of the plasmalemma.

(5) When measuring the $2A_{,,}$ parameter of the spin labelled fatty acids (I/12,3) (incorporated into the membranes of the plasmalemma rich microsomal fractions (PRMF) of rice and wheat roots, we observed that the structure (the $2A_{,}$ parameter) of the rice PRMF changed with decreasing pH, whereas no change could be observed in the case of the wheat PRMF.

(6) Increasing concentrations of H^+ and Ca^{2+} caused similar responses in the structure of the rice PRMF. The anomalous K^+ uptake of rice roots could be explained by the extraordinary increase of the ion fluxes (influx, efflux) due to the rearrangements of membrane lipids (cluster formation, lipid phase separation).

FUNCTION AND METABOLISM OF MEMBRANE LIPIDS IN PLANTS UNDER FROST STRESS

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Although the primary molecular events involved in the response of plants to low temperatures are not known in every detail, it is generally accepted that the thermotropic phase transition temperature of the structural lipids in cold sensitive plants lies at a higher value than in resistant plants. Chilling and frost resistant plants differ in several respects, one of these being their lower temperature limits. While the latter survive, after proper hardening, at rather low temperatures, the former suffer cellular damage at temperatures obviously higher than the freezing point of intracellular water. Extrapolation of the concept of homeoviscous adaptation of membrane fluidity observed with both prokaryotic and eukaryotic cells to frost resistant plants implies that membrane fluidity in increased is some way during the hardening process. However, senescence induces a decrease in the fluidity of membranes in almost every system studied.

Thus the following questions arise:

(1) Whether the hardening plants can, in some way, compensate for the effects of senescence:
(2) How do frost-resistant plants assure the optimum fluidity of their membranes (mainly plasma membrane) in the cold:
(3) What is the connection between a physical change in the membrane system in response to low temperature and changes in the physiological responses of intact plants:
(4) What is the accuracy of approximation of the molecular ordering of membrane lipids if our considerations are based only on analysis of bulk lipids extracted from the whole plant.

Because of the wide range of their survival capacity, cultivars of different frost hardness of the wheat *Triticum aestivum* L. were selected for our investigations. ESR and X-ray diffraction methods were applied to infer the existence of different lipid phase as well as to discern "melts" in the hydrocarbon zones of membranes.

SOLUBILIZATION AND GEL ELECTROPHORETIC ANALYSIS OF DIVALENT CATION DEPENDENT ADENOSINE TRIPHOSPHATASES

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The divalent cation dependent ATPase (Mg^{2+}-ATPase, Ca^{2+}-ATPase, $Ca^{2+}Mg^{2+}$-ATPase) play important roles in the biological systems and therefore their properties and functions have been widely studied [1, 2, e]. However, on the basis of our recent knowledge about the characteristics of these enzymes it is not possible to decide whether or not the different cation dependent ATPase represent a single protein.

Our aim was to separate these enzymes with gel electrophoresis by elaborating suitable solubilization and activity measuring procedures.

In the experiments rat brain cortical microsomes were used which are known to contain all the above mentioned ATPases [4, 5, 6]. On the basis of comparative kinetical analysis we have concluded that Nonidet P-40 non-ionic detergent treatment was the most effective for solubilization of divalent cation dependent ATPase (applied detergent concentration, 3.5 mg Nonidet P-40/mg protein at 0 °C, pH, 8.5 for 30 min). The active conformation of the enzymes did not change during the solubilization, concentration and storing procedures and thus these samples were suitable for further gel electrophoretic analysis.

As the result of gradient polyacrylamide gel electrophoresis (by using a modified DAVIS [7] technique, $T_{acrylamide}$: 5–25%) three distinct bands, stained for ATPase activity, could be detected. The protein band with the lowest R_f required Mg^{2+} for enzyme activity assays, while the bands with the highest R_f needed Ca^{2+} ions for full enzyme activity. A well expressed ATPase activity could be observed in the band with a medium R_f in the presence of either cations.

The polypeptide structure of the tree protein bands was analysed by SDS gradient ($T_{acrylamide}$, 6–22.5%) gel electrophoresis as well as by isoelectrofocusing. The results suggest that a definite distinction can be made between the protein structure of the Mg^{2+}-ATPase and the Ca^{2+}-ATPase.

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CHARACTERIZATION OF OPIATE RECEPTORS IN RAT BRAIN MEMBRANE PREPARATION

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Various pharmacological and biochemical data support the existence of multiple opiate receptors. It is well accepted that opiate alkaloids and enkephalins bind to different receptors. However, the correlation of the pharmacologically defined receptor subtypes (μ, δ, σ, χ, ε) and the biochemically defined high- and low-affinity sites is not known yet.

In the present study the characteristics of ^{3}H-nalaxone binding were examined at 23 °C in rat brain membrane preparation. The concentration curve was biphasic, Scatchard-analysis revealed the existence of a high- and a low-affinity binding site. In competition experiments classical opiates (morphine, naloxone) and enkephalins competed potently both for the high- and for the low-affinity ^{3}H-naloxone binding site. While naloxazone, a C-6 hydrazone derivative of naloxone had comparable affinity with naloxone (IC_{50}:5–10 nM), phenylhydrazone substitution yielded and analogue with much weaker affinity (IC_{50}:5 μM). If the membrane preparation was preincubated with nalaxazone, the high-affinity site was selectively and irreversibly blocked and the low-affinity site was not affected. Similar result was obtained if membranes were preincubated with the chloromethyl ketone derivative of D-Ala^2-Leu^5-enkephalin. Experiments in which membranes were preincubated both with naloxazone and enkephalin-chloromethyl ketone revealed that the two ligands acted partly through the same population of binding sites, partly through different ones. According to Pasternak [Life Sci. **29**, 843 (1981)] the high affinity site is common for enkephalins and opiates and this site mediates their analgetic action.

DESENSITIZATION AND SENSITIZATION OF β-ADRENERGIC RECEPTORS

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Investigations of the last ten years have proven that, beside the hormone -β-adrenergic receptor and adenylate cyclase, a third factor, the G/F or N protein has an important role not only in receptor-cyclase coupling but also in the sensitization and desenzitization of receptors.

Desensitization or sensitization is caused most frequently by high or low hormone levels in vivo. The chronic administration of certain drugs (agonists or antagonists), changes of ion or nucleotide concentration, or congenital variations in the synthesis or degradation of the protein components may also be involved.

Our own investigations started in 1975 [1] with in vitro systems, when we published that catecholamines potently activated the mammalian heart adenylate cyclase [2] and serotonin the muscle heart adenylate cyclase [1] when they had been treated previously with reserpine, i.e. endogenous amines were depleted. When we examined in details the hormone action in rabbit heart membrane preparations we found an increase in the number of β-adrenergic receptors and an increase of the isoproterenol activation of adenylate cyclase activity in the presence of guanine nucleotides after reserpine treatment. At the same time the basal activity of adenylate cyclase decreased in the reserpine treated hearts [3].

In other experiments we succeeded in increasing the number of β-adrenergic receptors with preincubation of adrenergic blocking drugs. Addition of guanine nucleotid increased this effect, while adenylcyclase activity remained unchanged [4].

It is concluded that in both cases the tightly bound high endogenous catecholamine level is responsible for the desensitization which can be reversed with reserpine treatment, or the receptor conformation is changed by them. Guanine nucleotides (GTP, GppNHp) are capable to act on the desensitized receptor-adenylate cyclase complex in two different manner, viz. (1) by promoting the dissociation of the hormone-receptor complex and (2) by activating the adenylate cyclase directly.

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INTACT MEMBRANE OF BRAIN CELLS IMPLY THE REGULATION OF β-ADRENERGIC RECEPTORS

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Kinetic analysis of hormone-receptor complex formation, as the initial event of the physiological response, may provide informations about the characteristics and function of the receptor.

β-adrenergic receptors were studied in intact cells of chick, rat and mouse embryonic brain in primary culture, in comparison with the receptors of broken cell preparations derived from the cultured cells or from the forebrain tissues used for the cultivation, by the specific binding of [^{3}H]-dihydro-L-alprenolol ([^{3}H]-DHA). We supposed that the differences in the binding kinetics of these preparations might provide informations on the role of the intact membrane in β-receptor function.

The [^{3}H]-DHA equilibrium binding to broken cell preparations of either chick, rat or mouse brain cultures or brain tissues was found non-cooperative with a Hill-coefficient $n = 1$, a K_D varying between 1–2 nM and a $B_{max} = 10^3$–10^4 sites/cell, in accordance with the data of other investigators.

