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Physiologia — Pathophysiologia

NEUROTOXICOLOGICAL INVESTIGATION OF THE NEW HUNGARIAN PESTICIDE TOXURAZINE®

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(Received September 30, 1981)

The new total herbicide Toxurazine® was subjected to general toxicologic and neurotoxicologic examinations. Rats were treated with 1/80, 1/40 and 1/20 of the acute oral LD_{50} for three months. The animals showed a decrease in body weight and a transitional anaemia. The product had a slight liver damaging effect. Renal function tests and urine analysis were negative. With time, the learning ability of animals tested in maze for 42 days became impaired dose dependently. At the end of the treatment, both at rest and on flashlight stimulation, as well as in complex EEG activity and in each frequency band there was a decrease in electric activity. In electroneuromyographic tests no effect damaging the peripheral nervous system could be detected. The hot plate method showed the reaction time of the animals to be slightly extended.

The results suggest that the compound should only be used in uncultivated areas.

In agriculture, a revolutionary change was induced by the advent of herbicides. The number of active ingredients is continuously increasing and in spite of the severe health protective measures, the plant protective compounds offer several possibilities for accidental or intentional intoxication. Plants treated with herbicides may represent a harm for the whole community caused by pesticide residues in foods. Therefore, the task of hygienic toxicology is to detect the toxicity of chemicals in the environment, to determine the conditions of their application, and to establish hygienic standards.

The present paper reports on the toxicological investigation and evaluation of the new herbicide Toxurazine®.

Materials and Methods

Examined product: TOXURAZINE® (Budapest Chemical Works)

Composition: 15% atrazine, 15% chlorinol, 30% aminotriazole, 40% vehicle.

Duration of the experiment: Three months.

Animals: Male and female CFY-strain rats of 150 g body weight from the random breed of LATI Gödöllő, Hungary.

Treatment: 1/80 (37.5 mg/kg/day), 1/40 (75 mg/kg/day), and 1/20 (150 mg/kg/day) of the oral LD_{50} of Toxurazine®.

Control: Vehicle (150 mg/kg/day).

Ten male and female animals each were used in all the groups. Toxurazine® was given to the animals mixed into their food (8 g/100 g body weight) daily.

General toxicological tests

1. Measurement of body weight once a week.
2. Haematological tests (total and differential blood counts, haemoglobin and haematocrit values) once a month.
3. Liver and kidney function tests [bromsulphophthalein (BSP) and phenolsulphophthalein (PSP)] after 90-day treatment [8].
4. Urine analysis at the end of the third month.

Neurotoxicological tests

1. Psychophysiological examinations by the maze method in the first six weeks [2]).
2. In the 12th week of the experiment electrocorticographic records were taken by an 8-channel EEG apparatus (EMG 4751/2 Type) at rest and with light stimulus loading, by silver electrodes implanted over the motor and visual cortex of rats anaesthetized with 40 mg/kg body weight pentobarbital. The EEG curve was separated into six different frequency bands by an EMG 5204/B type frequency analyser-integrator. [3, 4].
3. Electroneuromyographic examinations [7] were carried out by a Medicor MG-231 type electromyograph and an EMG 4767 type electrostimulator on the 12th week of the experiment. Concentric TE-1 type and bipolar FC1/2 type needle electrodes were used. Registration from the oscilloscope was done with a MFI-1 type photoregister. We determined the conduction velocity on the tail nerve fibres of rats anaesthetized with 40 mg/kg body weight of pentobarbital. The conduction velocity was defined as the quotient of the distance between the two registering electrodes per the difference of the latency period of action potentials evoked with supramaximal, 1 Hz, 0.2 ms stimuli.
4. Studies of the reactivity using the hot plate method [10]. In these tests the rats were placed on a hot surface of 56 °C and the time was measured until the animals had licked their paws in reaction to the heat. The changes were expressed in per cent values, so that the suppressive effect of the compound was considered 100% if the reaction time of the pretreated animals increased to 2.5 times the control value.

Results

General toxicological examinations

In the course of the 90-day subacute treatment, there was a dose dependent and significant ($p < 0.001$) lag in the gain of body weight from the 2nd month of treatment as compared to the controls (Fig 1).

In the haematological study there was no significant change between the treated and untreated animals. The haemoglobin and haematocrit values showed a decrease after 60 days of treatment. At the end of the 3rd month no sign of anaemia was detected.

In the liver tests 20 min after the i.v. injection of 50 mg/kg body weight BSP, PSP retention increased significantly ($p < 0.001$) in all the three treated male rat groups, while in the female rats a significantly increased retention ($p < 0.001$) was observed only after the two higher doses (Fig. 2).

In the kidney tests 45 min after the i.v. administration of 10 mg/kg body weight of PSP a serum level of 0.37 ± 0.02 mg/dl was found in the control male animals, and one of 0.47 ± 0.04 mg/dl in the females.

In the animals treated with vehicle and with different doses of Toxurazine® an insignificant dye retention was observed.

Urine analysis with N-Multistix in the 12th week of the experiment gave normal values. The same was the case with the Erlich test and the sediment.

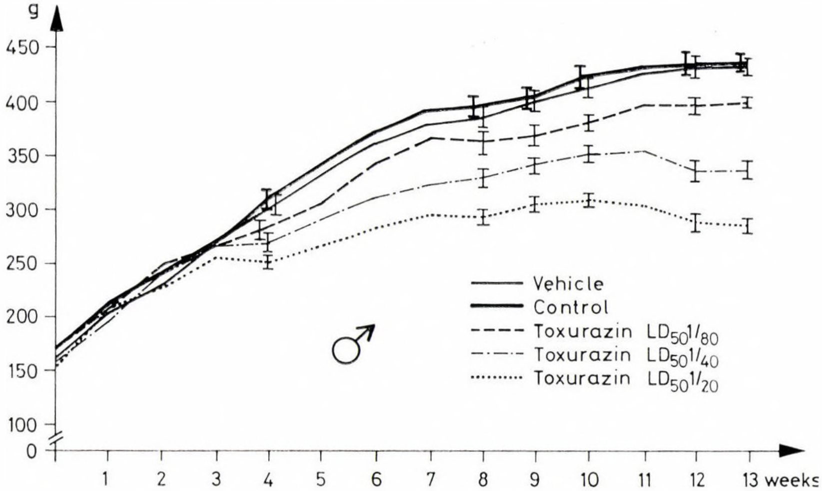

Fig. 1. Body weight curves

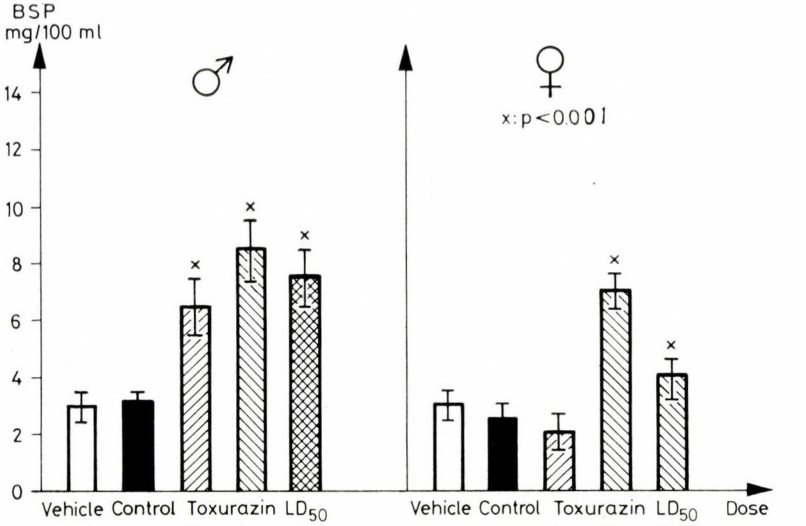

Fig. 2. Liver function tests

Neurotoxicological examination

The learning process was studied in a maze. Under the effect of Toxurazine® the running time of male and female animals increased dose dependently and significantly ($p < 0.001$) by the end of the experiment as compared to the controls and to the rats treated with vehicle (Fig. 3). The number of errors increased dose dependently and significantly ($p < 0.001$ in males;

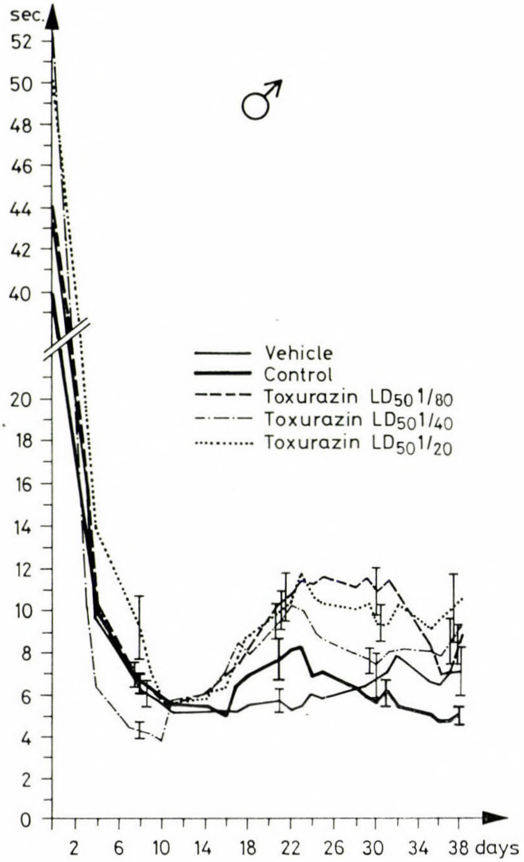

Fig. 3. Maze test: running time

$p < 0.01$ in females) in all the three treated groups on the 42nd day of treatment (Fig. 4).

In the EEG records taken at rest during the 12th week of treatment, both the bioelectric activity of the brain and the activity of each band decreased significantly in both the male and female groups as compared with the controls. Band analysis revealed in the animals treated with 1/40 and 1/20 of the LD_{50} of Toxurazine® a decrease of activity of the slow delta and theta waves, as well as of the alpha waves of medium frequency and of the fast $beta_1$ and $beta_2$ waves. In the gamma waves of the fastest frequency the activity was approximately the same as in the controls with the exception of the change caused by 1/40 of the LD_{50} (Fig. 5).

Under flashlight loading a similar tendency was observed as on the records taken at rest, only the changes were more definite (Fig. 6).

Electromyographic investigations showed that the reaction of the evoked muscle action potentials was normal. The amplitude and duration of muscle

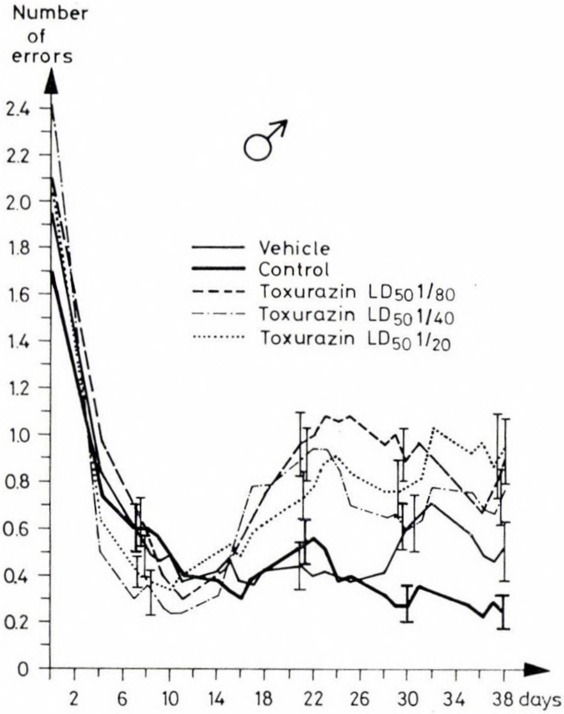

Fig. 4. Maze test: number of errors

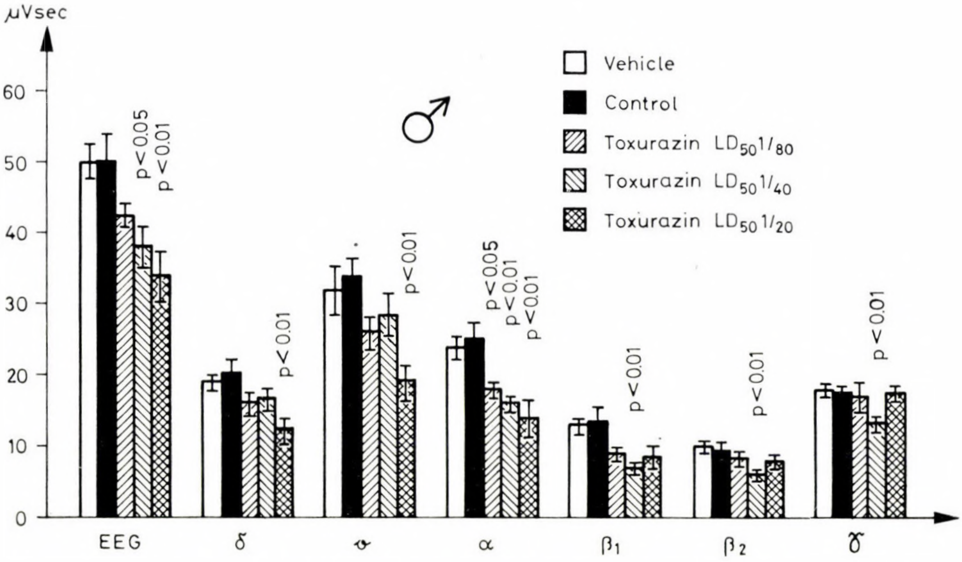

Fig. 5. EEG, graphic plotting of curves taken at rest

action potentials did not show alterations as compared to the controls. No decrease in maximum motor conduction velocity was observed (Fig. 7).

As to the reactivity, an increase in nociceptive reaction time was observed in the hot plate test after 3-month treatment. The most definite but still not significant effect was observed in the animals treated with 1/20 of the LD_{50} of Toxurazine® (Fig. 8).

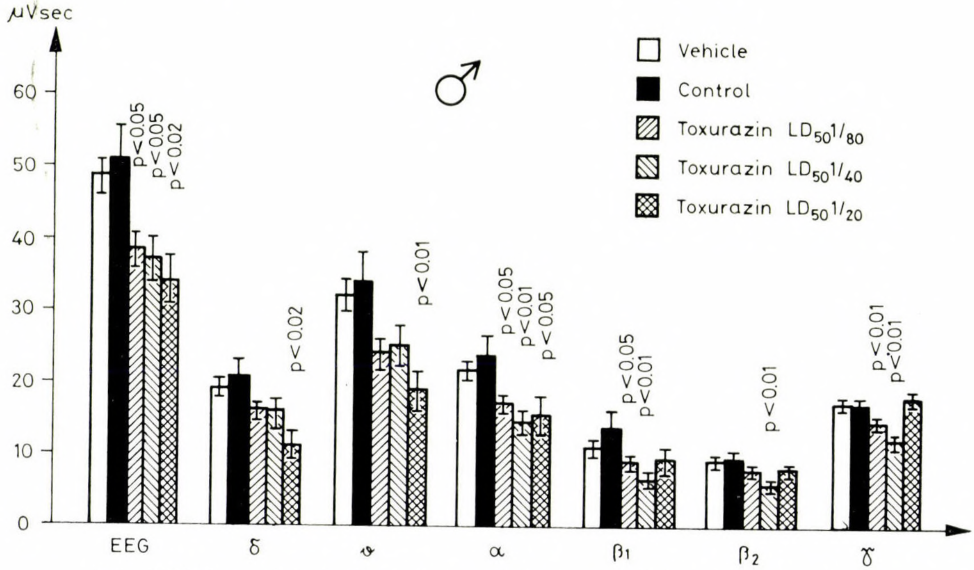

Fig. 6. EEG, graphic plotting of curves taken under 18 Hz flashlight stimuli

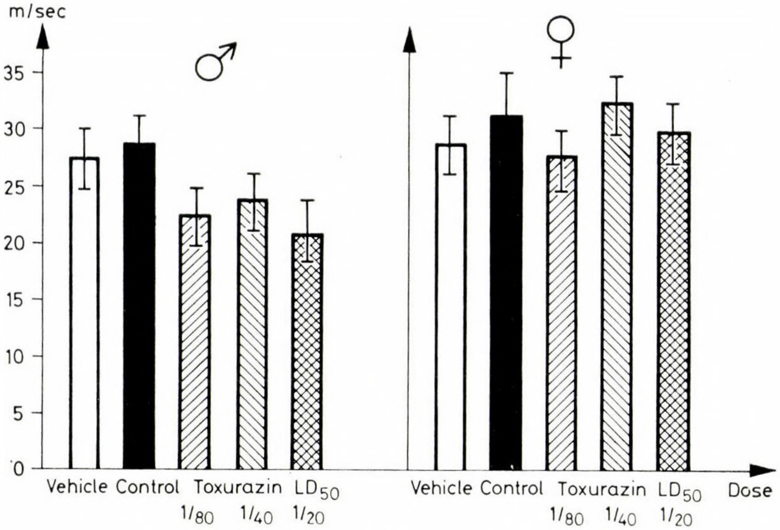

Fig. 7. Conduction velocity

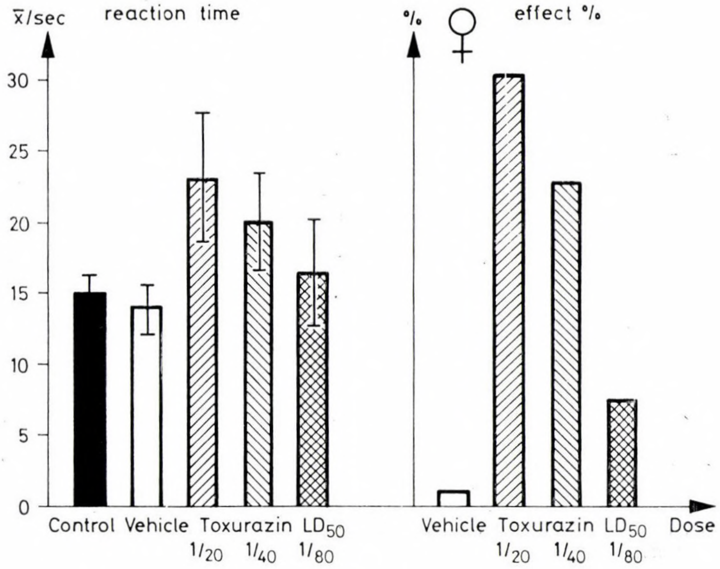

Fig. 8. Hot plate test: effect on reactivity

Discussion

According to the literature the three active ingredients of Toxurazine® have the following properties:

Atrazine: acute oral LD_{50} in rats: 3080 mg/kg body weight; percutaneous toxicity in rabbits: 7500 mg/kg body weight [6]. Atrazine has no allergic effect [11] or cumulative toxicity [9].

Chlorinol: acute oral toxicity in rats: 2100 mg/kg body weight; dermal toxicity in rats: 7100 mg/kg body weight [6]; moderate skin-irritating effect [1]. 3-month subacute treatment with 75 mg/kg body weight dose did not induce pathological changes in rats. [5]).

Aminotriazol: oral LD_{50} in rats: 1100–2500 mg/kg body weight; percutaneous toxicity: 10 000 mg/kg body weight [6]; moderate skin-irritating effect. A 68-week subacute treatment with 50 ppm daily did not cause changes in rats [9].

According to our results, the acute LD_{50} of Toxurazine® for the rat was 3000 mg/kg body weight. An 80% aqueous suspension caused a mild transitory skin inflammation. The product had no sensitizing effect. In the course of 12-week treatment we observed a loss of weight after the 3rd week of the experiment. A mild anaemia was seen after 2 months treatment but it disappeared by the 12th week. The product proved to have a liver-damaging effect. Kidney function test and urine analysis gave normal results.

Both the psychophysiological and electroencephalographic tests showed that the central nervous system was affected. An impairment of the learning ability was revealed by the maze test and an inhibition of brain activity by the EEG records. Still, in the electroneuromyographic experiments, by evaluating the parameters of each potential evoked by supramaximum stimuli and by determining the conduction velocity, no changes were observed after Toxurazine® treatment. In the hot plate test the nociceptive reaction time was slightly but not significantly lengthened. On the basis of the results Toxurazine® may be applied as a herbicide only in agriculturally uncultivated areas/ (beds of railroad, etc.), and in view of its toxicity strict safety measures must be applied during its use.

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ROLE OF A SECRETAGOGUE IMMUNOGLOBULIN IN GASTRIC ACID SECRETION

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Results of this study showed that in a considerable number of patients with duodenal ulcer a globulin of the IgG class was responsible for the enhancement of HCl secretion. This secretagogue globulin appeared to combine directly with the H_2 receptors of the parietal cells thereby increasing hydrochloric acid output without the formation of antigen-antibody complex.

In the genesis of peptic ulcer which is a multicausal disease the imbalance between the aggressive and defensive factors is of paramount importance [3, 7, 21]. In the pathogenesis of duodenal ulcer the aggressive factor, i.e. the overproduction of HCl is known to play a decisive role [3, 15, 32, 26, 62, 7]. In a number of patients with the active phase of this disease the secretion escapes regulation and feedback control. The gastric mucosa becomes hypertrophic and is the target of secretagogue and trophic stimuli of unknown origin [3, 15, 32, 56, 26, 7].

In animal experiments duodenal ulcer can be induced only by enhancing acid output by various means. Ulcer development can, on the other hand, be prevented by the intraluminal application of sodium bicarbonate solution [18, 27, 52].

In the corpus of the stomach the mucosa undergoes hyperplasia [17, 15, 54] thus the defensive factors are steadily strengthened along with the increase of aggressive ones and ulcers do not develop in this area. The self-digestion occurs primarily in such regions where the anatomical conditions favour such events, thus in the duodenum, due to the overproduction of HCl, the bulbus becomes markedly acidified for a long period [2, 26] and the conditions for H^+ back-diffusion are 45 times more favourable than in the corpus of the stomach [35, 25].

Since the aggressive supersecretion of HCl appears to be the key inducer of duodenal ulcer, we searched for a hitherto unexplored factor which is capable of stimulating the mucosa of the corpus of the stomach.

The immune system is known to play an important role in the direct or indirect control and modulation of many functions of the body [12, 13, 14, 50]. The immune apparatus can produce soluble molecules (lymphokines) of lymphocytic origin and immunoglobulins with stimulating effects such as LATS, TSI [1, 49, 54] which activate the target cells, eliciting their hyperfunction and hyperplastic responses. In the knowledge of these findings it seemed reasonable to search for a secretagogue factor in the immunoglobulin fractions of patients with duodenal ulcer.

Materials and Methods

We have first separated the total globulin fraction from the sera of patients with superacidity. The serum was subjected to dialysis in phosphate buffer (pH 5.8) for 48 hours and washed three times successively with distilled water to remove possible albumin and polypeptide contamination. The sediment was redissolved in TRIS buffer (pH 8.0). Control sera drawn from healthy subjects were treated in the same way. The globulin solution separated from 4.0 ml serum was lyophilized.

Effects of euglobulin solution were analysed using an *in vivo* rat stomach test (Ghosh–Schild 31) slightly modified, i.e. following the 1 hour "basal" secretion the human globulin was injected i.v. and the gastric secretion was continued to be monitored for an additional 8 hour period in Viadril anaesthetised rats. A cannula was introduced into the stomach through the cardia and another one via the pylorus. The stomach was perfused with 20 ml physiological saline in one hour through the upper cannula, then the HCl concentration in the fluid collected via the pyloric cannula was titrated (NaOH 0.01 N) using a microburette. A total of 75 bioassay procedures were performed utilizing the globulin fractions from 51 patients. In a control series 18 rats were treated either with buffer solution, saline, or with the same concentration of pooled globulin fractions obtained from healthy subjects, or were not treated at all.

From the sera of patients with secretagogue activity native IgG was purified using DEAE Sephadex A 50 in a batch method [22] and tested in the previously described bioassay system. The remaining total globulin fraction was also assayed in the same test system.

In the globulin fractions with or without secretagogue activity and in the sera immunoreactive *gastrin* concentrations were determined using the CIS RIA kit [38, 46].

Sections were prepared from the corpus and antrum of the rat stomach at a thickness of 6 μm in a *Pearse-Slee H* type cryostat. The sections were incubated in the presence of globulin or diluted IgG with secretagogue activity, then FITC labelled antihuman globulin was added and the binding of human globulin was detected by this immunofluorescent technique [48, 5]. The same studies were performed with globulins derived from healthy control subjects.

In another series of studies the sections were similarly incubated with the secretagogue globulins but then reincubated with antihuman globulin in the presence of separated [10] human lymphocytes and cooperativity [6, 53] was estimated. Thereafter the sections were fixed and subjected to differential staining as described by IRVINE [36].

The *in vivo* secretion test was also performed in cimetidine-treated rats. The drug was administered (4 mg i.v. or 10 mg into the jejunum) after the basal secretion had been recorded.

The *in vitro* immunofluorescent studies were also undertaken after preincubation of the rat stomach corpus sections with various concentrations of *cimetidine*.

The rosette formation of H_2 receptor bearing human lymphocytes was also examined by the addition of secretagogue and non-secretagogue globulins and various concentrations of *cimetidine* in an assay system described by KEDAR and BONAVIDA [40] as well as KÁVAI et al. [39].

Globulins containing antibodies against parietal cells isolated from sera of patients with pernicious anaemia were also tested in both the *in vivo* secretion test and *in vitro* immunofluorescent incubation system with and without *cimetidine* preincubation.

A total of 51 patients were subjected to routine physical and laboratory examinations including LE-cells [8], antinuclear antibody, Rose–Waaler test, organ directed (stomach and thyroid gland crude antigen) antibody titer [9, 57]. Clinical data and history, the course of the disease and laboratory values were grouped and evaluated according to carriers and non-carriers of secretagogue globulins.

Results

Results are shown in Fig. 1. The values of HCl secretion are referred to BAO taken as unit. In 25 out of 51 patients the globulins produced an apparent increase of secretion. The effect developed gradually, being the most pronounced at 3 and 4 hours. Values for control and negative series remained within the range of BAO. Reproducibility was checked by repetitive assays. There were 18 control cases. In the first 18 of the secretagogue positive cases the maximum HCl output is given in μg as compared with the controls (Table I). It is seen that in the control group acid production decreased to less than half of the initial value (2181 μg) whereas in the group treated with secretagogue globulin it increased more than 2.5fold (7713 μg).

Table I

Total HCl production of the rat stomachs in the control group (n=18) and in the group (n=18) treated with globulins having secretagogue effect

Hour	Control μg HCl	Secretagogue globulin μg HCl
Basal	4710	3002
3rd	2413	8180
4th	2181	7713

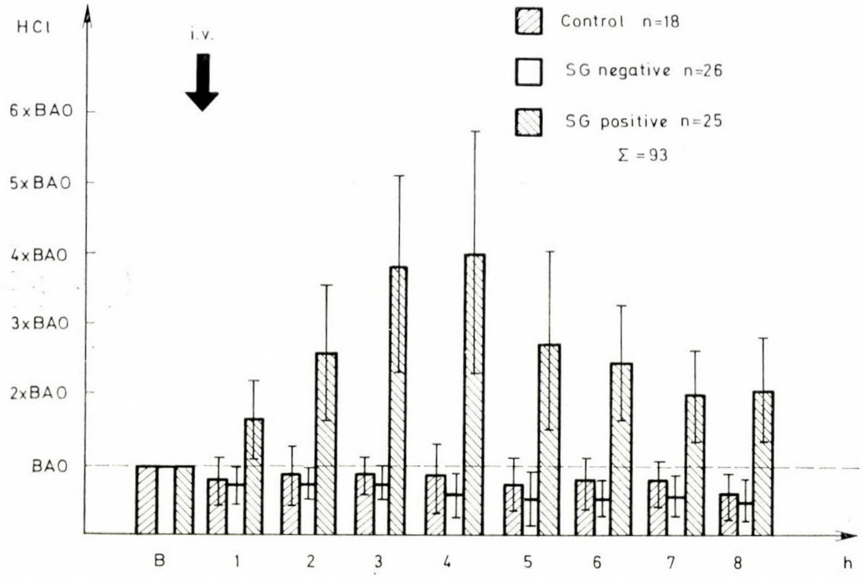

Fig. 1. HCl production of rat stomachs after i.v. administration of globulin derived from patients with duodenal ulcer in comparison with control ones. The values are referred to the "basal" secretion being taken as unit

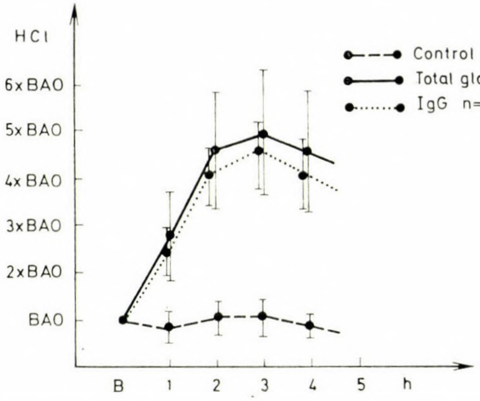

Fig. 2. Response of rat gastric HCl secretion, expressed in basal secretion units, to total globulins with secretagogue effect and to IgGs derived from the same patients

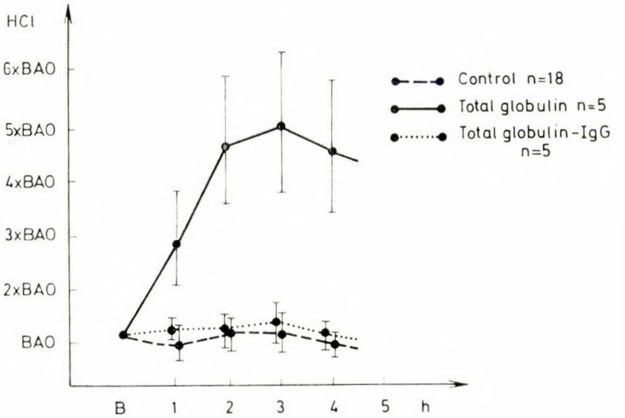

Fig. 3. Response of rat gastric HCl secretion, expressed in basal secretion units, to total globulin with secretagogue effect and total globulin free from IgG derived from the same patients

In the bioassay studies purified IgG or total globulin fractions from sera with secretagogue activity elicited similar secretory responses (Fig. 2). However, the IgG-free total globulin fraction had no secretagogue activity (Fig. 3). Generally, 20–25 mg of IgG was required to induce secretory response in rats weighing 200–250 g.

In the globulin fractions and sera with secretagogue activity immunoreactive gastrin was determined. No hypergastrinaemia was seen in these sera. In globulin fractions isolated from sera the gastrin concentration decreased to less than 25% of the initial value which is about 2% of the minimal effective dose. Gastrin concentrations in globulins and sera with no secretagogue action were also at this level (Table II).

Table II

Gastrin contents of sera of patients with duodenal ulcer and total globulins in those having and not having a secretagogue effect

IMMUNOREACTIVE GASTRIN pg/ml					
Secretagogue positive			Secretagogue negative		
Patient	Serum	Tot. glob	Patient	Serum	Tot. glob.
Sz. B.	100	11.5	O.I.	85	8.2
D.L.	80	9.2	V.L.	75	4.6
F.I.	100	23.5	B.J.	92	12.7
N.J.	60	27.5	A.G.	65	30.5
Gy.E.	75	25.3	K.Z.	75	48.8
P.J.	110	29.4	V.F.	100	53.8
Total	525	126.4		492	158.6
Mean	87.5	21.1		82	26.4
SEM	17.3	7.8		−11.3	19.6

Level of significance: secretagogue positive sera vs. tot. glob. $P < 0.001$
secretagogue negative sera vs. tot. glob. $0.05 > P > 0.02$

Upon incubation of rat stomach sections with secretagogue globulins we could show human globulin binding in the mucosa of the corpus (glandular part) using indirect immunofluorescent technique (Fig. 4). No such binding was observed in the antral region. By adding non-secretagogue globulins to the incubation medium binding failed to occur even in the corpus.

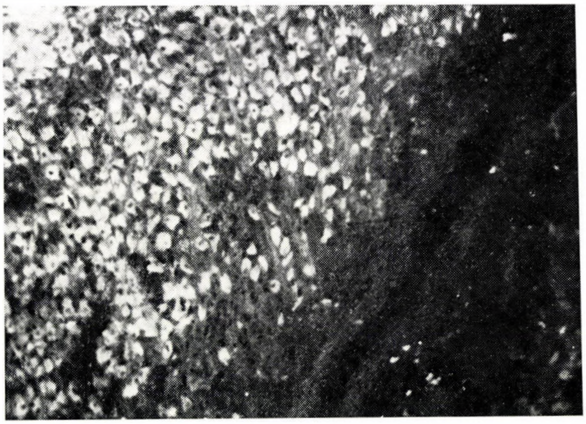

Fig. 4. Indirect immunofluorescent appearance of rat gastric corpus mucosa. Binding of the human globulin is seen in the glandular area

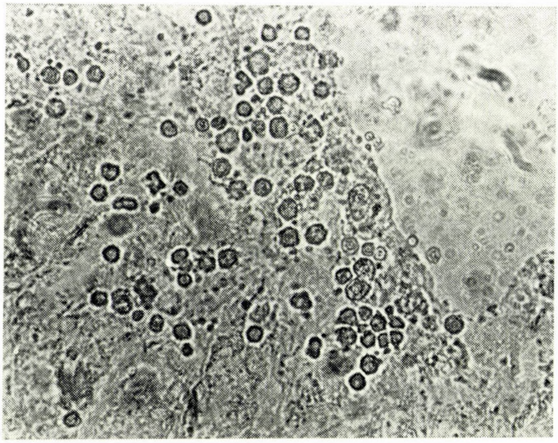

Fig. 5. Lymphocyte cooperation phenomenon on rat gastric corpus mucosa after incubation with globulin having a secretagogue effect, then with anti-human globulin and human lymphocyte suspension (10^7/ml)

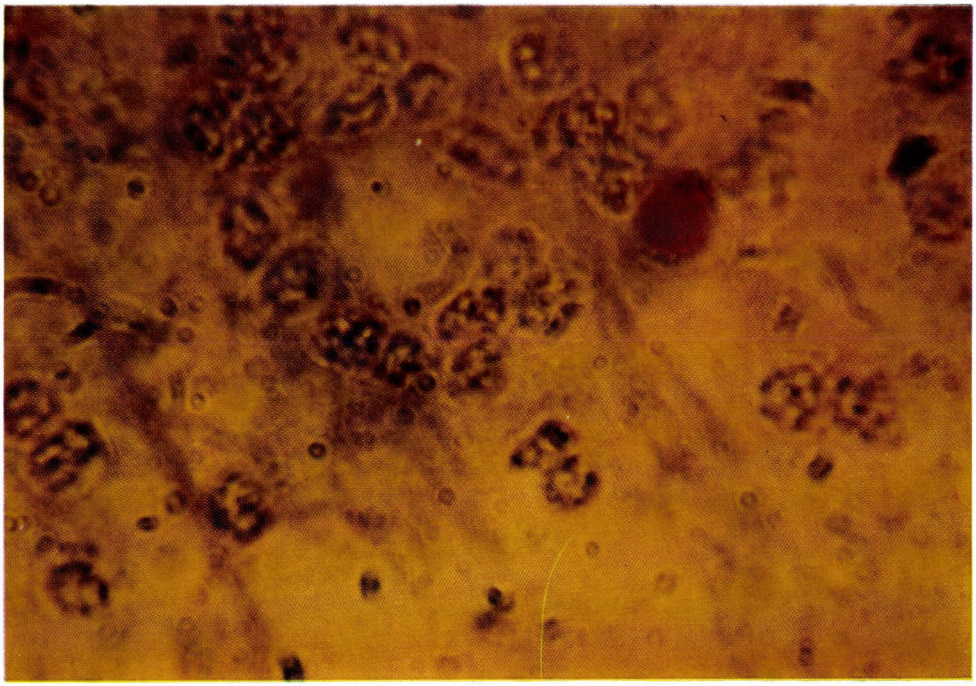

Fig. 6. Lymphocyte cooperation phenomenon on rat gastric corpus section stained by differentiation staining. The cooperation is seen with the yellow parietal cells

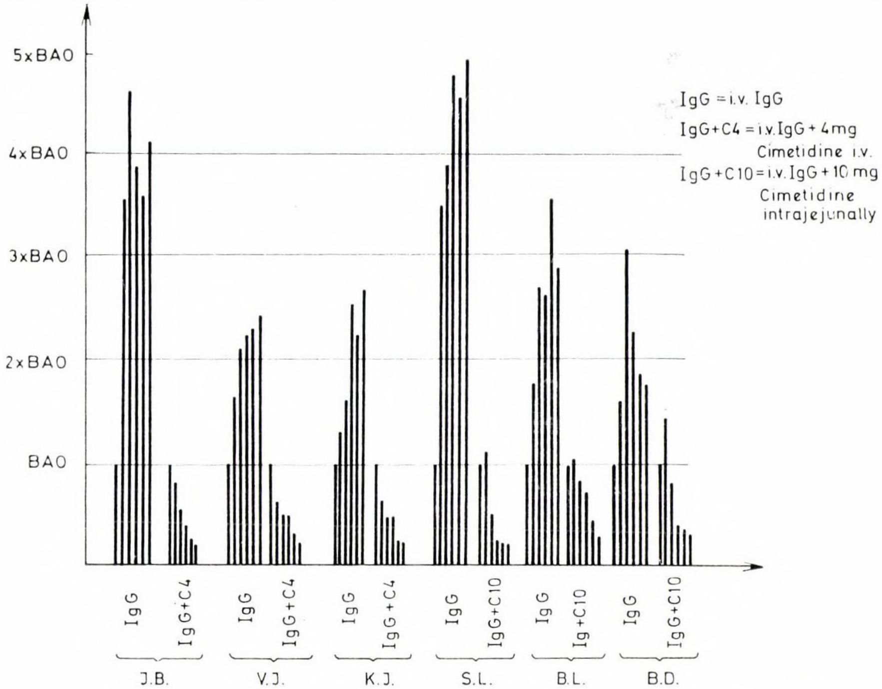

Fig. 7. Response of rat gastric HCl secretion after administration of IgG having a secretagogue effect and of simultaneously given cimetidine. The first columns represent the "basal" secretions, the subsequent ones the hourly values after i.v. administration of globulins or globulins and cimetidine

The sequential incubation of corpus sections with secretagogue globulin, antihuman globulin, and human lymphocytes resulted in cooperativity in the glandular part (Fig. 5). This indicates the binding of human globulins to the gastric mucosa. The antihuman globulin added to the system formed an *antigen-antibody complex* with the bound globulin, a number of lymphocytes being attached to this complex. After fixation and differential staining it became apparent that the lymphocytes were attached to parietal cells which stained yellow. (Fig. 6).

Cimetidine the H_2 receptor blocker administered to rats after the "basal" secretion period was found to prevent the secretory response induced by the secretagogue globulin (Fig. 7). Similar effect was observed in the *in vitro* system, viz. preincubation of the corpus mucosa sections with various concentrations of cimetidine inhibited the binding of the secretagogue globulins to the parietal cells (Fig. 8).

When secretagogue globulin was added to the *histamine-induced rosette* formation system the rosette forming activity of T-lymphocytes was markedly suppressed (Fig. 9). Similar effect was produced by *cimetidine*, but no change

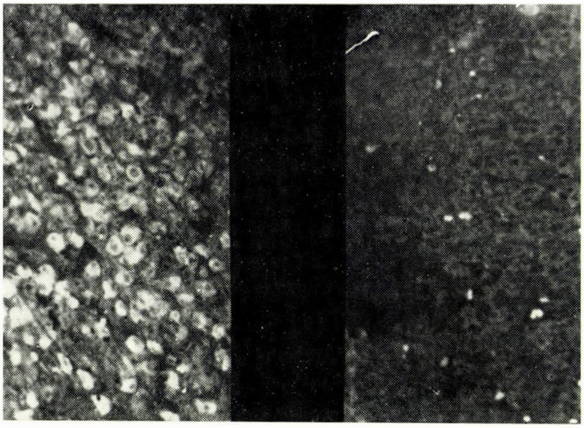

Fig. 8. Indirect immunofluorescent appearance of rat gastric corpus mucosa. The left field shows the secretagogue globulin binding, the right field the inhibition of binding with cimetidine

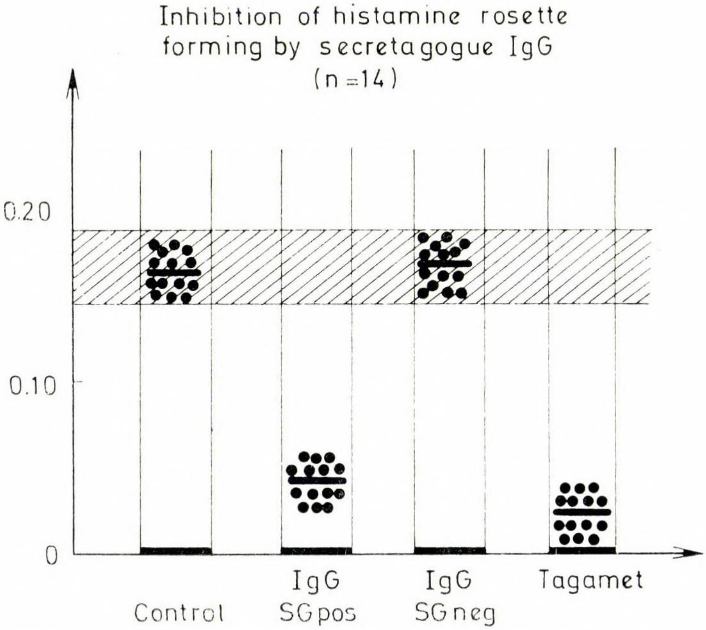

Fig. 9. Histamine rosette forming is inhibited by IgG with secretagogue effect as well as cimetidine IgG without any secretagogue effect does not show inhibition of rosetta forming

was seen in rosette formation upon incubation with non-secretagogue globulins.

In the bioassay studies the administration of globulins derived from patients with pernicious anaemia and containing the long known antibody

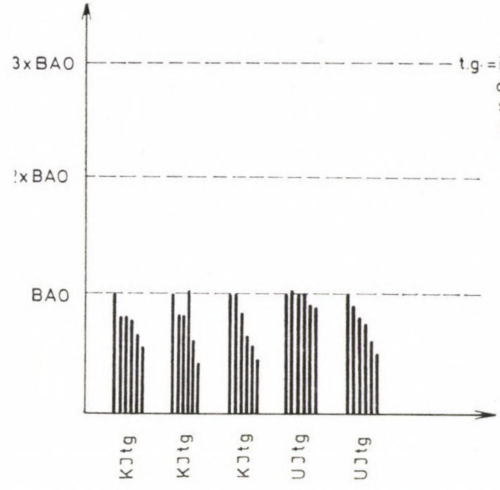

Fig. 10. Response of rat gastric HCl secretion, expressed in basal secretion units, to total globulin given i.v. derive from patients with pernicious anaemia. The first columns represent the "basal" HCl secretion, the subsequent ones the hourly values

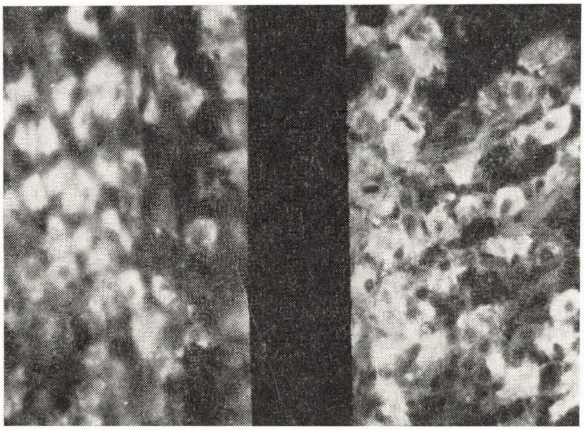

Fig. 11. Indirect immunofluorescent appearance of rat gastric corpus mucosa. The left field shows parietal cell antibody binding, on the right field also parietal cell antibody binding is seen which is not prevented by cimetidine

directed against parietal cell did not elicit any secretory response (Fig. 10). When this "true" antibody was incubated with the corpus mucosa section of the stomach in the presence of *cimetidine*, the H_2 receptor blocker failed to prevent the binding of the globulin to the cells (Fig. 11).

Upon evaluating the clinical results we have found differences between groups of secretagogue globulin carriers and non-carriers. Out of 25 carriers 3 patients had a record of previous perforations (Table III), 9 had macro-

Table III

Characteristics of the patients with secretagogue globulin (SG pos.) and without secretagogue globulin (SG neg.) in their sera

Characteristics	Groups	
	SG pos. n=25	SG neg. n=26
Perforation	3/25	2/26
Macroscopic gastrointestinal bleeding	9/25	1/26
Recurrence of G.I. bleeding	7/25	0/26
Association with Graves' disease	2/25	0/26
Association with rheumatoid arthritis	3/25	1/26
Association with chronic pancreatitis	1/25	0/26
Association with endarteriitis obliterans	1/25	0/26
Association with exudative pleurisy	1/25	0/26
Association with primary amenorrhea	1/25	0/26
Total associated immuno-pathological disease	9/25	1/26

Table IV

Clinical characteristics of the disease in the patients with secretagogue globulin (SG pos.) and without secretagogue globulin (SG neg.)

Characteristics	Groups	
	SG pos. n=25	SG neg. n=26
Stubborn persistency of symptoms	25/25	0/26
Good response to treatment	0/25	26/26
Need for parenteral or intragastric treatment	24/25	0/26
Cobalt irradiation of the stomach	1/25	0/26
Pyloric spasm	19/25	1/26
Pyloric stenosis	2/25	0/26
Resection of the stomach	4/25	0/26

Table V

The values of basal (BAO) and maximum acid secretion (MAO) stimulated by Peptalon *in patients with duodenal ulcer having a globulin with secretagogue effect (SG pos.) and without this secretagogue effect (SG neg.) in their sera*

Name	SG pos.		Name	SG neg.	
	BAO	MAO		BAO	MAO
N.J.	11.4	70.5	B.M.	6.6	14.5
V.Gy.	4.5	27.0	S.J.	7.9	28.5
F.B.	15.2	30.2	Z.J.	2.2	27.5
K.J.	10.2	20.2	O.I.	9.0	36.4
K.L.	13.8	62.4	V.L.	5.4	50.8
Sz.B.	8.4	25.6	A.G.	7.0	34.7
D.L.	9.0	26.0	K.Z.	7.0	31.2
B.J.	15.8	25.0	Sz.G.	6.0	32.4
F.J.	20.5	74.5	O.J.	8.7	31.6
N.J.	9.6	28.9	V.F.	10.3	19.9
Gy.E.	5.1	28.8			
P.J.	19.2	51.3			
F.L.	12.0	60.0			
T.I.	8.1	26.0			
T.S.	20.4	50.4			
Total	163.0	607.5		70.1	307.6
Mean	10.86	40.46		7.01	30.76
SEM	4.52	17.44		1.65	6.88

n=15 n=10

SG pos. BAO vs. SG neg. BAO = 4: 2.8 $(0.01 > P > 0.001)$
SG pos. MAO vs. SG neg. MAO = 2: 1.5 $(0.01 > P > 0.001)$

scopic gastrointestinal bleedings, 7 of them had recurrent episodes of melaena or haematemesis. In the non-carrier group, however, there was only one perforation and one bleeding on the record. Among the secretagogue globulin carriers several patients had records of earlier or concomitant diseases presumably of immunopathologic origin such as 2 cases of Grave‘s disease, 1 case of chronic pancreatitis, 1 case of endarteriitis obliterans, 3 cases of rheumatoid arthritis, 1 case of exsudative pleuritis and 1 case of primary amenorrhoea. This means involvement in 9 instances out of 25 patients, whereas in the non-

Table VI

Fasting serum gastrin levels (in pg/ml) of patients having globulin with a secretagogue effect (SG pos.) and without such a secretagogue effect (SG neg.) in their sera and as well as controls

SG pos.		SG neg.		Control	
name	gastrin	name	gastrin	name	gastrin
B.J.	110.0	V.F.	100.0	Sz.Gy.	63.5
Gy.E.	75.0	K.J.	75.0	Sz.J.	37.7
V.F.	100.0	B.J.	92.0	K.Gy.	31.5
N.J.	60.0	V.L.	75.0	N.J.	48.4
F.I.	100.0	O.I.	85.0	K.Gy.	33.1
Sz.B.	100.0	S.J.	74.7	Z.J.	70.0
K.L.	125.0	A.G.	65.0	L.A.	68.0
D.L.	80.0			M.F.	110.0
K.J.	41.0			L.I.	35.0
F.B.	35.0			Sz.I.	49.6
				M.J.	31.4
				S.L.	44.3
				S.J.	23.7
				S.G.	35.9
				V.I.	65.0
Total	826.0		566.7		747.4
Mean	82.6		80.9		49.8
SEM	25.7		11.6		17.5
n=10		n=7		n=15	

SG pos. vs SG neg. $P > 0.90$
SG pos. vs control $0.30 > P > 0.20$
SG neg. vs control $0.30 > P > 0.20$

-carrier group there was only 1 case of rheumatoid arthritis in addition to appendoctomy and influenza.

The time-course of the disease also differed in the two groups (Table IV). In the carrier-group for instance the disease was more aggressive in appearance, following onset the symptoms persisted for long and no periodicity was seen. In response to conventional treatments there was a slow recovery or no recovery at all. Parenteral and intragastric therapy was also applied, in one especially unresponsive case Co irradiation (1500 rad) was employed. There was a frequent occurrence of pyloric spasm and functional gastric retention (19 cases), 2 of them developing pyloric stenosis. Due to the inefficiency of conservative treatment 4 patients underwent surgery (also included are in this group 2 patients with pyloric stenosis). In the non-carrier group, however, patients were responsive to treatment and no complications were noted.

Values for HCl secretion were also different in the two groups (Table V). Although superacidity was present in all cases tested the mean BAO value was > 10 mmol HCl in the carrier-group and was ~ 7 mmol HCl in the non-carrier group.

Serum fasting *gastrin* concentrations did not differ between the carrier and non-carrier groups (Table VI).

Routine clinical and laboratory tests including LE-cell reaction and ANA did not reveal any differences between the groups.

Discussion

The HCl overproduction is known to play a key role in the pathogenesis of duodenal ulcer [3, 15, 32, 26, 7, 20]. The cause of this supersecretion is, however, not clear. Under such conditions the mucosa is in a state of hyperfunction being constantly stimulated, however, neither enhanced vagal activity [47, 31, 11] nor hypergastrinaemia [24, 44, 29, 61, 60] was seen. The secretagogue role of the intestinal phase has been confirmed in experimental animal studies [23, 37, 33, 42, 21, 42] but not in man [58]. According to some earlier observations a "gastrin-like" substance is responsible for the superacidity [16]. Yakimets and Bondár [63] reported on a substance in the thoracic duct lymph which is not identical with gastrin or histamine and stimulates gastric HCl secretion. Hansky et al. [34] found that out of 17 patients with duodenal ulcer and normal serum gastrin concentrations estimated by RIA the sera of 12 patients induced intense secretory responses in the rat stomach.

The immune system while exerting an overall surveillance over the integrity of the organism is directly or indirectly involved in almost all functions of the body [13, 14, 15, 12, 50]. Since the immune system can induce hyperplasia and hypertrophy in the thyroid gland [49, 55, 59], it is feasible that in certain instances it can produce hyperfunction (and hyperplasia) in the stomach, too. Considering the general mechanism of action in the immune system, trophic stimulation should be mediated by a humoral factor [5, 28, 4, 50] which is probably in the immunoglobulin fraction.

In the present study we have found that out of 51 patients with duodenal ulcer and apparent superacidity the globulins of 25 patients produced a marked secretory response in the stomach of anaesthetised rats *in vivo*. The onset of the response occurred with some delay, developed gradually, peaked at 3—4 hours and was reproducible. After purification of the globulins the secretagogue activity was linked to the IgG globulins. The acid secretion enhancing effect was not due to gastrin since the patients under study failed to have high serum gastrin levels and the gastrin concentrations in the globulin preparations were lower than the minimal effective dose required to induce a biological response. Using the indirect immunofluorescent technique the secretagogue globulin was shown to be bound to the corpus mucosa in the stomach, moreover the lymphocyte cooperation test indicated that the *parietal cells* were the targets for this type of globulin molecules. The H_2 receptor blocker cimetidine abolished the globulin-induced secretory response and the binding of the globulin to the parietal cells was also inhibited by cimetidine as revealed by the indirect immunofluorescent studies. It appears that the binding of the globulins to the parietal cells occurred via the H_2 receptors. When the affinity to the H_2 receptors of the secretagogue globulin was tested in a separate system the secretagogue globulin exerted a significant inhibition under such conditions, too.

Since the immunoglobulin, i.e. an antibody produced a stimulatory action, we tested the "classical" antibody directed against the gastric mucosa namely the globulin of patients with pernicious anaemia in the *in vivo* bioassay system and in the immuno-fluorescent study along with cimetidine. Results showed that the "true" antibody did not induce secretory response nor did cimetidine inhibit the binding of the antibody directed against the parietal cell.

On the basis of the present findings it appears that in some patients with duodenal ulcer and superacidity a humoral factor, an immunoglobulin, accounts for the enhanced secretion, through exerting secretagogue effect on the parietal cells by combinig with their H_2 receptors.

The clinical characteristics of the disease compiled from data of the 51 analysed patients seem to indicate that the secretagogue immunoglobulin carriers are genetically more susceptible to develop immunological "imbalance" such as autoaggressivity or autostimulation. This view is supported by the more frequent occurrence of immunopathological diseases in the history of a considerable number of these patients and the more aggressive, less periodical, thereapeutically unresponsive manifestations which were often associated with complications. The basal acid output (BAO) was markedly enhanced being 4-fold higher than normal and the stimulated secretion (MAO) was also higher than that in patients with no secretagogue globulins. Fasting gastrin concentrations and routine laboratory data did not significantly differ in the two groups.

Based on the present results in conjunction with the clinical symptoms we suggest that in certain instances of duodenal ulcer which is otherwise a

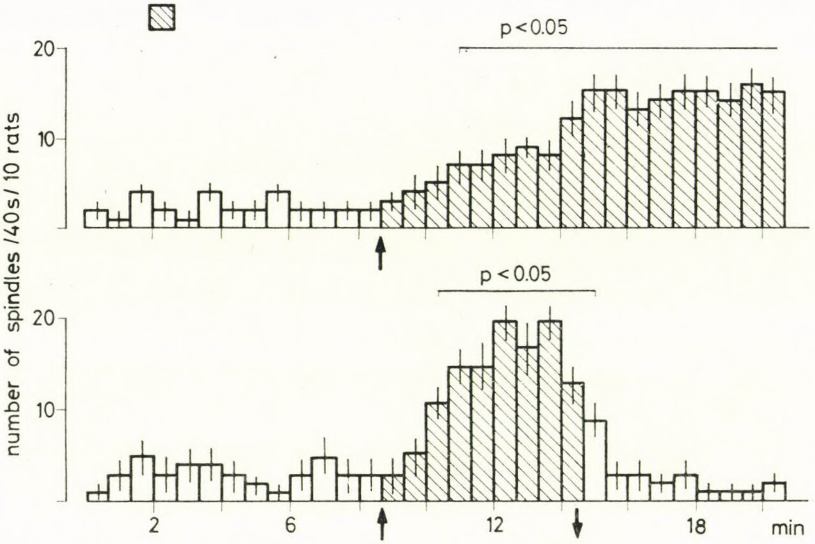

Fig. 6. **Increase of spindle activity in response to capsaicin microinjection (top, n=17) and thermal stimulation (bottom, n=10) in the posterior hypothalamus. Dotted columns denote the effect of stimulations. Small bars indicate standard error of means. Continuous lines mark significant differences in the number of spindles with respect to the minute before stimulation (Student's *t*-test)**

tions could not be obtained. Capsaicin injections into extrahypothalamic areas were also ineffective though stimulation in the thalamus (Fig. 1) sometimes induced slow wave activity.

Local heating in the PO–HYP resulted in EEG synchronization similar to that described for the capsaicin microinjections: high amplitude irregular delta waves and spindles appeared in a great number (Figs 5 and 6). The latency of the reaction was 1–2 min and the synchronized activity continued for about 1 min after termination of the stimulation. The effect of heating on spindle activity was most pronounced when the posterior hypothalamus was stimulated (Table I). In these experiments the increase of spindle activity was significant 1 min after the onset of heating (Fig. 4). Heating in the mid-hypothalamic area was less effective and the number of stimulations that facilitated spindles increased again when the preoptic region was heated. No relationship between the stimulated area and the slow wave activity was found. Extrahypothalamic heating without any effect was carried out in 5 rats.

Discussion

The mutual interaction between sleep and thermoregulation has repeatedly been reported. The thermoregulatory mechanisms are seriously altered during sleep [11, 22]. A significant deviation from the neutral ambient tempermulticausal disease the impaired function of the immune system might play a role. This abnormality is, however, not identical with that observed in autoimmune (autoaggresive) diseases.

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THERMAL AND CHEMICAL STIMULATIONS OF THE HYPOTHALAMIC HEAT DETECTORS: THE EFFECTS ON THE EEG*

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In acute immobilized rats, the effect on the EEG of thermal and chemical (capsaicin microinjection) stimulation of the warm sensors in the preoptic region, mid-hypothalamic area and posterior hypothalamus were studied. Both localized heating and capsaicin resulted in a sleep-like EEG with spindles and slow waves. Stimulation in the posterior hypothalamus was the most effective and stimulation in the mid-hypothalamus was the least effective in inducing spindle activity. Since capsaicin is regarded as a specific stimulant for the hypothalamic warm sensors, the results suggest that the EEG effect, and probably the sleep-inducing effect, of heat are mediated via the central thermoreceptors, and cannot be due to a non-specific activation of the basal forebrain hypnogenic mechanisms.

Drowsiness is a common experience in a mildly warm environment. Actually, local heating of the preoptic thermodetector area may induce EEG synchronization and sleep [2, 8, 12, 24, 25]. The question whether this deactivation is the result of the excitation of warm sensors or rather a non-specific activation of the basal forebrain hypnogenic mechanisms has not been settled. This problem was the subject of our experiments: in addition to local heating of the preoptic region-hypothalamus (PO–HYP), chemical stimulation of the heat detectors was applied to elicit slow waves and spindles in the EEG of acute immobilized rats. Capsaicin administered in a single small dose subcutaneously or into the preoptic region induces thermolysis via stimulation of the warm sensors [15, 16] while repeated treatment with the drug irreversibly damages thermoreceptors and severely impairs thermoregulation [16, 18, 19]. At least in the PO–HYP, the effect of capsaicin seems to be specific to the warm detectors [13, 17]. Based on these observations, the aim of the present experiments was to compare the EEG effect of capsaicin microinjection to that elicited by local thermal stimulation. To avoid the interference with anaesthetics which seriously modify both the EEG and the thermoregulatory responses [4], acute immobilized rats were used.

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Materials and Methods

Altogether 78 male CFY rats (300–450 g) were used. Under ether anaesthesia the trachea and the dorsal vein of the penis were cannulated. The skull was fixed in a stereotaxic apparatus. Recording electrodes were placed bilaterally over the frontal and parietal lobes. Wound edges and pressure points were infiltrated with procaine-HCl, and this procedure was repeated hourly during the experiments. The animals were immobilized with i.v. gallamine triethiodide and artificial respiration was introduced. The experiments were carried out in a heat box which maintained the colonic temperature between 35 °C and 36 °C. Stimulation was started 1 h after the anaesthesia had been discontinued.

The effect of capsaicin microinjection on the EEG was studied in 53 experiments. The drug was applied in a volume of 1 μl containing 10 μg capsaicin dissolved in ethanol and Tween 80 [14]. In 30 experiments the solvent without capsaicin was injected into the PO–HYP contralaterally to the stimulated area. The microinjector needle (a glass pipette about 30 μm in diameter) was lowered through a guiding cannula implanted 30 min earlier into the explored area. The drug was injected by means of a microdriver in 30 s.

Local heating of the PO–HYP was carried out in 46 experiments. A coil (resistance 400 Ω) described by Carlisle and Laudenslager [5] served as the heating electrode: a nicrothal wire, was wound around the tip of a stainless steel pin. The diameter of the electrode was 200 μm. Direct current of 180 mW (two-thirds of the coagulation power) was used to heat the coil. As determined in a separate experiment, this PO–HYP stimulation induced a 3–4 °C rise of the tail temperature in urethanized rats. Since in an animal more than one area was stimulated, the local PO–HYP temperature was not taken in order to decrease the tissue damage.

Three regions of the PO–HYP were stimulated; the preoptic area, mid-hypothalamus and the posterior hypothalamus (frontal planes 2.0, 0.0 and -1.6 in the stereotaxic atlas of Pellegrino and Cushmann [23]). The electrodes were aimed 0.5 to 2.0 mm lateral to the midline. In 20 experiments the electrode and cannula placements were verified histologically while in the rest of the rats, gross examination of the frontal sections of the brain seemed to suffice for localizing the electrode tracks. In some experiments extrahypothalamic regions were also stimulated; they are indicated in Fig. 1.

The EEG was recorded on paper and was visually evaluated in 40 s epochs. Increases in spindle and slow wave activity were treated as EEG synchronizations. The number of the spindles/40 s in the bifrontal derivation was used to characterize the effects. In addition, power density spectra were calculated from 40 s epochs in 15 experiments (7 with capsaicin and 8 with PO–HYP heating).

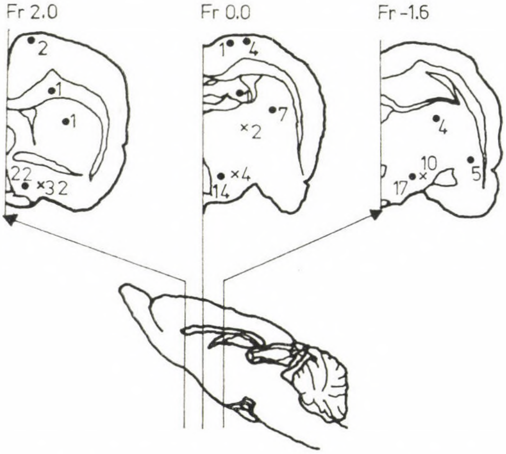

Fig. 1. Stimulation points in the brain. Dots: capsaicin microinjections. Crosses: thermal stimulations. Numbers denote the number of experiments

Results

The EEG of acute immobilized rats was characterized by a moderately synchronized activity, predominantly with waves of the theta range. High amplitude theta waves in the frontoparietal derivation often showed a regular pattern. This activity continued for several seconds, sometimes for minutes. It was regarded as a concomitant of the hippocampal rhythmic slow wave activity (RSA) since RSA often invades the parietal EEG in the rat [31]. It could easily be elicited by sensory stimuli. The EEG of the bifrontal derivation was flat with high frequency waves. Spindles were absent or extremely rare.

Capsaicin injected into the PO–HYP induced a powerful slow wave and spindle activity (Fig. 2) or a marked slow wave activity without spindles. Spindles appeared in a great number primarily in the bifrontal derivation. The frontoparietal derivations were dominated by high amplitude irregular delta waves resulting in a great increase of the power of delta range (Fig. 3). In response to sensory stimuli (touching the tail) this activity was immediately replaced by RSA over the parietal cortex and by fast waves over the frontal

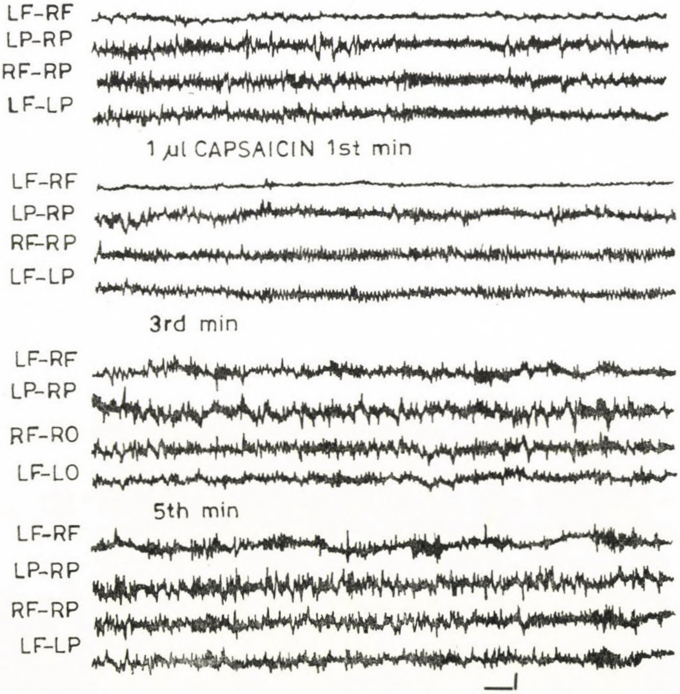

Fig. 2. EEG response to capsaicin injected into the preoptic region. Top traces show the EEG before the injection. LF and RF: left and right frontal. LP and RP: left and right parietal. Calibration: 100 μV, 1 s

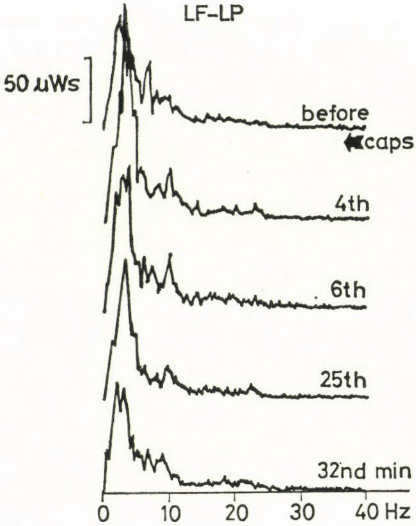

Fig. 3. Power density spectra obtained from 40-s records before and after capsaicin microinjection into the posterior hypothalamus

cortex. The synchronizing reaction to capsaicin had a latency of 1–4 min, lasted for 20–30 min, and then the original activity gradually returned. The EEG effects depended on the location of the cannula (Table I). Stimulation in the posterior hypothalamus resulted in the most consistent effect: an increase in spindle and slow wave activity was noted in each experiment. The rise in the number of spindles was gradual and significant from the second minute on (Fig. 6). In contrast with the posterior hypothalamic stimulation, capsaicin administered into the mid-hypothalamic area elicited spindle activity in one-third of the experiments. When the drug was applied into the preoptic region, the number of effective stimulations increased again. The latency of the response, however, tended to be longer in these cases than on stimulation of the posterior hypothalamus: it was close to 4 min. No definite difference between the areas studied was found with respect to the slow waves induced (Table I). In the 30 experiments where the solvent was injected alone, distinct reac-

Table I

Spindle (SP) and slow wave (SW) inducing effect of capsaicin microinjections and local heating in the PO-HYP. The effective/total number of rats is indicated for each frontal coordinate

	Fr: 2.0 preoptic region		Fr: 0.0 mid-hypothalamus		Fr: –1.6 posterior hypothalamus	
	SP	SW	SP	SW	SP	SW
Capsaicin	13/22	21/22	5/14	11/14	17/17	17/17
Heating	17/32	30/32	1/4	4/4	10/10	10/10

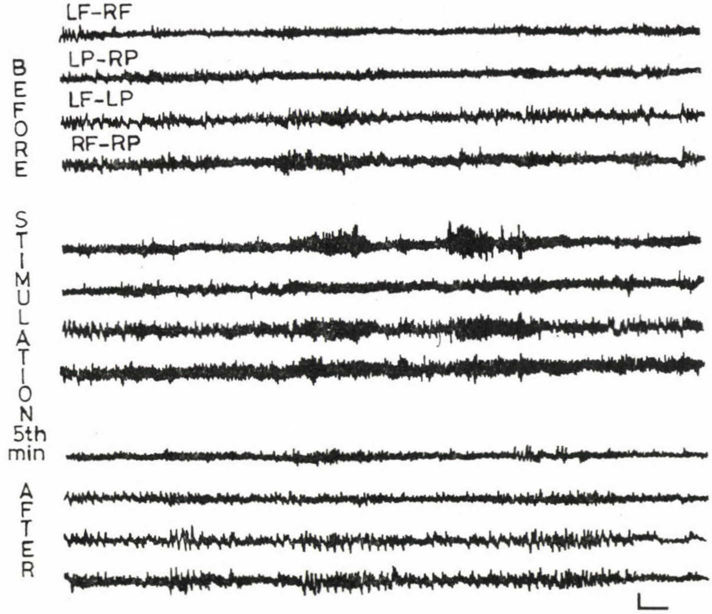

Fig. 4. **EEG response to thermal stimulation in the preoptic region. Calibration: 100 μV, 1 s**

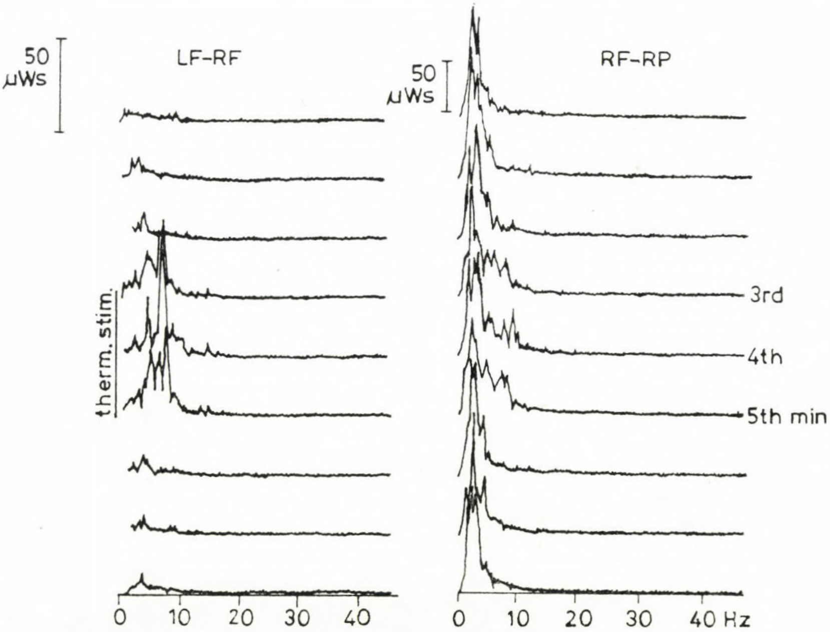

Fig. 5. **Power density spectra of 40-s cortical EEG records obtained before, during and after thermal stimulation in the posterior hypothalamus. Left side: values taken from bifrontal derivations. Right side: power spectra calculated from EEG records in the right fronto-parietal derivations. The three curves on the top were calculated from the EEG taken before stimulation, the next three curves marked by a vertical bar represent the EEG taken before stimulation. The three power spectra at the bottom were obtained after stimulation had been discontinued**

ature generally results in a reduction of sleep [20, 22]. However, when the rise in ambient temperature is moderate, total sleep or the percentage amounts of some sleep stages may increase. As to which stages are affected, quite different results have been reported depending on the method used (i.e. duration of heat exposure, temperature, recording time, preexposure adaptation, the rate of temperature variation, and the sleep scoring procedure) [10, 28, 29, 32]. On the one hand, sleep might be useful for thermoregulation since behavioural inactivity reduces heat production [27]. On the other hand, a primary behavioural response involving immobility to heat may facilitate the sleep process. Actually, a specific thermolytic behaviour of this type has been reported: it is a pronal extension of the body [6, 26, 29]. At more elevated ambient temperatures thermolytic reactions antagonistic to sleep are also activated: the rats groom vehemently and try to escape from the warm environment [6, 9].

The EEG synchronization obtained in acute immobilized animals should be interpreted cautiously as a phenomenon identical to that appearing during slow wave sleep. Still, in our experiments the synchronization seemed to be the result of the activation of sleep mechanisms. Comparing the two phenomena may therefore promote the understanding of these mechanisms. Local thermal stimulation in the preoptic region which induced a sleep-like EEG in acute immobilized cats, rabbits and rats [2, 8], facilitated sleep in freely moving animals too: cats fell asleep [24], the length of paradoxical phases increased [21]; in oppossums a sleep-like relaxation was found [25], in rats slow wave sleep increased [28]. It is not known whether these effects were due to the specific central thermodetectors. In the preoptic region, close to the thermodetector area, the existence of a powerful hypnogenic mechanism was reported [3, 30]; high frequency stimulation in this area induced sleep while lesions brought about insomnia. Heat may activate cells which have nothing in common with the thermodetectors [1]. Thus, a non-specific stimulation of the hypnogenic mechanisms as the reason for the sleep eliciting effect of heat could not be excluded. In this respect the experiments with capsaicin which specifically activates and, in the case of repeated treatment, damages the preoptic warm sensors, seemed to be decisive.

Our results showed that the effects of localized heating and capsaicin microinjections were strikingly similar: a significant increase of spindle activity and a definite slow wave activity, i.e. an EEG pattern closely resembling sleep. Some differences in the EEG responses with respect to the stimulated area were noted in both the thermal and chemical stimulation experiments. While slow waves could be elicited by stimulating any parts of the PO–HYP, the spindle-inducing points were not distributed uniformly. Since the intensity of the slow wave activity was not evaluated quantitatively in each experiment, particular importance should not be attributed to this divergency. As to the increase of spindle activity, positive responses were obtained more frequently from the posterior hypothalamus and preoptic region than from the mid-hypothalamic region. These differences in effectivity of the stimulation corresponded to the distribution of the warm sensors: they are more densely scattered in the anterior and posterior hypothalamus than in the mid-hypothalamic region, moreover, the characteristics of the anterior and posterior thermodetectors are also different [7]. Whether these differences may explain that spindle responses could be obtained more readily on posterior stimulation than on anterior one, is not clear. To our knowledge, in all the experiments but one where the interaction of the central thermodetectors and sleep was studied, the preoptic region was heated. The only exception [12], the experiments of Hemingway et al. corroborated our findings: in dogs, thermal stimulation of the posterior hypothalamus was more effective in eliciting sleep than was the anterior hypothalamus.

To summarize, both localized heating and capsaicin microinjections in the PO–HYP elicited slow waves and spindles in the EEG. The distribution of the spindle-inducing areas was identical for both kinds of stimulation. Since capsaicin, is regarded as a specific chemical excitant for the central warm sensors, it seems that these receptors were responsible for the EEG effects. The results are in favour of the view that the sleep facilitating effect of heat is mediated by the PO–HYP thermodetectors.

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THE ROLE OF DEGRADATION OF FETAL HAEMOGLOBIN IN THE ENERGY SUPPLY OF VERY LOW BODY WEIGHT PRE-TERM BABIES

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Low body weight premature babies born before the 32nd gestational week were studied to analyse the postnatal fall in plasma haemoglobin and in quantitative changes in amino acid levels. Red blood cells of premature low body weight infants were found to disintegrate more rapidly than those of mature newborns. Thin-layer ion-exchange chromatographic studies showed that amino acids originating from the degrading haemoglobin-F lead to rise in plasma amino acids. These amino acids might play a role as substrates for gluconeogenesis in the energy supply of low body weight premature babies during the special fasting state just after birth.

Perinatal medicine has experienced important new developments during the last decades. Modern gynaecological and neonatal care have resulted in the survival of an increasing number of very low birth weight babies. The same population of infants is, however, also responsible for a large proportion of newborn mortality as a result of immaturity (Fig. 1). It is of great importance, therefore, to learn more about the details of the physiology and adaptation of premature infants so that the unfavourable metabolic characteristics originating from the absence of the last intrauterine period in their development could be successfully influenced.

This study has been initiated by the clinical observation that immature very low birth weight babies develop marked anaemia during the first week of life. In addition to a detailed analysis of this anaemia, it has been aimed at investigating the role of the degrading products of haemolysed red blood cells in the process of neonatal metabolic alterations.

Materials and Methods

1. *Patients.* To measure haemoglobin (Hb) levels after birth, capillary blood samples were taken from 43 pre-term infants of low body weight. Gestational age was calculated on the basis of the last period of the mothers and checked by standard somatic and neurological tests of the newborns. Average gestational age was 29.7 weeks with a range of body weight of 750 to 1500 g (mean, 1225 g). In these patients no sign of general or specific organ bleeding could be observed and no transfusion was applied.

2. *Determination of Hb.* Serum Hb was measured as cyano-Hb by spectrophotometry using the Drabkin-reagent.

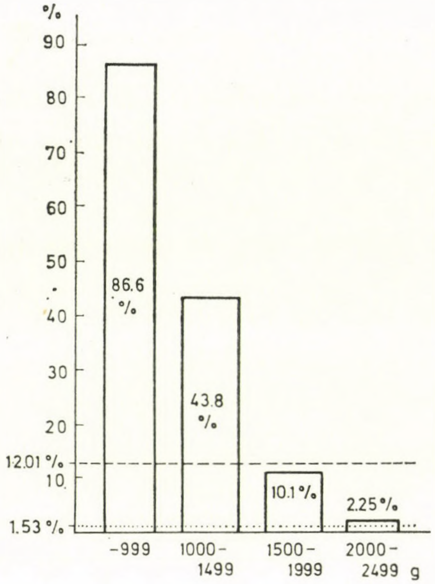

Fig. 1. Mortality rate of 0–6 days old newborn infants in Hungary in 1980 (given in %) as a function of birth weight. Dotted line shows mortality rate of all infants born alive, broken line shows mortality rate of pre-term infants weighing less than 3000 g (based on the estimation of the Central Statistical Office, Budapest, 1980)

3. *Withdrawal and storage of blood-serum samples.* Blood-samples were taken from peripheral veins of mature healthy newborns (n=5) and from very low birth weight premature newborns (n=8), the latter weighing 1050 g on the average with a gestational age of less than 32 weeks. After being left at room temperature for 30 min, the samples were centrifuged (10 000 rpm for 10 min) and stored at —20 °C for less than two weeks. Because of the small volume of the samples taken from infants of low body weight, the samples were pooled to get a volume of 3 to 4 ml (n=5–8).

4. *Extraction procedure.* 3 to 5 ml pools of serum samples were treated with 96% ethanol, after adjusting the pH to 2.0 with a 1 N-solution of HCl to remove proteins. The final concentration of ethanol was 70%. The supernatant of the centrifuged samples (see above) were dried with vacuum-distillation at 37 °C and the residue dissolved in 0.1 N HCl (1 ml).

5. *Thin-layer ion-exchange gel chromatography.* Thin-layer ion-exchange sheets measuring 200 mm × 200 mm were used (Fixion 50X–8, Chinoin). The sheets were equilibrated in a 0.02 mol Na-citrate buffer at a pH of 3.28 for 16 hours followed by drying at 25–30 °C for one hour. At a distance less than 10 mm, 100 μl samples of serum (see above) were transferred to these chromatographic sheets and run at a temperature of 55 °C in a mixture containing 0.35 mol Na-citrate at a pH of 4.15. This was followed by two drying periods at 50 °C for 40 min, interrupted by a spray of ninhydride solution. The density of the spots appearing after this procedure on the chromatogram, representing serum amino acids, were then measured with high-speed densitometry using various concentrations of arginine as standard.