In contrast, the [^{3}H]-DHA binding to living brain cells revealed the apparent positive cooperativity of the β-receptors. Fitting the Hill equation to the equilibrium binding data resulted in a B_{max} about 10^3–10^4 sites/cell, a K_D between 40–60 pM and a Hill-coefficient (n) more than unity, between 1–3, in the three studied objects. Apparent cooperativity of the binding reaction was supported also by the association kinetics resulting in the Hill-coefficient of $n < 1$, as well as by the kinetics of the competition with L-alprenolol supporting $n = 2.5$. The apparent positive cooperativity, associated with higher affinity of the β-receptor in intact cells and the lack of it in membrane fragments allow different explanations. One possibility is the conformational isomerization of the receptor in ligand-concentration-dependent fashion. Several processes in the intact membrane may participate in the maitenance of this regulation, physiological significance of which may be amplification of weak, modulatory stimuli.

LIPOSOMES: PREPARATION, INVESTIGATION AND APPLICATION IN MEMBRANE RESEARCH AND IN THE PRACTICE

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Liposomes were developed by biophysicist about fifteen years ago to model the lipid bilayer of biological membranes. At the early time they were used for investigating the physical structure and behaviour, while subsequently they served research in other disciplines too (e.g. biochemistry, physiology). Recently, the application of liposomes as drug carriers has become the main field of interest.

This lecture offers a short survey about the various methods of preparation and of characterization of liposomes. A separate part will deal with the preparation of lipids and the determination of purity of lipids. Emphasis will be laid on the methods which are more important, in drug technology. The size and their distribution homogeneity, surface charge, phase state, stability of liposomes can be characterized by the help of these methods. Some practical aspects will be mentioned, too.

In the part dealing with the application in basic research some examples will be quoted from the field of lipid-protein and liposome-cell interactions.

THE FATE OF LIPOSOMES IN BIOLOGICAL SYSTEMS

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The possible use of liposomes (phospholipid vesicles) as drug delivery systems has attracted wide interest in the past few years. The liposome-encapsulated drug has altered pharmacokinetics as compared to the free one, and in many cases this may lead to an increase in the therapeutic efficiency or to other advantagenous effects. The in vivo fate of the encapsulated drug is largely determined by that of the lipid capsule, until the drug remains within the liposomes. Consequently, for appropriate design of "pharmacosomes" the precise knowledge of the in vivo behaviour of liposomes seems to be essential. In this context we try to summarize the results concerning the in vivo fate of liposomes.

Reaching the circulation, liposomes interact with most of the cellular and non-cellular components of the plasma. These interactions may lead to an increase in membrane permeability, and the encapsulated drug may leak out of the liposomes. What weakens the stability of the liposome-membrane more than anything, is the interaction with high-density lipoprotein (HDL). In this case, the transfer of lecithin molecules to HDL is observable with formation of pores in the liposome membrane. To avoid the harmful outcome of the interactions, the liposome membrane has to be stabilized. The method of choice to stabilize the liposomes in vivo is the addition of cholesterol to lecithins in equimolar concentration, but the use of other stabilizer lipids like sphingomyelin, amino-sugar derivatives of elimination (clearance) of liposomes from the plasma varies largely depending on size, surface charge, lipid composition, mode of administration and surface modifications. The main organs of liposome-uptake are the liver and the spleen, to less extent the kidney, lung, bone-marrow, gut, lymph-nodes, etc. Liposomes are not capable of passing the blood-brain barrier. Within the tissues, liposomes are taken up mainly by the phago-

cytotic cells, i.e. by the Kupffer cells in the liver and by fixed macrophages in other tissues. A part of the liposomes can, however, reach the parenchymal cells, too. The tissue distribution may be modified to a small degree either by altering the mentioned liposome parameters, or by some ingenious method like the incorporation of target area-specific antibodies in the membrane of liposomes and concurrent saturation of the phagocytic cells by large, "empty" liposomes upon the administration of "targeted" liposomes.

The use of the "heat-sensitive" liposomes is also very promising, they can release much of the entrapped drug in the pre-heated tissue, the temperature of which corresponds to the phase-transition temperature of the liposomes.

Based on the above, it may be anticipated that pharmacosomes which are expected to act in the phagocytotic cells of the RES (RHS) may gain clinical use in the nearest future.

THE ROLE OF BIOLOGICAL MEMBRANES IN PSYCHIATRIC THERAPY

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A revolution in the treatment of psychiatric illnesses took place only three decades ago. Tranquillizers highly effective in the treatment of schizophrenia were first introduced and came into common use world-wide during the early 1950's. A few years later, during the 1960's, drugs were discovered which served in the treatment of affective psychosis. Lithium therapy, so important in the prophylaxis of affective psychosis was likewise introduced some thirty years ago. The use of drugs in psychiatric therapy is closely connected with the role of membranes. Studies have indicated that lithium is completely absorbed by the blood and within 2 hours 95% of it is excreted in the urine. Our measurements indicate that the fluid-serum ratio is about 25%, which is in keeping with references. Though lithium forms an important part of psychiatric therapy, it may represent a membranological problem for other organs as well, since lithium is excreted only by the kidneys thus, in case of long-term lithium therapy regular testing of renal function is very important. In our experience with patients undergoing treatment over a three-year period, lithium failed to affect glomerular filtration. Our finding concerning the absorption of phenotiazine, a transquillizer frequently used in clinical practice, differs in certain respects from the results on lithium. In our experiments performed on rat brain homogenate, when metophenazine largerthan (1.6×10^{-4} mol) was applied, there was a drop in the *in vitro* consumption of oxygen and in the utilization of oxygen in pyruvic acid. The increasing effect of frenolon on membrane

permeability was borne out by our experiments on rat brain synaptosoma. Addition of triton X-100 detergent to rat brain synaptosoma fractions in *vitro* increased the activity of glycolytic enzymes. All these observations point to the importance of detection, frequent control and monitoring of serum levels. The introduction of such a practice is in its infant stage abroad, as well, but particularly so, in our country. The instrumentation of necessary practices presents a financial problem; both gas-chromatography and liquid-chromatography are very expensive.

Our conclusion: (1). It is now possible to control whether a prescribed drug is actually taken by the patient or not. (2). Using this method we are able to eliminate the danger of excessive doses of the drug, and we can also prevent undesirable side effects or complications.

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Posters

ISOLATION AND PARTIAL CHARACTERIZATION OF HUMAN GRANULOCYTE CHEMOTAXIN FROM *ESCHERICHIA COLI* CULTURE FILTRATE

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Chemotaxis of human granulocyte was studied in vitro. Ethanol soluble agents were isolated from culture medium of *E. coli* bacteria. Sephadex G-10 gel chromatography of the medium yielded three main fractions two of which exhibited chemotactic activity. Partial characterization of one of the active fractions (molecular weight, appr. 3000) showed that it contained 10% protein, 20% carbohydrate and 35% lipid. It absorbed UV light, the 260/280 nm absorbance ratio being 1.03.

We suggest that these granulocyte chemotaxins have been derived from the envelope of dividing bacteria.

THE cAMP LEVEL OF POLYMORPHONUCLEAR LEUKOCYTES IN PSORIASIS VULGARIS

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In the previous studies the circulating polymorphonuclear leukocytes from patients with psoriasis vulgaris were found to be activated (enhanced chemotaxis, adherence, NBT reduction and *Candida albicans* killing). As these leukocyte functions are regulated by among others, the cyclic nucleotides and in the lesioned psoriatic epidermis the cyclic AMP level and/or beta adrenergic receptor responsiveness is diminished we measured the cAMP content of the polymorphonuclear leukocytes in psoriasis vulgaris with radioimmunoassay. The cAMP level of polymorphonuclear leukocytes from patients with psoriasis did not differ from that of the control cells. Thus other mechanisms may be implicated in the granulocyte activation.

POLYMORPHONUCLEAR LEUKOCYTE CHEMOTAXIS IN PSORIASIS VULGARIS

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The chemotactic responsiveness of the polymorphonuclear leukocytes from patients with psoriasis vulgaris was measured with the Boyden method. Zymosan activated, pooled, healthy donor serum was used as chemoattractant. The chemotactic responsiveness of the polymorphonuclear leukocytes of psoriatic patients was significantly higher than that of the granulocytes obtained from healthy individuals. The generation of lymphocyte derived chemotactic fator (LDCF) was found to be enhanced in psoriasis, too. As LDCF proved to be chemokinetic for the psoriatic granulocytes we assume that this factor is, at least partly, responsible for the polymorphonuclear leukocyte activation.