Results

In the first series of experiments Hb content of the blood taken from 43 very low birth weight infants born before the 32nd gestational week was compared with respective samples taken from mature newborns, in both cases the samples being taken at different times of the postnatal development; in both groups a postnatal fall in Hb concentration was observed (Table I).

Table I

Postnatal changes in blood haemoglobin levels in mature newborn infants (adapted from* MATOTH *et al.* [*11*]*) and in premature infants born before the 32nd gestational week (present studies)

Days	Term infants		Preterm infants under 32th weeks of gestation	
	Case No.	Hb, mmol l^{-1}	Case No.	Hb, mmol l^{-1}
1–3	57	11.74±1.55	19	9.87±0.62
4–5	22	11.24±0.99	9	9.50±1.18
6–7	27	10.93±1.43	5	8.38±1.24
Weeks				
1–2	32	10.74±1.43	22	8.26±1.18
2–3	11	9.69±1.61	8	8.63±1.30
3–4	17	8.82±1.30	15	8.07±1.30
4–5	15	7.89±0.99	8	7.89±0.81
5–6	10	7.39±0.93	8	7.58±0.62
6–7	10	7.45±0.93	3	6.64±0.74
7–8	17	6.81±0.68	2	5.34±0.50

In mature newborn infants a gradual and slow fall in Hb concentration was observed, with a slight fall (1.0 mmol l^{-1}) occurring within the first week [11]. However, in premature low body weight infants, Hb concentration fell by 1.62 mmol l^{-1} within the first week, with a marked decrease starting from day 4 and reaching the lowest level by the end of the first week. This marked degradation of Hb, together with the immaturity of the conjugating and excreting capacity of the liver, contributed to hyperbilirubinaemia which was the highest on day 7 in premature babies instead of day 4 as common for mature newborns. From week 5 to 6 onwards, the fall in Hb was more marked in premature babies than in mature neonates. This phenomenon could be explained by a rapid synthesis of Hb–A after the 36th gestational week, at the same time the synthesis of fetal Hb being already inhibited [4]. The concentration of fetal Hb started to fall rapidly at that time and total Hb concentration reached a critical low level by week 7 to 8 owing to the slow speed of Hb–A production.

The fate of globin deriving from Hb is practically unknown, but it is certainly split to amino acids. In the second part of this study the effect of globin catabolism on plasma amino acid levels was investigated.

According to other studies [1, 9] 70–90% of the Hb of cord blood taken from premature infants is fetal Hb (Hb–F). The less mature the newborn, the highest is the proportion of Hb–F in it's cord blood. The amino acid sequence of Hb–F [10] demonstrates that the dominant amino acids in one α and γ chain each are leucine, isoleucine, alanine, valine and lysine (Table II).

Table II

Relative incidence (mol%) of the individual amino acids, constituents of fetal haemoglobin

VAL	9.06	ASP	8.71	HIS	5.92
LEU	12.19	LYS	8.01	GLU	5.92
ILE	1.40	THR	6.62	TYR	1.74
SER	7.66	TRY	1.40	ARG	2.09
PRO	3.83	PHE	5.23	CYS	0.70
ALA	11.15	GLY	6.97	MET	1.40

An attempt was made to reveal a relationship between the changes in amino acid content and the composition of blood with the degrading products of Hb–F. In these investigations premature and mature newborn infants not previously given either exogenous protein or amino acids were studied. Plasma samples taken at day 7 from low body weight infants contained higher levels of amino acids (by several degrees of magnitude) than those of mature newborns of the same age (Fig. 2). The most marked rise occurred in tyrosine,

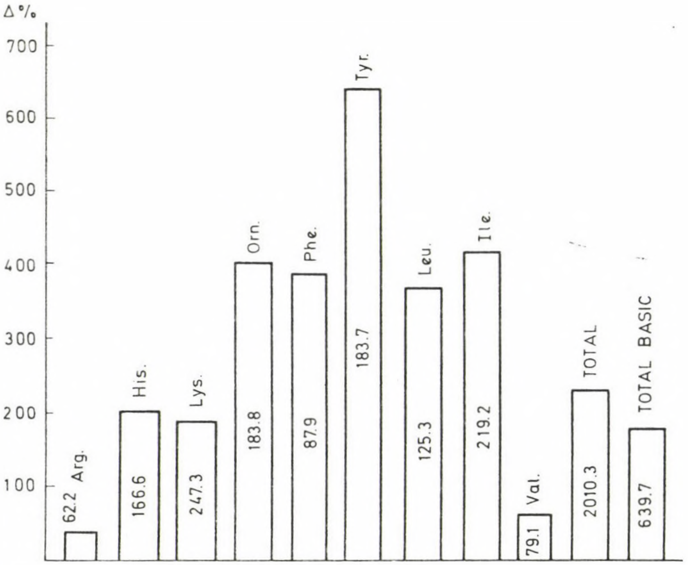

Fig. 2. Percentile difference (Δ%) of serum amino acid levels in very low body weight premature infants as compared with mature newborns in the 7th day of life. The numbers in the columns show amino acid levels in μmol 1^{-1}

leucine, isoleucine, phenylalanine and ornithine concentrations, with lysine and histidine levels being also significantly higher in the premature group than in the control one.

Discussion

Characteristic changes can be observed in serum levels of some metabolites in the course of neonatal adaptation. The fall in blood glucose concentration after birth indicates a gradual depletion of carbohydrate stores. In premature babies the level of hypoglycaemia is more marked [5]. A simultaneous rise in FFA level is a sign of substrate mobilization and usage from other sources [6, 7].

In very low birth weight premature infants the caloric needs cannot be met by oral alimentation, thus the obligatory fasting period is lengthened. This brings about a deterioration of the process of cardio-respiratory adaptation in premature infants. Consequently, parenteral (intravenous) alimentation is the method of choice after birth. The problems of parenteral feeding in newborns have been extensively dealt with by various groups of authors [2, 8, 12, 13, 14]. A positive nitrogen balance, essential for an adequate growth rate, can be achieved by a combined infusion treatment using glucose and amino acids. However, this method of feeding has some side-effects such as rises in non-protein nitrogen, ammonia, amino acid levels and aminoaciduria, all known to exert harmful effect on the newborn infant.

Very low birth weight premature infants have been shown to have higher amino acid levels than mature newborns, probably as a result of the degradation of globin derived from Hb–F. This latter opinion is supported by the present finding, in that the incidence of increased plasma levels of leucine, histidine, lysine constituents of Hb–F, was very high in premature babies (Fig. 4). The release of other amino acids (e.g. tyrosine, ornithine and phenylalanine), being partly or completely absent from Hb–F, can be ascribed to transformation of protein components in organs of the newborn during neonatal adaptation with the result of transient appearance of various amino acids in blood.

In the special intermediate metabolic adaptation of the newborn period the amino acids, shown to be released in the present study, might be used as an important energy source via metabolism in the gluconeogenetic pathway. This helps the otherwise more susceptible premature infant to reduce the load experienced at the beginning of extrauterine life.

Early amino acid infusions given to very low birth weight premature infants present an even greater danger than for mature newborns. Taken into consideration the perils of acidosis encountered as side-effect of infusions with synthetic amino acids [3], it can be concluded that in very low birth weight premature babies aged less than 2 weeks amino acid infusion should be accompained by monitoring of serum and urine amino acid levels.

In future studies the role of amino acids derived from Hb will be investigated and the origin of other amino acids should also be clarified.

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PROTEASES AND PROTEINASE INHIBITORS IN EXPERIMENTAL GLUCOCORTICOSTEROID MYOPATHY*

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The unknown enzymatic mechanism of enhanced protein breakdown in steroid myopathy was studied in functionally and biochemically different muscles of rabbits treated with dexamethasone for three weeks. After glucocorticoid administration the fast-twitch glycolytic semimembraneous muscle of treated animals was atrophied, whereas the weight of the slow-twitch oxidative soleus muscle was not altered. The specific activity of the lysosomal endo- and exopeptidases (cathepsin D, E, B and L, lysosomal carboxypeptidase A and dipeptidylpeptidase I) was increased about 2-fold in the atrophied white muscle. The activity of the cytosol enzyme Ca^{++}-activated neutral proteinase was also elevated, whereas that of the other cytosol endopeptidase, chymotrypsin-like enzyme, was unaltered. The level of alanine aminopeptidase was only slightly increased. On the other hand, there were no unequivocal changes in protease activity in the soleus muscle. These findings are in agreement with the known differences in glucocorticoid-sensitivity of the various muscles. Our results suggest that the lysosomal proteolytic system and the Ca^{++}-activated neutral proteinase may play an important role in the glucocorticoid-induced intracellular protein catabolism in muscle. The inhibitor capacities of cathepsin B and trypsin detectable in muscle cytosol were not altered after steroid treatment. Consequently, the increase in cathepsin B activity was not due to the loss of its inhibitor.

Introduction

It is a well-known fact that chronic treatment of animals [11, 14, 17, 50, 51] and humans [31, 10] with glucocorticoid hormones leads to muscle atrophy and weakness. Muscles containing a high proportion of fast-twitch glycolytic fibres are preferentially involved in glucocorticoid-induced atrophy [14, 17, 20, 33, 51].

Available data suggest that skeletal muscle is a target organ for glucocorticoids [33]. Excess amount of the hormones leads to a decreased rate of muscle protein synthesis [17, 34, 39, 43] and an enhanced muscle protein catabolism [10, 17, 51]. The enzymes responsible for the enhanced intracellular protein breakdown are not known. In 1967 BUCHANAN and SCHWARTZ could not detect any activation of acid hydrolases in muscles of cortisone-treated rats [6]. They measured non-protease enzymes in samples containing a mix-

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ture of hind-leg muscles. **Mayer** et al. [32] found an increased activity of the "myofibrillar" protease in glucocorticoid-treated rats. However, this activity is confined to mast cells [36, 54]. We, therefore, started a series of studies on different muscles of rabbits treated with the synthetic glucocorticoid dexamethasone. The activities of several lysosomal and extralysosomal protelytic enzymes derived from red and white muscles were measured, and the changes in the proteases were correlated with the changes in muscle weight.

In recent years some proteinase inhibitors of the muscles were discovered [42, 53]. It is possible that these inhibitors play a regulatory role in proteolysis. Therefore, the trypsin and cathepsin B inhibitor capacities of the muscle samples were also measured. A preliminary report of some of these findings has already been presented [35].

Materials and Methods

Male adult New Zealand rabbits weighing about 2500 g were used. Eighteen animals received intramuscular injections of dexamethasone (Oradexon, Organon OSS, Holland) into the foreleg triceps in a dose of 2 mg/kg every second day for a three-week period. Twelve normal, untreated rabbits were used as controls. The animals were bled, and the hindleg semimembraneous muscle, containing mainly fast-twitch glycolytic fibres and the soleus muscle containing mainly slow-twitch oxidative fibres [37] were excised, cleaned of connective tissue and minced with scissors. For enzyme measurements the muscles were homogenized in 10 volumes of a solution containing 2% KCl and 0.1% Triton × 100. For inhibitor and Ca^{++}-activated neutral protease (CANP) measurements the muscles were homogenized in 3 volumes of 25 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA–Na$_2$. The homogenization was performed using a MSE-blender (2 × 30 sec), then a glass-teflon potter (B. Braun Melsungen) for 30 sec. The tubes containing the homogenates were kept on ice during the procedure. The homogenates were centrifuged at 20 000 g for 20 min and the supernatants were used for measurements.

The activities of cathepsin D, E and B lysosomal carboxylpeptidase A (LCA) and dipeptidylpeptidase I (DAP I) were determined essentially as described by **Barrett** [1]. Cathepsin L activity was measured by incubating the muscle samples at 40 °C for 120 min in a final volume of 1.0 ml containing 50 mM potassium phosphate (pH 6.0), 0.5% azocasein and 5 mM 2-mercaptoethanol. In the blank tubes the reaction mixture contained 10^{-6} M Z–Phe–Phe–CH–N$_2$, a specific inhibitor of cathepsin L [27, 28]. CANP activity was assayed with azocasein as substrate at pH 7.5, in the presence of 10 mM CaCl$_2$ and 10 mM 2-mercaptoethanol as activators [7, 25, 40, 41]. Chymotrypsin-like protease (CLP) was measured with acetyl-Tyr-p-nitroanilide (1 mM) as substrate at pH 8.0. Alanine aminopeptidase (AAP) was assayed as described by **Farr** et al. [12], using Co^{++} ions (1 mM) for activation [22].

For measurement of inhibitor capacities the muscle supernatants were incubated with purified cathepsin B and trypsin for 10 min. Residual cathepsin B and trypsin activities were determined with benzoyl–D, L–Arg–P–nitroanilide at pH 6.0 and 7.5, respectively [1]. Cathepsin B was partially purified from rabbit liver by the method of **Barrett** [2]. Trypsin (Type IX) was purchased from Sigma Chem. Co., St. Louis. Protein was determined by the micro biuret method of **Goa** [16].

Results

The fresh weight of the white semimembraneous muscle of treated rabbits decreased by approximately 35%, whereas the weight of the soleus muscle was practically unaltered (Table I).

Table I

Muscle weights in g in control and dexamethasone-treated rabbits

Muscle	Control (12)	Treated (18)
Semimembraneous	8.83±0.69	5.69±0.53*
Soleus	1.74±0.12	1.70±0.16

Means ±S.D. In parentheses: No. of animals. $^*P < 0.01$.

Protease in the semimembraneous muscle

The activities of lysosomal endopeptidases, such as cathepsin D, E, B and L, and those of lysosomal exopeptidases LCA and DAP I, expressed per gram soluble protein, showed an about 2-fold increase in the semimembraneous muscle of treated animals (Table II). The level of the cytosol enzyme CANP was also significantly elevated. The activity of the other non-lysosomal endopeptidase, CLP, was not changed. There was a slight but significant ($P < 0.05$) increase in the activity of the main cytosol exopeptidase, AAP.

Table II

Protease activities in the semimembraneous muscle of control and treated rabbits

Enzyme	Control (12)	Treated (18)
Cathepsin D, nkat/g	19.2 ±2.1	34.8 ±3.8*
Cathepsin E, nkat/g	7.8 ±1.1	14.2 ±2.6*
Cathepsin B, nkat/g	1.50±0.29	3.35±0.39*
Cathepsin L, μg/s/g	1.58±0.18	3.32±0.36*
LCA, nkat/g	30.7 ±3.7	53.3±5.5*
DAP I, nkat/g	3.67±0.52	6.83±0.62*
CANP, ng/s/g	473 ±41	645 ±56*
CLP, nkat/g	1.75±0.13	1.69±0.19
AAP, nkat/g	100.2 ±41	125.8 ±8.1***

Means ±S.D. In parentheses: No. of animals. $^*P < 0.001$ $^{**}P < 0.01$
$^{***}P < 0.05$

Abbreviations: see Methods.

Protease in the soleus muscle

There were no significant differences in either the lysosomal or the extralysosomal protease activities in the soleus muscles of treated versus control rabbits (Table III).

Table III

Protease activities in the soleus muscle of control and treated animals

Enzyme	Control	Treated
Cathepsin D, nkat/g	28.7 ± 1.7	29.2 ± 1.3
Cathepsin E, nkat/g	13.7 ± 0.8	14.0 ± 2.0
Cathepsin B, nkat/g	2.83± 0.37	3.00± 0.29
Cathepsin L, μg/s/g	2.84± 0.27	2.88± 0.15
LCA, nkat/g	60.1 ± 7.2	63.3± 9.5
DAP I, nkat/g	7.50± 0.62	8.67± 0.79
CANP, ng/s/g	520 ±61	563 ±78
CLP, nkat/g	1.50± 0.13	1.45± 0.16
AAP, nkat/g	146 ± 9	152 ±11

Means ±S.D. Abbreviations: see Methods.

Changes in proteinase inhibitors

In control rabbits the trypsin and cathepsin B inhibitor capacities were higher in the soleus muscle than in the semimembraneous muscle (Table IV). After dexamethasone treatment the inhibitor capacities of both muscles showed a slight but no significant increase.

Table IV

Proteinase inhibitor capacities in semimembraneous and soleus muscles of control and treated animals

Muscle	Cathepsin B-inhibition (nkat/g)	Trypsin-inhibition (μkat/g)
Semimembraneous (C)	35.7±4.1	1.63±0.19
Semimembraneous (T)	39.3±4.9	2.04±0.24
Soleus (C)	58.4±7.0	3.46±0.42
Soleus (T)	68.9±8.8	3.62±0.47

Means ±S.D. C = control; T = treated.

Discussion

A prominent metabolic response to glucocorticoid hormone administration is the increased supply of amino acids from peripheral tissues to the liver, where they serve as gluconeogenic precursors [13]. Prolonged treatment with glucocorticoids results in a loss of body weight, a negative nitrogen balance [30] and muscle atrophy, which is more pronounced in fast-twitch glycolytic muscles [14, 17, 33]. The quantity of sarcoplasmic and myofibrillar proteins have been found to decrease in steroid myopathy [14, 21, 50]. Changes in lipid composition have also been described [19, 20]. The finding that the activity of the enzymes involved in glycolysis and the citric acid cycle are equally decreased suggests that the changes in muscle protein synthesis and degradation are more fundamental than those in the energy-supplying metabolic pathways [14]. The reduction of protein synthesis in steroid myopathy is due to the loss of RNA and to an inhibition of translation resulting from an impairment in peptide-chain initiation [39]. An increased rate of muscle protein breakdown after glucocorticoid administration has been demonstrated, too [17, 10, 51]. Tomas et al. [51] concluded that plasma concentrations of glucocorticoids within the normal range do not effect the rate of muscle protein breakdown, whereas excessive plasma concentrations of these hormones accelerate muscle protein breakdown [51].

The employed dexamethasone treatment resulted in typical steroid myopathy in the semimembraneous muscle, whereas the soleus muscle was practically unaffected [20]. In the atrophied semimembraneous muscles of the treated rabbits we observed an about 2-fold increase in the specific activity of the measured lysosomal protease (Table II). In recent years it has been established that lysosomes are present in skeletal muscles [3, 5] and play an essential role in the catabolism of myofibrillar and sarcoplasmic proteins [4, 5, 15, 46]. The inhibition of lysosomal proteinases with protease inhibitors of microbial origin can reduce muscle protein catabolism [48]. The main lysosomal endopeptidases, cathepsin D, B, L and H are capable of degrading myofibrillar proteins [42, 45]. The function of the lysosomal exopeptidases is to split the protein fragments formed by the endopeptidases [1]. Our finding of an equal increase in the activity of lysosomal endo- and exopeptidases points to the hyperfunction of the lysosomal proteolytic system in steroid myopathy and suggests an important role of lysosomal proteolysis in the development of atrophy. Buchanan and Schwartz [6] failed to observe activation of acid hydrolases in mixed fibre hindleg muscles of cortisone-treated rats [6]. Owing to the great differences in the hormone responsiveness of the individual muscles, their results cannot be regarded as evidence against the role of lysosomes in steroid myopathy. The enhancement of lysosomal proteolytic activity has also been observed in other conditions leading to muscle atrophy, e.g. after denervation [37, 49], in disuse atrophy [44], during starvation [3], in hyperthyroidism [9, 52], in vitamin-E deficiency [24] and in dystrophies [23, 26].

The activity of the cytosol enzyme CANP was also increased in the atrophied white muscle (Table II). The function of this proteinase may be the solubilization of myofibrillar proteins, facilitating their lysosomal uptake

[4, 8, 18, 40]. The enzyme is activated by an increase in the intracellular free Ca^{++} concentration [8, 18]. This activation of CANP requires concentrations of ionized calcium about 100 times higher than that assumed to be present in the activated muscle. However, the physiological level of Mg^{++} ions may reduce the Ca^{++}requirement of the enzyme [47]. Several authors have demonstrated the presence of an inhibitor of CANP in the muscle [18, 53]. This inhibitor may play a role in the regulation of CANP activity, too. Elevated levels of CANP in muscles have been observed in dystrophy [26] and hyperthyroidism [52], whereas no change has been found in denervation [26] and in disuse atrophy [44].

The specific activity of the other cytosol endopeptidase CLP was not altered in the atrophied muscle (Table II). Similar observation has been made in disuse atrophy of rabbit muscles [44]. AAP is the main aminopeptidase of the muscle cytosol [22]. Its activity was slightly elevated in the white muscles of treated rabbits (Table II). Little or no change in AAP activity has been found in other muscle atrophies [22, 26]. Our present knowledge about the functions of CLP and AAP is insufficient to permit a discussion of their significance in the increased muscle protein breakdown.

In our experiments the red soleus muscle of the treated rabbits was not atrophied (Table I). Accordingly, there were no unequivocal changes in the protease activities in this muscle (Table III). The resistance of the red muscle to glucocorticoid-induced atrophy may reflect differences in protein turnover and sensitivity to catabolic stimuli between red and white muscles [29, 34]. The differences in glucocorticoid receptor density cannot explain the differences in hormone responsiveness [43].

The alteration in inhibitor capacities was not significant in any of the muscles investigated (Table IV). This fact suggests that the increase in cathepsin B activity (Table II) was not due to the loss of its inhibitor. The possible role of cathepsin B inhibitor may be the neutralization of cathepsin B-released lysosomes to the cytosol. The trypsin inhibitor of the muscles was described by Waxman and Krebs [53]. Its function is as yet unknown.

The mode of protease activation during steroid myopathy requires further investigation.

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DEMONSTRATION OF FC AND C3B RECEPTORS ON RAT PERIKARYA

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Minced rat brain deprived of cerebellum was dissociated by trituration through stainless steel screen and nylon meshes, then by velocity sedimentation technique free-floating perikarya were separated from cell syncytia and cellular debris. The presence of Fc-receptor and C3b-receptor activities, as well as the absence of membrane-bound immunoglobulin and receptor for sheep red blood cells were demonstrated on separated perikarya of the rat brain.

The putative immune pathomechanism of demyelination diseases including multiple sclerosis has raised interest in the surface antigenic properties of brain cells. Interspecies normal brain cell surface antigens [2, 4, 9] and HLA antigens on human brain tumour cells [17] have been described. Cross-reactive T-lymphocyte normal brain cell membrane antigen, TH-1, was first detected in the mouse [14], then specific human TH-1 type antigens common to a subset of T lymphocytes and fetal brain have been reported [6, 16]. Recently, the demonstration of Fc receptors on microglia in cell cultures of rat brain [13], and in brain tissue sections from patients with multiple sclerosis [11] have suggested that the microglia may be related to cytotoxicity.

In the studies to be reported we examined the whole variety of rat brain cells separated *in vitro* on the basis of size, for the presence of cells with Fc receptors, C3b receptors, receptors for sheep erythrocytes and surface IgG.

Materials and Methods

2–6 months old Wistar rats were decapitated, their entire brain was removed, cleaned of connective tissue, and trimmed of cerebellum. The cerebral tissue was dissociated by trituration through stainless steel screen and nylon meshes of 74 μm aperture, and resuspended in sedimentation medium (SM). SM contained Eagle's minimal essential medium with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.1% sodium methyl cellulose. The pH was adjusted to 7.4 with sodium bicarbonate and Hepes. Suspensions were regularly agitated with a magnetic stirrer.

Cell separation was performed by velocity sedimentation at 4 °C [3]. The sedimentation chamber was loaded with 5 ml of SM containing 0.5% Ficoll, 10 ml of cell suspension with $4\text{–}5 \times 10^6$ cells/ml in SM, 10 ml of 0.5% Ficoll in SM, 30 ml of 0.5–2% linear Ficoll gradient in phosphate buffered saline (PBS), and 20 ml of 2% Ficoll in PBS. The filling procedure lasted 25–30 min. After 60 min sedimentation interval, 10 fractions of 7.5 ml were collected, and numbered in the order of collection. After separation the fractions were washed twice, centrifuged at 1500 rpm for 5 min, and resuspended in Ficoll-free SM. Every fraction was examined microscopically without fixation and staining.

Cell fractions were tested for viability by dye exclusion with 2% trypan blue in PBS at 4 °C, and then evaluated in 3–5 min by microscopic cell countings. Separated perikarya of fractions 3–7 were tested for Fc-receptor activity with rosette technique using human (0 Rh+) erythrocytes sensitized by IgG anti-Rh of the Ripley type [5]. Receptors for activated C3 (C3b) were tested by yeast-complement (YC) rosette technique [15]. Sedimented fractions were treated with fluoresceinated goat antiserum to rat gamma-globulin to detect membrane-bound surface IgG [7]. Formation of nonimmune rosettes (E-rosettes) was studied with sheep red blood cells [8].

Results

During velocity sedimentation at unit gravity the separation of particles takes place mainly according to size [10]. Large particles sediment faster and can thus be collected earlier. In each of the collected ten fractions particles of characteristic size could be observed. The fluid in the cone of the column 13.26 ml approx.) was taken up by the first two fractions containing mostly cell aggregates and syncytia. In fractions 9, 10 and partly in fraction 8, predominantly homogeneous round dense bodies, presumably free cell nuclei (approx. 7 μm in diameter), and further subcellular particles appeared. Discrete cellular particles—perikarya—were seen in fractions 3–7 (from 20 μm to 7 μm in diameter). High purity of characteristic cell types could not be obtained in any of the fractions after a single sedimentation step which proved to be slightly harmful to surface structures of the perikarya [12]. Thus cell separation resulted in three groups of dissociated particles: supracellular (fractions 1–2), cellular (fractions 3–7), and subcellular (fractions 8–10). Fractions 3–7 were taken for marker studies. Marker properties of cellular fractions are presented in Table I.

Table I

Marker properties of perikarya separated from the rat brain by velocity sedimentation

Fraction No.	Viability	EA–RFC	YC–RFC	sIgG	E–RFC
3	100 ± 0.0	14 ±5.8	10 ±0.5	—	—
4	93.3±4.4	11 ±7.2	9 ±1.0	—	—
5	83.3±11.1	1.2±0.7	6.8±1.8	—	—
6	96.6± 2.2	7.5±4.7	10 ±7.7	—	—
7	100 ±0.0	6.4± 4.8	17 ±4.1	—	—
Average of fractions 3–7	94.6±3.5	8.0±4.6	10.5±3.0	—	—

Viability of cells was based on trypan blue negativity. EA-RFC: EA rosette froming cells, YC-RFC: cells forming rosettes with complement-coated yeast particles, sIgG: surface-immunoglobulin tested by fluorescein-labelled goat antiserum to rat gamma-globulin, E-RFC: cells forming nonimmune rosettes with sheep erythrocytes. Values represent mean percentages ±S.E.M. Numbers of samples: n=4.

Fc and C3b receptors were present on separated perikarya of all rats investigated. The mean percentage of EA-rosette forming cells (EA–RFC) concerning all the cellular fractions was 8.0%. A mean of 10.5 per 100 perikarya formed yeast-complement rosettes (YC–RFC). Surface IgG and receptors for sheep erythrocytes were not detected on the perikarya, thus indicating the absence of B and T lymphocytes in the preparations.

Discussion

The efficacy of velocity sedimentation was found to depend on two main factors, cell streaming and formation of aggregates. Cell streaming was effectively reduced by choosing the suitable cell concentration of $4-5 \times 10^6$ cells/ml in the suspension layered onto the gradient. Clumping of cells was inhibited by adding 0.1% sodium methyl cellulose to SM. Accordingly, trypan blue staining cell viability was slightly affected after a single separation step. A more effective separation by discontinuous density gradient centrifugation caused a serious damage to the surface structure and viability of the perikarya [12].

To investigate surface markers of brain cells, we used freshly separated perikarya that allowed the simultaneous demonstration of Fc and C3b receptors, whereas NYLAND et al. [11] failed to detect complement receptors in human brain tissue sections from multiple sclerosis. Data are not sufficient to provide a detailed comparison of these findings.

The presence of Fc receptors was demonstrated not only on the microglia [11, 13] but also on neurones and myelin sheaths [1] suggesting a ubiquitous character of this marker in the nervous system. Detection of C3b receptors referred to the possible role of brain derived cells in effector mechanisms of immune and/or inflammatory processes via the complement system. Since fixation and staining could not be achieved using rosette techniques in wet preparations, further studies are needed for specification of Fc and C3b receptor positive perikarya.

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EFFECTS OF CHOLECYSTOKININ OCTAPEPTIDE SULPHATE ESTER AND UNSULPHATED CHOLECYSTOKININ OCTAPEPTIDE ON ACTIVE AVOIDANCE BEHAVIOUR IN RATS

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The effects of peripherally administered cholecystokinin octapeptide sulphate ester and its unsulphated form on the active avoidance behaviour of rats were studied. The acquisition of avoidance behaviour was impaired, while extinction was facilitated, following cholecystokinin octapeptide sulphate ester or unsulphated cholecystokinin octapeptide treatment. These peptides had no action on open-field activity. It is concluded that peripherally administered cholecystokinin octapeptide influences acquisition and extinction of active avoidance behaviour and this effect is unrelated to general motor activity of the animals.

Peripheral peptide hormones, earlier associated only with peripheral organs, have recently been discovered in the brain [for reviews, see 15, 16]. The peptide cholecystokinin (CCK) was first identified in, and isolated from, different brain areas of several species [3, 4, 5, 13, 14, 17, 19, 20, 22, 24, 25, 26, 27, 30, 34, 35]. There are a few data suggesting that CCK might act as a satiety signal [for review, see 21]. Subcutaneous injection of the COOH-terminal octapeptide of cholecystokinin (CCK-8) has recently been shown to produce antinociceptive effects, palpebral ptosis and sedation in mice, to inhibit their spontaneous rearing activity and to prolong the hexobarbital-induced sleeping time [37, 38].

In our earlier experiments it was shown that intracerebroventricular administration of cholecystokinin octapeptide sulphate ester (CCK-8-SE) modified the monoaminergic system in the brain [7, 11, 12, 31, 32, 33], whereas the unsulphated cholecystokinin octapeptide was practically ineffective [9]. It was also demonstrated that in CCK-8-NS and CCK-8-SE treated animals the acquisition of conditioned feeding behaviour was considerably impaired, while the latency of passive avoidance behaviour was increased as compared to the control [6, 8].

In the present paper the effects of peripherally administered CCK-8-NS and CCK-8-SE on the active avoidance behaviour and exploratory activity of rats have been studied.

Materials and Methods

1. *Animals*

CFY adult male albino rats, weighing 150–180 g at the beginning of the investigation were housed, 6–6 per cage, with free access to food and tap water. The animals were kept under controlled lighting conditions (12 h light, 12 h dark), the light period starting at 6 a.m. Experiments were carried out daily between 6 a.m. and noon.

2. *Apparatus*

(a) *Active avoidance behaviour.* The experimental apparatus was a bench-jumping conditioning box, measuring 45 × 25 × 45 cm, with a Plexiglass window in the front. A Plexiglass bench (13 × 9 cm) was fixed on one side of the box, 7 cm above the floor. The conditioning stimulus was the light of a 45 W bulb. The unconditional stimulus was an electric shock of 1.0 mA AC delivered to the grid floor.

(b) *Exploratory activity.* An open-field box measuring 60 × 60 cm, consisting of 36 squares measuring 10 × 10 cm each, was used to test the exploratory activity. The apparatus was illuminated by a 200 W bulb.

3. *Procedure*

(a) *Active avoidance behaviour*

Acquisition: an experimental session of 10 min was performed daily. Each session consisted of 10 trials with a mean intertrial interval of 60 s (range 50–70 s). The conditioning stimulus was presented for a maximum of 15 s. If the rat jumped onto the bench during the first 10 s (conditioned avoidance response, CAR), the conditioning stimulus was terminated. Failure of performance was associated with the unconditional stimulus in the third 5 s of the conditioning stimulus period. The avoidance training criterion was 80% or more CARs during three consecutive days.

Extinction: after having reached the criterion of learning, the animals were subjected to extinction. During extinction, the conditioning stimulus was not followed by electric shock (unconditional stimulus).

(b) *Exploratory activity*

Open-field exploratory activity was scored by counting the number of squares crossed, and the number of rearing, grooming and defecation activites during a 3 min session on three consecutive days.

4. *Treatment*

Cholecystokinin octapeptide sulphate ester (CCK–8–SE) and unsulphated cholecystokinin octapeptide (CCK–8–NS) were synthetized by Penke et al. [23]. Peptides were dissolved in physiological saline solution and given intraperitoneally in doses of 4 nmol/kg (5 μg/kg) and 400 nmol/kg (500 μg/kg) in a volume of 1.0 ml/kg. Control animals received physiological saline solution in a volume of 1.0 ml/kg. All treatments were carried out 1 h before the session.

5. *Statistical evaluation*

The nonparametric ranking tests of Kruskal–Wallis and Wilcoxon [36] were used for statistical analysis of data. A probability level of 0.05 or less was accepted as the level of significance.

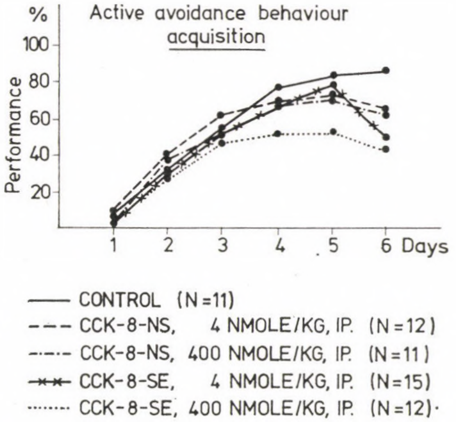

Fig. 1. Effect of two different doses of CCK–8–NS and CCK–8–SE on acquisition of active avoidance behaviour. Number of animals is shown in brackets

Results

CCK-8-NS and CCK-8-SE in a 400 nmol/kg dose (Fig. 1) resulted in a decline of the conditioned avoidance performance during the second three days of acquisition ($p < 0.05$ versus control). The 4 nmol/kg dose of these peptides had no effect on the acquisition.

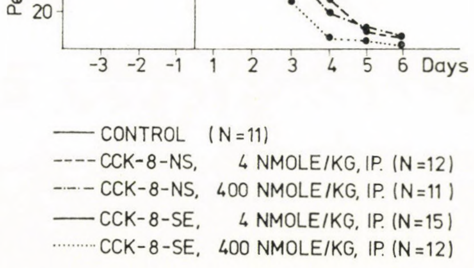

Fig. 2. Effects of two different doses of CCK–8–NS and CCK–8–SE on extinction of active avoidance behaviour

CCK-8-NS in 4 and 400 nmol/kg doses and CCK-8-SE in a 400 nmol/kg dose caused facilitation ($p < 0.05$) of the extinction (Fig. 2).

Neither CCK-8-NS nor CCK-8-SE treatment could change ambulation or the number of grooming, rearing and defecation activities in the open-field test (Table I).

Table I

Effects of intraperitoneal administration of cholecystokinin octapeptide sulphate ester and unsulphated cholecystokinin octapeptide in two different doses on the open-field activity of rats 1 h following injection

	Dose (nmol/kg)	Number of occasions on which activity was displayed*			
		Ambulation	Rearing	Grooming	Defecation
Control	0	29.0±4.0	4.2±0.7	2.8±0.3	2.8±0.3
CCK-8-NS	4	43.0±7.7	7.7±1.7	3.0±0.7	2.7±0.3
CCK-8-NS	400	34.0±5.0	6.0±1.0	2.7±0.7	2.7±0.3
CCK-8-SE	4	41.3±6.0	6.3±0.7	4.0±0.7	3.3±0.3
CCK-8-SE	400	22.3±4.3	4.0±0.7	2.0±0.7	1.9±0.3

* Mean value ±S.E.M. (n=10–12) during a 3 min session on three consecutive days

Discussion

CCK is one of the gastrointestinal peptides which have been identified in the brain. Although its physiological role is unknown, evidence has accumulated implying that this peptide might play a role in the central regulation of satiety. Several groups have shown that peripheral [21, 28] or central [2, 29] administration of CCK or related analogues inhibits eating behaviour in fasting animals. In our previous experiments it was demonstrated that intraperitoneally administered CCK-8-NS and CCK-8-SE were able to damage the acquisition of conditioned feeding behaviour and to facilitate the extinction of conditioned feeding behaviour [6]. On the other hand, these peptides increased the latency of passive avoidance behaviour of rats, showing that CCK interfered with retrieval and/or consolidation processes [8].

In the present study both CCK-8-NS and CCK-8-SE slightly diminished the acquisition and facilitated the extinction of the active avoidance behaviour of rats, showing that these CCK octapeptides also interfere with fear-motivated behaviour. The action of these peptides on peripheral organs (pancreas, gut, etc.) could have caused discomfort, which might have affected the motor activity. However, this can be ruled out, since the peripheral effects of CCK-8-SE are much stronger than those of CCK-8-NS [1, 18] and in our study there were no differences in any of the parameters of the open-field activity. On the other hand, these peptides display similar actions on the active avoidance behaviour following their intracerebroventricular administration [10]. The similarity between the influence of peripherally and centrally administered CCK-8-SE and CCK-8-NS suggests that these peptides interact with central nervous mechanisms independent of the route of administration.

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EXTRAVASCULAR CIRCULATION OF PLASMA PROTEINS

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The escape of radioiodinated serum albumin (RISA) from the circulation and lymphatic albumin transport was investigated in anaesthetized rabbits. The fraction of RISA escaping each hour from the circulation was 0.0932±0.0075, lymphatic albumin transport in the thoracic duct was 0.0389±0.0026 in the hepatic lymph trunk 0.0115±0.016, in the intestinal trunk 0.0122±0.0037 and in the renal lymphatics 0.0185±0.0021. About 78% of the lymph and 91% of albumin transported by the thoracic duct originated from the abdominal and renal lymphatics. The ratio of albumin escape from the circulation versus lymphatic return was 2.36. From the first slopes of the lymphatic RISA activity curves the albumin escape rates were calculated and found to be 1.89 in the liver, 2.32 in the kidney, 0.69 in the intestine and 0.20 g h^{-1} kg^{-1} tissue weight in the leg (skin). The lymph vessels returned 17% of the escaped albumin, from the liver about 12% from the intestines and almost all from the kidneys. A very strong correlation (r=0.996) was found between lymph to plasma albumin concentration ratios and the first slopes of the RISA equilibration curves, proving that protein concentration in the lymph is determined by the rate of protein escape from the capillaries and that the rates obtained from the first slopes of the RISA cpm/g albumin in lymph per RISA cpm/g albumin in plasma equilibration curves are a measure of capillary permeability to protein.

A substantial fraction of plasma proteins is located in the interstitial fluid outside the vascular system, and there is a constant exchange between the intravascular and extravascular protein pools. It has been generally assumed that the mechanisms involved in the process are the leakage of protein molecules from the capillaries by a flow dependent (convection) process or by some mechanism independent of water flow (diffusion or vesicular transport) and subsequently the retransport into the circulation of the escaped macromolecules by the lymphatic system [3]. The escape of plasma proteins from the circulation was estimated by the kinetic analysis of the early disappearance of labelled serum proteins from the circulating plasma. Comparison, however, of the albumin transported by the lymphatic system has revealed that only a fraction of the escaped albumin is returned by the thoracic duct lymph which represents at least 80% of total body lymph flow. It has been calculated that in the dog thoracic duct albumin transport is equal only to 46.6% of the amount leaving the circulation [10].

On the other hand it has been shown [10, 15, 17] that labelled albumin introduced into various tissues or serous cavities and enzymes escaping from injured cells are removed not only by the lymphatics but also by the blood stream. These observations have demonstrated that macromolecules may pass from the tissues into the blood capillaries. It has been assumed that the large molecules move in the direction opposite to convective flow by diffusion or vesicular transport. Consequently the net flux of macromolecules is equal to the difference between outward movement and inward movement and it is determined mainly by the transvascular concentration gradient of the respective molecule. The lymphatic transport of macromolecules is essentially equal to their net flux. There are, however, no data about the relationship between the escape of macromolecules from the circulation and their lymphatic return. The calculations cited previously are based on data obtained in different groups of animals in experiments not aimed at the solution of this question. In the present investigations the escape of labelled protein from the circulation and the lymphatic protein transport were therefore studied simultaneously in the same group of animals. In other animals the contribution to transcapillary protein exchange was studied in regions where protein leakage from the capillaries is known to be important.