EFFECT OF IMMUNE COMPLEXES FROM PATIENTS WITH SLE ON THE CHEMOTAXIS OF HUMAN MONOCYTES

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The effect of immune complexes (IC) precipitated from SLE sera with polyethylene glycol and gel filtration was examined on the chemotaxis of helathy monocytes. Even at a low protein concentration (1 μg/ml) IC-s inhibited the monocyte chemotaxis. The IC-s from patients with SLE nephritic syndrome caused a significantly larger inhibition in comparison to the IC-s patients without any renal manifestation. No correlation was seen with the activity of the disease. The inhibitory effect of the IC-s on monocyte chemotaxis correlated with their inhibitory effect on Fc receptor activity, suggesting a relationship between the chemotactic and the Fc function of monocytes. Analysis of the IC-s by enzyme-labelled immunoassay revealed no correlation between the quantity of the IgG, C_3 or DNA-anti-DNA complexes in the IC samples and the effect on monocyte function.

THE EFFECTS OF BENZYLADENINE, NAPHTYLACETIC ACID AND GIBBERELLIC ACID ON THE K/Na TRANSPORT IN WHEAT

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Winter wheat was grown for 3 weeks in the presence of benzyladenine (BA), naphtylacetic acid (NAA) and gibberellic acid (GA) combined with optimum and low K-supplies in the complete nutrient solution. During the growth of seedlings, the contents of macroelements (N, P, K, Na, Ca and Mg) and water, K and P uptake and the activities of ion-activated ATPase and acidic phosphatase were monitored.

Growth was stimulated by all the applied hormones. The greatest effect was shown by GA given in a concentration of 0.1 μmol/dm^3. The uptake of K and P decreased while the K/Na selectivity increased due to the decrease of Na uptake. In BA treated plants the ion-independent ("basic") ATPase activity increased parallel with the elimination of the ion-dependent ATPase activity, whereas GA stimulated both the ion-independent and ion-dependent ATPase activities. When BA and GA were combined the effect of the former predominated.

These results support that some of the transport properties including K/Na selectivity of plants may be controlled by hormonal treatments.

PHOTODESTRUCTION IN BENZONITRILE-TREATED CHLOROPLAST MEMBRANES

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The effect of 3,5-disubstituted 4-hydroxy-benzonitriles was investigated in vivo and in vitro on chloroplast ultrastructure and on photodestruction of chlorophylls and chlorophyll-protein complexes (CPC) of thylakoids.

The polyacrilamide gel electrophoretic separation of CPC-s isolated from chloroplasts of wheat seedlings treated for 96 hours and greened for by illumination with 5000 lux showed that bromoxynil, a widely used agent for weed control in cereals, was ineffective. However, 3-nitro-5-bromo-4-hydroxy-benzonitrile decreased the amount and chlorophyll a/b ratio of photosystem I. CPC, and increased the relative amount of light harvesting CPC-s, having low chlorophyll a/b ratio. The electron microscopical pictures and their morpho-

metrical analysis proved that the membrane structure of the treated plastids became similar to that of the shade-adapted plastids (i.e. increased granum membrane area, wider grana with decreased number of thylakoid).

During 3 hours of illumination with 5000 lux the photobleaching of chlorophyll was enhanced by all 3,5-disubstituted 4-hydroxy-benzonitriles in suspension of isolated spinach chloroplasts. The photosystem II CPC was the most sentitive to these in vitro treatments.

IN VIVO MODIFICATION OF LIPID CONTENT AND FATTY ACID COMPOSITION OF CHLOROPLAST MEMBRANES BY PYRIDAZINONE HERBICIDES

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The lipid content and fatty acid composition of chloroplast membranes exhibit unique features as compared to other membraneous organelles derived from animal and plant cells. The structural and functional significance of this special lipoid pattern is not well understood.

In our investigations we tried to cause alterations in the lipid and fatty acid composition of chloroplast membranes during the light-dependent chloroplast differentiation (greening) by the application of three pyridazinone herbicides (SAN 6706, SAN 9789, SAN 9785).

Both inhibitory and stimulatory actions of the herbicides were observed.

COMPOSITION AND PHYSICAL STATE OF CUCUMBER LEAF PHOSPHOLIPIDS AS AFFECTED BY SUBOPTIMAL GROWTH TEMPERATURE

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During frost hardening augmentation of lipids and concomittant increase of phospholipid content have been observed in many plant species [1, 2].

In this study the effect of suboptimal temperature (20/10 °C) was studied on cucumber lines differing in growth at suboptimal temperature.

Total phospholipid (PL) content of low-temperature tolerant and sensitive lines increased at suboptimal air temperature. When the soil was heated to 16 °C, PL content of the leaves was decreased. The percentage phosphatidylcholine (PC) of the total phospholipids of the sensitive lines grown at 20/10

°C was 45% and it was 37% with soil heating. Tolerant lines contained 3 to 10% more PC under the same conditions.

Total sterol (ST) to total phospholipid (PL) ratio may affect the phase transition temperature of membranes [3]. ST/PL ratio (at 20/10 °C, soil heated) was remarkably lower in the tolerant lines compared with the sensitive ones.

The percentage linoleic acid (18:3) of the PL fatty acids from the sensitive line was lower (40%) at 23/20 °C than at 20/10 °C (55%). When the soil was heated a significant increase in the level of 18:3 and a decrease of 18:2 were observed in the tolerant lines while the sensitive line remained at the same level. A similar relatively high level of 18:3 was found in the fatty acids of the different phospholipid classes of plants grown at 20/10 °C. Phosphatidylcholine and phosphatidylethanolamine specifically contained more linoleic acid and less linoleic acid in the tolerant lines than PC and PE of the sensitive line suggesting that a high degree of unsaturation of these phospholipids is a prerequisite for adaptation to suboptimal growth temperature.

The correlation between changes in PL composition and the physical state of isolated phospholipids was determined with electron spin resonance (ESR). When the sensitive line was grown at 20/10 °C instead of 23/20 °C, the phospholipid phase separation temperature (T_c) was 5 °C instead of 12 °C, indicating that a functional adaptation to low temperature occurred. Phospholipid vesicles of tolerant lines grown at 20/10 °C showed a higher T_c than the sensitive control at 20/10 °C. A higher T_c seems to be correlated with a higher growth capacity at low temperature.

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MEMBRANE DAMAGING TREATMENT OF NYSTATIN SENSITIVE AND RESISTANT *CANDIDA ALBICANS* CELLS

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The effect on 42-K and 24-Na influx and efflux respectively, of antifungal polyenic macrolides (*Nys*tatin, *Am*photericin *B*, *Pim*aricin) and a detergent (*C*ethyl *P*yridinium *B*romide) was studied on a *Nys* sensitive ("S") Candida albicans and on a strain ("R") of induced *Nys* (16x) resistance (and ergosterol-less mutant).

Results indicate that:

(*i*) The barrier functions of the plasma membrane were destroyed only by *CPB*.

(*ii*) *Nys* in concentrations capable to inhibit the grwoth of "S" or "R" cells (sens = S-MIC, res = R-MIC), did not destroy the barrier functions of the membrane. It caused, however, ion loss in preloaded cells, while ion influx was not inhibited, even its rate was enhanced. On "R" cells, obviously, S-MIC of Nys failed to alter spontaneous ion leakage, although ion influx was slightly enhanced. All these processes were influenced by the extracellular presence of both K^+ and Na^+.

(*iii*) The $50\times$ S-MIC of *AmB* and $10\times$ S-MIC of *Pim*, increased ion uptake processes slightly, while not being capable of inducing efflux in preloaded cells.

Our results refer to mobility increase, inducible only with *Nys*, of carriers of the K-influx as well as Na-efflux pumps, but even by using this polyene nothing indicated hole or pore induction. The resistance, however, can be diminished with high (R-MIC) dose of *Nys*, i.e. both a considerable ion influx increase and an ion leakage are inducible, however, the question arises whether both "S" and "R" cells respond by the same mechanism.

EFFECT OF GONADOTROPIN RELEASING HORMONE ON THE ADENYLATE CYCLASE ACTIVITY OF AVIAN GRANULOSA

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The activity of adenylate cyclase in purified preparation of hen granulosa was investigated by measuring the production of cyclic AMP during a 20 min incubation at 30 °C. Gpp/NH/p, the nonhydrolyzable analogue of GTP, stimulated enzyme activity in a dose related manner (K_{act}, 1.06×10^{-7} M). Ovine LH and, to a lesser extent, ovine FSH also activated adenylate cyclase in the presence of half maximally stimulating concentrations of Gpp/NH/p (10^{-7} M). Gonadotropin releasing hormone (10^{-10}—10^{-4} M) failed to significantly affect basal or gonadotropin promoted adenylate cyclase activity or the production of cyclic AMP by intact granulosa cells. Progesteron production, on the other hand, was enhanced by GnRH (10^{-8}—10^{-6} M). It is suggested that in chicken granulosa cells, as in mammalian pituitary cells, the adenylate cyclase/cyclic AMP system is not a mediator of GnRH action.

THE EFFECT OF D-PENICILLAMINE ON THE MICROSOMAL CYTOCHROME P-450

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D-Penicillamine (D-Pa) is used in our department in the therapy of neonatal hyperbilirubinemia and in the prevention of retrolental fibroplasia. Based on our previous studies we have suggested that D-Pa may influence the haem metabolism. Since cytochrome P-450 is generally present as the dominant haem protein of microsomes, it was of interest to investigate the effect of D-Pa on this enzyme system.

Experiments were carried out in newborn and adult CFY rats. D-Pa treatment did not cause any changes in adult animals. In newborn rats we found the following.

1. The hexobarbital sleeping time was shortened by D-Pa treatment;
2. The amount of cytochrome P-450 was greater in the livers of treated animals;
3. Electron microscopy revealed an enrichment of the endoplasmic reticulum in hepatocytes of treated rats.

This age-related difference in the action of D-Pa was also demonstrated in our previous studies.

MACROPHAGE ACTIVATING EFFECT OF NATURAL OLIGOPEPTIDES

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The effect of some neuropeptides on the effector functions of rat peritoneal macrophages (PM) was investigated. Angiotensin II (At II), vasopressin (Vp), RH-LH and TRH did not affect the expression of Fcu and Fcγ receptors of PMs. However, at high concentrations (10^{-5}—10^{-6} M) the Fcγ receptor mediated rosette formation was diminished by At II, Vp and LH-RH, while the subsequent phagocytosis of ^{51}Cr-SRBCs was enhanced. In contrast, oligopeptides (OP) enhanced the rosette formation and inhibited the incorporation at 10^{-7} M concentrations. The C3b receptor mediated rosette formation and

phagocytosis were augmented by the above OPs. In addition, OPs failed to alter the IgM mediated processes of PMs. The phagocytosis enhancing effect induced by OPs was completely abolished under the effect of 10^{-5} M indomethacin, and a medium containing 5 mM EGTA inhibited the phagocytosis suppressing effect of OPs at low concentrations. Benzoyl-tyrosyl-aethyl-ester (BTEE) was able to abolish both the phagocytosis enhancing and suppressing effects of OPs through inhibition of macrophage-peptidase having a key position in the activation.

Chemiluminescence of PMs was induced by OPs, but BTEE diminished the activity of "respiratory burst". The intralysosomal chymotrypsin and elastase activity was significantly inhibited by OPs, and the intracellular killing capability was also lowered in the presence of them. TRH did not induce any alterations in target cells.

Summarized: It was found that a number of neuropeptides were able to induce PM-activation associated with enhanced incorporation and lowered lysosomal enzyme activity as well as killing capability. These effects were mediated by increased PG synthesis, Ca^{2+}-influx and H^+ transport into the cells.

REGULATION OF MEMBRANE RECEPTOR ACTIVITY ON RESIDENT AND PROVOKED PERITONEAL MACROPHAGES

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The regulatory role of cyclic nucleotides on the effector functions of both resident and thioglycollate-elicited rat peritoneal macrophages (PM) was studied. Cyclic nucleotides exerted the same effect on the two types of PMs. Extracellular cAMP as well as PGE_2 enhanced Fcμ receptor mediated phagocytosis and oxidative burst while intracellular protease activity (assessed by the use of fluorogenic substrates) was decreased by the same agents. Extracellular cGMP as well as $PGF_{2\alpha}$ enhanced the Fcγ receptor mediated phagocytosis, chemiluminescence and intralysosomal protease activity. cAMP or PGE_2 inhibited, while cGMP and PGF_2 augmented, the intracellular killing of fungi in thioglycollate-elicited PMs.

The C3b receptor mediated rosette formation and phagocytosis were sensitive to extracellular cyclic nucleotides only to a lower extent.

CHARACTERIZATION OF MACROPHAGE ACTIVATION INDUCED BY Ca IONOPHORE A 23187 AND VALINOMYCIN

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Ca ionophore A 23187 and valinomycin know to induce primary K efflux appeared to activate rat peritoneal macrophages (PM) as revealed by spreading inhibition, aggregation and enhanced nitro blue tetrazolium reduction. The effect of valinomycin was detected also in the presence of 5 mM EGTA. In contrast, both drugs inhibited markedly the Fcμ receptor mediated functions, whereas the IgG2a mediated processes were only decreased. The C3b receptor mediated phagocytosis appeared to be more resistant to the drugs affecting cation transport.

Both ionophores induced significant chemiluminescence and inhibited the intralysosomal elastase and chymotrypsin activity in PMs. The intracellular killing capability of PMs was markedly inhibited after incubation with Ca ionophore and slightly inhibited or unchanged after valinomycin treatment.

The membranes of PMs observed by polarized light microscopy revealed strong radially-positive birefringence indicating the tangentially extended carbohydrate chains on the membrane surface. On the contrary, the membranes of PMS after exposure to ionophores displayed a discontinous weakly birefringent mosaic-like pattern. These optical phenomena suggest a lower degreee of the orientation and/or a loss of carbohydrates of the cell coat as compared to the control cells.

MEASUREMENT OF INTRACELLULAR PROTEASE ACTIVITY WITH FLUOROGENIC SUBSTRATES IN LIVING PHAGOCYTIC CELL SUSPENSION

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A number of methods are known for the determination of protease activity released from macrophages or granulocytes. In the present study the intracellular (intralysosomal) trypsine, chymotrypsine and elastase activities were measured with fluorogenic substrates (benzoyl-I -arginine-β-naphthyl-

-amide, L-phenylalanine-β-naphthylamide and L-alanine-β-naphthyl-amide) in living cell suspensions.

Our method is based on the principle that substrates are able to penetrate the membrane surrounded lysosomes and after the enzymatic effect the fluorescent β-naphthylamide disperses homogeneously in the cytoplasma and in the medium.

First, the optimal experimental conditions were elaborated. RPMI 1640 medium has a high absorbance at 338 nm (excitation wavelengh of β-naphthylamide), therefore the determinations were carried out in the lowest essential amounts of this medium buffered with Tris-HCL and supplemented with L-glutamine.

Enzyme activity depends on the cell number. We found a linear relationship between the protease activities and cell number in a range of 10^6–10^7 cell/ml. The fluorescence of released β-naphthylamide was not disturbed by cell density in the above mentioned range. The protease activity of living cell suspensions did not change during the first five hours of incubation.

In addition, during this period we could not detect significant enzyme activities in the medium. The soybean trypsin inhibitor failed to inhibit protease activity in living cell suspension, althought ε-amino-caproic acid decreased enzyme activity. These findings prove that by our method the intracellular protease activity was determined.

THE EFFECT OF THYROXINE ON THE MN AND PMN PHAGOCYTES

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In hyperthyreosis an enhanced activation of the immune system was described. The activation of effector cells (macrophages, granulocytes) may be related to the direct effect of the hormone on oxygen consumption and on ATPase activity of the membrane. However, it cannot be excluded that the process is related to the conversion of T_4—T_3 when $NADPH^+$ gives H^+ to the enzyme deiodase. Fcγ receptor activity of human monocytes and red macrophages measured by rosette formation and phagocytosis of tracer-red blood cells increased, while ADCC activity decreased after *in vitro* incubation with thyroxine. Depressed intralysosomal protease activity measured by fluorogenic substrates as well as decreased superoxide anion production (chemiluminescence) were responsible for the inhibition of ADCC. When thyroxine was ad-

ministered *in vivo*, rosette formation increased but phagocytosis was blocked or it did not alter. Thus, the relatively low incorporation means an energy loss for the cell. Fcγ receptor mediated phagocytosis of thyreoidectomized rats decreased and rosette formation was nearly totally blocked, therefore high relative incorporation was observed. Intralysosomal protease activity decreased in macrophages of thyreoidectomized as well as thyroxine-treated rats. In contrast, the spontaneous chemiluminescence was increased only after thyroxine administration. The fact that GSH protected the in vitro blocking effect of T_4 on the chemiluminescence indicates that the in vitro effect thyroxine is in close connection with the T_4–T_3 convertase enzyme activity. According to our conclusions the reaction of effector cells differs depending on whether the hormone is administered *in vitro* or *in vivo*.

DETERMINATION OF ANTIGEN COMPONENTS OF IMMUNE COMPLEXES AS WELL AS SOLUBILIZED HUMAN Fc RECEPTORS BY LASER NEPHELOMETRY

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In our present study the amounts of immune complexes (I. C.) as well as the proportion of human thyreoglobulin (hTG) of I. C. was determined by the use of laser nephelometry in sera of patients with Basedow-Graves' disease. This method based on flocculation is well practicable with addition of antigen excess to achieve inhibition of PEG-precipitation. The system was used for quantitative as well as qualitative analysis of antigen components of I. C. in general.