It has been pointed out already by STARLING [9] that the regional differences observed in the protein concentration of lymph may result from differences in capillary permeability. The rate of exchange of injected labelled protein or other macromolecules between plasma and lymph varies also from tissue to tissue. The equilibration is more rapid in the regions where the protein content of lymph is relatively high [1, 5, 6, 11, 12, 13]. These findings are also consistent with differences in blood capillary permeability to macromolecules. Consequently, the movement of the labelled protein molecules from plasma to lymph may reveal data on the permeability characteristics of regional capillaries.

Materials and Methods

The experiments were made on rabbits of both sexes with an average body weight of 3.36 ± 0.25 kg. The animals were anaesthetized with pentobarbital (35 mg/kg) additional anaesthetic being injected whenever necessary. The animals were divided into 3 groups. In Group 1 the thoracic duct was cannulated by a polyethylene tube on the left side of the neck. In these animals prenodal crural lymph was also collected in the second hour of the experiment. In Group 2 of the animals the hepatic lymph trunk in the liver hilum and the intestinal trunk were simultaneously cannulated. In these animals leg lymph was collected in the 4th hour. In Group 3 renal lymph was collected. This was done by two different methods. In about half of the animals, in contrast to the situation in the dog, the renal lymph vessels unite into a single duct. In these animals one of the renal lymph trunks, usually the left, was cannulated with a plastic tube and the total flow rate of renal lymph was calculated by multiplying the collected amount by two. In animals where the renal lymph trunk could not be cannulated the abdominal thoracic duct was ligated below and above the orifice of the renal lymphatics and a cannula was introduced into this part of the duct. All other lymphatics joining the cannulated part of the duct were tied off. No difference was detected between the results obtained by the two methods of lymph collection therefore the data were pooled in a single group. In all experiments lymph was collected in 30-min periods for 4 hours. Radioiodinated serum albumin (RISA) was introduced in a single intravenous bolus at the beginning of the lymph collection and plasma samples were obtained also at half hourly intervals.

Total protein concentration in plasma and lymph samples was estimated according to Lowry. Albumin was estimated by microzone electrophoresis. Radioactivity was measured in a well-type Na/Tl scintillation detector. Plasma and lymph activities were normalized by dividing with total injected radioactivity.

Total circulating albumin mass was calculated from the plasma albumin concentration and circulating plasma volume. The latter was estimated from injected total radioactivity and plasma activity extrapolated to zero time. In the subsequent periods eventual changes of plasma volume were calculated from arterial blood haematocrit. Albumin escape rate from the circulation was obtained by the analysis of the exponential curve of the disappearance of RISA from the circulation. Lymphatic return rates were calculated from lymphatic albumin concentration, the hourly output of lymph and circulating albumin mass. All values are computed for 1 kg body weight.

The results in the tables and figures are means ±SEM.

Results

Escape of radioiodinated albumin. The slope of the plasma decay curve (K_i) was 0.0932±0.0075 in Group 1 with cannulated thoracic duct, in Group 2 (cannulated abdominal vessels) it was 0.0931±0.0026 and in Group 3 (cannulated renal lymphatics and ligated abdominal thoracic duct) 0.0863±0.0052. Thus in the rabbit about 9% of the introduced RISA and by inference the same fraction of the circulating albumin mass leaves the circulation each hour.

Lymph flow and lymphatic albumin return. Lymph flow rates fluctuated during the 4 hours of observation but the differences between the periods were not significant (Fig. 1). The average hourly thoracic duct lymph output was 2.36±0.32 ml h^{-1} kg^{-1}, hepatic lymph flow was 0.493±0.135 ml h^{-1} kg^{-1}, the intestinal flow 0.618±0.165 ml h^{-1} kg^{-1} and finally renal lymph flow was 0.743±0.137 ml h^{-1} kg^{-1}. The total output from the 3 cannulated regions was 1.86 ml min^{-1} kg^{-1} or 78.6% of the thoracic duct lymph flow. Thoracic duct albumin transport was calculated as being 39.8±3.8 mg h^{-1} kg^{-1}; 10.4±2.9 mg h^{-1} h^{-1} was transported by the hepatic lymph trunk, 11.0±1.6 was obtained from the intestinal trunk and 14.7±1.2 was transported by the

Table I

Extravascular circulation of plasma proteins

	Thoracic duct	Hepatic trunk	Intestinal trunk	Renal lymphatics
N	8	11	12	16
Lymph flow, ml h^{-1} kg^{-1}	2.36 ±0.23	0.493 ± 0.059	0.618 ±0.055	0.743 ±0.070
Total protein, g/l	35.0 ±2.9	48.6 ± 3.8	33.3 ±4.0	37.8 ±2.2
Albumin, g/l	18.8 ±1.5	22.4 ± 0.8	16.2 ±1.1	21.5 ±1.2
L/P albumin	67.9 ±1.8	87.7 ±10.5	63.3 ±7.6	80.5 ±8.2
Albumin transport, mg h^{-1} kg^{-1}	40.15 ±2.87	11.05 ± 1.61	10.45 ±2.87	14.70 ±1.26
Albumin transport/circulating albumin	0.0389±0.0185	0.0122± 0.034	0.0115±0.041	0.0185±0.0029

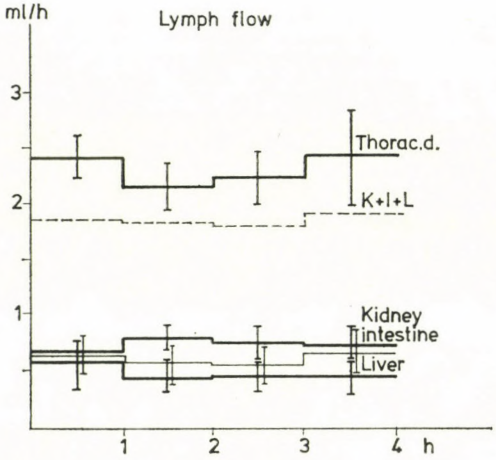

Fig. 1. **Lymph flow rates in thoracic duct, renal lymphatics, and intestinal and hepatic trunks of anaesthetized rabbits**

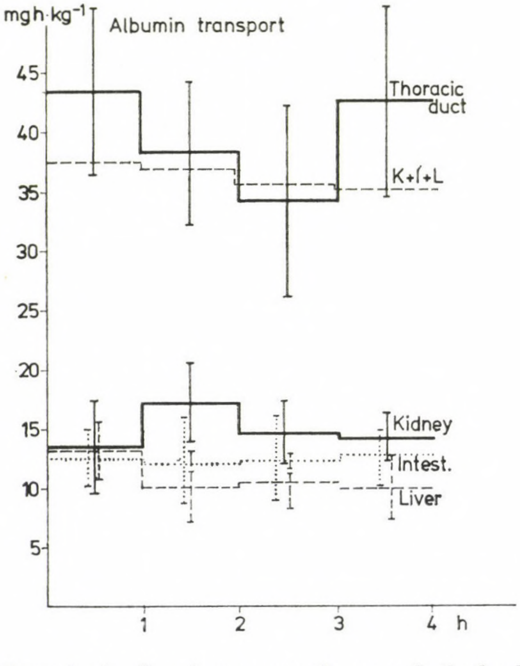

Fig. 2. Lymphatic albumin transport in anaesthetized rabbits

renal lymphatics (Fig. 2). The total albumin transport in the 3 regions was 36.2 mg h^{-1} or 90.9% of the thoracic duct transport. Obviously the comparison of the data obtained from 3 different groups of animals may lead to errors, as will be discussed later.

The rate of thoracic duct return of albumin was calculated as being 0.0389 ± 0.0026, i.e. an amount corresponding to nearly 4% of the total circulating albumin mass was retransported by the thoracic duct each hour. The transport rates in the hepatic, intestinal and renal lymph vessels were $0.022 \pm \pm 0.0016$, 0.0115 ± 0.0037 and 0.0185 ± 0.0021, respectively, adding up to a cumulative rate of 0.0422, which is higher than the albumin transport in thoracic duct lymph. The discrepancy is due to the differences in circulating albumin mass. The animals of Groups 2 and 3 had lower serum albumin concentrations and plasma volumes than those of Group 1. The differences are greater than can be accounted for by the individual differences between the animals and they are probably due to the more traumatising procedures required for the cannulation of abdominal and renal lymph vessels.

Equilibration of labelled albumin in lymph. The appearance of RISA in lymph is shown in Fig. 3. The rise of lymphatic activity was very fast in the hepatic lymph, renal and intestinal lymph being slower. The slowest increase of activity was observed in crural lymph which originates from the skin

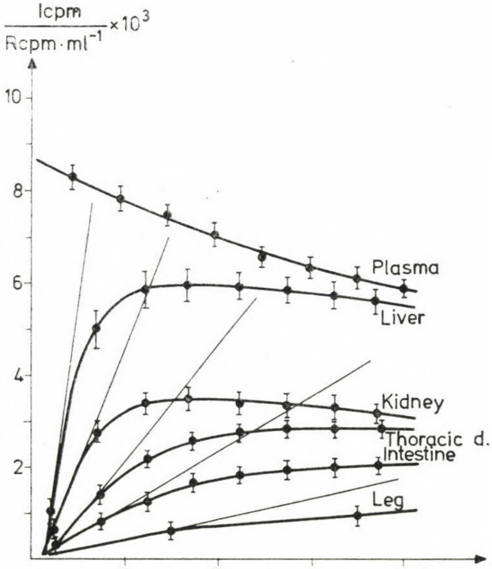

Fig. 3. **Appearance of RISA in lymph after a single bolus intravenous injection; activity curves and first slopes of the curves**

The same order was evident in the equilibration, i.e. the increase of specific radioactivity. Figure 4 shows the increase of lymphatic specific activity as a function of plasma specific activity (cpm g albumin^{-1} in lymph/cpm g albumin^{-1} in plasma).

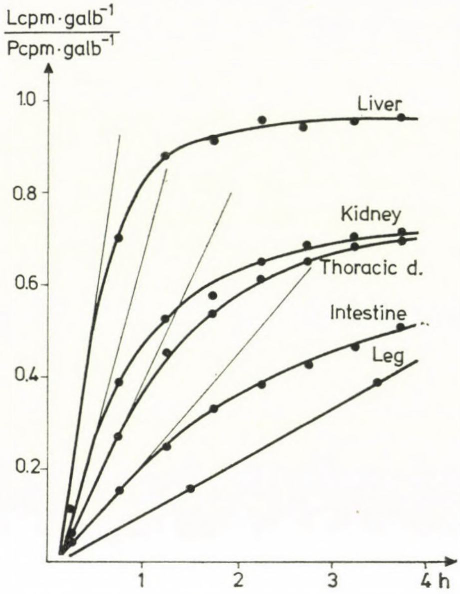

Fig. 4. Equilibration of RISA in the lymph (cpm g albumin^{-1}) and first slopes of the curves

Equilibrium was not attained even in the liver, where the escape of the labelled albumin was very fast. At the end of the 4th hour of the experiment the specific activity ratio in the liver lymph was 0.94, in the mixed thoracic duct lymph 0.66, in the intestinal one 0.40, in the renal lymph 0.67 and, finally, 0.36 in the leg lymph.

Discussion

Relationship between protein escape and lymphatic return. Most methods of lymph collection probably lead to an underestimation of lymph flow. Liver lymph is, for example, collected in the hepatic hilum. Several lymph vessels run, however, upwards with the hepatic veins. It was estimated in the dog that about 30% of liver lymph is transported by vessels other than the hepatic lymph trunk [16]. Furthermore not all of the intestinal lymph is carried by the main intestinal trunk. Several small vessels can be seen in the mesentery not joining this duct. From their number and caliber we estimated that they may carry as much as 20 to 30% of the intestinal lymph. In the rabbit the division of the thoracic duct in two branches is not uncommon [4] and there may also be several small branches joining the veins. In the present experiments the subsidiary branches were ligated and the main trunk cannulated, nevertheless some vessels may have escaped detection. However, average thoracic duct lymph flow rate in the present study (2.17 ml h^{-1} kg^{-1}) is similar to that reported in the literature (2.2 ml h^{-1}) for anaesthetized rabbits [8], and it is not without significance that the majority of authors observed very similar flow rates in a variety of animals ranging from rat to horse [7]. It cannot be excluded that not all lymph is collected in every animal but it can be safely assumed that the values presented here are not a gross underestimate of the true thoracic duct lymph flow and albumin transport. It should be added that about 20% of the lymph formed in the body is not collected by the thoracic duct but is carried to the circulation via other vessels (right duct, right cervical trunk, etc.).

The escape of labelled albumin from the circulation is usually calculated in the early stage, i.e. in the first 30 to 90 min after its injection. This seems to be justified since labelled molecules accumulate progressively in the interstitial fluid. The analysis of plasma decay curves after a bolus injection shows, however, that there is only a single exponential slope up to the time when equilibration is attained between the intravascular and extravascular albumin pools. This is reached in different animal species in 8 to 24 hours. The equilibrium is, however, a dynamic one, i.e. the outward movement of albumin is equal to its return by the lymph plus the inward movement by diffusion and/or vesicular transport. In the present study lymphatic return is eliminated by cannulating the thoracic duct, while the inward movement of albumin molecules begins from the moment they appear in the extravascular fluid. Accordingly, calculations made from the exponential plasma activity curves may underestimate the outward movement of albumin molecules. The initial stage of the plasma activity curve has been plotted therefore on a linear scale and it was estimated that in the first 30 to 60 min the hourly escape rate may be about 0.131, i.e. about 40% higher than calculated conventionally from the exponential curve. Accordingly, both outward movement of albumin from the circulation and total lymphatic return might be underestimated by as much as 20 to 40%. The ratio of 2.36 for albumin escape to lymphatic return obtained in the present study may be therefore very nearly correct. In the dog this ratio calculated by a different method from data compiled from various experiments and from the literature attained 2.15 [10]. This means that in the normal animals less than half of the plasma protein escaping from the capillaries is returned by the lymphatic system.

Regional capillary permeability and extravascular circulation. The analysis of regional transcapillary exchange is doomed by several difficulties. Only the amount of protein transported by the lymphatics can be measured. In the present study lymph flow per kg liver weight was 14.3 ml h^{-1}. This is about the half of the value observed in dogs [2, 14]. Accordingly, 316 mg kg^{-1} albumin is transported every hour from the liver. Albumin leakage is surprisingly high in the kidney. At a lymph flow rate of 116 ml h^{-1} per kg organ weight albumin escape was 2319 mg h^{-1} (Table II).

Table II

Albumin movement into the tissues and lymphatic removal in g h^{-1} kg^{-1} tissue weight

Tissue	K_L	Escape	Lymphatic removal
Liver	0.00855	1.89	0.32
Kidney	0.00469	2.32	2.16
Intestine	0.00122	0.69	0.085
Leg	0.00037	0.20	(0.008)*

* From "missing" thoracic duct lymph.

It is difficult to estimate the mass of gastrointestinal tissue drained by the intestinal lymph trunk. Assuming that this corresponds to about 10 to 15% of body mass lymph production would be 4 to 6 ml h^{-1} per kg and albumin transport around 85 mg h^{-1} kg^{-1}.

Assuming that thoracic duct lymph not accounted for by hepatic, intestinal and renal lymph production comes chiefly from muscle and skin which represent about 50% of the body mass, albumin transport from these tissues would be as a rough approximation 8 mg h^{-1} kg^{-1}. That the basic assumption is not totally unfounded is corroborated by the fact that the albumin concentration of the lymph "missing" from the thoracic duct is 11.7 g/l and the actual concentration found in leg lymph is 12.6 g/l.

The appearance of the labelled albumin in the lymph might be a measure of regional capillary permeability. However, the rate of indicator appearance declines with time. This is a consequence of the accumulation of labelled albumin in the extravascular fluid and of the decrease of the transcapillary concentration gradient. The first slopes of the lymphatic concentration curves (K_L) were therefore obtained by linear extrapolation (Table II, Fig. 3). Assuming that the concentration of labelled protein in lymph and tissue fluid are equal, the amount of albumin moving from the circulation into the tissue can be calculated as follows

$$\text{Alb. mg h}^{-1}\,\text{kg}^{-1} = K_L\,M_A\,V$$

where M_A is the circulating albumin mass in mg and V is the extravascular interstitial fluid volume in ml per kg organ weight.

Unfortunately no data are available about the extravascular interstitial fluid volume in the individual tissues of the rabbit. As an approximation the values for rats obtained from another study were used. Table II shows that the computed albumin escapes into the tissue fluid. It can be concluded that the amount of protein escaping from the capillaries per unit tissue weight is similar in the liver and the kidney, while in the liver only about 17% of the escaping albumin is carried away by the lymph vessels. In the kidney the lymphatics transport almost all albumin filtered or diffusing into the tissue fluid. Albumin escape rate in the intestines is about one third of the renal or hepatic values and the lymph vessels transport again only about 12% of the albumin moving out from the blood capillaries.

The crural lymph vessels carry mostly skin lymph. Albumin escape calculated from the appearance of RISA in the lymph is about 10 times slower than in the liver and kidney. The comparison of albumin escape into the leg (skin?) with the lymphatic albumin transport calculated from the "missing" thoracic duct lymph is not made because it would be loaded with many unverifiable assumptions.

In the 4 hours of the experiment full equilibration of radioiodinated albumin with the unlabelled molecules is approached only in the liver lymph. In other regions the R_L/R_P is lower, and it decreases in the same order as the L/P albumin concentration ratio. This proves only the obvious: the greater the permeability of the capillaries, the higher will be the protein concentration in the lymph flowing from the organ. The analysis of the curves in Fig. 4 shows, however, that the rate of R_L/R_P equilibration decreases with time. In the course of the analysis the first slopes of the curves were considered. A non-linear correlation with a hyperbolic regression line was found between the first slopes and the L/P albumin concentration ratios, accordingly a linear correlation exists between the L/P ratios and the reciprocals of the slopes, i.e. of the equilibration times (T). The correlation coefficient of this

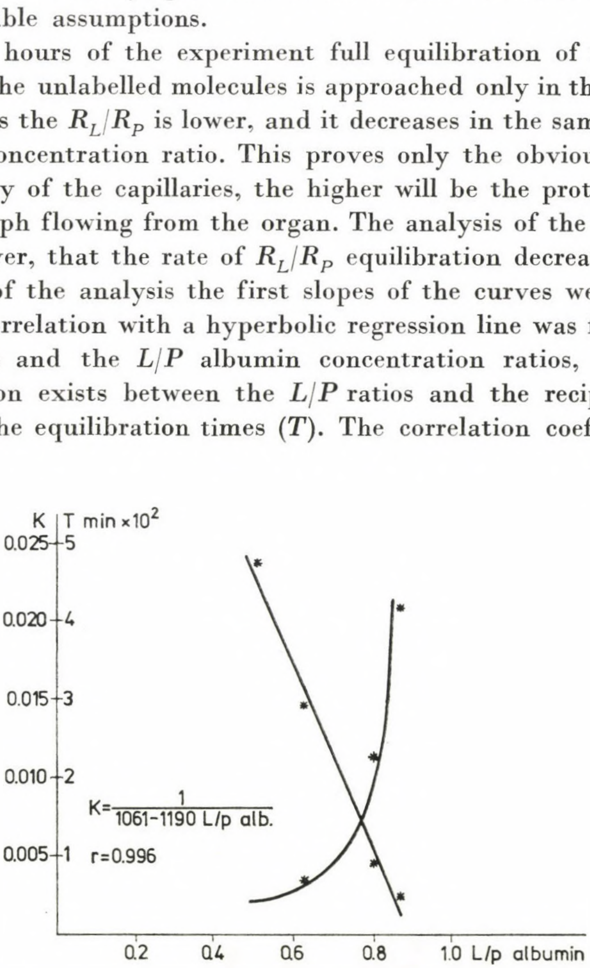

Fig. 5. Relationship between equilibration constants (K) obtained from the first slopes in Fig. 4 and the L/P albumin concentration ratios. The straight line shows the relationship between the equilibration times (T) and the albumin concentration ratios

relationship was extremely high ($r = 0.996$). The reciprocal of the rate constant K_L is equal to the time (T) during which equilibrium is attained if the equilibration would proceed at the initial rate. The extremely close correlation means that the protein concentration in lymph (and tissue fluid) is determined by the rate of protein movement from the capillaries and that the rate constant obtained from the first slope of the equilibration curves is a measure of the capillary permeability to albumin.

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PHARMACOLOGICAL EVALUATION AND MOLECULAR WEIGHT DETERMINATION OF ACETYLCHOLINESTERASE INHIBITING FRACTIONS IN THE VENOM OF *DENDROASPIS ANGUSTICEPS*

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The three true cholinesterase inactivating factors isolated from the venom of *Dendroaspis angusticeps*, when tested on isolated pharmacological test objects showed potentiation of contractile responses to endogenously released as well as exogenously applied acetylcholine. The fractions are distinct and separate polypeptides which were confirmed by the determination of their molecular weight and electrophoretic mobility.

In our previous study we have shown that the whole venom of *Dendroaspis angusticeps* possesses anticholinesterase activity. Column chromatography of the venom yielded three fractions with anticholinesterase activity with maximal inhibition against true cholinesterase.

In the present paper, attempts were made to characterise by molecular weight determination the three fractions which have been shown to be distinct compounds. Anticholinesterase activity of each fraction has been further tested on suitable pharmacological test preparations.

Materials and Methods

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis

Gel electrophoresis [5] was carried out in the presence of sodium dodecyl sulphate (SDS) using standard molecular weight markers (BDS). Sample loads of the crude venom or the fractions were 600 μg/tube. The electrophoresis was done at 8 mA per tube for 3 hours at 4 °C with bromophenol blue as tracker dye.

The gels were stained with Kenacid blue R and destained with 7.5% glacial acetic acid. Mobility was expressed as the ratio of $\frac{\text{the distance moved by the protein} \times 100}{\text{distancemoved by the tracker dye}}$

Electrophoretic mobilities were plotted against $\log_{10}$ of the molecular weight of the marker standards (BDH product No. 44223 2U). By comparing the mobility of the protein fraction with those of the markers, a close estimate of the molecular weight of the unknown protein was obtained.

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* The work reported here was undertaken in partial fulfilment of the requirements for the degree of PhD of University of Nairobi.

Pharmacological experiments

The isolated cholinesterase inhibiting fractions were tested on isolated rabbit jejunum, rectus abdominis muscle of the frog and coaxially stimulated guinea pig ileum [3].

The guinea pig ileum was mounted between two coaxially situated platinum electrodes. Parameters of electrical stimulation were: frequency, 0.2 Hz; pulse width, 2 msec; voltage, 90 V. The duration of stimulation was 2 min.

Results

SDS polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed on the whole venom as well as on fractions displaying inhibitory activity on mouse brain cholinesterase. These fractions obtained by column chromatography on CM–Sephadex C-25 were designated D_aIV (T_{24}), D_aVI (T_{24}) and T_{39}.

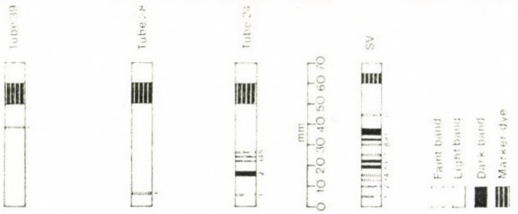
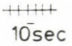


Fig. 1. SDS polyacrylamide gel electrophoresis of anticholinesterase fractions of the venom of *Dendroaspis angusticeps.* Bands are numbered sequentially from the point of application of the whole venom or fraction. Whole venom or fraction load was 600 μg per tube. Electrophoresis was carried out at 8 mA per tube for 3 hours



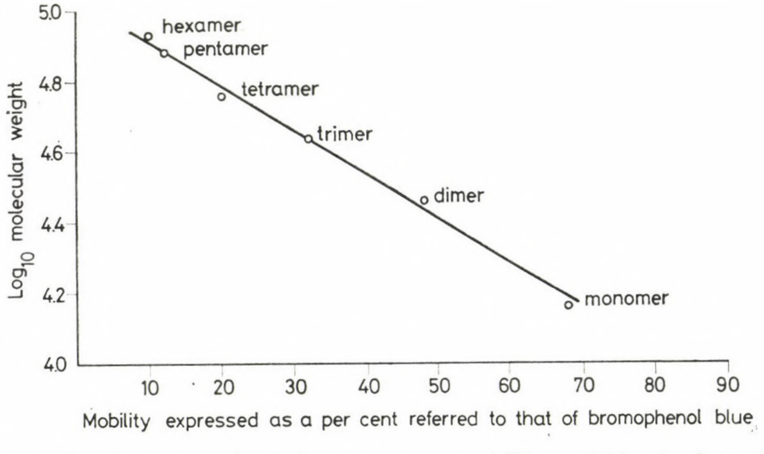


Fig. 2. Semi-log plot of molecular weight against percentage mobility on SDS gels of standard molecular weight markers (MW 14 300–71 500). Mobility expressed as percentage referred to that of bromophenol blue (tracker dye). Molecular weights of the markers: monomer 14 300; dimer 26 600; trimer 42 900; tetramer 57 200; pentamer 71 500; hexamer 85 800

Eleven bands were obtained after electrophoresis of the whole venom. Fraction D_aIV showed one major dark band while D_aVI and T_{39} produced one band each (Fig. 1).

Table I shows the molecular weight values of these proteins (from each fraction). These values were obtained from the standard curve in Fig. 2 plotted from the results obtained by electrophoresis of standard molecular weight markers.

Table I

Molecular weight of cholinesterase inhibiting fractions obtained by column chromatography of the crude venom of Dendroaspis angusticeps

Cholinesterase inhibiting fractions	Percentage mobility	Molecular weight
D_aIV		
Band 1	13	70 800
Band 2	25	50 100
Band 3	37	34 700
Band 4	40	30 900
Band 5	43	28 800
D_aVI	13	70 800
T_{39}	65	14 300

The fractions D_aIV, D_aVI and T_{39} obtained by column chromatography of the venom were tested on the above preparations. The period of incubation of each fraction with the tissue was 20 min.

Contractions of the rabbit jejunum were elicited by acetylcholine (0.01 μg/ml). The contractile response to acetylcholine was again tested after addition of the fractions D_aIV (4.8 μg/ml), D_aVI (1.5 μg/ml) and T_{39} (1.5 μg/ml). There was potentiation of the contractile reponse after addition of each of the fractions (Figs 3*A*, 4*A*, 5*A*).

Similarly, fractions D_aIV (12 μg/ml), D_aVI (0.8 μg/ml) and T_{39} (1.5 μg/ml) potentiated the response of coaxially stimulated guinea pig ileum (Figs 3*B*, 4*B*, 5*B*).

Contraction of the frog rectus abdominis muscle was elicited by acetylcholine (0.05 μg/ml). The contractile response to the same dose of acetylcholine was potentiated after addition of each of the fractions D_aIV (23 μg/ml), D_aVI (23 μg/ml) and T_{39} (20 μg/ml) to the organ bath (Figs 3*C*, 4*C*, 5*C*).

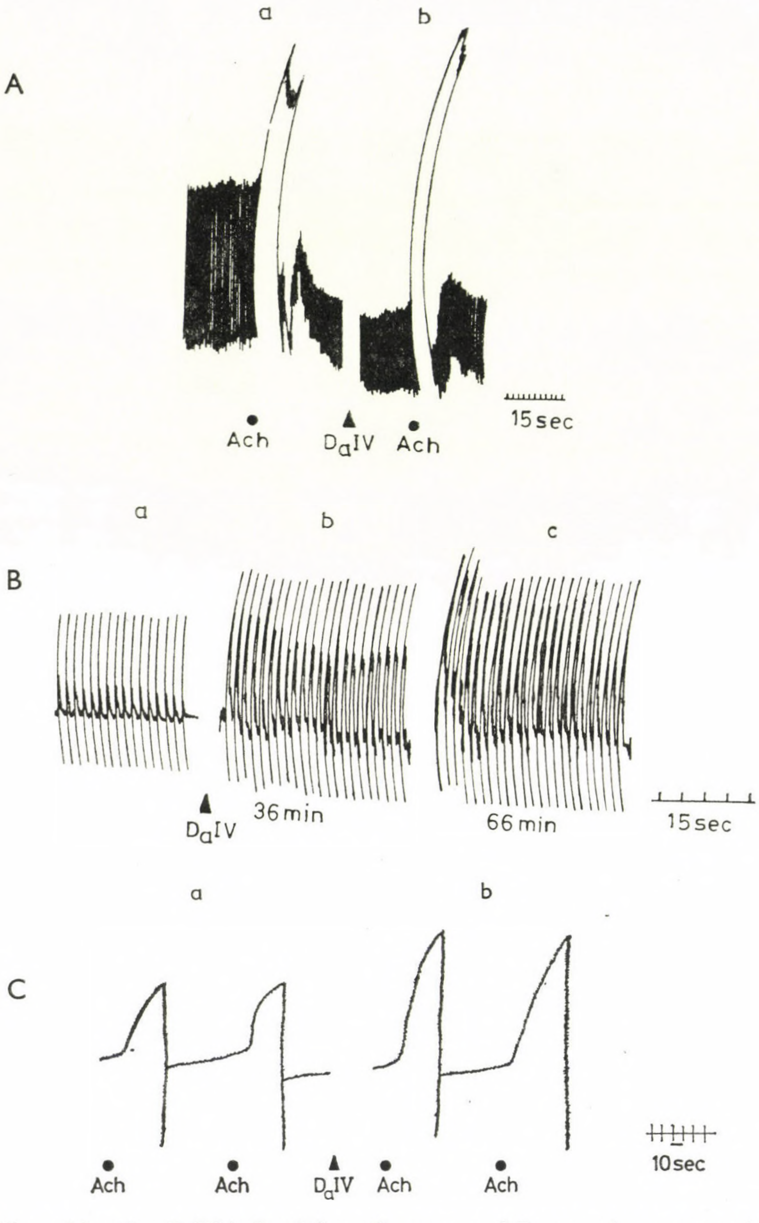


Fig. 3. Effect of fraction D_aIV isolated from the venom of *D. angusticeps* on coaxially stimulated guinea pig ileum, acetylcholine induced contractions of| rabbit jejenum and frog rectus abdominis muscle. *A*(*a*) Acetylcholine (0.01 μg/ml) induced contractions of rabbit jejunum. (*b*) Potentiation of contractile response to acetylcholine 20 min after addition of fraction D_aIV (4.8 μg/ml). Volume of bath, 10 ml. *B*(*a*) Contractions of coaxially stimulated guinea pig ileum (*b*, *c*) potentiation of contractile response after addition of D_aIV fraction (12 μg/ml). Volume of bath, 10 ml. Parameters of stimulation: frequency 0.2 Hz, pulse width 2 msec, voltage 90 V. Duration of stimulation 2 min. *C*(*a*) Acetylcholine (0.05 μg/ml) induced contractions of frog rectus abdominis muscle. (*b*) Potentiation of contractile response to acetylcholine 20 min after addition of fraction D_aIV (23 μg/ml). Volume of bath, 20 ml

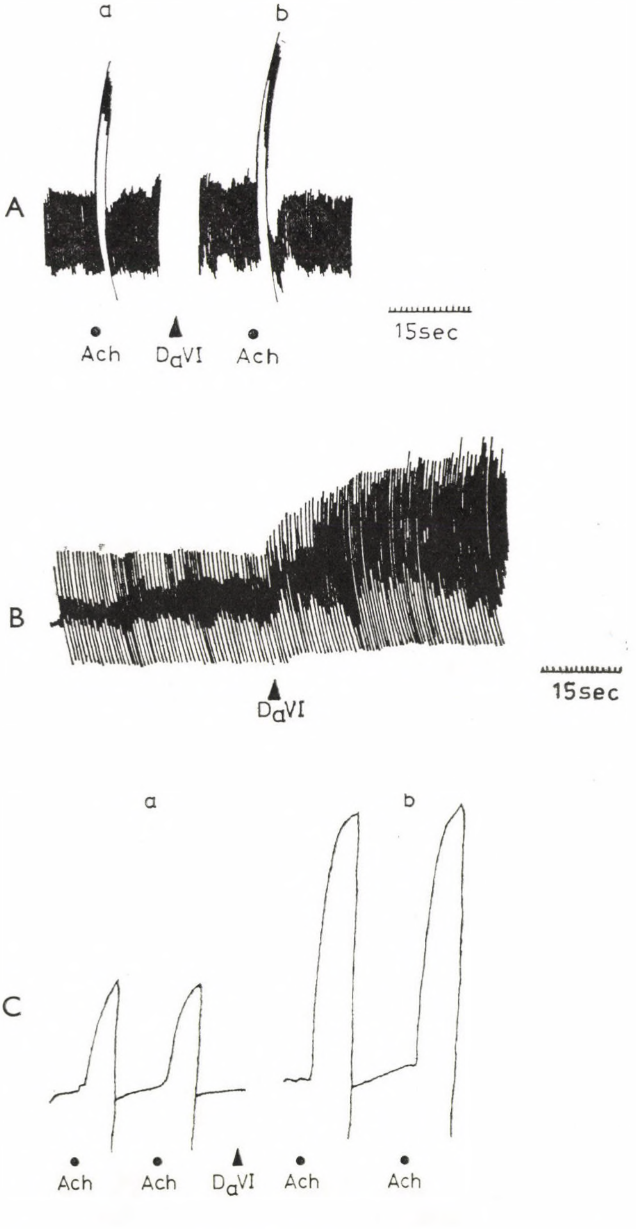


Fig. 4. Effect of fraction D_aVI isolated from the venom of *D. angusticeps* by column chromatography on the acetylcholine induced contractions of frog rectus abdominis muscle, isolated rabbit jejunum and coaxially stimulated guinea pig ileum. *A*(*a*) Acetylcholine (0.01 μg/ml) induced contractions of rabbit jejunum. (*b*) Potentiation of contractile response to acetylcholine 20 min after addition of fraction D_aVI (1.5 μg/ml). *B*(*a*) Contractions of coaxially stimulated guinea pig ileum. (*b*, *c*) Potentiation of contractile response after addition of fraction D_aVI (0.8 μg/ml). Parameters of stimulation same as in Fig. 3*B*. *C*(*a*) Acetylcholine (0.05 μg/ml) induced contractions of frog rectus abdominis muscle. (*b*) Potentiation of contractile response 20 min after addition of fraction D_aVI (23 μg/ml)

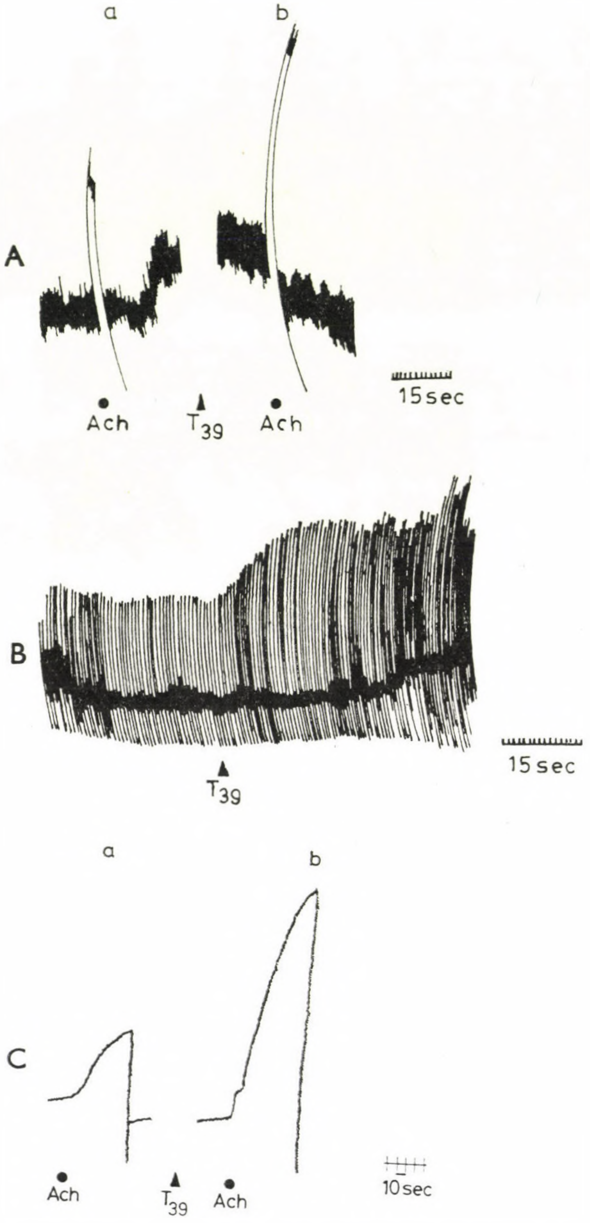


Fig. 5. **Effect of fraction T_{39} isolated by column chromatography from the crude** venom *(D. angusticeps)* **on acetylcholine induced contractions of frog rectus abdominis muscle, isolated rabbit jejunum and coaxially stimulated** guinea pig ileum. *A*(*a*) Acetylcholine (0.01 μg/ml) induced contractions of rabbit jejunum. (*b*) Potentiation of contractile response to acetylcholine 20 min after addition of fraction T_{39} (1.5 μg/ml). *B*(*a*) Contractions of coaxially stimulated guinea pig ileum. (*b*, *c*) Potentiation of the response after addition of fraction T_{39} (1.5 μg/ml). Parameters of stimulation the same as in Fig. 3*B*. *C*(*a*) Acetylcholine (0.05 μg/ml) induced contraction of frog rectus abdominis muscle. (*b*) Potentiation of contractile response 20 min after addition of fraction T_{39} (20 μg/ml)

Discussion

The study confirms the cholinesterase inhibiting activity of various fractions of the venom of *Dendroaspis angusticeps*. The fractions potentiated the contractile response of exogenously applied acetylcholine on the guinea pig ileum and the frog rectus abdominis muscle as well as the endogenously released acetylcholine in the coaxially stimulated guinea pig ileum. The potentiating effect of these fractions on the pharmacological preparations could be explained on the basis of inhibition of acetylcholinesterase enzyme.

An acetylcholinesterase inactivating factor was found in the venom of *Naja atra* [1]. It is now known that the inactivating factor corresponds to one of the cardiotoxins contained in the venom [2]. This has been confirmed in our earlier work where fraction T_{39} has been shown to be cardiotoxin [4].

These fractions are distinct and separate polypeptides with varying cholinesterase inhibiting activity, which has been confirmed after determination of their molecular weights. Of them, D_aIV had the highest molecular weight while T_{39} the lowest. Their percentage mobility was also different with T_{39} having greater mobility (65%) than D_aVI and D_aIV (Band 1) which possessed the least mobility (13%), being a heavier polypeptide.

Acknowledgement

We thank the Dean's Committee for the grant (670–072) which supported this work. We also gratefully acknowledge the photographic work of Mr. E. Njogu.

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IDENTIFICATION AND ISOLATION OF THREE ACETYLCHOLINESTERASE INACTIVATING FRACTIONS IN THE VENOM OF *DENDROASPIS ANGUSTICEPS*

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Three fractions were isolated from the venom of *Dendroaspis angusticeps* by column chromatography on CM–Sephadex C–25. All the three fractions were shown to possess acetylcholinesterase inhibiting activity. The toxicity of the fractions as tested on mice were variable. Although the toxic signs were identical, fraction D_aVI was highly lethal (LD$_{50}$ 1.9 μg/g) whereas fractions D_aIV and T_{39} were less lethal, the LD$_{50}$ being 3.6 μg/g and 4.1 μg/g respectively. The three fractions significantly inhibited true acetylcholinesterase to the extent of 91–95%.