Furtermore, Fc receptors shed from human mononuclear leukocytes as a result of temperature shift were also detectable in the presence of human IgG by laser nephelometry. The PEG precipitation under these experimental circumstances depended on the Fc receptor to IgG ratio in the system. Thus, the various amounts of Fc receptors shed from leukocytes of patients with autoimmune disease are detectable. However, the Fc parts of IgG molecules were blocked by preincubation with anti-human IgG(Fc) groat serum therefore Fc receptors failed to precipiatate IgG. In parallel experiments the inhibitory effect of solubilized Fc receptors on rosette formation was also measured and we found a good correlation between the above mentioned methods.

Summarized: laser nephelometry thus applied seems to be suitable for the detection of antigen components of I. C. as well as the shedding rate of Fc receptors from mononuclear cells in various autoimmune diseases.

ROTATIONAL DYNAMICS OF MEMBRANE PROTEINS IN FROG SCIATIC NERVE

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The translational and rotational motion of protein in membranes, the distribution of internal patterns of motion, their frequency and energy characteristics are in strong correlation with the biological function of the membranes. The segmental flexibility and the rotational motion of protein domains were studied by —SH directed spin labels using conventional and saturation transfer EPR technique. It was found that denaturation, change in temperature, oxidizing and reducing agents, different drugs pertrub primarily the lipid region of the membranes, after the conformational state of the proteins. This fact supports the assumption that the protein segments where the —SH-groups are located play a central role in the ionic transport processes across the membrane.

LIPID-PROTEIN INTERACTION IN FROG SCIATIC NERVE

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A rapid and effective method has been elaborated for spin-labelling of the lipid region of the membranes and the —SH-groups of the membrane proteins in the sciatic nerve of the frog. The excitatory properties were not changed after the incorporation of the fatty acids by this method. The experiments, in which spin-labelled —SH reagents were used suggest that a mobile ($\tau_2 \sim 1$ ns) protein-segment of hydrophylic environment could be responsible for the mechanism of the action potential. It has been revealed that at least those —SH-groups to which the spin labels are bound exhibit an anisotropic static distribution. This static distribution and also the mobility of the protein altered even during perturbation in the lipid phase, as perturbing agents e.g., benzyl-alcohol, n-butanol and halothane were used.

Thus our experiments provide direct evidence that the conformational state and the stability of the membrane proteins are principally influenced by the changes in the dynamic state of the lipids.

EFFECTS OF PHLORRHIZIN ON THE MUSCLE MEMBRANE

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Our earlier results [Acta physiol. Acad. Sci. hung. **52**, 41–51 (1978)] showed that phlorrhizin significantly decreased the frequency of membrane potential oscillation induced by cevadine. According to our recent experiments the frequency decreasing effect of phlorrhizin fails to ensue if the total Cl^- content of the incubation solution is substituted for non-penetrating anions (glutamine, isethionate or sulphate).

The effect of 2 mmol/l phlorrhizin on the passive electrical characteristics of the sartorius muscle was investigated. It was established earlier that the resting membrane resistance of the muscle incubated in normal Ringer decreased by $^2/_3$. There is a decrease of similar degree even in Na-free (choline) Ringer solution. However, the decrease of the membrane resistance fails to occur in Cl^- free (glutamate) Ringer.

Data mentioned above suggest that phlorrhizin influences the Cl^- conductance.

PHOSPHATE TRANSPORT IN ISOLATED SYNAPTOSOMES

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Isolated synaptosomes serve as useful models for studies on transport processes through the plasma membrane of nerve cells. In the present paper the transport of phosphate ions, their distribution between mitochondria and cytoplasm, and their possible role in depolarization-induced calcium uptake have been investigated. The following results were obtained:

(1) Freshly isolated synaptosomes contain 12.8 ± 3.05 nmol phosphate per mg protein. Disrupting synaptosomes 0.7–3.1 nmol phosphate was found to be associated with mitochondria. Disregarding any phosphate binding the above values give an intracellular phosphate concentration of 2–6 mM.

(2) Depolarization-induced calcium uptake is not accompanied by any net phosphate movement.

(3) Within 60 min 0.25 nmol ^{32}P-phosphate appears in organic compounds in the presence of 0.5 mM external phosphate at 30 °C.

(4) In phosphate-free medium synaptosomes release 2 nmol phosphate per mg protein per 60 min. This process is stimulated by the protonophor FCCP to 10 nmol per mg per 60 min.

ROLE OF THE REDOX STATE POTENTIAL IN THE REGULATION OF MUSCLE ACTIVITY

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Direction of changes of the redox state potential (E_0') in muscles stimulated indirectly depends on the stimulatory frequency in vivo. Slow frequency evoking acute redosis, while stimulation with high frequency resulting in oxidosis. These changes are frequently followed by compensatory redox alterations, e.g., acute oxidosis evoked by stimulation with low frequency is followed by a compensatory redosis. When the muscle is stimulated with series of impulses of similar frequencies, the compensatory redox changes will sum up, in other words, the basic redox state will be shifted. This might explain the differentiation of the embryonic muscle tissues, according to the following scheme: If the embryonic tissue receives only stimulatory impulses of low frequency → acute redosis → compensatory oxidosis → increased catabolism → red type muscle evolves.

INVESTIGATION OF TRANSPORT PROCESSES OF EXCISED AND INTACT WHEAT ROOTS

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It is known that the ion uptake process of excised roots may differ considerably from that of intact plants under specific conditions. The reason of this has not been cleared yet. Earlier, the influx of ions was studied mostly in excised roots, and other investigations connected with transport were not taken into consideration. This interesting question of methodological importance has been investigated using 7 day-old wheat (GK Szeged) seedlings grown in water culture. Our results are the following: (1) ATPase enzyme activity of excised roots was more inhibited by 0.01 mmol 2,4-D than the ATPase of intact plant. This inhibitory effect was stronger in plants grown under low salt conditions (0,5 mmol $CaSO_4$) than in plants grown in diluted nutrient solution. (2) The Viets-effect and effects of different stress factors could be observed in

excised roots and intact plants as well, while the Ca^{2+}-stimulated K^+-uptake and K^+-content was higher in the latter. (3) By our opinion, the differences in the transport processes of intact plants and excised roots can be explained by the common effects of root cutting and the different environmental stress factors.

HEPATOBILIARY TRANSPORT OF CHOLEPHILIC ORGANIC ACIDS AND THEIR EFFECT ON MITOCHONDRIAL RESPIRATION

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(1) Indocyanise green, rose bengal, sulfobromophthalein (BSP) and bromcresol green are excreted by the bile at low rates (0.1–0.5 μmol/kg/min). They inhibit bile production and the biliary excretion of amaranth. These organic acids, unlike those with high biliary excretion rates (2–5 μmol/kg/min), choleretic effect and low potency to inhibit the biliary excretion of amaranth, depressed the respiration of isolated liver mitochondria at lower concentrations (5–50 μM) than those found in the liver after iv. administration. (2) A correlation was found between the inhibitory effects of bile acids (litocholic acid, chenodeoxycholic acid, deoxycholic acid, cholic acid, dehydrocholic acid) on the biliary excretion of amaranth and on the respiration of hepatic mitochondria. (3) Taurocholic acid reduced the effects of BSP to inhibit mitochondrial respiration and the excretion of BSP-glutathione conjugate, and increased the biliary excretion of total (unconjugated plus conjugated) BSP.

These observations suggest that toxic effects on mitochondria of cholephils may be responsible for their low biliary excretion rates as well as for their strong potency to inhibit bile production and hepatic transport of amaranth.

EFFECT OF HYPERGLYCAEMIA ON THE SUGAR TRANSPORT OF THE SMALL INTESTINE

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The intestinal transport of sugars (glucose, 3–0–methylglucose, β-methyl--D-glucose) was investigated in the isolated musosa of guinea pig. Sugar transport was increased by hyperglycaemia. The serosal to mucosal flux was only

slightly enhanced as a result of the increase of paracellular permeability. The mucosal-serosal transport was also increased due to the stimulation of transcellular transport processes. The calculated flux coefficients indicate that both the liminal and the basolateral membranes may be involved in the increased sugar transport induced by hyperglycaemia.

INTERCELLULAR JUNCTIONAL FORMATIONS IN RAT SKIN TUMOURS INDUCED BY METHYLCHOLANTHRENE: A FREEZE-FRACTURE STUDY

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The occurrence of different intercellular junctions in several epithelial skin tumours induced by methylcholanthrene was investigated using thin sections and freeze-fracture replicas. The primary tumours were basal cell carcinomas; desmosomes and gap junctions were detectable in them. In the subsequent tumours a squamous component evolved. In the squamous carcinomas tight junctions were also observed in addition to desmosomes and gap junctions. Metastases developed merely from the squamous carcinomas. In the metastatic, undifferentiated carcinomas solely desmosomes could be identified. Our data suggest a possible connection between the absence of gap junctions and the metastasizing capacity of tumours.

FREEZE-FRACTURE HISTOCHEMICAL INVESTIGATIONS OF THE GRANULE MEMBRANES OF HUMAN POLYMORPHONUCLEAR GRANULOCYTES. THE OCCURRENCE OF FILIPIN-STEROL COMPLEXES

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Normal human granulocytes isolated by Uromiro-dextran sedimentation were investigated by means of freeze-fracture technique. Before freeze-fracturing, the cells were fixed with 2.5% glutaraldehyde in the presence of 150 μg/ml filipin (Upjohn Co). It was found that the membranes of most azurophil granules contained numerous filipin-sterol complexes while the majority of specific granule membranes lacked complexes. In granulocytes investigated without

filipin pretreatment the distribution and density of the intramembrane particles were about the same on the membranes of both type of granules. Therefore, the different particle densities could not contribute to the different appearance of the filipin-sterol complexes observed. These results indicate that in the human polymorphonuclear granulocyte the membranes of the azurphil granules contain more cholesterol than those of the specific granules.

FUNCTIONAL AND ULTRASTRUCTURAL ALTERATIONS OF MURINE BLOOD CELL MEMBRANES AFTER NEUTRON + GAMMA IRRADIATION IN VIVO

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Following mixed neutron+gamma irradiation of mice by 0.5–4.5 Gy (gamma component appr. 30%), platelets, lymphocytes and erythrocytes were separated at various time-points. Functional alterations of plasma membranes were tested by lectin-binding technique, the ultrastructural appearance of cells was studied by scanning and transmission electronmicroscopy. The experimental data show that the amounts of ^{3}H-concanavalin-A bound to the plasma membranes increased as a function of the radiation dose within 3 hours following radiation treatment. The most sensitive reactions were observed with lymphocytes and platelets as in these cases 0.5 Gy already initiated well detectable increases in lectin-binding. This functional perturbation of plasma membranes exhibited an oscillatory character. Micromorphological observations revealed tendencies of erythrocytes to develop into spherocytes, smoothening of lymphocyte surfaces and dilatations of their intracellular membranes as well as a degranulation of platelets.

ALTERATIONS IN LECTIN-BINDING FUNCTION AND SURFACE CHARGE OF PLASMA MEMBRANES UPON THE EFFECT OF IONIZING RADIATION

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Primary human embryonic fibroblasts were submitted either to x-irradiation with 2.5 Gy or to β-irradiation from tritiated water (HTO, 37 kBq per ml) for 1 to 60 min. The lectin-binding of plasma membranes was tested by ^{3}H-

concanavalin A, changes of negativ surface charges were followed by cationized ferritin. Experiments revealed that the amount of ^{3}H-concanavalin A bound to the cell surface increased already 1 minute after x-irradiation while after 10 and 60 min lectin-binding reached a level of appr. 50% above that of unirradiated control cells. In contrast, ^{3}H-concanavalin A binding did not change during the 1st minute of β-irradiation, 10 min afterwards, however, a sharp increase of 100% above the control was observed followed by a decrease of 45% below the control level at 60 min of HTO treatment. A similar radiation-induced perturbation of plasma membrane could also be demonstrated by cationized ferritin. Its binding expressed in number of particles per μm^2 was decreased to 41% of control 10 min after X-irradiation, 1 hour later, however, it returned to the level of unirradiated control cells and did not change further during the observation period of 24 hours. Both the con A and cationized ferritin bindings, though changes of opposite directions were observed, proved to be sensitive techniques for the demonstration of the radiation-induced early and temporary perturbation of plasma membranes.

THE EFFECTS OF 4-AMINOPYRIDINE ON CALCIUM MOVEMENT AND THE CHANGES OF THE MEMBRANE POTENTIAL IN THE PINCHED-OFF NERVE TERMINALS FROM RAT CEREBRAL CORTEX

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The aminopyridines, especially 4-aminopyridine (4-AP), greatly increase the number of transmitter quanta released in response to a single stimulus in electrophysiological experiments. The effect of 4-AP was similar when applied in pharmacological experiments, i.e. the release of acethylcholine from guinea pig ileum myenteric plexus was greatly enhanced by electrical stimulation in the precense of Ca^{2+}.

In our present experiments we have investigated the in vitro effects of 4-AP on $^{45}Ca^{2+}$ movements and changes of the membrane potential in the pinched-off nerve terminals by biochemical and biophysical techniques.

Initial calcium entrance into the isolated nerve endings was greatly increased in the presence of 4-AP which had different kinetical characteristics as compared to the calcium uptake in the absence of 4-AP. The fast initial phase of the calcium entrance culminated in the first 15 s after depolarization in the presence of 4-AP. In the absence of 4-AP the calcium entry peaked in 45 s after depolarization. The degrees of calcium accumulation in the treated and

untreated synaptosomes were equal during this time interval (45 s). Similar results have been obtained in biophysical studies using the potential sensitive fluorescent dye for monitoring the changes in membrane potential evoked by 4-AP treatment. In potassium rich medium the final level of synaptosomal membrane potentials was not altered in the presence or absence of 4-AP but the kinetics of the processes was different. The initial part of the synaptosomal membrane depolarization process was slower in the presence of 4-AP, (τ-4-AP, 42 s; τ-control, 18 s). Since the initial calcium entry into the nerve terminals was increased, during this higher calcium influx the rate of membrane depolarization decreased.

Summarizing our biochemical and biophysical data it may be concluded that the major effect of 4-AP on isolated presynaptic nerve endings is based on alterations of calcium movement across the plasma membrane. In the presence of this drug the rate of calcium entrance is grossly increased by K^+ depolarization, i.e. a rapid extensive calcium influx occurs. This higher calcium current may well be the basis of the "chemical potentiation" of transmitter release caused by 4-AP.

FUNCTIONAL COOPERATION OF MICROSOMAL MONOOXYGENASE ENZYMES DURING INDUCTION

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Microsomal cytochrome P-450-linked monooxygenase system is of great importance in the microsomal enzyme system.

After inducation of the supramolecular monooxygenase enzyme, complex changes in the composition and activity as well as functional cooperation occurred.

It is supposed that by altering the ratio of the components, induction will result in altered functional relations between each other and the membrane.

PHOSPHOLIPID-HAEMOGLOBIN INTERACTION IN HAEMOGLOBIN CONTAINING LIPOSOMES

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Encapsulation of haemoglobin (Hb) into liposomes (haemosomes) offers a possibility to elaborate a universal blood substitute capable of ensuring the oxygen supply transitionally, first of all in emergency cases. Regarding the

applicability, it is crucial that Hb remain stable both structurally and functionally within the lipid capsule. However, our experiments have revealed that Hb undergoes a denaturation process in liposomes, and it transforms into haemichrome. In case of haemosomes prepared by the "ether injection" method the denaturation of Hb is a fast process ensuing during the preparation, while in case of "hand-shaken" haemosomes the haemichrome formation is only slow, which can be detected only during storage, after a few days. The rate of denaturation was found to depend on the temperature, lipid composition and the met-Hb level. The analysis of the lipids extracted from haemosomes indicated increased hydrolysis and autoxidation of phosphatidylcholines. The fast haemichrome formation could be inhibited by free radical scavenger, inert gases and by the use of sphingomyelin, however the slow denaturation could not be prevented.

It is suggested that the denaturation of Hb is due to changes in the quaternary structure of Hb and to the increased autoxidation of the haeme, the two process acting concurrently.

BIOCHEMICAL AND IMMUNOHISTOCHEMICAL LOCALIZATION OF γ-GLUTAMYL TRANSPEPTIDASE IN NEURAL TISSUE

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The γ-glutamyl transpeptidase (γ-GT) has been suggested to be involved in the transport process of amino acids across the plasma membrane. Recently another possible function γ-GT in the CNS has been suggested by work in our laboratory.

In the present work the subcellular and cellular localization of γ-GT has been studied. About 95% of the total enzyme activity was bound to the particular fractions. The highest specific activity was detected in the fraction containing microvessels.

The specific activity γ-GT in microsomal and plasma membrane fractions was higher, while it was lower in synaptosomal plasma membranes than in the homogenate. Considerable differences of γ-GT activity were detected in neural tissue cultures.

The astroblast cultures exhibited the highest specific enzyme activity and it was lower in rat neuronal (mixed) cultures, while no measurable activity was observed in pure chicken neuronal cultures.

The biochemical findings were confirmed by indirect immunohistochemical studies using fluorescein isothiocyanate (FITC) and horseradish peroxidase antibody conjugates. The highest labelling was observed in the capillary endothelial cells in brain tissue slices. In tissue cultures, the round small astrocyte cells were intensely labelled by FITC.

From these results it can be concluded that of γ-GT activity is localized in glial cells and part in neuronal cells, however, the highest activity is probably exhibited by epithelial cells. The higher γ-GT activity of glial and epithelial cells and their physiological function in CNS may support the idea of a presumed role of this enzyme.