In the course of our experiments on *Dendroaspis angusticeps* venom it was found that the whole venom reversed the blocking effects of d-tubocurarine on the isolated rat phrenic nerve diaphragm.

The present work was undertaken to establish whether *D. angusticeps* venom possesses anticholinesterase activity. Three cholinesterase inactivating factors have been isolated by column chromatography and their cholinesterase inhibiting property has been characterized by a biochemical method.

Materials and Methods

Column chromatography

The venom was fractionated by column chromatography on CM–Sephadex C-25 according to the method of Patel and Excell [9]. Samples of 100 mg of the venom dissolved in 0.005 M sodium phosphate buffer (pH 6.0) were applied to the equilibrated resin in a column of 30 × 2 cm. Elution was carried out at room temperature with a two-stage solium phosphate linear gradient buffer system. The flow rate was 30 ml per hour and 7.5 ml fractions were collected after passage through a UV analyzer (280 nm) connected to a potentiometric recorder (LKB system).

The protein content of each fraction was determined [5].

Cholinesterase inhibiting activity

True cholinesterase was obtained from the homogenate of mouse brain [6]. Whole brains removed from three freshly decapitated mice were homogenized in 0.25 M sucrose solution at 4 °C. After centrifugation at 4000 r.p.m. for 20 min, the solution was decanted from the cell debris.

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[*] This work reported here was undertaken in partial fulfilment of the requirements for the degree of PhD of University of Nairobi.

A 0.2% (v/v) brain supernatant was utilized for the experiments. Pseudocholinesterase was obtained from the plasma of adult rats after bleeding, a 2% (v/v) solution of plasma being used. The assessment of cholinesterase inhibiting activity of the venom was carried out by the method of ELLMAN et al. [2]. Enzyme activity was determined by adding 0.1 ml of the enzyme solution to the reaction flask containing 4.9 ml of sodium phosphate buffer (0.2 M: pH 8.0) of 5,5-dithiobis-2 nitrobenzoic acid (DTNB: 0.01 M). The hydrolysis was started by adding 0.1 ml of 0.05 M acetylthiocholine and snake venom to the reaction flask. Control experiments without the venom were performed on a flask containing only the enzyme. The absorbance at 412 nm was determined at intervals of 2 min up to 6 min.

Percentage inhibition by the venom was calculated from the residual cholinesterase activity. The cholinesterase activity in the control experiments was considered 100%.

Measurement of LD_{50}

The toxicity of each fraction was assayed in mice (National Health Laboratory strain: 17–23 g) by i.p. injection. The required concentration of the toxic dose was made up in normal saline and the volume of injection did not exceed 0.1–0.2 ml. The LD_{50} and slope function were calculated by the method of LITCHFIELD and WILCOXON [4].

Desiccated whole venom obtained from Baringo Snake Farm (Nakuru, Kenya) was kept in a referigerator at 4 °C prior to use.

Statistical analysis of the data and the level of significance was determined at 99% confidence limits. Values of P greater than 0.01 were considered not significant.

Results

Column chromatography

Column chromatography of the venom (100 mg) on CM–Sephadex C-25 using phosphate buffer for elution produced a profile with nine peaks as illustrated in Fig. 1. Protein recovered as estimated in all fractions was 84% of the total proteins present in 100 mg of the venom. Among the nine fractions isolated, fraction D_aIV (Tube 24) constituted 29% of the total proteins followed by fractions D_aVI (15%) and T_{39} (2%) corresponding to tubes 28 and 39, respectively. The LD_{50} and slope function values of these fractions (Table I)

Table I

LD_{50} and slope function of the crude venom and fractions of Dendroaspis angusticeps on mice

Fraction	LD_{50}(ip) in mice(μg/g)	Slope function	Potency ratio*
Crude venom	2.4(2.2–2.6)	—	1
D_aIV	6.3(5.2–7.5)	1.23(1.13–1.34)	0.4
D_aVI	1.9(1.3–2.8)	1.71(1.47–1.98)	1.3
T_{39}	4.1(3.2–5.2)	1.32(1.19–1.46)	0.6

* The potency ratio of crude venom in mice was considered 1.
Figures in parentheses denote 95% confidence limits.

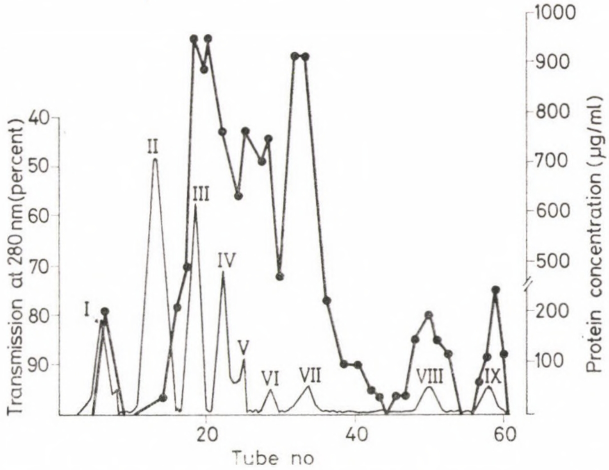

Fig. 1. Column chromatography of *Dendroaspis angusticeps* venom on CM–Sephadex C-25. Elution pattern of *D. angusticeps* venom (100 mg) on a CM Sephadex C-25 column (30 × 1.0 cm) using a two-stage sodium phosphate buffer system. Gradient: (*i*) 0.005 m (pH 6.0)–0.2 M (pH 7.3); (*ii*) 0.2 M (pH 7.3)–0.5 M (pH 8.0). Elution was carried out at room temperature. Shaded areas represent fractions D_aIV, D_aVI possessing anticholinesterase activity. Fraction T_{3a} showed zero absorbance but contained 100 µg/ml of protein (see text for explanation)

showed that fraction D_aVI exhibited the highest toxicity compared with the rest of the fractions. The toxic symptoms produced in mice by each fraction were almost identical. Flaccid paralysis and convulsions preceded death from respiratory failure within a few hours.

Cholinesterase inhibiting activity

Rat plasma, 0.1 ml in a total volume of 5.0 ml hydrolysed acetylthiocholine iodide (0.05 M) at the rate of 0.090 6 absorbance units per min at 412 nm. Ellman [2] showed that the extinction coefficient for a molar solution of the coloured anion formed in the reaction between released thiocholine and DTNB to be 1.36×10^{-2}. Thus the rate of hydrolysis of the substrate is given by the formula:

$$\text{Rate of hydrolysis} = \frac{\Delta A \times V}{1.36 \times 10^{-2}}\ \text{nmol/min},$$

where ΔA is the rate of change in absorbance units and V is the volume of the solution in ml. Thus the rate of hydrolysis of acetylthiocholine by 2% rat plasma is 33.3 nmol/min.

Similarly 0.2% of mouse brain homogenate hydrolysed acetylthiocholine (0.05 M) at the rate of 0.190 absorbance units per min. Thus the rate of hydrolysis by 0.2% mouse brain homogenate is 69.8 nmol/min.

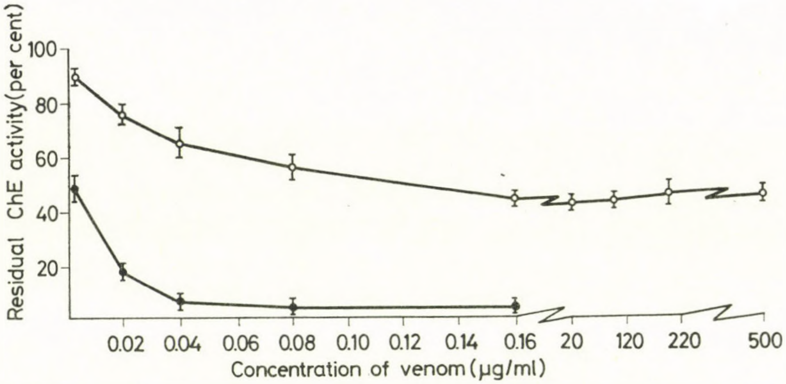

Fig. 2. **Effect of snake-venom on the *in vitro* hydrolysis of acetylthiocholine by rat plasma and mouse brain homogenate. Ordinate: residual cholinesterase activity (%). Abscissa: concentration of venom (μg/ml). △ —— △ hydrolysis of acetylthiocholine by 2% rat plasma (pseudocholinesterase); ▲ —— ▲ hydrolysis of 0.2 mouse brain homogenate (true cholinesterase). Each point represents mean of 18 estimations; horizontal bars denote ±SE**

Whole snake venom at different concentrations inhibited the hydrolysis of acetylthiocholine by rat plasma or mouse brain homogenate (Fig. 2).

Table II shows that the whole venom at a concentration of 0.16 μg/ml inhibited cholinesterase activity in rat plasma by 57% whereas cholinesterase activity in the mouse brain homogenate was inhibited to a maximum of 94%. The enzymic activity in rat plasma was not completely abolished even when high doses (500 μg/ml) of the venom were used. The three fractions exhibited maximal inhibition of true cholinesterase at concentrations as low as 0.04 μg/ml (Table III). The residual true cholinesterase activity of these fractions was significantly different ($p < 0.01$) from the controls. The control experiments were performed without addition of the venom or fraction, the buffer acting as control.

Table II

Inhibition of true and pseudocholinesterases (ChE) by whole venom of Dendroaspis angusticeps

	Rate of hydrolysis of acetylthiocholine* nmol/min + SE		Mean residual ChE activity* % + SE		Percent inhibition of total activity	
	True ChE	Pseudo ChE	True ChE	Pseudo ChE	True ChE	Pseudo ChE
Controls	69.8±5.1	33.3±2.0	100	100	—	—
Whole venom (0.16 μg/ml)	4.1±0.4**	14.2±0.7**	5.9±0.5	42.6±2.3	94	57

* Rate of hydrolysis of acetylthiocholine in the controls was taken as 100%.
** Values were significantly ($P < 0.01$) different from the controls.

Table III

Inhibition of true cholinesterase in the mouse brain homogenate after treatment with fractions isolated by column chromatography of the crude venom of Dendroaspis angusticeps on CM-Sephadex C-25

No. of estimations	Fraction (0.04 μg/ml)	Rate of hydrolysis of acetylthiocholine (nmol/min ±SE)	Mean residual true cholinesterase (%±SE)	Percent inhibition of total activity
18	Controls*	69.8±5.1	100	—
12	D_aIV	5.5±0.4	7.9±0.6	92
12	D_aVI	3.6±0.3	5.2±0.4	95
12	T_{39}	6.0±0.4	8.6±0.5	91

* Values were significantly different ($P < 0.01$) from the controls which were performed with the buffer only.

Discussion

The discrepancy between our results and those of BARRETT and HARVEY [1] could be attributed to the use of intact chick biventer cervicis muscle as their source of true acetylcholinesterase. It is generally known that in many species and particularly in the chick the enzyme activity decreases about 20-fold between hatching and adulthood [7, 8]. Besides the acetylcholinesterase enzymes found in avian tissues particularly those of the chick show intensitivity to specific inhibitors of true and pseudocholinesterase [3].

In the present study, whole venom inhibited only true cholinesterase. There was residual hydrolysing activity in the rat plasma and the percentage inhibition of total activity of the enzymes was 65% in the presence of a large amount (500 μg/ml) of the venom. Activity of the enzyme in mouse brain homogenate was considerably reduced and high percentage inhibition (94%) was obtained with very low concentration (0.16 μg/ml) of the venom. Ion exchange chromatography of the whole venom yielded three fractions which in very low doses were shown to exhibit maximal inhibition of acetylthiocholine by mouse brain homogenate.

Fraction T_{39} had near zero ultraviolet absorbance but a considerably high protein concentration. The fraction is probably deficient in aromatic amino acids (tyrosine, tryptophan and phenylalanine) and hence shows low absorbance [10].

Mouse brain homogenate [6] exhibits exclusively true cholinesterase activity and hydrolyzes acetylcholine at a higher rate than other choline esters. On the other hand, rat plasma [7] contains large amounts of propionylcholinesterase the properties which differ in various respects.

The significant difference in the LD_{50} of these fractions confirms that the fractions have a distinct toxicity of its own which is not dependent on other substances present in the venom.

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Pharmacologia

EFFECT OF CALCIUM CHLORIDE ON GROSS BEHAVIOURAL CHANGES PRODUCED BY CARBACHOL AND ESERINE IN CATS

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The effect of calcium chloride injected into the cerebral ventricles of group-housed unanaesthetized cats upon vocalization (rage, hissing and snarling), fighting (attack with paws and claws, defense with paws and claws and biting), mydriasis, tremor and clonic-tonic convulsions produced by carbacol and eserine injected similarly was investigated. Calcium chloride depressed or almost completely abolished the vocalization and fighting due to carbachol and eserine. On the other hand, mydriasis, tremor and clonic-tonic convulsions evoked by carbachol and eserine were not significantly changed by calcium chloride. It is apparent that calcium chloride can "dissociate" vocalization and fighting from autonomic and motor phenomena such as mydriasis, tremor and clonic-tonic convulsions caused by carbachol and eserine. Calcium chloride inhibited the vocalization and fighting produced by carbacol and eserine most probably by a nonspecific stabilizing action on central muscarinic cholinoceptive sites. These results further support the view that calcium ions in excess have an atropine-like action also in the central nervous system.

Cholonomimetics and anticholinesterases injected into the hypothalamus [10, 23, 24, 31] or into the cerebral ventricles [1, 2, 4, 5, 13, 14, 16, 20, 21] of cats are known to evoke emotional behaviour and aggression associated with autonomic and motor responses. In addition, it was found that these drugs injected into the cerebral ventricles of conscious cats elicited aggressive behavioural phenomena mainly by an action on central muscarinic cholinoceptive sites [1, 4, 5]. Similarities in the ganglionic action of calcium and atropine have already been pointed out [30]. Moreover, it was shown that the sensitivity of the longitudinal muscle of guinea pig ileum to muscarinic drugs depended on the concentration of calcium in the bathing medium [3, 7]. In the light of these observations it seemed worth to investigate whether calcium in excess possess also an atropine-like action on central muscarinic cholinoceptive sites.

Materials and Methods

Cats of both sexes weighing between 2 and 4 kg were used in the experiments with injections being made into the left lateral ventricle. A Collison cannula, described by **Feldberg** and **Sherwood** [12], was screwed aseptically into the skull through a hole which was drilled and taped 7 to 8 mm from the zero line and 4 to 5 mm from midline during pentobarbitone sodium (35–50 mg/kg i.p.) anaesthesia. The lower end of the cannula shaft was made of polythene tubing with a side opening 1 mm from its closed tip and positioned with the lumen towards the foramen of Monro. Dye studies post-mortem indicated that the injected material passes from the lateral ventricle into the third and fourth ventricles. Postoperatively, penicillin was administered intramuscularly and an interval of 5 days had been allowed to elapse before the cats were used for the experiments.

The substances injected into the cerebral ventricles were dissolved in sterile and pyrogen-free 0.9% sodium chloride. The solutions were injected by hand from a 1 ml syringe under aseptic conditions in a volume of 0.1 ml over a period of 15–20 seconds and washed with 0.1 ml of saline under the same conditions as the substances. All the tested animals, thereafter, were observed for a period of 4 hours and intermittently for 24 hours.

In this study vocalization, fighting, mydriasis, tremor and clonic-tonic convulsions were observed, measured and scored in a wire mesh cage measuring 110 × 130 × 150 cm. On the day of the experiment the cats were habituated for at least 1 hour before the intracerebroventricular injection of drugs. The duration of vocalization, fighting, mydriasis and tremor was measured and clonic-tonic convulsions were scored by two experienced observers. Both observers were blind with regard to the applied drugs. The correlation coefficient for these checks ranged consistently between 0.92 and 0.98.

Statistical analysis was performed for the duration of vocalization, fighting, mydriasis and tremor using analysis of variance, while for the appearance of clonic-tonic convulsions the chi-square test was used.

The applied compounds were: calcium chloride, carbachol chloride and eserine sulphate. The drug doses and concentrations refer to the salts.

Results

The fighting behaviour is best studied when several cats are kept together in the same cage [4, 5]. Singly-housed cats present only vocalization without fighting [4, 5]. Therefore in these experiments group-housed (4–6) cats were used.

In control experiments injections of 0.3 ml of 0.9% NaCl into the cerebral ventricles of 4 unanaesthetized cats did not produce any visible behavioural, autonomic or motor changes. However, seldom a transient miaowing was noted.

Carbachol, eserine and aggressive behaviour

The most impressive effects of intracerebroventricular carbachol (0.01–0.03 mg) and eserine (0.4–1 mg) are various emotional changes (restlessness, irritability, rage, fear and threat) and aggression (attack, defense and fighting).

In carbachol and eserine treated cats any sudden movement of the cat often triggered the attack, the aggression occurring even when the other cats remained motionless. The animals crouched, trembled, retracted their ears, jumped, hissed and snarled, and attacked each other with paws and claws. Intermittent periods of adynamia lasting from a few seconds to a few minutes occurred after enhanced motor activity. Finally, the cats savagely bit each other, although in the initial part of the attack the animals clearly used their

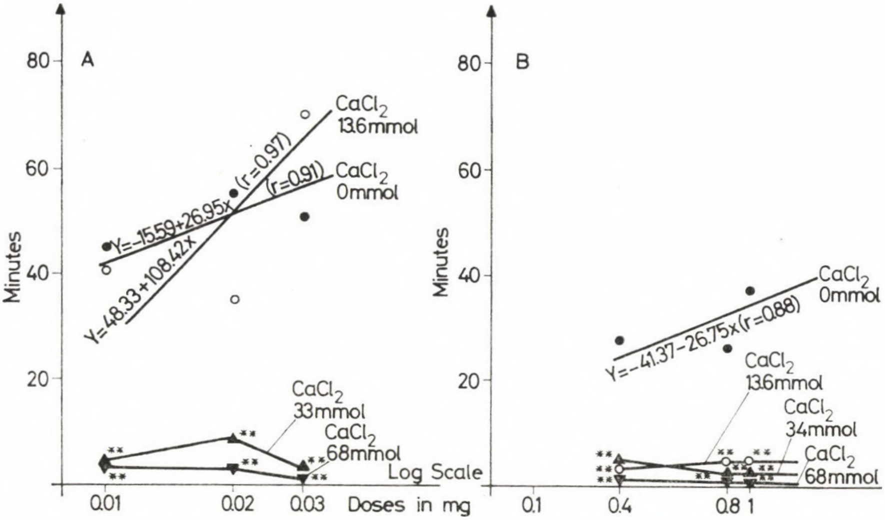

Fig. 1. The effect of calcium chloride on vocalization caused by carbachol (*A*) and eserine (*B*). In *A*, ● —— ● various single doses of carbachol, ○ —— ○ calcium chloride in concentration of 13.6 mmol plus carbachol, ▲ —— ▲ calcium chloride in concentration of 33 mmol plus carbachol and ▼ —— ▼ calcium chloride in concentration of 68 mmol plus carbachol were injected into the cerebral ventricles of unanaesthetized cats. In *B*, ● —— ● various single doses of eserine, ○ —— ○ calcium chloride in concentration of 13.6 mmol plus eserine, ▲ —— ▲ calcium chloride in concentration of 34 mmol plus eserine and ▼ —— ▼ calcium chloride in concentration of 68 mmol plus eserine were injected into the cerebral ventricles of unanaesthetized cats. Ordinate, duration of vocalization in minutes. Calcium chloride was injected 15 to 20 minutes prior to intracerebroventricular carbachol (*A*) or eserine (*B*). Each symbol represents the mean of four experiments. Each cat was used only once for the experiment. $^{**}P < 0.01$

claws. Vocalization (rage, hissing and snarling) and fighting (attack with paws and claws, defense with paws and claws and biting) were the main characteristics of aggressive behaviour. As shown in Figs 1 and 2, vocalization and fighting were dose-dependent and long lasting.

Carbachol, eserine and autonomic and motor effects

The aggressive behaviour produced by carbachol (0.01–0.03 mg) and eserine (0.4–1 mg) injected into the cerebral ventricles of unanaesthetized cats was associated with autonomic (mydriasis, salivation, piloerection, dyspnoea, defecation and urination) and motor (scartching, circling, tremor, rigidity and weakness with adynamia) phenomena. Of these symptoms mydriasis and tremor were the most characteristic ones. However, mydriasis (Fig. 3) and tremor (Fig. 4) produced by interacerebroventricular carbachol and eserine were less dose-dependent while lasting longer than the vocalization and fighting produced by these drugs.

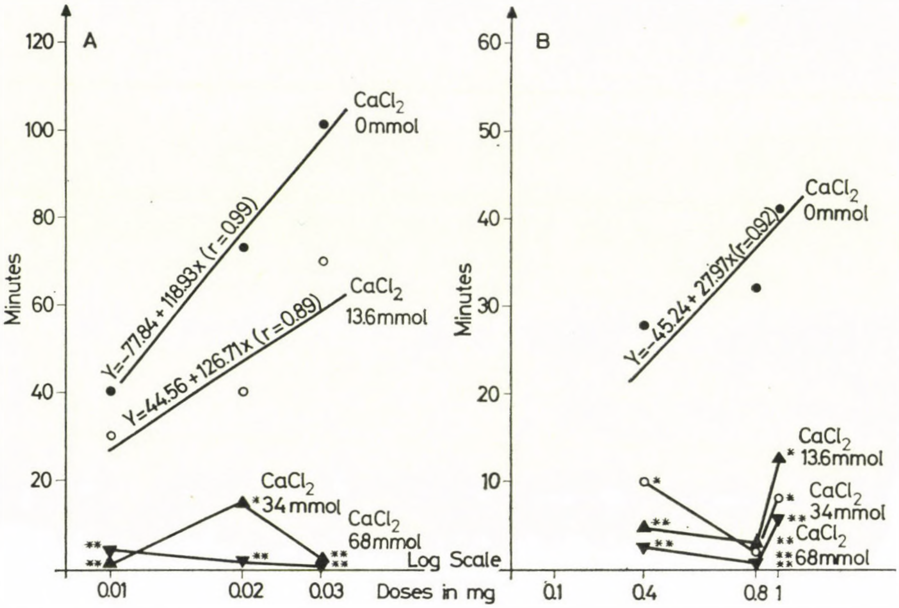

Fig. 2. The effect of calcium chloride on fighting caused by carbachol (*A*) and eserine (*B*). In *A*, ● —— ● various single doses of carbachol, ○ —— ○ calcium chloride in concentration of 13.6 mmol plus carbachol, ▲ —— ▲ calcium chloride in concentration of 34 mmol plus carbachol and ▼ —— ▼ calcium chloride in concentration of 68 mmol plus carbachol were injected into the cerebral ventricles of unanaesthetized cats. In *B*, ● —— ● various single doses of eserine, ○ —— ○ calcium chloride in concentration of 13.6 mmol plus eserine, ▲ —— ▲ calcium chloride in concentration of 34 mmol plus eserine and ▼ —— ▼ calcium chloride in concentration of 68 mmol plus eserine were injected into the cerebral ventricles of unanaesthetized cats. Ordinate, duration of fighting in minutes. Calcium chloride was injected 15 to 20 minutes prior to intracerebroventricular carbachol (*A*) or eserine (*B*). Each symbol represents the mean of four experiments. Each cat was used only once for the experiment.
$^{*}P < 0.05$, $^{**}P < 0.01$

Carbachol only in doses of 0.03 mg and eserine in doses of 0.8 and 1 mg injected into the cerebral ventricles of unanaesthetized cats evoked clonic-tonic convulsions (Fig. 5).

Effect of calcium chloride on vocalization and fighting produced by carbachol and eserine

In this series of experiments calcium chloride (13.6–68 mmol) was injected 15 to 20 minutes before carbacol (0.01–0.03 mg) and eserine (0.4–1 mg) into the cerebral ventricles of unanaesthetized cats. The vocalization as well as the fighting response to carbachol and eserine were inhibited by calcium chloride (Figs 1 and 2). Calcium chloride in low concentrations had no effect on the vocalization and fighting produced by carbachol ($P > 0.05$), while depressing the vocalization ($P < 0.01$) as well as fighting ($P < 0.05$) evoked

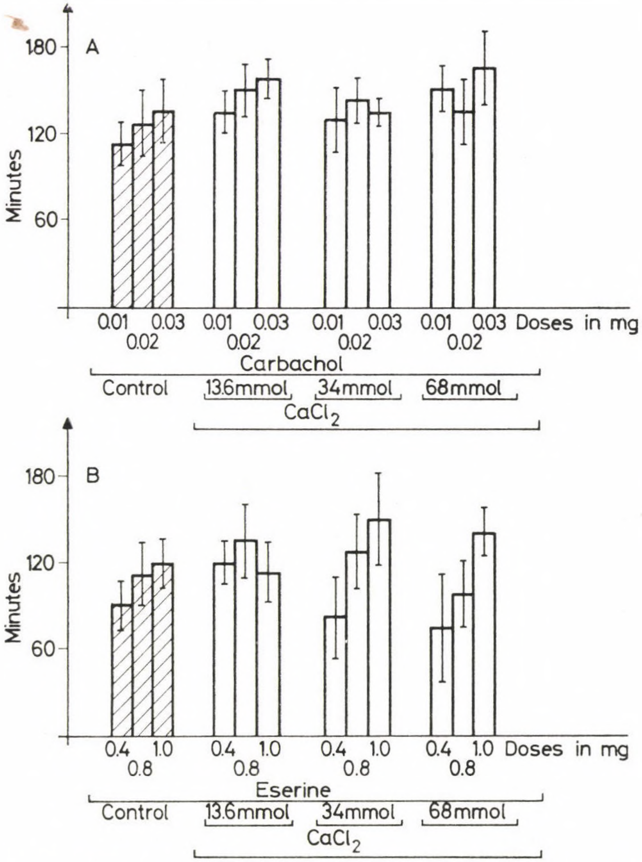

Fig. 3. The effect of calcium chloride on mydriasis caused by carbachol (*A*) and eserine (*B*). Ordinate, duration of mydriasis in minutes. The first three columns (hatched) in *A* and *B* represent the control experiments. Calcium chloride was injected into the cerebral ventricles of unanaesthetized cats 15 to 20 minutes prior to intracerebroventricular carbachol (*A*) or eserine (*B*). Each column represents the mean ±S.E.M. of four experiments. Each cat was used only once for the experiment

by eserine. By raising the concentration of calcium chloride to 68 mmol the vocalization and the fighting caused by carbachol and eserine were almost completely abolished ($P < 0.01$).

Effect of calcium chloride on mydriasis, tremor and convulsions caused by carbachol and eserine

Mydriasis (Fig. 3) tremor (Fig. 4) and clonic-tonic convulsions (Fig. 5) produced by intracerebroventricular carbachol (0.01–0.03 mg) and eserine (0.4–1 mg) were not significantly ($P > 0.05$) altered when calcium chloride (13.6–68 mmol) was injected into the cerebral ventricles of unanaesthetized cats 15 to 20 minutes prior to the administration of these drugs.

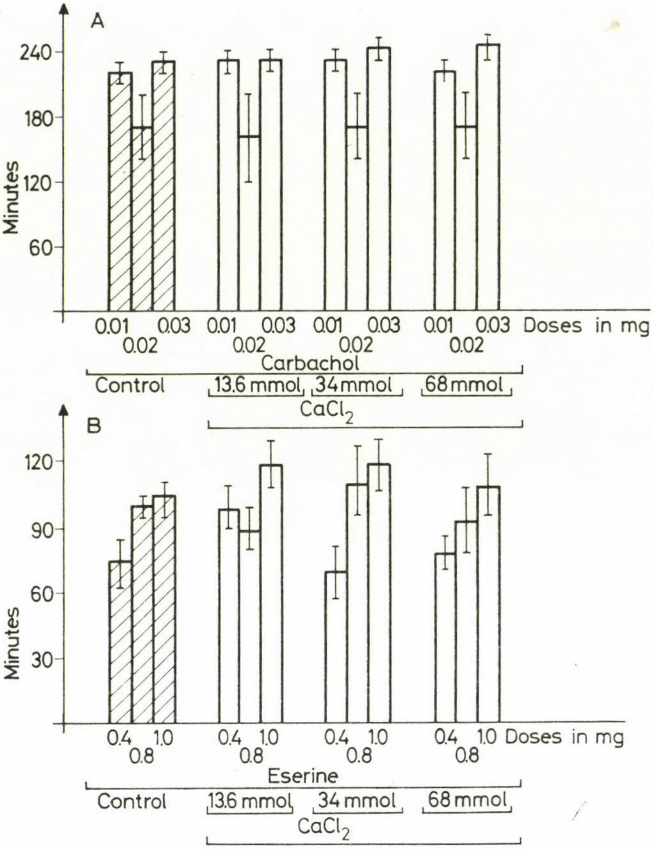

Fig. 4. **The effect of calcium chloride on tremor caused by carbachol (*A*) and eserine (*B*). Ordinate, duration of tremor in minutes. The first three columns (hatched) in *A* and *B* represent the control experiments. Calcium chloride was injected into the cerebral ventricles of unanaesthetized cats 15 to 20 minutes prior to intracerebroventricular carbachol (*A*) or eserine (*B*). Each column represents the mean ±S.E.M. of four experiments. Each cat was used only once for the experiment**

Discussion

The effect of calcium ions on the nervous system are complex and numerous [28]. The most important of these effects are the stabilization of electrogenic membranes and the participation in the neurotransmitter release [30]. The action of calcium chloride in the present experiments is, therefore, best to analyze considering transmitter release and postsynaptic membrane functions.

KUNO and RUDOMIN [19] reported that an increase in the calcium level had no significant effect on the amount of acetylcholine release, measured in the effluent from the perfused lumbosacral cord. Furthermore, BJEGOVIĆ,

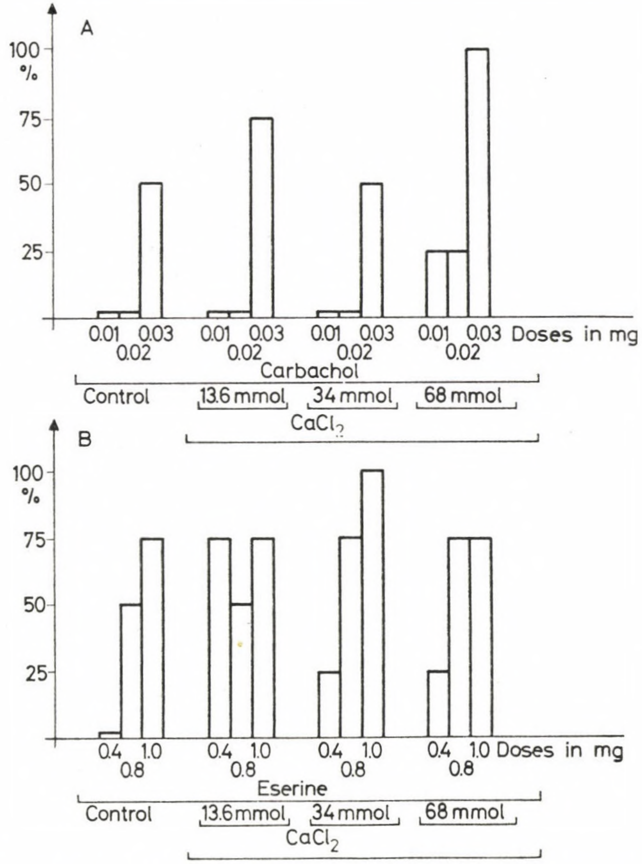

Fig. 5. The effect of calcium chloride on convulsions caused by carbachol (*A*) and eserine (*B*). Ordinate, percentage of cats showing convulsions. The first three columns in *A* and *B* represent the control experiments. Calcium chloride was injected into the cerebral ventricles of unanaesthetized cats 15 to 20 minutes prior to intracerebroventricular carbachol (*A*) and eserine (*B*). Each column represents the mean of four experiments. Each cat was used only once for the experiment

RANDIĆ and PADJEN [6] obtained variable results with solutions containing twice the normal concentrations of calcium chloride on the acetylcholine release from the stimulated and unstimulated cortex of the cat. In the present experiments calcium chloride in excess inhibited vocalization and fighting, while it had no significant effect on mydriasis, tremor and clonic-tonic convulsions produced by carbachol and eserine. If calcium chloride acted through increased release of acetylcholine, vocalization, fighting, mydriasis, tremor and clonic-tonic convulsions evoked by carbachol and eserine would have been enhanced. Alternatively, diminished release of acetylcholine due to excess calcium could contribute to the inhibitory effect of the ion. Since the predominant site of action of carbachol and acetylcholine is the postsynaptic membrane it is hard to believe that the inhibitory effect of calcium chloride in excess was due to the insufficient release of acetylcholine.

The predominantly postsynaptic site of action of calcium in the central nervous system has already been pointed out [17, 18, 29]. In addition, an increase in the amount of calcium in the cerebrospinal fluid depresses the autonomic reflexes [21] and produces a sleep-like state in freely moving cats [2, 15]. An atropine-like action of calcium in the sympathetic ganglia has also been established [30]. Moreover, it was shown that the sensitivity of the contractile response in the longitudinal muscle of guinea-pig ileum to muscarinic drugs was reduced when the bathing fluid contained increased amount of calcium [3, 7]. Similarly in the present experiments when the concentration of calcium chloride in the cerebrospinal fluid was elevated the carbachol- and eserine-induced vocalization and fighting responses were depressed or almost completely blocked. On the other hand, the increase of the calcium concentration in the cerebrospinal fluid failed to affect significantly mydriasis, tremor and clonic-tonic convulsions. Of equal interest is that the elevation of magnesium in the cerebrospinal fluid in unanaesthetized cats depressed or abolished the vocalization and the fighting evoked by intracerebroventricular injections of carbachol and eserine [25]. However, carbachol- and eserine--induced tremor was not appreciably changed [26]. By contrast, atropine blocked the tremor caused by carbachol injected into the cerebral ventricles and into the caudate nucleus of the cat [1, 8]. In this context it should be mentioned that no change was found in the tremorigenic action of tremorine after simultaneous intracaudate injections of atropine and tremorine [9]. An interesting finding is that intracerebroventricular magnesium blocked the convulsions caused by eserine, but had no effect on convulsions caused by carbachol [25, 26].

The antagonism by calcium chloride of vocalization and fighting produced by carbachol and eserine appears to be related to an action on the central atropine sensitive cholinoceptive sites. As reported previously atropine blocked the vocalization and fighting responses evoked by carbachol and eserine [27]. However, atropine in suitable doses blocked the convulsions, but not the vocalization and the fighting caused by carbachol and eserine [27]. In high doses atropine abolished vocalization, fighting as well as the clonic-tonic convulsions produced by carbachol [1]. In the present experiments calcium depressed or abolished the vocalization and the fighting responses, but not the convulsions elicited by carbachol and eserine. Similarly calcium in excess has been shown not to significantly change the sensitivity of the end-plate region of striated muscle to applied acetylcholine [11]. One of the possible explanation is that pharmacologically heterogenous cholinoceptive sites are present in the central nervous system and that only some of these sites are sensitive to the blocking action of calcium. The finding that calcium inhibited the cholinoceptive sites in the central nervous system, which are also inhibited by atropine, indicates that these sites are probably sensitive to the inhibitory action of nonspecific neuronal stabilizers.

Acknowledgement

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Recensiones

A. H. Adrian, E. Helreich, H. Holzer, R. Jung, O. Krayer, R. J. Linden, F. Lynen, P. A. Miescher, J. Piiper, H. Rasmussen, A. E. Renold, U. Trendelenburg, K. Ulrich, W. Vogt, A. Weber (eds)

Reviews of Physiology, Biochemistry and Pharmacology

Vol. 89. Springer Verlag, Berlin, Heidelberg, New York 1981. 254 p., 39 figures. Price US $ 43.80

One could say that each review published in this series belongs to the standard texts within the field. As in the earlier ones, in the present volume, too, the topics are of current interest and they offer valuable information for both the specialist and for those in teaching who wish to become acquainted with recent aspects of old problems.

There are three reviews in the present volume. The first by Ph. D. Snashall and J. M. Hughes deals with lung water balance, a subject important in clinical physiology. It gives a sound theoretical introduction which includes pressure relationships, and one finds a critical evaluation of the techniques available for measurement of lung water. The next chapter by D. D. Bikle, R. L. Morrissey, D. T. Zolock and H. Ramussen is a most needed review on the intestinal response to vitamin D. It summarizes the recent data in this rapidly expanding subject and the reader is told that apart from the accepted effect of vitamin D derivatives on the appearance of calcium-binding proteins in the intestinal epithelium there are some effects of the vitamin-D hormone independent of de novo mRNA synthesis. The chapter ends with a novel model of vitamin D action on the gut and it includes an impresive list of 476 references. The third review by P. D. di Pramero discusses the energetics of muscular exercise. It begins with a historical introduction and after this there is a rigorous physical treatment of efficiency. The greatest part of the paper however, deals with the whole-animal and human approach including oxygen debt. The list of references after each review includes full titles of the quoted papers.

The book begins with the obituitary of Walter Wildbrandt, a pioneer in biological membrane transport, written by E. Heinz, giving for future generations a felicitous picture of this great man of science.

A. Fonyó

E. Altenähr, W. Böcker, G. Dohm, W. Gusek, Ph. U. Heitz, G. Klöppel. H. Lietz, H. Mitschke, W. Saeger, H. J. Schäfer, J.-J. Staub, H. Steiner (eds)

Pathologie der endokrinen Organe

Springer Verlag Berlin, Heidelberg, New York 1981. Two volumes, 1309 p., 669 figures. Price DM 780 approx. US $ 409.50

The two volumes are published in the series "Spezielle pathologische Anatomie" (eds: W. Doerr and G. Seifert). This comprehensive handbook was written by twelve authors, all outstanding pathologists in the Federal Republic of Germany and Switzerland. The two main features of this handbook are the functional view of endocrine disorders and the presentation of data obtained with up-to-date morphological methods (electronmicroscopy, immunohistology, cytochemistry). Emphasis is laid on human pathology. Experimental data are quoted only exceptionally for the explanation of complicated relationships between various organs or regulatory processes.

The first eight chapters recall the traditional classification of endocrinology: pituitary gland, pineal body, thyroid gland, parathyroid gland, endocrine pancreas and diabetes mellitus, adrenal cortex, adrenal medulla, and vegetative ganglia. The 9th chapter deals with the physiology and pathology of diffuse endocrine cells, i.e. hormone production by non-endocrine organs. The next chapter is an excellent survey of pluriglandular endocrine disturbances with special emphasis on autoimmune diseases. The last chapter deals with paraneoplastic endocrine syndromes. Each chapter begins with an anatomical, embryological and histological introduction. This is followed by a concise description of present knowledge on hormone synthesis, the mode of hormone action, and the regulation of hormone secretion by the gland in question. The latest discoveries of endocrinology including e.g. the molecular anatomy of peptide hormones, may be found in these subchapters. In this way the book is a very useful handbook of physiology for pathologists. The subsequent subchapters deal with pathological disorders according to conventional classifications (developmental disorders, circulatory disorders, metabolic diseases, hyperplasia, tumours etc.).

The handbook is illustrated with excellent photomicrograms.

A. SPÄT

A. AZZI, U. BRODBECK, P. ZAHLER (eds)

Membrane Proteins. A Laboratory Manual

Springer Verlag, Berlin, Heidelberg, New York 1981. 256 p., 76 figures. Price US $ 22.30

This small paperback volume contains the protocols of the FEBS-SKMB course on membrane proteins organized in Bern in 1980. The Swiss courses have gained international reputation in the last years and it is a valuable contribution to international research activity that techniques employed have become available in booklet format also for those who could not participate in person. The single chapters are written by scientists of international reputation, all of which being familiar with the method described.