THE SUBCELLULAR DISTRIBUTION OF CHOLECYSTOKININ AND ITS APPEARANCE DURING ONTOGENESIS IN THE RAT BRAIN

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Several teams have reported the presence of immunoreactive cholecystokinin (CCK) in the brain of different species. On the basis of the presented results the following questions will be answered.

Which is the subcellular distribution of CCK in the rat brain cortex?

When does CCK first appear and how its concentration changes during ontogenesis?

Using bioassay measurements, we have found more than 50% of the total CCK activity in the synaptic structures, mostly in the synapsis rich membrane fragments. About 10 percent is localised in the nuclear fraction and 30 percent in the postmitochondrial supernatant. CCK appears already in the embryonal life and its amount increases from a low level continously up to 25th–30th days partum. The amount of CCK in the brain of the pregnant animals did not change during pregnancy and the placenta did not contain CCK activity during any phase of pregnancy.

The specific subcellular distribution of CCK in the rat brain cortex and the published data that neuronal membranes contain CCK receptors support the idea that CCK participates somehow in the neurotransmission.

COMPARATIVE STUDY ON CATION UPTAKE AND ^{3}H-OUABAIN BINDING IN DIFFERENT REGIONS OF RAT MYOCARDIUM

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^{86}Rb- and ^{42}K-uptake were studied in resting and stimulated myocardial strips isolated from the basic, middle and apical regions of the left and right ventricles. The initial rate of ^{86}Rb-influx was about twice, and ^{42}K-influx about three times higher in the right ventricle than in the left ventricle and septum. ^{42}K uptake decreased gradually from the basis toward the apex in the right ventricle. There were also regional differences in ^{86}Rb-influx of the right ventricle: the lowest value was found in the middle part and Rb-influx rate increased toward the basis and apex. In the left ventricle and septum, however, ^{42}K- and ^{86}Rb-influx showed no regional differences. The ouabain sensitive cation uptake also showed marked regional differences; the highest uptake rate was found in the apical region, and the lowest values at the basis in both ventricles. Specific ^{3}H-ouabain binding capacity of plasmalemma preparations from various part of the left and right ventricle revealed also regional differences in agreement with cation-uptake.

COMPARATIVE STUDIES ON THE EFFECT OF ANTIARRHYTHMIC DRUGS INFLUENCING Ca^{2+} TRANSPORT PROCESSES IN CARDIAC, SKELETAL AND SMOOTH MUSCLE

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In spite of the fact that different antiarrhythmic drugs are widely used in the clinical practice, the exact molecular mechanism of their action remains to be clarified. Presumably their cardiological effects are related not only to monovalent but also to divalent cation transport processes. Therefore we studied whether Ca^{++}-transport of the sarcoplasmic reticulum could be influenced by antiarrhythmic compounds (benzcyclan, quinidine, lidocain and pindolol); furthermore, we examined what kind of differences could be detected in their

action using heart, skeletal and smooth muscle preparations. Benzcyclan and quinidine inhibited in a dose dependent manner both Ca^{++}-binding and Ca^{++}-uptake as well as Ca^{++}-ATPase activity of sarcoplasmic reticulum prepared from heart tissue, while lidocain and pindolol proved to be ineffective. In contrast to heart tissue only benzcyclan influenced the Ca^{++}-transport in skeletal muscle exerting an ionophor-like effect: it decreased Ca^{++}-uptake and potentiated Ca^{++}-ATPase activity. Although in smooth muscle preparation benzcyclan and quinidine acted similarly as in the cardiac tissue, pindolol enhanced the Ca^{++}-uptake only in this tissue.

Our data indicate that the antiarrhythmic drugs tested have different effects on Ca^{2+}-transport processes in various types of muscles.

EFFECT OF AGENTS INFLUENCING CONTRACTILITY ON MONOVALENT CATION BALANCE OF CARDIAC TISSUE

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The aldosterone antagonists exert a direct action in the heart by promoting the transport of K^+ into the cells without affecting the $Na^+ + K^+$-ATPase activity or its inhibition by ouabain (Somogyi et al. 1977).

In the present study the action of drugs influencing contractility of the cardiac muscle (lidocain, Halidor and verapramil) was studied on the K^+ balance of isolated Langendorff heart preparations from guinea pigs as well as on the plasma membrane preparations from pig heart tissue. These compounds did not influence K^+ balance of the isolated heart in any concentrations tested. On the other hand, when they were applied prior to or simultaneously with ouabain, the loss of K^+ from heart cells due to ouabain administration was lowered in a dose dependent manner. These drugs caused a dose dependent inhibition of $Na^+ + K^+$-ATPase and its partial reactions.

It can be concluded that in cotrast to the aldosterone antagonists, these compounds can influence be binding of ouabain. Their effect excerted on the $Na^+ + K^+$-ATPase does not seem to be specific, as their concentrations capable of inhibiting the enzyme was several orders of magnitude higher than that of ouabain.

EFFECT OF ACROLEIN ON ELECTROMECHANICAL COUPLING OF SKELETAL MUSCLE

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Recently it has been established by Japanese authors that acrolein applied in millimolar concentrations prevents the mechanical responses of muscles to electrical square wave impulses. In the present experiments it was found that this agent dissolved both in Na^+ and in Na^+ free Ringer's solution elicited a transient depolarization of the membrane and a contracture of the muscle, respectively .These were followed by relaxation and then repolarization depending on conditions of incubation.

After treatment of the muscle with acrolein its ability to contract practically ceased, while contracture could be still evoked by coffeine. In Ringer's solution, when depolarization in response to acrolein started to develop, a series of action potentials, i.e., firing could be observed which was accompanied by simultaneous seizure of the muscle.

From these results it has been concluded that a reduction of chloride conductance may be involved in these phenomena.

ROLE OF ADP IN THE REGULATION OF ATP-ASE IN ACTOMYOSIN SYSTEM

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ATP was measured by luciferin-luciferase and turbidity was registered by spectrophotometer during superprecipitation of rabbit skeletal muscle actomyosin elicited by ATP. The correlation between the changes of ATP level and turbidity was studied in relation to the concentration of ADP added together with ATP. It was found that during phasic superprecipitation [cf. Csabina et al. Muscle Res. Cell Motility, **1**, 512 (1980)] changes in the rate of decrease of ATP concentration and turbidity were synchronous and both depended on the concentration and ratio of ATP and ADP. For the explanation of these phenomena a model was constructed in which the phases were related to complexes of different composition for myosin, actin, ATP and ADP. In response to an increase of ADP concentration a special complex of the dimer myosin molecule evolved in which one myosin head was bound to actin and ADP, and the other one to ATP only. The conformation of the two myosin heads changed alternately, resulting in a moderate increase of turbidity (first phase of superprecipitation), and in a controlled level of actin activation.

VESICULATION AND K—Na TRANSPORT IN STORED HUMAN ERYTHROCYTES AND IN ATP-DEPLETED FRESH RED CELLS

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Cell shape, vesicle release, ATP content and K-Na transport capacity were followed in red cells collected in ACD and stored under blood banking conditions, as well in fresh cells depleted at 37 °C through glucose deprivation. Reversibility of changes were tested by 37 °C (rejuvenation). During storage, discocyteechinocyte transformation, membrane vesiculation and decrease of ATP-level occured progressively. Also a marked decrease in ouabain-sensitive components of K-Na fluxes was found. A major fraction of released vesicles was membrane-bound red cell fragments, with a ruthenium red and Con-A-peroxidase stained cell coat. Higher acetylcholinesterase and lower Ca^{2+} and ouabain-sensitive ATP-ase activities were measured in the vesicles than in the ghosts. By SDS-electrophoresis, practically each type of membrane protein was demonstrated with a marked increase of the 55–58 000 Mw fraction; the presence of high Mw complexes was also apparent. Rejuvenation restored ATP content and ion-fluxes to the normal level and lowered achinocytosis considerably. After ATP-depletion, similarities were found as regards morphology and enzyme activities of released vesicles, but a conspicuous difference was demonstrated in membrane composition: abundant Band 3 protein and some spectrin contamination were observed. Rejuvenation resulted in shape reversion of depleted cells, neverteless neither ATP content nor active pump mechanisms were completely restored.

In conclusion it is emphasized that there are essential differences between the mechanisms of storage changes and short-term ATP-depletion of red cells.

CHARACTERIZATION OF HUMAN K562 LEUKEMIA CELLS AFTER HAEMIN ADMINISTRATION

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Human leukemia K562 cells kindly provided by Prof. G. Klein, Stockholm, were treated with 46 μM haemin (Koch-Light). To follow the kinetics of the inducation of haemoglobin synthesis, the binding of ^{125}I-labelled transferrin, Fe uptake from ^{59}Fe-labelled transferrin and acetylcholinesterase activity were measured until the 7th day of treatment. On the 5th day, when haemoglobin

reached the highest level, the binding of ^{125}I-transferrin, ^{59}Fe uptake and acetylcholinesterase activity were also significantly elevated. The HbX was the most abundant haemoglobin synthetized in haemin stimulated cells.