The first part deals with analytical techniques including two-dimensional electrophoresis, quantitative detergent-electrophoresis, two-dimensional thin-layer chomatography and the combination of gas liquid chromatography and thin-layer chromatograpy as applied for the study of membrane components, proteins as well as lipids. In the second part methods of protein isolation are described with particular emphasis on affinity chromatography. Reconstitution techniques for various transport systems are discussed in the third part. Some of them give minute details which are essential for successful reconstitution but are usually omitted from other publications for lack of space. This section includes experiments with the pure acetylcholine receptor, the mitochondrial ADT/ATP carrier, the purple membrane and the anion transporter protein of the red blood cell membrane. Modification techniques are given in the fourth part, including crosslinking. Various other techniques for the investigation of membranes are described in the next two sections, including spin-labelling, fluorescence spectroscopy, potentiometric dyes and circular dichroism measurements. Essential references are given after each method.

The reviewer considers this book not only useful but even indispensable in laboratories where graduate students and post-doctoral fellows do membrane research. Many of the protocols may even be of value to senior staff members who finished their studies at a time when many of these methods had not yet been known.

A. FONYÓ

I. J. CHOPRA

Triiodothyronines in Health and Disease

Monographs in Endocrinology. Vol. 18. (Ed.: GROSS, F.). Springer Verlag, Berlin, Heidelberg
New York 1981. 145 p., 76 figures and 18 tables

The new analytical techniques, mainly the specific radioimmunoassays of the 1970s revealed several iodothyronines in human biological fluids, including reverse triidothyronine (rT_3), diidothyronines, acetates of thyroxin (T_4 and T_3 etc.). This monograph gives an overview of thyroid physiology and biochemistry, with special emphasis on the production, biological effects and analysis of triiodothyronines. Special attention is paid to the following problems: 1. Is T_4 a prohormone of T_3 or is it also a hormone? 2. Is rT_3 a biologically effective product? 3. What factors control the monodeiodination of T_4 to T_3 and rT_3? 4. What factors are responsible for the changes in the ratio of the concentration of T_3 and rT_3 in various physiological and pathological conditions? 5. What is the potency of various triiodothyronines in suppressing TSH?

The monograph contains nine chapters as follows: Description and history; Transport of iodothyronines; Molecular structure and biological function of triiodothyronine (by V. Cody); Concentration of T_3 and rT_3 in serum and other biological fluids; Kinetics of peripheral metabolism and production rates of T_3s; Sources of T_3s; Control of production of T_3s from T_4; Routes of T_3s metabolism; Biological effects of iodothyronines.

The monograph contains about 500 references to studies conducted between 1969 and 1978. It provides an excellent introduction into modern thyroid physilogy for physiologists and clinicians.

A. Spät

Robert M. Dowben and Jerry W. Shay (eds)

Cell and Muscle Motility

Plenum Press, New York and London 1981. XIV + 400 p., 115 figures and 7 tables. Volume I.

This is the first member of a new multivolume series envisaged to present recent advances in the study of various aspects of the motile systems. The book contains 10 chapters written by outstanding experts of the field.

Chapter 1: Contractile function as a determinant of muscle growth by Radovan Zak. Chapter 2: Studies of sarcomere length by optical diffraction by Roger McCarter. Chapter 3: The sarcoplasmic reticulum of skeletal and cardiac muscle by Giuseppe Inesi. Chapter 4: Myosin phosphorylation: A biochemical mechanism for regulating contractility by Roger Cooke and James T. Stull. Chapter 5: Fine-structural and related aspects of nonmuscle-cell motility by Ian K. Buckley. Chapter 6: The role of intermediate (10-nm) filaments in the development and integration of the myofibrillar contractile apparatus in the embryonic mammalian heart by John W. Fuseler, Jerry W. Shay, and Howard Feit. Chapter 7: Creatine kinase and intermediate filaments in cultured mammalian cells, by Barry S. Eckert, Stephen J. Koons, and C. Richard Zobel. Chapter 8: Polymorphic assemblies of tubulin by Paul R. Burton. Chapter 9: Action structure in fibroblasts: Its possible role in transformation and tumorigenesis by Patricia F. Maness. Chapter 10: Nuclear magnetic resonance studies of muscle constituents in living tissue by C. Tyler Burt.

The book supplies important of information for those interested in the vast field of cell and muscle motility. The book can be recommended to all researchers and workers in cell biology, biochemistry, physiology, molecular biology, biophysics and microbiology.

E. Varga

H. L. König, A. P. Krueger, S. Lang and W. Sönning

Biologic Effects of Environmental Electromagnetism

Springer Verlag, New York 1981. 332 p., 1942 illustrations. Price US $ 75.70

This volume is an important member of the series "Topics in Environmental Physiology and Medicine" edited by K. E. Schaefer.

The book covers nearly all the diverse topics related to the biological effects of natural as well as artificial environmental electromagnetic fields, electric and magnetic fields, air ions and other phenomena of atmospheric electricity, being the first comprehensive treatment of these subject. It even deals with parapsychological observations, heliobiology etc., luckily for the volume's reputation only in the Appendix. Perhaps some other chapters like

"Project seafarer" and "The divining road phenomenon" would also have their place in the Appendix rather than in the neighbourhood of such well-developed topics as e.g. "The electromagnetic environment", "Electrophysical forces of natural origin", "Biological activity of the material accessible and useful to a wide array of readers including public health officials and researchers, physicians, biologists, engineers, veterinarians, city planners and architects, environmentalists, meteorologists, and all specialists involved in the production and utilization of electric and electromagnetic energies. The basic statements and conclusions of the book should became an integrative part of our general culture.

E. Monos

Peter, J. Morgane and Jaak Panksepp (eds)

Behavioural Studies of the Hypothalamus

Handbook of the Hypothalamus. Vol. 3. Part A.
Marcel Dekker, New York, Basel 1980. 499 p. Price SFr. 195,00

This part of the Handbook of the Hypothalamus deals with the role of the hypothalamus in regulating body temperature, water and energy balance, feeding and drinking behaviour and alertness. Chapter 1 by J. A. Boulant gives an excellent, well illustrated, thorough survey of the neurophysiological basis of the hypothalamic control of thermoregulation. In Chapter 2 neurochemical mechanisms of this control are summarized by R. D. Myers in a very critical and excellent way. The two chapters are well coordinated. Chapter 3 by T. L. Powley and his coworkers is concerned with the role of the hypothalamus in energy homeostasis. In this well written chapter disturbances produced by ventromedial and by lateral hypothalamic interventions are reviewed. Chapter 4 by S. F. Leibowitz discusses noradrenergic, adrenergic, dopaminergic, serotonergic, cholinergic, histaminergic, GABA-ergic and peptidergic systems in relation to the control of feeding and drinking behaviour, as well as water-electrolyte excretion. The complexity of the control system and the integration of multiple neurotransmitter systems are emphasized. Description of the anatomy of the various neurotransmitter systems does not seem to be necessary in this chapter, because it has already been dealt with in Volume 1. The last chapter by E. T. Rolls deals with the activity of hypothalamic and related neurones in alert animals.

All research workers and clinicians interested in the homeostatic systems will find this volume an extremely useful, stimulating and a very important source of references.

B. Halász

INDEX

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PHARMACOLOGIA

RECENSIONES

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ACTA PHYSIOLOGICA

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РЕЗЮМЕ

НЕЙРОТОКСИКОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ НОВОГО ВЕНГЕРСКОГО ПЕСТИЦИДА ТОКСУРАЗИНА

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Авторы выполнили исследование общих и нейротоксикологических свойств нового тотального пестицида токсуразина. Препарат вводился крысам перорально в течение трех месяцев, о количестве 1/80, 1/40 и 1/20 части значения ЛД$_{50}$. У животных наблюдалось замедление прибавления в весе. Отмечалась умеренная анемия. Препарат оказывал слабое повреждающее действие на печень. Результаты исследования функции почек и анализа мочи были отрицательными.

С увеличением числа опытов прогрессировало дозозависимое снижение способности к обучению, как показали результаты 42-дневных опытов в лабиринте. После исследований, выполненных в покое или при мигающем с разной частотой свете, в конце кормления понизилась электрическая активность как в комплексной деятельности ЭЭГ, так и в отдельных ее участках.

Электронейромиографические исследования не выявили повреждающего действия пестицида на периферическую нервную систему.

Определенное методом «*hot plate*» латентное время реакции несколько удлинилось. Результаты показывают, что исследуемый препарат можно применять только на не обработанных сельскохозяйственных участках.

РОЛЬ ИММУНОГЛОБУЛИНА-СЕКРЕТАГОГА В СЕКРЕЦИИ ЖЕЛУДКОМ СОЛЯНОЙ КИСЛОТЫ

Ш. ДОБИ и Б. ЛЕНКЕИ

Результаты приводимых в статье экспериментов позволяют сделать вывод, что, при язве 12-перстной кишки, в значительной части случаев, за избыточную секрецию соляной кислоты является один глобулинсекретагог из группы IgG, который связывается с периетальными клетками таким образом, что образует с ними не комплекс антиген-антитело а присоединяясь к H_2 клеточным рецепторам, стимулирует отделение соляной кислоты.

ТЕРМИЧЕСКОЕ И ХИМИЧЕСКОЕ РАЗДРАЖЕНИЕ ТЕПЛОВЫХ РЕЦЕПТОРОВ ГИПОТАЛАМУСА: ВЛИЯНИЕ НА ЭЭГ

Г. БЕНЕДЕК, Ф. ОБАЛ МЛ., З. ЛЕЛКЕШ и Ф. ОБАЛ

В опытах на иммобилизированных крысах изучали действие термического и химического (микроинъекция капсаицина) раздражений преоптической области, среднего гипоталамического отдела и заднего гипоталамуса. Как местное согревание, так и введене капсаицина вызывали на кривой ЭЭГ изменения, напоминающие таковые во время сна (веретена и медленные волны). Наиболее эффективным с точки зрения появления веретен было раздражение заднего гипоталамуса, раздражение же средней части гипоталамуса наименее эффективным. Поскольку капсаицин считают специфическим возбудителем тепловых сенсорных образований гипоталамуса, то результаты настояших опытов указывают, что влияние тепла на ЭЭГ и, вероятно, на наступление сна тоже передается через центральные терморецепторы, а не является следствием не-специфического активирования гипногенных механизмов базального переднего мозга.

РОЛЬ РАСПАДА ФЕТАЛЬНОГО ГЕМОГЛОБИНА В ЭНЕРГЕТИЧЕСКОМ ОБМЕНЕ У НЕДОНОШЕННЫХ ДЕТЕЙ, РОДИВШИХСЯ С ОЧЕНЬ МАЛЫМ ВЕСОМ

Я. БОКАИ, М. ИДЕИ, Й. ГРОФ, Б. БЮКИ и Я. МЕНЬХАРТ

Авторы, беря за основу клинические наблюдения, исследовали постнатальное, уменьшение уровня гемоглобина у недоношенных детей, родившихся перед 32-й неделей беременности с очень малым весом, а также определяли в связи с этим количество аминокислот в сыворотке крови.

Они установили, что эритроциты у незрелых, недоношенных детей, имевших малый вес при рождении, имеют более высокую скорость распада в первую неделю постнатальной жизни, чем у зрелых доношенных новорожденных. С помощью метода тонкослойной ионообменной хроматографии показали, что концентрация плазменных аминокислот повышается отчасти за счет аминокислот, появляющихся при распаде фетального гемоглобина.

Принимая во внимание послеродовое особое «голодное» состояние обмена веществ, авторы предполагают, что аминокислоты, появляющиеся вследствие распада фетального гемоглобина, могут играть роль — путем метаболизации через глюконеогенез — в снабжении энергией недоношенных детей, родившихся с низким весом.

АКТИВНОСТЬ ИНГИБИТОРОВ ПРОТЕАЗ И ПРОТЕИНАЗЫ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ МИОПАТИИ, ВЫЗВАННОЙ ГЛИКОКОРТИКОИДОМ

И. ШОХАР, И. НАДЬ, Л. ХЕЙНЕР, З. КОВАЧ и Ф. ГУБА

Мы исследовали неизвестный энзиматический механизм усиленного белкового распада, который наблюдается под воздействием стероидной миопатии, вызванной введением дексаметазона в течение трех недель, в функционально и биохимически различных скелетных мышцах кролика. После введения гликокортикоидов, быстросокращающаяся полуперепончатая мышца, имеющая гликолитический тип обмена веществ, атрофировалась, в то же время вес камбаловидной мышцы, с медленным типом сокращения и окислительным обменом веществ, не изменился. Специфическая активность лизосомных эндо- и экзопептидаз (катепсин D, E, B и L, лизосомная карбоксипептидаза A и дипептидилпептидаза 1) увеличилась приблизительно в два раза в атрофированной белой мышце. Активность действующей в цитозоле и активированной Ca^{2+} нейтральной протеиназы тоже повысилась, в то же время активность другой цитозольной эндопептидазы — химотрипсиноподобного энзима — не изменилась. Эти данные хорошо согласуются с уже известной различной чувствительностью к гликокортикоидам мышц разного типа. Полученные результаты позволяют сделать вывод, что лизосомная белковорасщепляющая система и активированная Ca^{2+} нейтральная протеиназа играют важную роль в процессе внутриклеточного расщепления белков, индуцированном гликокортикоидами. Определяемая в цитозоле ингибиторная активность, направленная против катепсина B и трипсина, под воздействием обработки не изменилась. Из этого следует, что активность катепсина B в белой мышце со стероидной миопатией повысилась не из-за изменения концентрации ингибитора.

ОБНАРУЖЕНИЕ Fc И C3в РЕЦЕПТОРОВ НА ПЕРИКАРИОНАХ У КРЫС

А. ПЕРЛ, Б. ФЕКЕТЕ, П. ГЕРГЕЙ и А. КОВАЧ

После продавливания через стальную и нейлоновую сетки лишенной мозжечка ткани головного мозга крыс, в полученной тканевой суспензии авторы отделили, с помощью скоростного осаждения, свободные перикарионы от клеточного синцития и клеточных обломков. В выделенных из мозга крыс перикарионах они отмечали активность Fc и C3в рецепторов, однако связанный с мембраной иммунгобулин и рецепторы эритроцитов барана обнаружить не удалось.

ВЛИЯНИЕ СУЛЬФАТНОГО ЭСТЕРА ОКТАПЕПТИДА ХОЛЕЦИСТОКИНИНА И НЕ СУЛЬФАТИРОВАННОГО ОКТАПЕПТИДА НА АКТИВНОЕ ОБОРОНИТЕЛЬНОЕ ПОВЕДЕНИЕ КРЫС

М. ФЕКЕТЕ, М. БОКОР, Б. ПЕНКЕ и Д. ТЕЛЕГДИ

Мы изучали влияние, оказываемое на активное оборонительное поведение крыс сульфатным эстером октапептида холецистокинина и не сульфатированным холецистокинином, при периферическом их введении. Оба пептида отрицательно влияли на процесс выработки оборонительного поведения, усиливая его угашение. *Open-field* активность под воздействием пептидов достоверно не изменялась. На основании этих данных можно думать, что введенный периферически октапептид холецистокинина оказывает влияние на процесс выработки активного оборонительного поведения и на его угашение не через общую двигательную активность животных.

ФАРМАКОЛОГИЧЕСКАЯ ОЦЕНКА И ОПРЕДЕЛЕНИЕ МОЛЕКУЛЯРНОГО ВЕСА ФРАКЦИЙ В ЗМЕИНОМ ЯДЕ *DENDROASPIS ANGUSTICEPS*, КОТОРЫЕ ИНГИБИРУЮТ АЦЕТИЛХОЛИНЭСТЕРАЗУ

Я. ВАНГАИ, К. ТХАИРУ, Б. С. БХАРАЙ и Б. В. ТЕЛАНГ

На изолированном фармакологическом тест-объекте авторы изучали три фактора, обладающих способностью ингибировать натуральную ацетилхолинэстеразу. Факторы усиливали сокращающее действие ацетилхолина, как освобождающегося эндогенного, так и экзогенного.

Факторы являются различными полипептидами, что подтверждается их разным молекулярным весом и неодинаковой электрофоретической подвижностью.

ОПРЕДЕЛЕНИЕ И ВЫДЕЛЕНИЕ ТРЕХ ФРАКЦИЙ, ИНАКТИВИРУЮЩИХ АЦЕТИЛХОЛИНЭСТЕРАЗУ В ЗМЕИНОМ ЯДЕ *DENDROASPIS ANGUSTICEPS*

Я. ВАНГАИ, К. ТХАИРУ, Б. С. БХАРАЙ и Б. В. ТЕЛАНГ

С помощью хроматографии на колонках СМ-Сефадекс G 25 авторы изолировали три фракции, которые обладают способностью инактивировать ацетилхолинэстеразу.

Признаки токсичности были одинаковыми, но процент летальности был разным, при применении разных фракций. Фракция D_a VI вызывала высокую летальность (LD_{50} 1,9 мкг/г), две другие фракции более низкую; у фракции D_a IV LD_{50} была 6,3 мкг/г, у фракции T_{39} LD_{50} 4,1 мкг/г. Все три фракции очень значительно — в 91—95% ингибировали натуральную холинэстеразу.

ЭКСТРАВАСКУЛЯРНЫЙ ОБОРОТ ПЛАЗМЕННЫХ БЕЛКОВ

ДЬ. САБО и Ж. МАДЬЯР

В экспериментах на наркотизированных кроликах авторы изучали исчезновение меченного радиоизотопом йода (^{131}I) альбумина из кровяного русла и появление его в лимфе. Исчезающая из кровообращения пропорция меченного альбумина составляла 0,0932 ± 0,0075, возвращающаяся через грудной проток фракция тогда же равнялась 0,0389 ± 0,0026, транспорт через лимфатические сосуды печени составлял 0,0115 ± 0,0016 в час, через лимфатические сосуды кишечника 0,0123 ± 0,0037 и лимфатические сосуды почки — 0,0185 ± 0,0021. 78% жидкости и 91% альбумина, транспортируемых через грудной проток, происходят из брюшных и почечных лимфатических сосудов. Соотношение между альбумином, покидающим кровяное русло, и альбумином, возвращающимся обратно через лимфатические сосуды, составляло 2,36.

Определение количества меченого альбумина из первого наклона *(slope)* кривых активности показало, что из разных органов в течение часа выходит следующее количество альбумина: из печени 1,89 г.кг$^{-1}$, из почки 2,32 г.кг$^{-1}$, из кишечника 0,69 г.кг$^{-1}$ и из конечности (кожа) 0,2 г.кг$^{-1}$. Лимфатические сосуды уносят 17% альбумина, выходящего из печени, 12%, выходящего из кишечника, и почти все количество альбумина, выходящего из кровеностных капилляров в почке. Выявилась довольно тесная корреляция ($r = 0,996$) между коэффициентами концентрации альбумина в лимфе и плазме и первыми наклонами установившихся кривых активности меченого альбумина в лимфе. Последнее указывает на то, что концентрацию белков в лимфе определяет скорость выхода белков из капилляров и что первый наклон кривой активности, рассчитанной на 1 г альбумина в лимфе/плазме действительно является мерой капиллярной проницаемости.

ВЛИЯНИЕ ХЛОРИСТОГО КАЛЬЦИЯ НА ЗАМЕТНЫЕ ИЗМЕНЕНИЯ ПОВЕДЕНИЯ У КОШЕК, ВЫЗВАННЫЕ КАРБАХОЛОМ И ЭЗЕРИНОМ

Д. Б. БЕЛЕШЛИН, Р. САМАРДЗИЧ, Ш. К. КРСТИЧ и М. ШТРБАЧ

Авторы изучали действие хлористого кальция, введенного в мозговой желудочек ручной бодрствующей кошки, на реакции, вызванные введением также в желудочек мозга карбахола и эзерина: звуковые (шипение, урчание), нападения (нападения, при котором пускаются в ход лапы и когти, оборонительная реакция, когда кошка ударяет лапой, а также пускает в ход лапы и когти), расширение зрачков, дрожание (тремор) и тонико-клонические судороги. Хлористый кальций уменьшал или почти полностью отменял вызванные карбахолом и эзерином звуковые реакции, но не оказывал достоверного влияния на мидриаз, тремор и тонико-клонические судороги. Очевидно, что хлористый кальций может «отличать» вокализацию и атакующую реакцию от вызванных карбахолом и эзерином моторных (тремор, тонико-клонические судороги) и автономного (мидриаз) явлений. Хлористый кальций скорее всего тормозил вызванные карбахолом и эзерином вокализацию и атакующую реакцию путем не-специфического стабилизирования центральных холинэргических рецепторов мускаринового типа. Полученные результаты по-прежнему поддерживают взгляд, по которому ионы кальция в больших количествах обладают атропино-подобным действием и на центральную нервную систему.

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STATEMENT

HUNGARIAN PHYSICIANS FOR THE PREVENTION OF NUCLEAR WAR

On 12 March 1982 six hundred Hungarian Physicians, research workers and outstanding representatives of the health service of the country met in the assembly hall of the Hungarian Academy of Sciences to sign the Statement launching the movement of Hungarian doctors for the prevention of nuclear war. The number of those signing the document is steadily increasing. It should be realized, however, that participation in the movement means more than just signing a statement, it must become integral part of our every-day medical practice.

In early 1981 a committed group of American, British and Soviet doctors initiated an international movement to prevent nuclear war. This movement has been expanding ever since. Today, doctors from 25 countries are united in it to achieve a goal which cannot be a matter of indifference to any doctors who sincerely believes in his calling.

The steadily growing accumulation of nuclear weapons and missiles and the increasing destructive power of the newer and newer weapons is threatening all of mankind and the whole global environment with catastrophe. We, who have taken the Hippocratic Oath to devote all of our knowledge and ability to curing our fellow-man, to alleviating pain and suffering and to preventing disease must feel that it is our obligation to take position against this, the greatest threat to human life. We are the people who are best acquainted with the immediate and delayed destructive effects of nuclear war. The 14 kiloton uranium bomb dropped on Hiroshima in 1945 killed 100,000 people and destroyed 60,000 buildings. Today 60,000 strategic and tactical nuclear weapons are known to exist, and their combined destructive power in one million times stronger than the bomb dropped on Hiroshima.

In 1970 the United States had sufficient strategic weapons to have been able to destroy every city in the Soviet Union with a population of over 100,000 fifty times over, while the Soviet Union had twenty times the amount sufficient to destroy the cities of the United States. Despite this the nuclear arms race has continued to accelerate, which, most recently, has been combined with an extremely rapid improvement in quality, i.e. in accuracy in aiming.

Today there are six countries (the United States, the Soviet Union, Great Britain, France, China, and India) which have nuclear weapons that have been officially accounted. It is most probable that other countries, i.e. Pakistan, South Africa, Israel, Argentina, Brazil and Spain also have a nuclear striking force. Taiwan and South Korea can easily become new members of the group of countries with nuclear weapons.

Proclamation of the doctrine that "limited" nuclear war is possible and that in this case the loss in civilian lives can be kept to an "acceptable" level, while buildings and large-scale industrial bases can be protected is an irresponsible and anti-humane deed. The history of our own century has twice proved that "limited" war in Europe is impossible, even with conventional weapons. The international and socio-political realities of today even more strongly preclude the tenability of maintaining a possible conflict with nuclear weapons within a "limited" framework.

The geographic and military policy situation of our country, which has suffered heavily in destructive war, is such that a so-called "limited" nuclear war in Europe would wipe our ten million people from the face of the Earth.

But even without the outbreak of war, a good part of the world's economic resources are being swallowed up by preparations for this mass destruction, which is in direct opposition to rational human thinking. The arms race is preventing the wonderful achievements of science, technology, biology and medicine from being used to the benefit of mankind. Even in the most advanced industrial countries, it is seriously reducing the funds available for welfare and culture. Today, when we have been able to synthetize the genetic material which controls the production of human hormones and other proteins, developed synthetic vaccine against infectious hepatitis, today when we are able to cure a high percentage of childhood leukemia and we are on the verge to disclose the mechanism of malignant transformation at a molecular level, today when gene transfer may become a reality in the treatment of inherited diseases, today when infectious diseases could be eradicated if financial means were available, increasing military expenditure diverts scarce resources from medicine and public health needs.

We bear an even greater responsibility if we think of the health situation of the developing countries, which make up two-thirds of the world. Smallpox could be eradicated by a single day's military expenditure budget. Malaria, which kills millions of people and which up till now has resisted prophylactic measures, could be eliminated with the world's three week military expenditure. To conquer lethal infections, blindness and other widespread but preventable diseases caused by lack of food, sanitation and housing, requires the cooperation of every doctor in the world, not only in actual treatment but also in fighting to raise the necessary financial means for prevention. In many countries of the world serious social inequality, and poverty destroying body and soul alike, are also sources of conflicts, increasing thereby the threat of nuclear war.

The threatening horror of nuclear war damages the human psyche and destroys normal, cultured human relations. The feeling of defencelessness in face of danger, the continuous threat to one's own future and the future of one's family undermines the faith in the sense of individual life and respect for human values. The atmosphere of fear and misconfidence incites to brutal and aggressive actions, and limits the possibilities for peaceful human coexistence.

There is but a single effective medicine against all this: action. And the sense to this action, to this fight lies in the fact that in the 20th Century, the role and significance of public opinion has increased. Can we, doctors, influence public opinion? The undersigners of this statement feel that we can.

We, doctors, have taken an oath to treat all patients in need, to alleviate all pain, and to prevent diseases if we can, irrespectively of political or religious belief and nationality. There are no national frontiers between doctors, we speak a common language. We have the chance to inform one another and our co-workers in health service. We can educate our patients about the medical consequences of nuclear war. Together with the international movement of doctors we call upon everyone to act for peace and for a reduction in nuclear arms. We take a position calling upon the United Nations Organization to pass a resolution banning the use of nuclear weapons just as it had banned the use of toxic gases and biological weapons. We, doctors must explain the almost inconceivable mass of death and environmental destruction caused by nuclear war. We must clearly see that the situation of the small percentage of physicians who may survive will be the most shamefully depressing. There can be no worse nightmare to a doctor who loves his calling than to stand helplessly among the dying, among the people suffering inconceivable pain of burns, blasts, and of consequences of radiation disease. There will be no hospitals, no water, no transport and no drugs. Doctors will not even have the possibility to ease pain or help slowly agonising people to a peaceful death. They have to stand without any hope to prevent long term fatal consequences of increased malignancy, malformations and other genetic defects.

By signing this statement every one of our colleagues and health workers can become a member of this movement. We have but a single request. Before signing this statement think over whether or not you will become a true comrade-in-arms, based on conviction. Join us if you have truly understood the goals of our movement and wish to help us actively, so that, in cooperation with peace-loving people we be able to raise a barrier against this irrational nuclear arms race and guarantee a peaceful, secure and creative life for the generations living today and those to follow us.

Budapest, March 1982

Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 60 (3), pp. 107–120 (1982)

Physiologia—Pathophysiologia

CARDIOVASCULAR REACTIONS FROM HYPOTHALAMIC SELF-STIMULATION IN THE RAT

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Arterial blood pressure (BP) and heart rate (HR) were continuously recorded during lateral hypothalamic self-stimulation with optimal stimulus parameters in rats. Hyper-hypotensive, hypertensive and hypotensive reactions were observed during separate self-stimulation, and the biphasic type considerably prevailed over other types of reactions. During the single cycles of self-stimulation hyper-hypotensive and hypertensive reactions with different heart rate reactions also occurred. In this case the biphasic type was seen in 75% of all reactions, whereas the hypotensive type occurred in 25% of the cases. The main type of BP reaction (92%) during continuous self-stimulation for 60–240 min was a gradual increase of the mean BP level from 15 to 40 mm Hg against initial values ($p < 0.001$), that depended on the duration of self-stimulation. Changes of HR were more variable: tachycardia and bradycardia were seen approximately with the same frequency. Comparative analysis of cardiovascular reactions during hypothalamic SS and escape reactions provoked by ventromedial hypothalamus stimulation revealed some peculiarities of autonomic manifestations during positive and negative emotional reactions. The results of this comparison raises the question of a stress-reaction during self-stimulation behaviour.

In a number of studies [1, 3, 6, 10, 12, 17, 25, 30, 32, 34, 50] it was shown that self-stimulation (SS) is always accompanied by definite peripheral autonomic and hormonal responses which may be connected with a rewarding effect of stimulation. This finding pointed to a simultaneous and rather complicated involvement in SS behaviour of the sympathetic and parasympathetic systems.

Ángyán showed [1, 2, 3, 4, 5, 6] a close relationship between changes of autonomic functions in particular arterial blood pressure and lever pressing behaviour. A close correlation was observed also between the autonomic and behavioural effects of SS from different brain areas [6].

The biological significance of positive and negative emotional reactions [8, 9] and the fact that negative reactions may cause cardiovascular disturbances [7, 39, 40] has called for a study of the cardiovascular reactions during hypothalamic SS in the rat and to compare this with haemodynamic changes during negative emotional reactions.

Methods

A total of 32 male Wistar rats weighing 200—300 g was used in the experiments. Bipolar nichrome electrodes of 0.3—0.5 mm tip diameter were implanted in the area hypothalamica lateralis according to the coordinates of the THOMPSON atlas [42].

The electrical stimulus consisted of monophasic square waves of 50—100 imp/s frequency, 0.7—1.8 pulse duration and 0.3—0.6 pulse train duration ("Physiovar", Alvar-Electronic, France). The stimulus voltages used were minimal for stable SS, usually 2.75—5 V. For arterial blood pressure recording a chronic polyethylene catheter was tied in the abdominal aorta via the caudal artery [38] one day before the SS experiment. EMT-34 pressure transducer and Mingograph-81 polyphysiograph (Elema-Schönander, Sweden) were used.

Recording of cardiovascular reactions was performed during SS behaviour in a standard Skinner box 6—8 days after implantation of the electrodes.

In the interval of 1 min for a period of 2—5 hours the mean of the values of the single waves of arterial blood pressure (BP) and their maximal and minimal differences (index of dispersion), mean BP, heart rate (HR) and lever pressing rate (PR) were analysed.

After the experiments the animals were sacrificed by and overdose of ether. Localization of the electrode tips was determined in 30—60 μm slices by routine histological techniques.

Results

SS behaviour for the analysis of cardiovascular reactions was studied from several aspects.

Single voluntary lever pressing separated from previous and subsequent lever pressings by more than 4 s was considered a separate SS. Separate SS were seen throughout the whole experiment, but most frequently they occurred at the beginning of the session.

SS behaviour consisted mostly of bursts, i.e. groups of consequent lever pressings divided by free intervals. The bursts were called single cycles of SS. The number of lever pressings per cycle amounted from 4 to 120, with intercycle intervals from 4 to 45 s.

Voluntary lever pressing behaviour for 2—5 hours was considered a continuous SS. Frequency of SS rate varied in different rats from 600 to 4200 lever pressings per hour (mean, 1527 lever pressings per hour).

A substantial increase in locomotor activity, urination and defecation were observed during SS. Ejaculation was seen in 75% of the cases. No change in SS rate was observed during 1—3 hour sessions if a constant stimulating current was used.

According to the character of SS extinction after switching off the current the animals were divided into two groups. In the first group a rapid decline of the lever pressing rate occurred after 4—10 non-reinforcements. In the second group the extinction was slower, the rats continued to press the lever for 5—7 min without reinforcement, making about 100—200 of non-reinforced reactions in this period. During SS session, these animals when put in the opposite side of the cage have tried to return to the lever. Lever pressing rate in this group was lower than that of the rats with slow extinction curve. No correlation was observed between SS extinction rate and the time of electrodes being in the brain.

Following the increased locomotor activity that is typical of SS behaviour, a decline of this activity was observed after 2—4 min of current switching off. Slow and forced breathing, sometimes active grooming was seen in this period.

Normal levels of BP and HR in conscious rats before SS training were, for BP; 115.96 ± 1.34 mm Hg (SD = 6.56): and for HR: —428.38 ± 7.22 beats/min (SD = 34.40). Individual variations of HR (coefficient of variability C = 8.26%) was higher than those for BP (C = 5.65%).

Cardiovascular reactions during separate SS

In 19 rats 534 cardiovascular reactions were studied and three types of BP response were observed.

Hyper-hypotensive reaction with 10—45 mm Hg hypertensive phase and 8—30 mm Hg hypotensive phase was observed in all rats in 84% of all cases (Fig. 1.A).

Hypertensive reaction of 5—40 mm Hg and 1.5—2 s time of peak reaching was observed in 11% of all cases (Fig. 1.B).

A hypotensive reaction of 5—10 mm Hg was seen in 2% of all cases. In 3% no BP reaction was seen during SS with optimal parameters of current used.

No differences between BP reactions were seen during elicited single stimulation and voluntary single lever pressing.

Cardiovascular reactions during the single cycles of SS

In 19 rats 612 single cycles were studied and two different types of BP response were revealed.

The first biphasic type BP response (Fig. 2.a) was found in all of the 19 rats. In this type BP increased during all periods of lever pressing and decreased after cycle termination. The pressure was 10—50 mm Hg and the depressor phase varied from 5 to 30 mm Hg and lasted from 3 to 240 s (usually 4—8 s).

Two opposite HR responses of biphasic type BP reaction were observed. In 11 rats the HR decreased to 3—18% of the initial level before the beginning of the cycle and reinstated for 8—120 s in the intercycle period. In all cases HR reinstating time was longer than the BP one. The second HR response was tachycardia and this type of reaction occurred in 9 rats.

The second hypertensive type of BP response (Fig. 2.b) was observed in 16 rats. The BP increase varied from 5 to 50 mm Hg. Bradycardia and tachycardia were also seen in this type of BP response.

Considering the shape of the hypertensive reaction, two main types of BP response were analysed. In some cases the peak BP increase occurred at

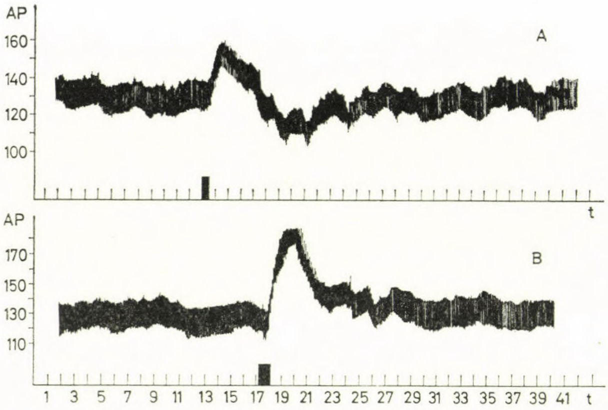

Fig. 1. Arterial blood pressure (AP) reactions during the separate self-stimulation. (Abbreviations: AP: in mm Hg; t: time in s; A: hyper-hypotensive reaction; B: hypertensive reaction)

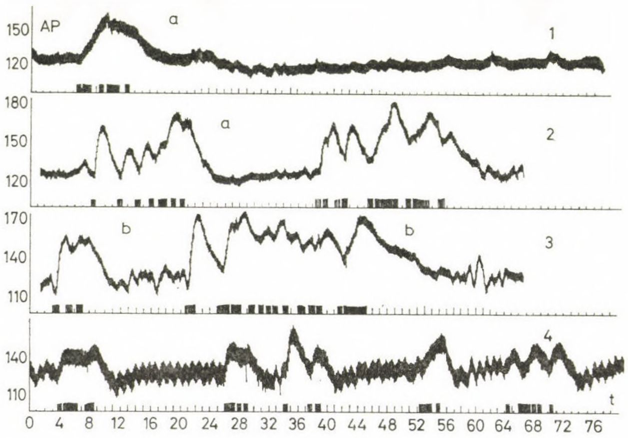

Fig. 2. Arterial blood pressure (AP) reactions during the single cycles of self-stimulation. (Abbreviations as in Fig. 1.)

the beginning of the cycle. In others a gradual summation of single BP reactions occurred and the peak BP coincided with the maximal rate of lever pressing or it was near the end of the cycle.

In most cases the two types of BP response alternated in SS behaviour. The biphasic type of BP response prevailed. It was seen in 75% of all reactions, whereas the monophasic hypertensive type occurred in 25% of the cases.

As the magnitude of BP changes depended on the current level, in 6 rats 224 cardiovascular reactions were analysed with different rewarding currents.

With a threshold current for SS behaviour, only 10—20 mm Hg BP hyper-hypotensive fluctuations were observed without any change of the mean level.

With a 1.5—2 threshold current the SS rate increased to the maximal level and BP reaction types became more obvious. In these cases the hypertension increased parallel with the increase of the current.

Increasing the voltage of the current to 2—4 times the threshold level caused in most rats a decrease in the response rate or in some rats a termination of SS behaviour. It was accompanied by an increase of the hypertensive phase and a stabilization or decrease of the hypertensive phase. In this period the rate of biphasic BP reactions decreased. The peak HR always occurred at a weaker current than peak level of BP.

An increase of the current to 4—6 times the threshold level caused the termination of level pressing in all rats and stimulation of these regions produced a defence-like reaction.

It could be shown that in the majority (80%) of the experiments changes in the polarity of the stimulating current did not significantly influence SS behaviour and its cardiovascular reactions. But 10% of the residual cases showed quantitative and qualitative alterations of the cardiovascular reactions without marked changes in lever pressing rate and the SS pattern. In the other 10% of these residual cases changes of the current polarity led to a decrease in the lever pressing rate or a termination of SS. In the latter case neutral, ambivalent, exploratory and defence-like reactions were caused by elicited stimulation, then hypertensive reactions prevailed over hyper-hypotensive reactions and indicated also an increase in their duration.

Cardiovascular reactions during continuous SS

BP and HR were analysed during SS behaviour for 60—240 min under constant current parameters optimal for stable SS.

The main type of BP reaction (22 rats 92%) revealed a gradual 15 to 40 mm Hg increase in the BP level as compared with the initial values (Fig. 3.A), accompanied by 40—70 mm Hg fluctuations. This BP type showed three different HR reactions; first, a decrease in (7 rats); second, an increase in (7 rats); third, variations without changes in the mean HR in 8 rats. Statistical comparison between mean BP before SS training and those of different periods of SS behaviour showed significance ($p < 0.05$) from the 16th min of SS (Fig. 3.B). Subsequently mean BP increased to 142 ± 5.4 mm Hg in the 96th min of SS ($p < 0.001$). BP variation indices of a SS period were significantly higher ($p < 0.001$) than with prestimulation controls. A mean BP variation in the 96th min SS of 34.94 ± 3.1 mm Hg was measured to a control value of 9.89 ± 2.14 mm Hg. Mean HR at various moments during the SS period did not differ significantly from the control.

After switching off the current the time of BP recovery compared with control values varied from 6 to 40 min or more whereby from the 16th min

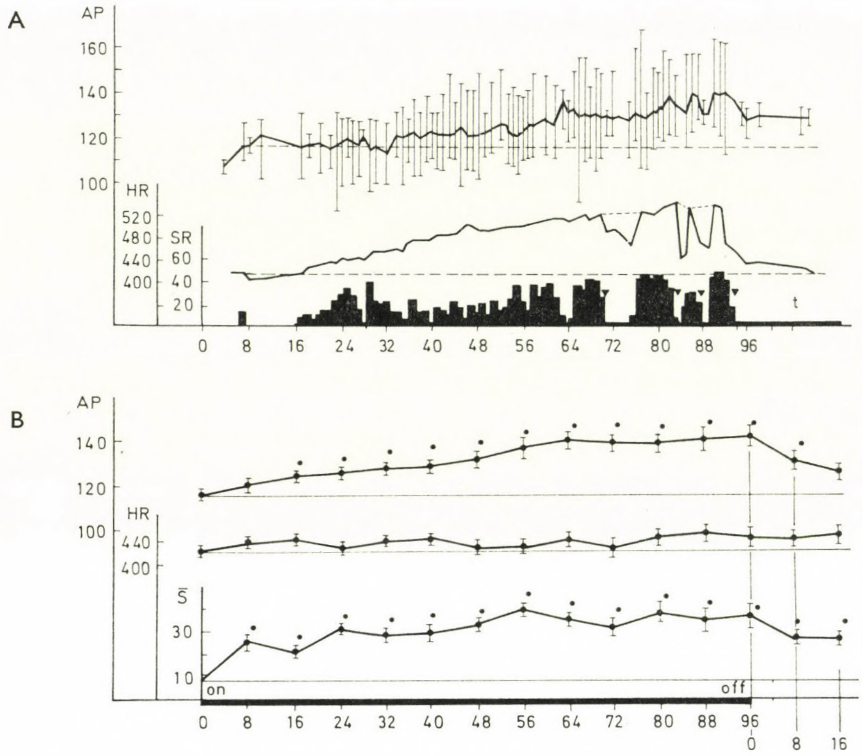

Fig. 3. Arterial blood pressure (BP) and heart rate (HR) reactions during continuous selt-stimulation. A) Individual reactions. (Abbreviations: SR-self-stimulation rate; t: time in min; ▼: artificial termination of self-stimulation; --- initial levels of AP and HR); B) Results of statistical analysis. (Abbreviations: S-fluctuations of AP in mm Hg; on off-time of self-stimulation; ■: statistical significance of the differences; ($p < 0.05$))

mean BP did not significantly exceed the control level in contrast to the BP variation values of 25.62 ± 3.9 mm Hg ($p < 0.05$).

Two rats demonstrated different haemodynamic reactions during continuous SS.

In the first rat (Fig. 3) which showed burst-like lever pressing, BP decreased to 9—95 mm Hg combined with variations of 40—70 mm Hg, while HR increased from 430 to 540 beats/min.

The second rat (Fig. 3.B) characterized by a high and rather constant lever pressing rate BP increased during 25 min to a peak of 185—190 mm Hg. Simultaneously with this BP increase the HR decreased from 480 to 350/min. At peak BP the SS behaviour was terminated. During the following 4 min, BP decreased to 155—150 mm Hg then it decreased slowly to 145—140 mm Hg by the 70th min after the termination of SS. In this period elicited stimulation was not priming for SS resumption.

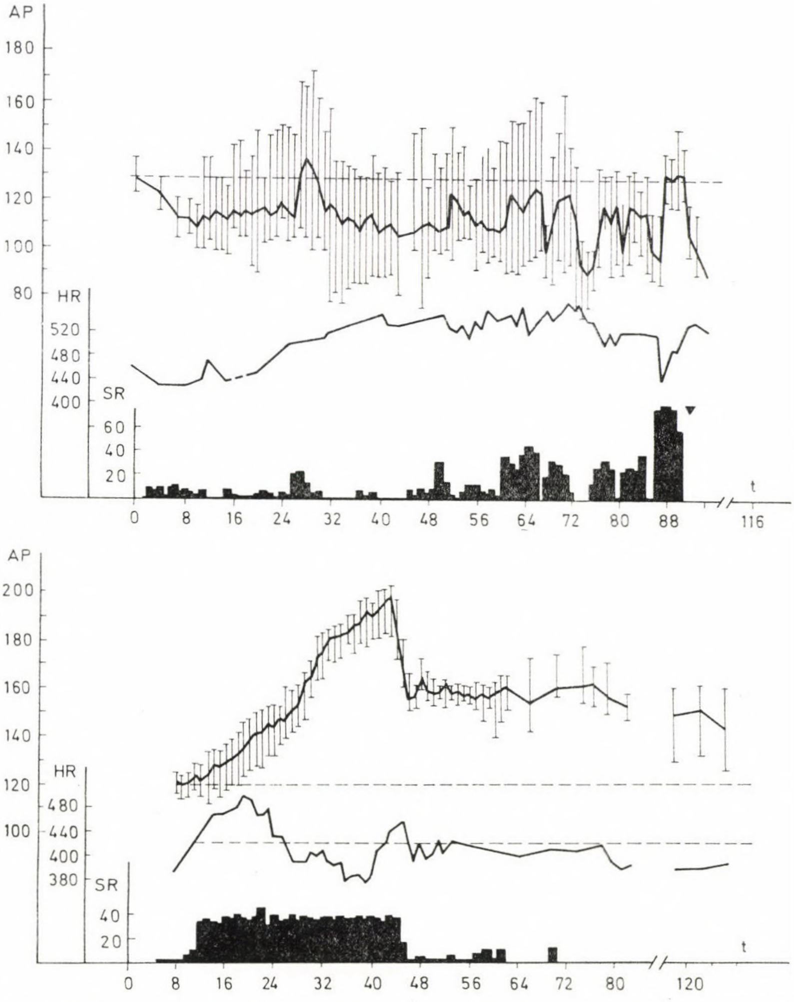

Fig. 4. **Arterial blood pressure (AP) and heart rate (HR) reactions during continuous selfstimulation. (Abbreviations as in Fig. 3.)**

Discussion

The present results support earlier reports that SS elicits definite cardiovascular responses [1, 3, 6, 10, 12, 21, 25, 30, 34, 50].

A study of SS from several aspects allowed to reveal typical cardiovascular reactions during separate SS, single cycles and continuous SS behaviour.

The change of BP was the most stable index of cardiovascular reaction, the main type of which was on increase of BP of different duration and stability. HR changes were more variable; tachycardia and bradycardia were seen approximately with the same frequency. This may be one of the reasons of the conflicting results reported concerning the effect of SS on HR [21, 24, 29].

Considering the shape of BP reaction, the hypertensive phase produced by a separate SS was followed by a hypotensive phase. This type considerably prevailed over hypertensive reactions.

Two types of BP response were observed in a case of single cycle SS behaviour, but in this case a biphasic type of BP response prevailed over the hypertensive one at the beginning and during the first hour of SS session.

The gradual increase from 20 to 40 mm Hg of the mean BP level depended on the duration of SS. It was the most typical response in the case of continuous SS. If continuous SS resulted in a more expressed BP increase (60—75 mm Hg), a termination of SS behaviour was observed.

Ángyán [1, 2, 3, 4, 5, 6] connected an SS displaying cyclic bursts with hypertensive dynamics. In our experiments the current was weaker than that applied by Ángyán and the BP reaction was weaker too. Still, in each case SS behaviour was cyclic. Thus the cyclic pattern of lever pressing cannot be ascribed to BP fluctuations alone.

The correlation between mean BP and the duration of continuous SS, and the fact of termination of lever-pressing behaviour in the case of a rapid and considerable increase of BP allows to assume an essential role of the mean BP level in determination of the duration of continuous SS. This variable increase may, however, reflect deviations of the levels of different biologically active substances produced regularly by self-stimulation.

One of these substances might be corticosteroids, the blood levels of which increased during SS [18, 33, 41, 44]. Intravenously administered corticosteroids gradually decreased the rate of lever pressing behaviour [13] and increased the mean BP [16].

Our results concerning cardiovascular reactions during hypothalamic SS and escape reaction evoked by ventromedial hypothalamus (VMH) stimulation in the rat [50] allow to compare the haemodynamic changes in these opposite emotional reactions.

Both a separate SS and a short stimulation of VMH produced hypertensive reactions similar in magnitude and duration accompanied by two different types of behaviour. In the case of separate SS the hypertensive reaction was followed by a hypotensive phase in 84%. In the case of escape reaction the biphasic type of BP response appeared only in 23%. During short stimulation of VMH the main type of BP response was a hypertensive reaction with a prolonged descending curve.

A similarity in the magnitude, duration and shape of the BP response was shown in the case of single cycles of SS and periodical stimulation of VMH by similar current parameters. A biphasic BP response, typical of the single cycles of SS in the case of escape reactions was, however, practically absent.

Continuous stimulation of VMH (current parameters and stimulation sequence as in the case of typical SS) for 2—4 hours led to an increase of 20—40 mm Hg in mean BP. The intensity of this increase depended on the duration and parameters of stimulation.

In the case of aversive stimulation an increase in current voltage, pulse train duration and quantity of stimulation led to more pronounced hypertensive reactions. Transitory hypertension produced by VMH stimulation has been earlier described [39, 40].

In the case of rewarding stimulation negligible changes in the current parameters caused a suppression of lever pressing behaviour. Elicited stimulation of these positive sites was accompanied by the same behavioural and haemodynamic changes as were observed during escape and exploratory behaviour. If continuous SS was accompanied by a more pronounced increase in mean BP, a termination of lever pressing behaviour occurred. Elicited stimulation used in this period was not priming for the resumption of SS behaviour and was similar to aversive and exploratory reactions.

Thus, the results of these experiments indicate that the most important feature of SS behaviour is the active choice and constant control by the animal according to the applied current and the intervals between self-stimulations. Any quantitative and qualitative changes of these conditions which take place during replacement of natural SS in experimenter-produced "SS" and during artificial stimulation of the positive brain sites make it impossible for the animal to control its own state and the moments of each stimulation.

In this case the emotional reaction is inverted and leads to characteristic alterations of the haemodynamic reactions produced by stimulation. Numerous data concerning the autonomic and hormonal responses to artificial stimulation of emotional positive brain sites [11, 12, 22, 25, 43] support this idea. It might therefore be assumed that there is no reason to consider artificial stimulation of the positive brain sites even if they had previously been tested as SS sites, as a model of positive emotional reaction.

Relations between behaviour and current stimulation in this case differ principally from natural brain reinforcement which is realized in SS behaviour.

In the case of natural rewarding behaviour the following may be assumed.

Priming stimulation of the reward site of the brain led to appearence of the drive and as a manifestation of this drive to exploratory behaviour which caused the first lever pressing and brain rewarding stimulation. Then, after brain stimulation accompanied by autonomic and hormonal responses different in duration, a comparison between this drive and the former brain rewarding stimulation is made. Feedback mechanisms from different homeostatic levels probably play an essential role in this stage. As first suggested by Valenstein [46, 47] and shown experimentally by Ángyán [1—6].

After suppressing the drive by effects of stimulation, restoration of the drive occurred and was now followed by purposeful searching of the lever as the source of reward.

The time interval between lever pressings and other behavioural indices of SS such as the duration of lever pressings, cycles and continuous SS are determined by the animal according to its available drive.

Modification of the experimental conditions during SS (sexual indifferent partner, sexual significant partner, escape reaction of partner) led to significant changes in the rate and pattern of SS [23, 25, 49]. Artificial limitation of animals motility led to marked modifications of SS. Thus, (unpublished data) rats previously taught to receive rewarding stimulation by nasal contact with special circuites after some time of natural SS, were immobilized in a box with free access to the circuiter. This led to the termination of SS in each animal. Interestingly, in these cases an artificial stimulation caused behavioural and haemodynamic changes similar to escape reactions. It was found that after a definite time interval only few animals reassumed SS but at weak current. In this case the rate of lever pressings was 5—20 times lower than that of natural SS behaviour.

The comparison of electrophysiological, hormonal and autonomic reactions with opposite emotional reactions raises the question of a stress-reaction during SS behaviour.

During SS, forced artificial stimulation of the positive sites, active avoidance and stimulation of the negative sites of midbrain tegmentum were revealed with similar changes in NA and DA concentration in the brain [22, 23, 28, 37], corticosteroid levels [28, 33, 41, 44] and the corticosteroid-sensitive hepatic enzyme tyrosine-aminotransferase [28].

Stimulation of the lateral hypothalamic area, the main positive brain site [27] as in the case of VMG stimulation at the main negative brain site [27] led to similar changes of the plasma ACTH and corticosteroid concentrations [18, 19].

During SS the typical manifestations of the stress-reaction such as lymphoid tissue involution, acceleration of blood coagulation and suppression of immune processes [20] were described.

Long-term stimulation of the negative and positive sites of the hypothalamus in rabbits led to a similar increase of adrenal weight with hypertrophy and hyperplasia of all its layers [36].

It has been shown that activation of the same regions of the hypothalamus may provoke different behavioural patterns [15, 47]. In certain conditions it was possible to elicit SS behaviour with high lever pressing rate by activation of the VMH, the stimulation of which also led to stress- reaction manifestations [31, 39, 40].

It is possible to transform SS behaviour into ambivalent and negative emotional reactions by slight changes in the parameters of the current and by modification of the experimental conditions [27, 35, 49].

Thus it is possible that stress-reactions developing during SS by their autonomic and hormonal reactions are similar to stress-reactions developing in the case of natural conflict situations and during stimulation of the negative brain regions.

It is known that under natural conditions positive emotional reactions in contrast to negative ones cannot lead to arterial hypertension and cardiovascular disturbances [7, 40].

These facts can be explained by Anokhin's biological theory of emotions [8, 9] according to which the natural positive emotional reactions are always short in duration and appear after terminating the behavioural act when the goal is achieved.

Therefore, they cannot be summarized in time. During SS the animal can control the parameters of the reward. Furthermore, SS behaviour is suppressed regularly by definite changes in the main homeostatic indices that exclude the possibility of development of pathological reactions.

Apparently, the central neurochemical organization of opposite emotional reactions may show differences, thus explaining the fact that alternations of the positive and negative emotional reactions under natural conditions exclude the pathological consequences of the negative emotional reactions. Therefore, probably, the main mechanism of the organism's stability to emotional stress consists in a constant alternation of negative and positive emotional reactions [8, 9, 40].

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ELEVATED OPIOID ACTIVITY IN SERA OF CHRONIC SCHIZOPHRENICS

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The effect of native serum samples from schizophrenic and control patients on the electrically induced contractions of the isolated mouse vas deferens (MVD) preparations was investigated.

It was demonstrated that only the samples of schizophrenic origin elicited a naloxone dependent inhibition on the contractions of the MVD preparations, while sera from healthy individuals and those from non schizophrenic but mentally ill patients proved to be ineffective in this respect.

By using ultrafiltration and gel chromatographic techniques, four fractions disclosing MVD related biological activity could be separated from schizophrenic samples. Chemical analysis revealed an elevated quantity of ninhydrin and Lowry positive materials as well as of unidentified carbohydrate components in the active fractions. Molecular mass of the serum ingredients carrying opioid activity was found to range between 0.5 and 5.0 KD. It is speculated that new appearance or the accumulation in the sera of several, and partly at least, unknown peptides and glycopeptides disclosing opioid activity might be characteristic of schizophrenia.

Wagemaker and Cade [14] reported in 1977 on the successfull hemodialysis of patients with chronic schizophrenia. The effectiveness of the treatment seemed to indicate, that the development and/or the maintenance of the schizophrenic symptoms might be causally related to endogenous substances demonstrable also in the serum, which penetrate through the common dialysing membranes. After the observation by Wagemaker and Cade, several investigations with considerably divergent results were carried out to check the validity of this hypothesis and to identify the substances postulated to be specific for schizophrenia.

In the present paper, we report on the results obtained by partial purification, biological and chemical characterization of a group of serum components seemingly characteristic of schizophrenia.

Materials and methods

Collection of samples

Serum samples were collected from chronic schizophrenics (n = 51) from non schizophrenic but mentally ill patients (n = 38) and from healthy individuals (n = 43). The exact diagnosis of the patients was known neither to the person who collected nor to those who carried out the analysis of the samples.

Ultrafiltration of serum samples

30 ml portions of sera were ultrafiltered through an Amicon YM 5 membrane (nominal limit of permeability: 5 KD) at 4 °C and at a pressure of 4.10^5 Pa. Then, the protein free ultrafiltrates were concentrated on an Amicon UM 05 membrane (nominal limit of permeability: 0.5 KD) under the conditions specified above.

Gel chromatography

3 ml portions of the Amicon YM 5 ultrafiltrates were chromatographed on a Sephadex G-25 (SG-25) fine gel column previously equilibrated with normal saline solution (0.9% NaCl). The volumetric parameters of the column were as follows: $V_t = 61.7$ ml; $V_k = 21.0$ ml; $V_b = 21.6$ ml and $V_x = 19.1$ ml. The separated fractions were characterized by their distribution coefficients (K_{av}), by their biological activity (v. inf), and by some of their chemical properties. Flow diagram demonstrating the steps of the separation is shown in Fig. 1.

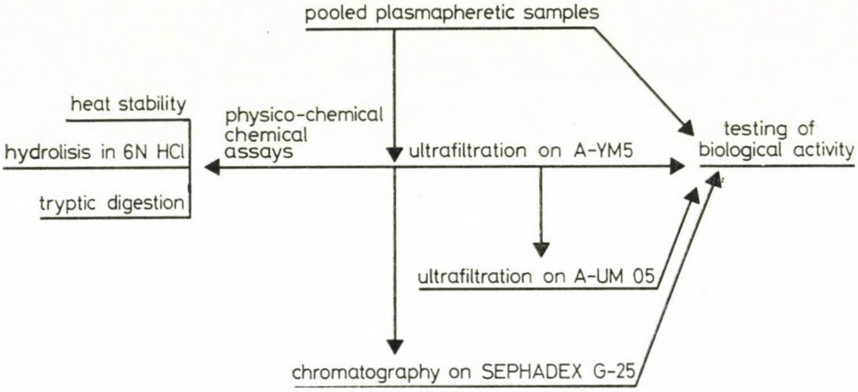


Fig. 1

Physico-chemical characterization of the active components

Heat resistance: 1 ml portions of the YM 5 ultrafiltrates were boiled for 5 h centrifuged, and the supernatants (90 μl) tested for their biological activity.

Acid hydrolysis: 0.5 ml portions of the YM 5 ultrafiltrates were hydrolyzed by 6 N HCl for 36 hours. After the HCl had been carefully removed by repeated azeotropic distillations, the samples were reultrafiltered (desalted) on an Amicon UM 05 membrane. The retentates were dried (vacum-distillation at 37 °C), and subsequently diluted by 0.5 ml distilled water. This was used for testing posthydrolytic biological activity of the samples.

Trypsin digestion: was performed as described in DÉVÉNYI and GERGELY's monograph [4]. Enzyme protein was removed by boiling the samples for 10 min followed by centrifugation (10 min, 10^4 rpm, room temperature). The supernatant was used for testing biological activity.

Chemical analysis: total and free alfa-amino-nitrogen (ninhydrin reaction), and total carbohydrate [12] content as well as the amount of LOWRY positive materials [9] were determined both in the YM 5 ultrafiltrates and the SG-25 fractions.

Measurement of the biological activity

Biological activity of the native serum samples and of their fractions obtained by ultrafiltration and by gel filtration was tested under in vitro conditions by using electrically stimulated vas deferens preparations isolated from 20 g male mice (CFLP strain). The organ bath consisted of Mg^{2+}-free Krebs' solution (37 °C) which was continuously bubbled through with a gas mixture of 95% O_2 + 5% CO_2. Contractions of the preparations were evoked by square-wave impulses of 70 V amplitude and of 2.2 ms duration generated in every 12 s. Volume of the samples used for testing biological activity varied between 45 and 250 μl.

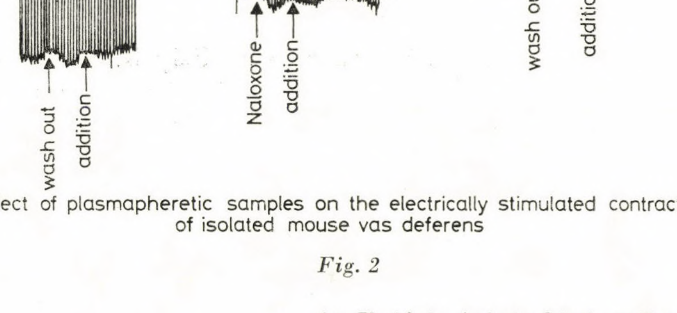
Results

Electrically induced contraction of the MVD preparations were decreased by 50% by native sera collected from schizophrenic patients in a concentration of 0.01 ± 0.012 ml serum per ml (ID_{50}). The 50% inhibition could be antagonized by 0.18 ± 0.018 μmol per ml naloxone, a morphine antagonist. In sharp contrast to this, native serum samples derived from healthy individuals or from patients with other neuropsychiatric diseases did not affect the MVD preparations even in a concentration as high as 0.1 ml sample per ml (Fig. 2).

Ultrafiltration experiments revealed that, while YM 5 ultrafiltrates displayed full biological activity, UM 05 ultrafiltrates were completely devoid of such activities. This is interpreted that biological activity is bound to serum components with molecular mass ranging between 0.5 and 5.0 KD.

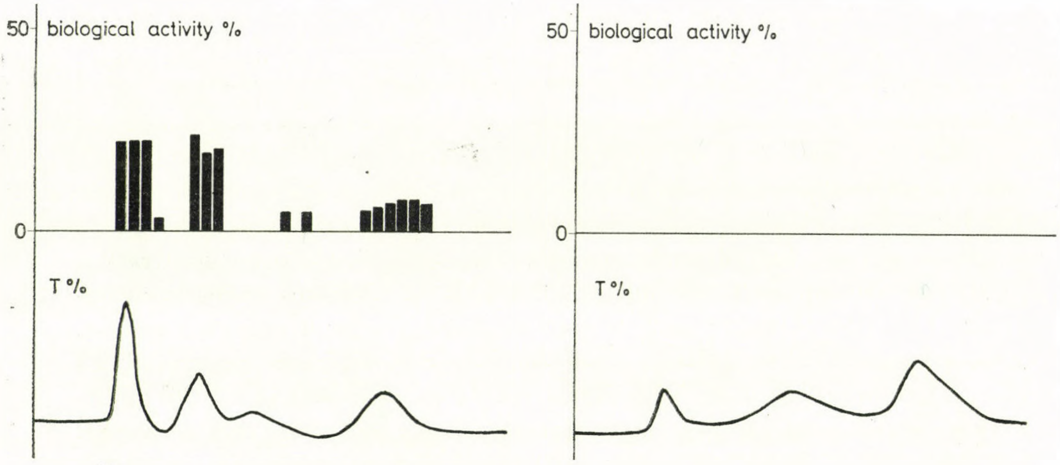
Biological activity in YM 5 ultrafiltrates was completely lost following acid hydrolysis.

It was a conspicuous finding, that the amount of Lowry positive materials was approximately five times higher (25.4 ± 12.7 μmol/l) while that of the total carbohydrate was approximately 17.5 times higher (52.2 ± 5.2 mmol/l) in YM 5 ultrafiltrates of schizophrenic origin than the Lowry positive material (5.2 ± 2.4 μmol/l) and the total carbohydrate (3.0 ± 1.2 mmol/l) content of similar ultrafiltrates derived from healthy individuals.



Effect of plasmapheretic samples on the electrically stimulated contractions of isolated mouse vas deferens

Fig. 2



Elution conditions:

V_t : 47.2 ml

L: 30.7 cm

diameter: 0.7 cm

detection: 254 nm

eluens: 0.9 NaCl (bacterium free)

flow rate: 0.6 ml/min

fraction volume: 4 ml

sample volume: 3 ml

Separation of schizophrenic sample on SEPHADEX G-25 column

Fig. 3

According to the gel-chromatographic analysis (Fig. 3), at least four serum fractions are to be made responsible for the biological activity detectable in the unseparated native sera. All of them absorb light at 254 nm, but they have different molecular weight and physico chemical properties (K_{av} values of the four SG-25 fractions are as follows; I: 0.00; II: 0.51; III: 0.84; IV: 1.59). Though each SG-25 fraction contained ninhydrin, and Lowry positive materials as well as carbohydrates, their relative amount varied from fraction to fraction. Carbohydrate was most abundantly present in fraction II, while fraction I was the richest in the Lowry positive materials.

Discussion

THÖLEN et al. reported in 1960, that haemodialysis, introduced to fight uremic intoxication in a patient with chronic schizophrenia, was effective not only in alleviating the uremic symptoms but also the schizophrenic ones [13]. This fascinating finding, however, remained unobserved in the literature until WAGEMAKER and CADE published similar results in 1977 [14]. Based on their observations, the latter authors raised the possibility that schizophrenia might be causally related to the appearance or the accumulation of some diffusible metabolites in the body fluids of chronic schizophrenics which can effectively be removed by haemodialysis. This was seemingly supported by PALMOUR's observation [11] who reported on the presence of an elevated amount of leu-endorphin in the hemodialysate of a few schizophrenics. However, several subsequent attempts have failed to demonstrate an evident improvement of schizophrenic symptoms by haemodialysis [5, 7, 8, 10,]. Similarly inconsistent results were obtained in experiments aimed at demonstrating an elevation in the quantity of known opioid peptides of one kind or another in the body fluids of schizophrenic patients [1, 2, 3, 10]. In most experiments published in the literature radioimmunoassay (RIA) techniques were used for quantitative detection of individual opioid peptides in sera or CSF. However, it is worth to recall, that quantitative determination of any substance by RIA is based on a reaction taking place between strictly determined chemical region of the interacting antigen with known chemical structure and the antibody molecules, and that such a reaction has nothing to do with the biological activity of the molecule to be determined. Thus, while RIA methods are suitable for the quantitation of individual opioid peptides with already known chemical structure, they can used neither for simultaneous detection of several materials disclosing opioid activity, nor for detection of substances with unknown chemical structure. Thus, when the detection of some kind of biological activity in a given biological fluid is the primary aim of an experiment, and the experimentator is uninterested in knowing the number and sort of the materials responsible for such an activity, it is better to replace RIA techniques with less specific methods capable of detecting biological activity instead of chemical structure.

In the experiments presented here, we gave up the specificity provided by the RIA techniques and also the possibility to determine individual substances in sera of schizophrenics. Instead, we wanted to know whether an elevated serum opioid activity is or is not an accompanying feature in schizophrenia, irrespective of the sort and number of molecules carrying such an activity. This led us to use MVD preparation, and naloxone, a specific opioid antagonist for monitoring opioid activity in the samples tested.

By using MVD preparations, it could be demonstrated that all sera of schizophrenic origin disclosed a naloxone dependent (opioid) biological activity, while those derived from non schizophrenic psychiatric patients or from healthy individuals, did not. The fact that YM 5 ultrafiltrates maintained, but UM 05 ultrafiltrates lacked opioid activity indicated that the molecular mass of the serum component(s) carrying the opioid activity ranged between 0.5 and 5.0 KD. It was also a characteristic finding, that both Lowry positivity and total carbohydrate content of YM 5 ultrafiltrate of schizophrenic origin surpassed significantly those found in the non schizophrenic samples. By SG-25 chromatography, YM 5 ultrafiltrates of schizophrenic origin could be resolved into four fractions disclosing MVD related biological activity. All of these fractions displayed Lowry and ninhydrin positivity and one fraction was positive also for carbohydrate. The observation, that sera from schizophrenic patients regularly disclosed elevated opioid activity may help in explaining inconsistencies of the results obtained by quantitative determination of opioid peptides in the body fluids of schizophrenic patients by RIA techniques. The finding namely, that the consistently elevated opioid activity in sera of chronic schizophrenics, was not accompanied by consistent elevation in the concentration of known opioid peptides determined by RIA technique might be interpreted, that elevated serum opioid activity is to be attributed to an elevated quantity of probably several endogenous opioid substances partly with unknown chemical structure. This is supported by our present observation, that schizophrenic sera could be resolved by SG-25 chromatography into at least four serum components disclosing opioid activity. Ninhydrin, Lowry and partial carbohydrate positivity of the separated serum fractions indicate, that opioid activity detected in sera of schizophrenics might be mediated by several peptides and/or glycopeptides also including presently unknown ones. Further purification and structural identification of these serum components are in progress in our laboratory.

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QUANTITATIVE AND QUALITATIVE CHANGES IN SERUM OLIGOPEPTIDE COMPONENTS OF PATIENTS WITH END STAGE MALIGNANT TUMOUR DISEASE

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A number of changes in peptide components could be demonstrated in sera of patients with end stage malignant diseases. Total and free alfa amino-N content of such sera was shown to be significantly higher than those in sera of patients with no sign of malignancy. In addition, a significant increase was found in one of the serum fraction obtained by Sephadex G-25 chromatography. This increase was shown to be due to an increase in the amount of one of the isotachophoretically separated serum components of anionic character as well as to the appearance in sera of tumour bearing patients of two additional isotachophoretic components never detected in non tumour bearing patients. Based on their chromatographic behavior as well as on observations made in earlier experiments, the peptide nature of the two isotachophoretic serum components seemingly characteristic of sera of tumour bearing patients is highly probable.

Introduction

The end stages of malignant tumour diseases are associated with clinical symptoms (cachexia, CNS disturbances etc.) which indicate their toxic origin and which are, in many respects, resembling those observed in other endogenous intoxications, (advanced stages of renal and hepatic failure). It would, certainly have both practical and theoretical significance to prove the correctness of this hypothesis and to recognize its biochemical background as well as those features of the latter which are common with those observed in other endogenous intoxications.

Literary data suggest that quantitative and qualitative changes take place in the spectrum of serum oligopeptides in end stage malignant tumour patients [1, 2, 3, 5, 6, 7, 8, 9].

However, neither the exact chemistry of these oligopeptides nor their potential role they might play in induction or maintenance of toxic symptoms associated with end-stage malignant diseases have satisfactorily been elucidated. Even the methodology applicable to the preparation and exact analysis of these particular serum components has not been acceptably delineated.

The present experiments the first in a series that are designed to see whether changes in serum peptide components are or are not taking place that are characteristic of tumour bearing patients; whether these changes are or are not responsible for the toxic symptoms at the molecular level and finally to see whether the changes observed can or can not be used for diagnostic purposes. In the present paper results obtained by simple chemical methods, by Sephadex chromatography and by isotachophoresis (ITP) of sera from patients with end stage malignant tumour diseases and from those with no sign of malignancy will be reported.

Materials and methods

1. *Collection and storage of samples*

Blood samples were obtained from the cubital vein of patients in the last stage of malignant tumour diseases ($n = 18$) and of patients with no clinical sign of tumour disease ($n = 17$). The samples were kept at room temperature for 30 min and then centrifuged (10 min, 10^4 rpm, room temperature). The samples then were frozen and kept at -20 °C until processing or 2 weeks at most.

2. *Acid ethanol precipitation*

Of the samples 10 ml portions were mixed with 96% ethanol pH = 2.0 under continuous stirring until final ethanol concentration reached 80%. The precipitate was removed by centrifugation and the sediment was redissolved in 8 ml 80% ethanol and centrifugation was repeated. The ethanol supernatants were mixed and kept at 40 °C overnight. The fine precipitate was removed by filtration. The clear ethanol filtrate was concentrated to approximately 2 ml in vacuum at 37 °C and then filled up to 10 ml by distilled water. The suspension was centrifuged (15 min, 10^4 rpm, 4 °C). The clear supernatant was stored at -20 °C.

3. *Gel-chromatography*

Of the samples prepared as described above, 60 μl portions were analysed by chromatography in a 6×940 mm SEPHADEX G-25 (SG-25, superfine) gel column. Volumetric parameters of the column were determined by routine techniques. Conditions of the elutions were as follows eluent: 0.9% NaCl; flow rate: 0.17 ml/min; detection: 206 nm; paper speed: 120 mm/h. The fractions separated on the column were characterized by their K_{av} values.

4. *Ultrafiltration*

Ultrafiltration was introduced with the aim to prepare a sample that, quantitatively and exclusively contains only those components of the acid ethanol and water soluble serum fraction whose molecular mass are ranging between 0.5 and 5.0 KD. In order to achieve this goal, the serum fraction mentioned above was subjected to ultrafiltration through two membranes with different permeability. As a first step, the serum fraction was ultrafiltered (4 °C, 4 atm, N_2-gas) through an Amicon YM 5 membrane (nominal limit of permeability: 5.0 KD). By repeting fourtimes the ultrafiltration through this membrane, when dilution with water alternated with reconcentration of the samples, the components with a molecular mass of less than 5.0 KD could be practically quantitatively transferred into the pooled ultrafiltrates. This was indicated by the binding, that less than 2% of the amount of such components could be detected in the retentate discarded. As a subsequent step, the pooled YM 5 ultrafiltrates were quantitatively ultrafiltered through an Amicon UM 05 membrane (nominal limited of permeability: 0.5 KD). Repeated ultrafiltrations were carried out also through this membrane. By this procedure, a retentate could finally be obtained in which the amount of components with a molecular mass of less than 0.5 KD was a fraction of 1% only. As the final UM 05 retentate was concentrated to one fifth of the original volume of serum, concentration of serum ingredients with a molecular mass between 0.5 and 5.0 KD was about fivefold as high in this retentate as that of those in the original serum.

Isotachophoresis (ITP)

30 μl portions of the acid ethanol soluble and ultrafiltered serum fraction were analysed on a LKB 2127 Tachophor equipped with a 0.5×630 mm capillary and a thermal and UV (254 nm) detector. The leading electrolyte was a solution containing 5 mmol/l Cl^- and 0.5 g% hydroxylpropylmethylcellulose (HPMC). The solution was titrated to pH 4 by beta-alanine. The terminating electrolyte was a 5 mmol/l capronic acid solution. The analysis was carried out with constant current (65 μA) and alternating voltage (5—30 kV). The Joule-heat generated by different changes in the field strength of the individual materials of different mobility was detected by a thermocouple built in the capillary. The transmittance of each component was detected continuously by an UV detector at 254 nm. The paper speed was 3 cm/min. The effective mobility ($m_{eff} = m_e \times pK$; where m_e is the electrical mobility of the material) of the mobile fractions was expressed in percents of the effective mobility of the terminating electrolyte. The relative change in the quantity of the individual fractions ($\Delta\%$) was calculated on the basis of the length of the thermal steps and it was expressed in per cents of the control.

Chemical analysis

Free (F) and total (Σ) alfa-amino N (αaN) content of the samples were determined by the ninhydrin reaction performed before and after an alkaline hydrolysis and the bound (B) αaN content calculated (BαaN = αaN-FαaN) carbohydrate content was determined by the orcin-sulphuric acid reaction. Alkaline hydrolysis was performed in NaOH (2.5 mol/liter) at 90 °C for 2.5 h.

Results

1. *αaN and carbohydrate content of blood serum preparations* (Fig. 1)

The αaN content of the acid ethanol and water soluble serum components was significantly higher in patients with malignant tumour ($\bar{x} = 14.6 \pm 4.32$ mmol/l; $p < 0.05$) than in patients having no malignant tumours ($\bar{x} = 8.2 \pm 3.31$ mmol/l). The same was true for the FαaN content of these

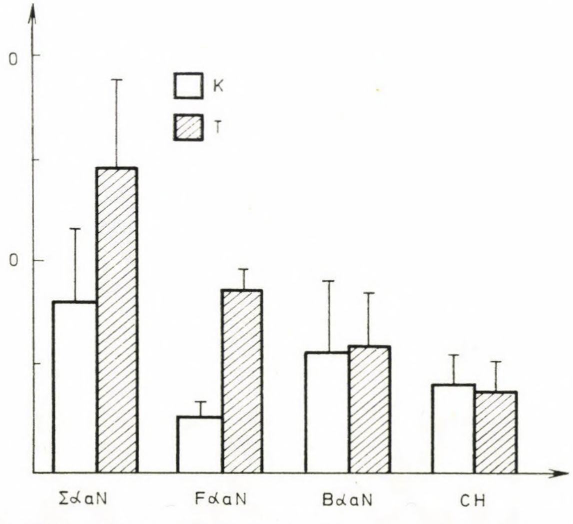

Fig. 1. Total (Σ), free (F) and bound (B) α-aminonitrogen (αaN) and carbohydrate content of blood serum originating from patients in the last stage of malignant tumour disease (T) and from patients with no sign of malignancy (C)

fractions tumour: ($\bar{x} = 8.7 \pm 0.83$ mmol/l; control: ($\bar{x} = 2.5 \pm 0.75$ mmol/l; $p \leqslant 0.01$). On the other hand the BαaN content was not significantly different in the two groups investigated. Tumour: $\bar{x} = 5.8 \pm 2.53$ mmol/l; control: $\bar{x} = 5.7 \pm 3.33$ mmol/l; $p < 0.9$. There was no difference between the two groups in the blood serum carbohydrate content either. Tumour: $\bar{x} = 3.7 \pm 1.31$ mmol/l; control: $\bar{x} = 4.1 \pm 1.25$ mmol/l; $p < 0.5$.

2. *The SG-25 fractions of blood serum preparations*

The acid ethanol and water soluble blood serum preparation could be separated into three fractions (A, B and C) by SG-25 chromatography. The size of the B fraction with $K_{av} = 0.77 \pm 0.026$ (determined by planimetry) was significantly larger in patients with malignant tumour ($\bar{x} = 95.0 \pm 35.96$ cm^2) than in those with no clinical sign of malignancy. ($\bar{x} = 43.4 \pm 3.07$ cm^2) (Fig. 2).

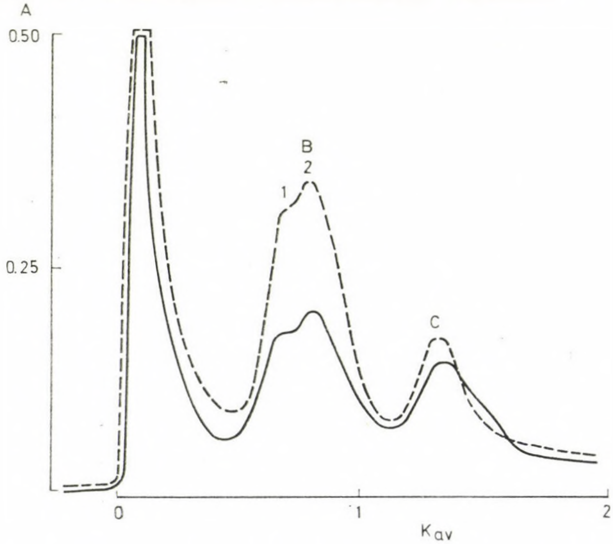

Fig. 2. SG-25 chromatographic profile of blood serum from patients with (- - - -) and without (——) tumour. The A, B_1, B_2 and C fractions were eluted from the column at a K_{av} value of 0, 0.67 ± 0.023, 0.77 ± 0.026 and 1.49 ± 0.051, respectively (ordinate). Abscissa: absorbance at 206 nm

3. *Results of isotachophoretic analysis* (Fig. 3)

The acid ethanol and water soluble blood serum fractions of patients with no malignant disease could be resolved by ITP into three components (1, 2 and 3) with anionic properties at pH 4.0 (pI > 4). The mobility of these was as follows. *Component 1:* $36.9 \pm 0.85\%$; *component 2:* $50.3 \pm 1.06\%$;

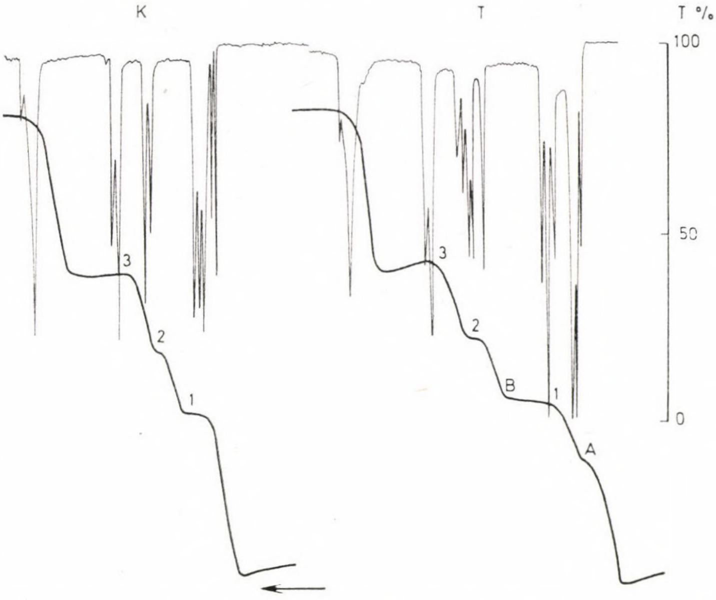

Fig. 3. Isotachophoretic pattern of blood serum fractions separated by alcoholic extraction and ultrafiltration. Upper half of the figure illustrates transmittance (T%) and the lower half the thermal steps of the zones

component 3: 67.0 ± 0.99%. The corresponding blood serum fraction of patients with malignant tumour contained two additional isotachophoretic components (A and B), which, in the present system behaved as anions. The mobility of these two components, was A; 27.1 ± 0.54% and B; 40.1 ± 0.80%. In addition to this qualitative difference, there was a quantitative difference in the sera between the two groups investigated. The amount of component 1 calculated from the length of the thermal step, was significantly larger in patients with malignant tumour ($\Delta\% = 85$; $p \ll 0.01$) than in tumour-free patients.