Subcutaneous injection of K562 cells into immune-suppressed mice resulted in solid tumour formation. Surface markers and karyotype by G banding were identical in the K562 solid tumour and in the cells of the tissue culture. The characteristic cytogenetic markers were similar to those published earlier (Klein et al. 1976) though further rearrangements occurred. Three copies of chromosomes 11 and 16 (coding for the β-like and the α-like globin genes, respectively) and Philadelphia chromosome could be detected.

Based on the present observations and some recent data the question is raised wether haemin treatment results in a genuine erythroid differentiation process or whether it simply increases the rate of synthesis of specific proteins produced in minute amounts in the untreated cells as well.

ACETYLCHOLINESTERASE ACTIVITY OF ERYTHROCYTES AND LYMPHOCYTES DERIVED FROM AGED NORMAL AND DEMENTED SUBJECTS

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Acetylcholinesterase (AchE) as a typical membrane enzyme is a suitable marker for changes occurring on cell surfaces. Although it is characteristic to the brain and muscle, it is also detectable on various blood cell membranes in different amounts. Decreased AchE activity of the brain was reported in various mental diseases. Moreover, certain myopathies are characterized by low AchE activity both in the muscle and in the erythrocytes. Since blood cells are readily available, it would be a convenient diagnostic tool if the known alterations of the brain tissue during normal and mentally altered aging process were reflected in AchE activity of the erythrocytes and lymphocytes too. Our results show that AchE activity of a given cell is dependent on its age: the more immature the cell, the higher its activity is. On the other hand, AchE activity of the blood cells is decreasing with normal aging of the subject. The differences between the AchE activity of the normal control group and the group of healthy elderly people was significant and a significant difference could also be demonstrated between the normal controls and the aged demented patients while the aged healthy and demented group did not differ significantly from each other in respect to their blood cell AchE activity. These results are in good agreement with other data on blood cell membrane alterations during the normal aging process.

THE TURNOVER OF FATTY ACIDS, CHOLINE AND ETHANOLAMINE IN EHRLICH ASCITES TUMOR CELLS AT VARIOUS STAGES OF TUMOR PROLIFERATION

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The kinetics of uptake and turnover of ^{14}C-palmitic acid and ^{14}C-oleic acid was examined in vitro in the various fatty acid esthers of Ehrlich ascites tumor cells. A differences was found both between the quantity of uptake and the turnover kinetics in the cells at various stages of tumor growth. A greater amount of ^{14}C-fatty acid was incorporated into the phospholipids of cells derived from the earlier, proliferative period of tumor growth, than those of the later period, moreover the release was found to be also somewhat greater. The incorporation into neutral lipids was similar in both cell populations, therefore a lower ^{14}C-phospholipid/neutral lipid ratio was characteristic in the cells from the late period of tumor growth. At each time of incubation this ratio (^{14}C-PL/NL) was found to be < 1, and the highest ^{14}C activity could be measured in the phosphatidylcholine fraction.

The data point to the existence of at least two independent free fatty acid pools in the cells as suggested by the kinetics of uptake and release of ^{14}C fatty acids. One of them can be characterized by a rapid exchange between the cell fatty acid esthers and the surrounding medium. The other one, however, must have a longer life time, since a part of ^{14}C activity was found to be associated to the various fatty acid esthers during the whole period of investigation (13 hours).

The rate of de novo PC and PE biosynthesis, as measured by the incorporation of ^{14}C-choline and ^{14}C-ethanolamine, was also greater in the cells derived from the earlier period of tumor growth. The activity of the methylation pathway, as measured by the ^{14}C-ethanolamine incorporation into PC and SM fractions, was found to be decreased in the cells from the late period of tumor growth. Presumably the lack of the methylation pathway gives rise to the changes in the PC/PE ratio and the decrease of the SM content in these cells.

Acta Physiologica Hungarica, Volume 62 (3—4), pp. 287—288 (1983)

Book Reviews

E. Chazov, V. Smirnov, N. S. Dhalla (Eds.)

Advances in myocardiology, Volume 3

Plenum Medical Book Company, New York and London 1982, 656 pages.

This new volume of the series contains part of the proceedings of the Tenth Congress of the International Society for Heart Research, which was held in Moscow on September 23–29, 1980, and has been devoted to Richard J. Bing and Alexi Chernukh for their inspiration in bridging the gap between the basic and clinical aspects of cardiology as well as for their pioneering work on cellular metabolism in studying the pathophysiology of heart diseases.

The volume consists of two sections. The first section provides a comprehensive analysis of recent advances in cardiac physiology and pharmacology. There are 25 chapters in this section, dealing with the mechanical, ultrastructural and electrical properties of cardiac muscle as well as with cardiac adrenoceptors and the optimal calcium concentration for cardioplegic solutions. The five chapters written by Hungarian authors discuss the effect of prostacyclin on cardiac transmembrane currents (R. Markó et al.); the drugs affecting both the tetrodotoxin-sensitive sodium mechanism (K. Kelemen et al.) and the calcium dependent slow depolarization mechanism (V. Kecskeméti et al.); the effect of potassium canrenoate on norepinephrine metabolism and potassium ionic currents (Z. Antaloczy et al.); and the pharmacological evaluation of electrical events in the myocardium (L. Szekeres).

The second section on cardiac membranes and metabolism contains 34 chapters that provide information on complex processes involved in energy production energy utilization, cation transport, and regulation of metabolism in the myocardium. One chapter in this section dealing with the glycogen-containing lysosomes and glycogen loss in the cardiocytes of embryonic and neonatal mice is written by a Hungarian author (Sz. Virágh).

It would be difficult to discuss only a single one of these chapters in the framework of this review. It should, however, be stated that the majority of the authors have presented new experimental data. There is an index in the book, facilitating quick orientation.

G. Pogátsa

E. Flückiger, E. del Pozo, K. von Werder

Prolactin

Monographs on Endocrinology, Springer–Verlag, Berlin–Heidelberg–New York, 1982
224 pages with 54 figs. 14 tables. Price DM 96.—, approx: US $ 44.70

Prolactin was discovered in 1928 and has caused since that time several intriguing problems in human physiology. Does it exist at all or do we attribute some effects of growth hormone to a non-existing compound? What is its function in the male? And an up-to-date question: in physiological concentrations does it stimulate or inhibit gonadal functions? The existence of prolactin as a hormone different from growth hormone was borne out only 40 years after its discovery and its physiological role in the human — apart from that in lactation — is still mysterious. Nevertheless, with the aid of specific radioimmunological methods, more and more pathological conditions have been revealed to be associated with hyperprolactinaemia. The postulated role of prolactin in several sexual dysfunctions has stimulated research in this field for several years and the monograph at issue gives an excellent survey of the results of physiological biochemical and clinical works accomplished in recent years.

The first chapter deals with the synthesis, fate and actions of prolactin. Data on chemistry and synthesis of the different forms of prolactin, its level in various body fluids, its metabolism as well as its actions on genital and extragenital tissues are summarized in the chapter. The pertaining literature is almost complete and data obtained in animals are compared with those obtained in the human. In view of the several contradictory observations published in the literature it is not surprising that in several cases the reader cannot draw any conclusion from a multitude of data.

The second chapter, dealing with the control of prolactin secretion, included data on secretory patterns, demonstrated and postulated regulatory factors and mechanisms and discusses in detail the dopaminergic mechanisms inhibiting prolactin secretion. This chapter also describes the action of some drugs acting via serotoninergic, cholinergic of GABA-ergic mechanisms on prolactin secretion.

The chapter dealing with the physiology and pharmacology of lactation describes all the known hormonal and neural factors preparing the mammary tissue for lactation during pregnancy and maintaining lactation after delivery. An up-to-date review of the pharmacological control of lactation is given.

The second half of the monograph deals with the pathological conditions associated with hyperprolactinaemia.

Chapter 4 deals with nontumoural hyperprolactinaemia while Chapter 5 describes pituitary prolactinomas. The pathology, illustrative clinical cases and therapeutic attempts of all known hyperprolactinaemic forms are described here.

The monograph contains the literature published till the end of 1980 and a few references from 1981 are also included. It is textbook very useful in all respects for physiologists teaching the biology of reproduction and for clinicians seeing patients more and more frequently with troubles of prolactin secretion.

A. SPÄT

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Schmidt, R. F.: Fundamentals of Sensory Physiology. Springer Verlag, New York–Heidelberg–Berlin 1978.

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