When ITP and SG-25 gel chromatography were used in combination it was found that the isotachophoretic serum components from tumour-free patients (components 1, 2 and 3) and those from patients with malignant tumour (components A, 1, B, 2 and 3) were equally eluted from the gel column at the $K_{av} = 0.67$ value.

Discussion

As it was stated in the introduction, the primary aim of these investigations was to see whether qualitative and/or quantitative changes in serum oligopeptides characteristic of end stage malignant diseases can or cannot be detected. It was demonstrated that quantitative changes in FαaN and $\Sigma\alpha$aN content of the ethanol and water soluble serum fraction could be detected in patients with end stage malignant diseases in comparison to appropriate controls. Similarly, quantitative changes could be shown in one of the serum fractions obtained by SG-25 chromatography. However, since similar changes also can be detected in other pathological conditions, for example in uremic intoxication [4] these changes cannot be regarded as characteristics of patients with end stage malignant diseases. On the other hand, the qualitative changes detected by isotachophoresis in acid ethanol and water soluble serum fraction of tumour bearing patients seem to be a characteristic feature of sera of such patients. These isotachophoretic components were shown to be eluted in a SG-25 chromatographic fraction the peptide content of which had been demonstrated in earlier experiments [4]. As the appearance on the isotachophoretogram of these components appears to be characteristic of end stage tumour bearing patients they deserve particular attention in subsequent investigations. Studies aimed at confirming present results in a larger number of patients and at characterising further the chemical and physicochemical properties of these serum peptide components as well as attempts to isolate them in a chemically pure form are in progress in this laboratory.

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INTERMEDIATE MOLECULAR WEIGHT SUBSTANCES IN SERA OF PSORIATIC PATIENTS

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In the experiments presented here, serum samples were collected both from psoriatic (PS) ($n = 8$) and non-psoriatic (NPS) patients ($n = 8$) and were analyzed by the combination of fractional precipitation (80% ethanol, pH 2) and gel filtration techniques as well as by chemical methods. It was demonstrated that, in comparison to the non psoriatic samples, concentration of the free alfa-amino group bearing and the Lowry positive components as well as total carbohydrate content was significantly elevated in the acidic ethanol soluble serum fraction of psoriatic samples. In psoriasis, the quantity of serum components with UV light absorbing capability at 206 nm, and with a molecular mass between 0.3 and 5.0 KD (estimated by chromatographic criteria) also was elevated in one of the fractions obtained by Sephadex SG-25 chromatography. Results presented in this paper indicate, that psoriasis is accompanied by changes in quality and in quantity of middle size molecular weight serum components with chemical properties suggesting their peptide — and/or glycopeptide-like character.

Introduction

Several aspects of the molecular mechanisms connected to the induction of psoriasis, a multifactorial disease have already been studied in considerable details. The presently available, and mostly symptomatic therapy of the disease is based on the results of these studies. One of the therapeutical approaches introduced recently is haemodialysis of psoriatic patients. This sort of therapy is based on the hypothesis that the induction and/or the maintenance of some of the psoriatic symptoms might be attributed to some unidentified endogenous substances of 0.3—5.0 KD molecular mass, whose elevated serum concentration could be ascribed to their increased production or their accumulation in body fluids of psoriatic patients. Haemodialysis, by removing these components from the serum would alleviate some of the symptoms characteristic of psoriasis [1, 3].

In order to check the validity of this hypothesis, blood serum fractions were analyzed by fractional precipitation and SG-25 chromatography as well as by chemical analysis of serum samples collected both from psoriatic and non psoriatic patients.

Materials and methods

Collection and storage of samples

Blood was sampled from the cubital vein of psoriatic (PS) ($n = 8$) and non psoriatic (NPS) ($n = 8$) patients. The blood samples were kept at room temperature for 30 min, and centrifuged thereafter (10^4 rpm, 10 min, room temperature). The serum samples were stored at -20 °C until processing.

Acid ethanol extraction of samples

To 5 ml portions of the samples, 96% (v/v) ethanol (pH 2.0) was added under continuous stirring, until the final concentration of the ethanol reached 80% (v/v) in the samples. The precipitate was removed by centrifugation, the sediment redissolved in twofold volume of 80% ethanol (pH 2.0) and recentrifuged. The procedure was repeated three times. The ethanol supernatants of psoriatic and non psoriatic origin were separately pooled and kept at -4 °C overnight. The fine precipitate regularly formed under the cooling period was removed by filtration, and the filtrate concentrated in vacuum (37 °C) to 50% of the original volume of the native serum.

Gel chromatography

The samples prepared as described above were chromatographed on a Sephadex G-25 (SG-25) column (column size: 0.6×94.0 cm; elution fluid: 0.9% NaCl; flow rate: 0.17 ml/min; detection: 206 nm; paper speed: 6 cm/h). Elution peaks appeared on the chromatograms were characterized by their K_{av} values. Samples were applied to the column, in volumes of 250 μl and were washed into the gelby 150 μl of normal saline (0.9 NaCl) solution. The amount of serum components absorbing at 206 nm was estimated by calculating the area covered by the chromatographic peaks and was expressed in cm^2.

Chemical analysis

Free (F), bound (B) and total (Σ) alfa-aminonitrogen (αaN) content of the samples were determined by the ninhydrin reaction performed both before and after alkaline hydrolysis. Total carbohydrate content was determined by the orcin sulphuric acid reaction [4], and the peptide content by FOLIN's method as modified by LOWRY et al. [2].

Results

In psoriasis, BαaN content ($\bar{x} = 10.9 \pm 4.07$ mmol/liter) of the acid ethanol soluble serum fraction was practically identical to that of the non-psoriatic patients ($\bar{x} = 9.2 \pm 3.82$ mmol/liter; $p > 0.5$). The same was true for the BαaN content released by alkaline hydrolysis (PS: $\bar{x} = 7.3 \pm 3.08$ mmol/liter; NPS: $\bar{x} = 6.0 \pm 2.92$ mmol/liter; $p > 0.5$). On the other hand, the FαaN content of PS samples ($\bar{x} = 3.6 \pm 0.83$ mmol/liter) was significantly higher ($\Delta\% = 44\%$; $p < 0.05$) than that of the sera from NPS patients ($\bar{x} = 2.5 \pm 0.67$ mmol/liter).

The amount of the Lowry positive components in PS samples ($\bar{x} = 27.2 \pm 9.38$ mmol/liter) was significantly higher ($\Delta\% = 78\%$); $p < 0.05$) than that in the NPS samples ($\bar{x} = 15.2 \pm 6.89$ mmol/liter). The same was true for the carbohydrate content of the samples (PS: $\bar{x} = 7.7 \pm 2.04$ mmol/l; NPS: $\bar{x} = 4.1 \pm 1.25$ mmol/liter; $\Delta\% = 86$; $p < 0.01$) (Fig. 1).

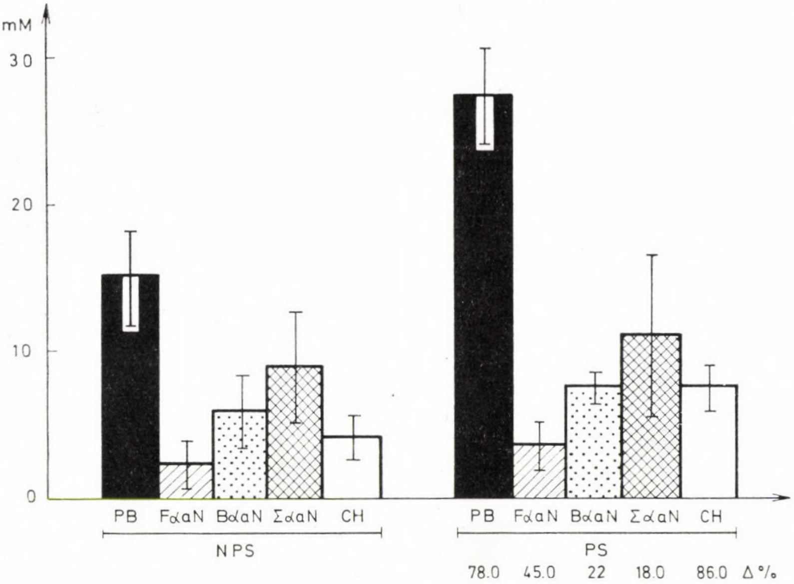

Fig. 1. **Chemical composition of the acid ethanol soluble serum fraction collected from non psoriatic (NPS) and psoriatic (PS) patients. PB: Lowry positive materials; F, B and αaN: free, bound and total alpha-amino-nitrogen; CH: carbohydrate**

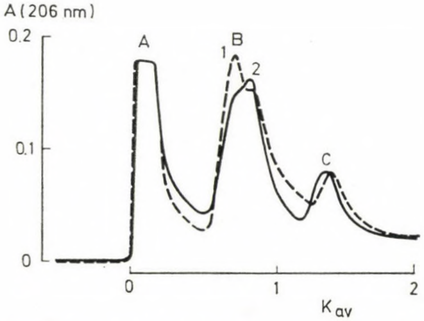

Fig. 2. **Sephadex G-25 gel filtration profile of serum preparations of psoriatic (- - -) and of non psoriatic (——) origin. Ordinate: absorbance at 206 nm. X-axis: K_{av} = average distribution coefficient. A, B and C represent individual fractions**

The amount of serum components represented by one of the UV (206 nm) absorbing SG-25 peaks characterized by a $K_{av} = 0.68 \pm 0.023$ (indicating a distribution in the effective permeation volume of the column, and thus a nominal molecular mass ranging between 0.3 and 5.0 KD) was higher by 26% ($p < 0.02$) in the PS ($\bar{x} = 48 \pm 8.2$ cm^2) than in the NPS samples ($\bar{x} = 38 \pm 10.6$ cm^2). It was also a consistent finding that, while B_2 was the dominant subfraction of fraction B in NPS samples, it was subfraction B_1 which dominated in the PS samples (Fig. 2).

Discussion

The observation that some of the psoriatic symptoms could be beneficially influenced by haemodialysis [1, 3] led to the assumption, that substances of endogenous origin with molecular size allowing their diffusion through commonly used haemodialysis membranes might be participating in molecular etiology of the disease. The present study performed on a small group of psoriatic patients lends some support, though of a limited value only, to such a hypothesis. It was namely demonstrated, that concentration of the substances with ninhydrin, Lowry and carbohydrate positivity in the acid ethanol soluble serum fractions from PS samples, surpassed significantly those found in the corresponding fraction of NPS samples. It was also shown, that quantity of the serum components with an UV light absorbing capability at 206 nm, and with a molecular mass between 0.3—5.0 KD (by chromatographic criteria) was also higher in one of the SG-25 fractions of PS origin than in that of the NPS samples. The data presented also indicated, that the substances with an estimated molecular weight that allows their penetration through dialysis membranes, and the concentration of which are elevated in psoriasis disclosed peptide and/or glycopeptide-like character might be the serum components whose removal by haemodialysis from the serum of psoriatic patients has been postulated and connected to the improvement of the disease in earlier experiments. Studies aimed at characterizing the chemistry of these serum components as well as clarifying the role they might play in molecular etiology of the disease are in progress in this laboratory.

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ON THE CYTOPROTECTIVE ACTION OF SULFHYDRYL-CONTAINING SUBSTANCES

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Sulfhydryl substances (cysteine, glutathion, cysteamine and BAL) provide protection against experimental gastric ulcer induced by indomethacin while being ulcerogenous in stress-ulcer.

Experimental data indicate the necessity to distinguish between "real" and "occasional" cytoprotective drugs.

Recently SZABÓ [5], SZABÓ et al. [6] and KONTUREK et al. [3] have published data indicating that cytoprotection is a property not only of prostaglandin analogues (PGs), as it was proposed by ROBERT [4] who first described this phenomenon, but several other compounds of various structure and property may exert such an effect.

According to ROBERT [4] the protective action of PGs is due to increased mucus secretion in the stomach.

SZABÓ et al. [6] suggest that the cytoprotective action may be related to the presence of sulfhydryl groups, since the gastric mucus contains reduced glutathion in high concentration [2].

Substances containing sulfhydryl groups are capable of chemically binding various free radicals and so they may influence the physical and chemical properties of the gastric mucus.

Since these data suggest the importance of sulfhydryl groups in the pathomechanism of gatsric ulcer we investigated the effect of four sulfhydryl-containing substances [cysteine, glutathion; dicaptol (BAL) and cysteamine] on experimental gastric ulcer of the rat.

Materials and methods

Female Wistar rats of 200–230 g body weight were used, and prior to experiment they were fasting for 24 hours, but they received water ad libitum. The animals were assigned in groups, each one consisting of 20 animals. A total of 260 rats were used.

a) *Indomethacin-ulcer*

The animals received 30 mg/kg indomethacin suspension intraperitoneally at the beginning of the experimental period; they were killed 4 hours later and their stomach was removed.

b) *Stress-ulcer*

The animals were immobilized, lying on their back; they were killed 24 hours later and their stomach was removed.

The removed stomach was opened in both experimental series and the structural changes of the mucosa were evaluated by "ulcer-index" (U.I) [1].

The chemicals were of usual purity and the appropriate amount of their aqueous solution was administered orally by gastric tube. Dicaptol (BAL) was injected intraperitoneally thus avoiding the possible local action of the oily solvent material on the gastric mucous membrane.

The experimental design was as follows:

Table I

Experimental design

	Indomethacin-ulcer	Stress-ulcer
	Treatment (single dose)	
	0. min and 2nd hour	0. min, 6th, 12th and 18th hour
Control	none	none
Cysteine	100 mg/kg p.o.	100 mg/kg p.o.
Glutathion	100 mg/kg p.o.	100 mg/kg p.o.
Dicaptol (BAL)	10 mg/kg i.p.	10 mg/kg i.p.
Cysteamine	100 mg/kg p.o.	100 mg/kg p.o.

Mean values ±S.E.M. were calculated in all animal groups. Statistical analysis was performed by Student's two-paired *t*-test and significant differences were assumed when the probability was less than 5%. The experimental design was the same in the so-called complementary experiments, with the exception that 200 mg/kg glutathion was administered.

Results

The experimental results are demonstrated in Fig. 1 and in Tables II and III.

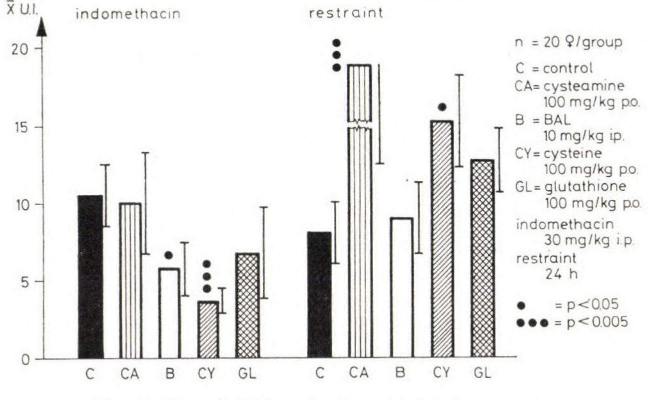

Fig. 1. Detailed description see in the text

Table II

Effect of sulfhydryl-containing substances on experimental gastric ulcer of the rat

	Indomethacin-ulcer U.I., $\bar{x} \pm$ S.E.M. (Δ%)		Stress-ulcer U.I., $\bar{x} \pm$ S.E.M. (Δ%)	
Control	10.4 ± 2.06	(100.0)	7.9 ± 1.99	(100.0)
Cysteamine	9.9 ± 3.32	(95.2)	29.4 ± 6.39***	(372.2)
Dicaptol (BAL)	5.7 ± 1.80*	(54.8)	9.0 ± 2.25	(113.9)
Cysteine	3.6 ± 0.82***	(34.6)	15.2 ± 3.11*	(192.4)
Glutathion	6.6 ± 2.91	(63.5)	12.6 ± 2.14	(159.5)

n = 20/group
* = $p < 0.05$ vs control
*** = $p < 0.005$ vs control

Table III

Complementary experiment

	Indomethacin-ulcer U.I., $\bar{x} \pm$ S.E.M. (Δ%)		Stress-ulcer U.I., $\bar{x} \pm$ S.E.M. (Δ%)	
Control	14.4 ± 2.73	(100.0)	12.4 ± 1.13	(100.0)
Cysteamine 100 mg/kg	6.6 ± 2.08	(45.8)	21.6 ± 1.19*	(174.2)
Glutathion 200 mg/kg	2.6 ± 1.05***	(18.1)	27.9 ± 1.14***	(225.0)

n = 10/group
* = $p < 0.05$ vs control
*** = $p < 0.005$ vs control

Discussion

From the experimental results the following conclusions can be drawn;

1) The suggestion of Szabó [5] that sulfhydryl groups in the mucous membrane of the stomach may exert protective action was verified only in part of the experiments. Indomethacin-induced ulcer was significantly inhibited by BAL and cysteine, but the effect of glutathion was only trend-like.

2) The above mentioned protective action could not be shown if the ulcer was induced by immobilization (stress), the addition of sulfhydryl-containing drugs being even ulcerogenic. This was statistically significant in the case of cysteine and cysteamine.

3) It was shown in both ulcer types that cysteine was more effective than glutathion if similar doses were used. As far as their chemical structure is concerned cysteine is a simple amino acid, while glutathion is a cysteine containing tripeptide. It is possible that the difference in effectivity is due to the relatively lower amount of sulfhydryl groups per molecular weight. This

assumption was further emphasized in the complementary experiment where the effect of 200 mg/kg glutathion was compared to that of 100 mg/kg cysteine. Glutathion was as effective as cysteine in this case. The ratio of the molecular weight of the two substances is 1.00; 1.75.

4) There was a remarkable difference between indomethacin induced and stress-ulcer as far as their protection is concerned. Sulfhydryl containing substances *inhibited indomethacin-ulcer* but they potentiated the *ulcerogenic* effect in the case of *stress-ulcer*. This experimental result provides further data for the difference of the pathomechanism of various experimental ulcers and thus their direct extrapolation to the human disease and to its treatment needs extreme caution.

5) On the basis of the present results it seems substantiated a differentiation to be introduced between *"real" and "occasional" cytoprotective substances.* According to our opinion *"real"* cytoprotective substances are those materials which exert protection in *all kinds* of experimental ulcer models, while *"occasional"* cytoprotective agents give protection *only in one* experimental ulcer type. The term "cytoprotection" should however be maintained for the description of agents which exert protection in a dose *not* capable of influencing gastric secretion. According to this classification we consider prostaglandin-analogues as "real", and the sulfhydryl-containing substances as "occasional" cytoprotective agents.

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MODIFICATION OF THE CYTOPROTECTIVE EFFECT OF PROSTACYCLIN ON GASTRIC EPITHELIAL CELLS OF RATS BY COLCHICINE

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The effect of prostacyclin (PGI_2) and colchicine on fundic gastric mucosa of sexually mature female Wistar rats was investigated histochemically after restraint stress. Under PGI_2 treatment the ulcer index decreased significantly; this effect was moderately inhibited by colchicine. The mucus content of epithelial cells was decreased during restraint and was increased by PGI_2 treatment. This protective action was also inhibited by colchicine. The nuclear volume of fundic epithelial cells increased significantly in restraint and decreased following colchicine application. These phenomena were not influenced by PGI_2.

According to our results the cytoprotective effect of PGI_2 is not accompanied by nuclear volume changes.

In experimental animals cell proliferation is blocked in the course of restraint stress, even before erosions appear. DNA synthesis is inhibited, chromosomes do not duplicate, mitotic activity and cell divisions are absent. These changes together with the acid secretion of the stomach result in the development of gastric erosions after the application of stress in the rodent [4, 5, 6, 7].

For several years there have been numerous attempts to block these ulcerogenic processes. In one of these studies the so-called "cytoprotective" effect of prostaglandins has been discovered [8].

In the present study the effect of prostacyclin (PGI_2) on the nuclei of fundic epithelial cells was examined by histochemical methods.

Materials and methods

Wistar female rats weighing 150—250 g were used; for 24 hours prior to the experiment food was restricted while water was allowed ad libitum. The animals were assigned into the following groups (Table I):

100 μg/kg PGI_2 was given i.p., solved in 0.05 M tris-buffer [1].

Colchicine (Alkaloida, Hungary; lot. No: 14770668) was given i.p. in aqueous solution in a dose of 50 μg/kg.

In the 24th hour of the experiment the animals were killed, their stomach removed and the gastric mucosal damage ("Ulcer Index") scored as follows: each mm^2 lesion represented 1 point, bleeding: further 5 points, perforation: further 10 points (2). The stomach was fixed in 10% neutral formol-saline, embedded in alcohol and paraffin. Mucus staining was done for periodate reactive glycyoproteide (PRGP) with the PAS method, for acid mucopolysaccharide (AMPS) with the Alcian blue method. For karyometry slides stained by Feulgen's method

Table I

Experimental Design

Group	n	Treatment (at the start of the experiment and in the 6th, 12th and 18th hr)
1.	15	None (Control)
2.	15	Restraint stress for 24 hours
3.	15	Restraint stress + PGI_2
4.	10	Restraint stress + Colchicine
5.	10	Restraint stress + PGI_2 + Colchicine
6.	10	PGI_2
7.	10	Colchicine

were prepared. Measurement of mature fundic surface epithelial cells was done according to HINTZSCHE [3] with the help of the formula $4\,a^2b\pi/3$. Data were given in μ^3. Each group represents 900—1000 nuclei, i.e. 100 cells per animal.

The mean values ±S.E.M. were calculated and analysed statistically using Student's *t*-test for paired data.

Results

Experimental results are given in Tables II through IV as well as in Figs 1 through 6.

Table II

Effect of restraint stress, PGI_2 and colchicine treatment on gastric ulcer of rats

Group	n	U.I.□	ΔU.I.%
1.	15	—	—
2.	15	12.2 ± 1.76	100
3.	15	3.8 ± 0.58*	31.1
4.	10	11.7 ± 1.38	95.9
5.	10	8.2 ± 0.97	67.2
6.	10	—	—
7.	10	—	—

* = $p < 0.05$ vs. restraint (Group 2)
□ = ulcer index

Normal AMPS content can be seen in Fig. 1.

The mucus content decreased markedly in restraint stress (Fig. 2).

An elevated mucus production is shown after PGI_2 treatment during restraint (Fig. 3).

Table III

Changes of mucus content of fundic epithelial cells

Group	n	PRGP	AMPS
1.	15	+4/15	+2/15
2.	15	+4/15	—8/15
3.	15	*+10/15*	*+13/15*
4.	10	—5/10	—5/10
5.	10	—5/10	—5/10
6.	10	*+8/10*	*+9/10*
7.	10	—4/10	—3/10

\+ increase of mucus content of the fundic epithelial cells
— decrease of mucus content of the fundic epithelial cells

Table IV

Changes of nuclear volume of fundic mature epithelial cells

Group	n	Nuclear volume ±S.E.M. μ^3
1.	9	176.41 ± 7.19
2.	9	333.72 ± 25.91***
3.	9	329.41 ± 36.6**
4.	9	147.29 ± 6.8*
5.	10	139.49 ± 3.40***
6.	10	181.73 ± 5.39
7.	10	176.17 ± 10.94

* $p < 0.02$ vs. control vs. control]
** $p < 0.005$ vs. control
*** $p < 0.001$ vs. control

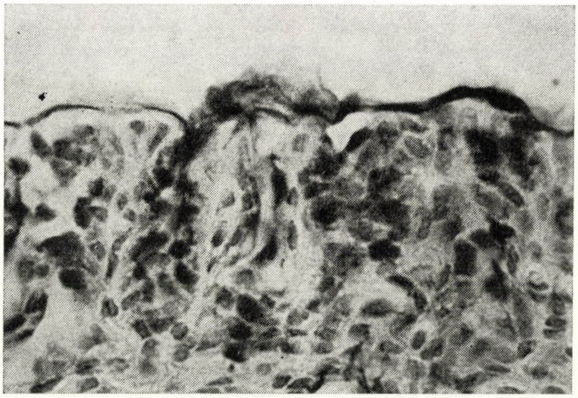

Fig. 1. A normal AMPS content can be seen in the fundic epithelial cells. Alcian blue, ×160

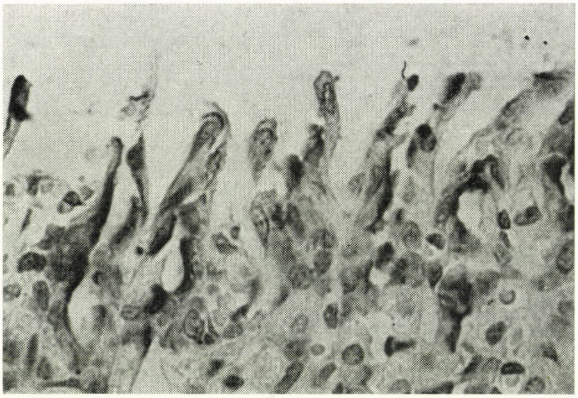

Fig. 2. There is no mucus staining of the fundic epithelial cells. Alcian blue, ×160

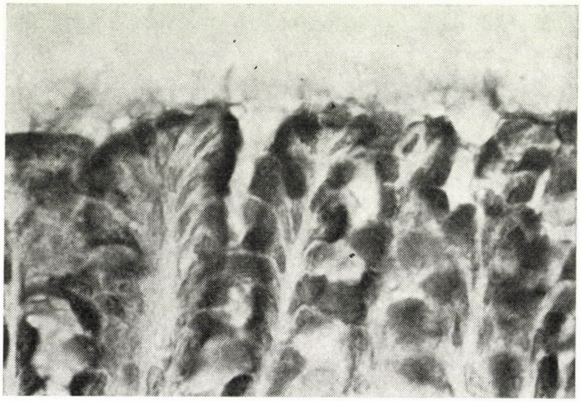

Fig. 3. An increased mucus production is shown after PGI_2 treatment. Alcian blue, ×160

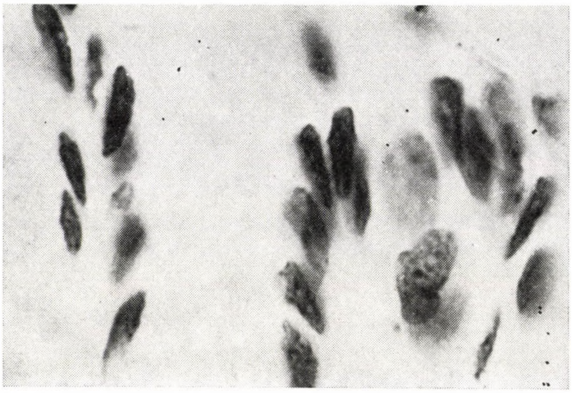

Fig. 4. Control: well maintained nuclear structure of fundic epithelial cells. Feulgen, ×390

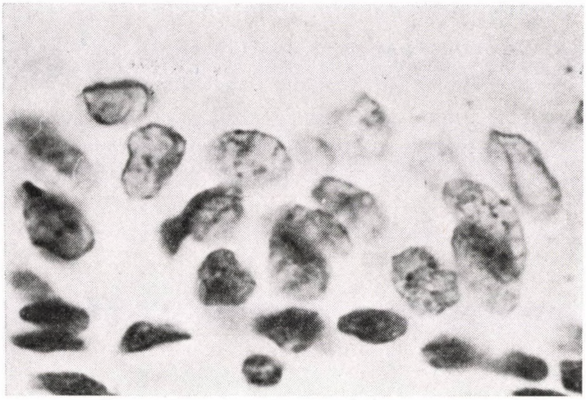

Fig. 5. There is a marked enlargement of nuclei. Feulgen, ×390

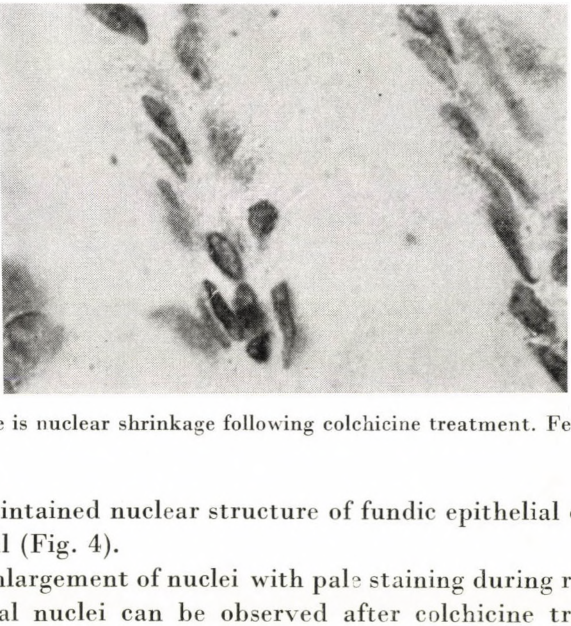

Fig. 6. There is nuclear shrinkage following colchicine treatment. Feulgen, ×390

A well maintained nuclear structure of fundic epithelial cells is shown in a control animal (Fig. 4).

There is enlargement of nuclei with pale staining during restraint (Fig. 5).

Small, oval nuclei can be observed after colchicine treatment during restraint (Fig. 6).

Discussion

The stimulating effect of prostaglandin analogues on mucus production is well known [9]. Our results with PG-I_2 confirm these observations. The enlargement of the nuclei of fundic epithelial cells can be explained by an increased sodium influx. This is primarily caused by degenerative processes. We have no explanation for the decrease of nuclear volume after colchicine treatment. The karyometric data show, however, [1] that the application of PG-I_2 in itself does not increase nuclear volume, [2] neither does it influence the significant nuclear swelling caused by stress and, [3] that it does not significantly affect the nuclear shrinkage following colchicine treatment.

According to our results the cytoprotective effect of PG-I_2 is not accompanied by nuclear volume changes.

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THE RELATIONSHIPS BETWEEN INDOMETHACINEINDUCED GASTRIC ULCER, ULCER PROTECTION BY CIMETIDINE AND PROSTACYCLIN AND THE cAMP SYSTEM OF THE GASTRIC FUNDIC MUCOSA IN THE RAT

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Gastric ulcer was provoked by indomethacine (20 mg/kg s.c.) in rats. The ulcer protection by prostacyclin and cimetidine as well as the changes of tissue cAMP level in the gastric fundic mucosa — during ulcer-provocation and ulcer protection — were studied.

The animals received prostacyclin (125, 250 and 500 μg/kg) and cimetidine (2.5 and 50 mg/kg) together with indomethacine. Evaluation of the results was undertaken 4 hours after the administration of the provoking agent. The number and severity of the ulcers as well as the cAMP level of the gastric fundic mucosa were measured.

The following results were obtained:

(1) cAMP level of the gastric fundic mucosa remained unaltered at the time of ulcer provocation; (2) cimetidine and prostacyclin reduced the number and severity of the ulcers in a dose-dependent manner; (3) cAMP level of the gastric fundic mucosa was reduced after cimetidine and prostacyclin treatment in a dose-dependent manner, the extent of which however did not show any correlation with the degree of ulcer-preventive action.

The experimental results indicate that

(1) the development of indomethacine-induced gastric ulcer is independent of the ATP — adenylate cyclase — cAMP system of the gastric fundic mucosa; (2) the ulcer protective action of cimetidine and prostacyclin is independent of tissue cAMP system of the gastric fundic mucosa in this model.

Clinical observations have shown that nonsteroidal anti-inflammatory agents induce gastrointestinal bleeding by causing damage to the gastric mucosa [1].

Djahanguiri [2, 3] induced gastric ulcer by indomethacine. Indomethacine-induced ulcers develop in the glandular stomach (fundus) [2, 3]. It is well known that indomethacine inhibits prostaglandin synthetase thereby reducing the synthesis of prostaglandins [13].

In our earlier experiments we analysed in detail the biochemical changes in the gastric fundic mucosa of pylorus-ligated animals [6, 7, 8], of pylorus-ligated + adrenaline and salicylate treated animals [9], of stress-animals [9]

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and in animals treated by 0.6 M HCl, 0.2 M NaOH, 96% ethanol and 25% NaCl [9]. We found that during the development of gastric mucosal lesion the functional equilibrium between gastric mucosal energy-producing systems (ATP — membrane ATPase — ADP and ATP — adenylate cyclase — cAMP) deteriorated, leading to significant changes in the neural, hormonal and drug regulation of the two energy producing systems [7, 9, 11, 12].

In an attempt to extend these earlier studies the biochemical analysis of an ulcer model was undertaken in which the prostaglandin synthesis was inhibited without ligation of the pylorus. The most optimal model for such an analysis seemed to be the indomethacine-induced gastric ulcer. The purposes of the present study were: (1) to analyse the role of the ATP — adenylate cyclase — cAMP system of the gastric fundic mucosa in the development of indomethacine-induced gastric ulcer; (2) to analyse the ulcer-protective action of cimetidine and prostacyclin and its relationship with the tissue cAMP changes of gastric fundic mucosa.

Materials and methods

Experiments were carried out on CFY-strain rats of both sexes weighing 180 to 210 g No food was given for 24 hours before the experiments but water was allowed ad libitum.

On the morning of the study the animals received 20 mg/kg indomethacine subcutaneously. The animals were killed 4 hr thereafter.

The number and severity of ulcers were determined according to the score-system described previously [4].

The cAMP level of the gastric fundic mucosa was determined by radioimmunoassay (Becton Dikinson, Orengeburg, USA). The results were expressed for mg protein content. The protein content was determined by the method of LAYNE [5].

Drug administration was carried out at the time of indomethacine treatment. Cimetidine (Tagamet; Smith, Kline and French Laboratories, Ltd.) was dissolved in physiological salt solution. Prostacyclin (CHINOIN, Budapest) was dissolved in tris-buffer (pH 8) immediately before administration.

The results were expressed as mean ±S.E.M. Statistical analysis of the data was carried out by Student's *t*-test and by the Mann—Whitney method, the latter being used for the analysis of the severity of ulcers.

Results

Tissue cAMP content of the gastric fundic mucosa remained unaltered during the development of indomethacine-induced ulcer (Fig. 1).

Cimetidine reduced the number and severity of ulcers as well as the tissue cAMP-level of the gastric fundic mucosa in a dose dependent manner (Fig. 2). There was no correlation between the extent of ulcer-protection and of cAMP-level changes.

Prostacyclin reduced the number and severity of ulcers. Tissue cAMP level of the gastric fundic mucosa decreased as well, however there was no correlation with the ulcer-protective action (Fig. 3).

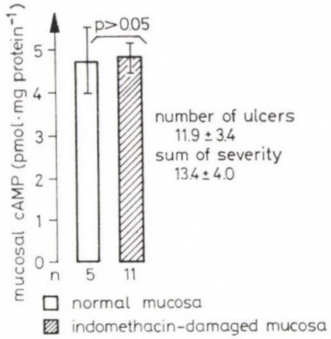

Fig. 1. Tissue cAMP-levels in the gastric fundic mucosa of rats in the control (NaCl-treated) and indomethacine-treated (20 mg/kg, s.c.) groups (mean ±S.E.M.). The number and severity of lesions (ulcers) demonstrated in the right side of the figure represent the results obtained in the indomethacine-treated group, since there were no ulcers found in the NaCl-treated control group. "n" represents the number of animals examined

Fig. 2. Effect of cimetidine on the number and severity of gastric ulcers provoked by indomethacine as well as on the cAMP content of the gastric fundic mucosa. The animals in the control group were treated by physiological salt solution (cimetidine was dissolved in NaCl as well). The numbers in parenthesis represent the number of animals

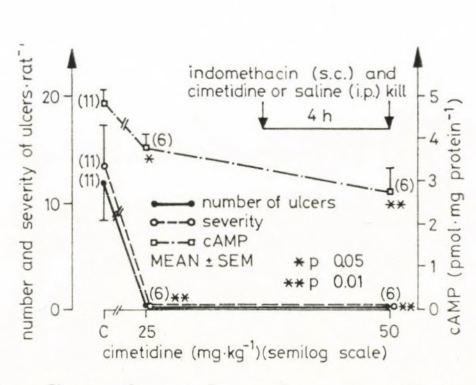

Fig. 3. Effect of prostacyclin on the number and severity of indomethacine-induced gastric ulcer as well as on the cAMP content of the gastric fundic mucosa. Control animals received 1 ml Tris-buffer (pH 8). The symbols are indentical to those of Fig. 2

Discussion

Indomethacine-induced gastric mucosal lesions are well reproducible and easy to evaluate. The exact mechanism of such ulcers is not known, nevertheless tissue hypoxia as an etiological factor can be excluded [3].

It is well known that indomethacine inhibits the prostaglandin synthetase thereby reducing the synthesis of prostaglandins [13]. Previous studies showed that the transformation rate of ATP — adenylate cyclase — cAMP system decreased significantly in the gastric fundic mucosa of animals with ligated pylorus [7, 8], with ligated pylorus and treated by adrenaline [9] or salicylate [9]. Furthermore it was decreased in animals during the development of gastric mucosal lesions evoked by the administration of 0.6 M HCl, 0.2 M NaOH, 96% ethanol and 25% NaCl [9].

In all these models tissue level of cAMP was increased during the protection of the gastric mucosal damage [4, 9, 10, 11]. During the development of stress ulcer gastric mucosal cAMP-level increases [9], while it decreases in the case of ulcer-protection [9].

The present experiments showed that tissue cAMP-level of the gastric fundic mucosa remained unaltered during the development of indomethacine-induced gastric ulcer. At the same time cimetidine and prostacyclin decreased the number and severity of indomethacine-provoked ulcers in a dose-dependent manner and tissue cAMP-level of the gastric fundic mucosa reduced by both drugs in parallel with the ulcer-protective action; however, the extent of protection did not correlate with the decrease of tissue cAMP-level.

These investigations show that gastric mucosal lesions may develop in the absence of changes of the gastric mucosal cAMP system, which is in sharp contrast to the results of our previous investigations. On the other hand, however, the present data demonstrate that ulcer protection can be achieved irrespective of significant cAMP-level reduction. Nevertheless, it is essential to note that the normal cAMP-level of the gastric fundic mucosa obtained during the development of ulcer protection was reduced. Prostacyclin enhances the ATP—ADP and ATP—cAMP transformation [4, 12] in ulcer models in the absence of prostaglandin synthesis inhibition. Thus the effect of prostacyclin on the energy systems depends on prostaglandin synthesis, while that of cimetidine is not.

The present and previous results indicate that (1) gastric mucosal lesions may develop as a consequence of various biochemical mechanisms; (2) normal gastric fundic mucosal cAMP-level represents normal tissue metabolism during the development of ulcer and ulcer-protection, while normal cAMP-level alone is not the sole index of the integrity of the gastric fundic mucosa.

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EFFECT OF THE Ca^{2+} CHELATORS EDTA AND EGTA ON SINOATRIAL-NODE ACTIVITY AND HEART IRRITABILITY

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The effect of the Ca-chelators EDTA and EGTA on sinoatrial activity and heart irritability was studied in dog experiments by perfusing the artery supplying the sinoatrial node. Beside the usual ECG recordings left and right atrial and left and right ventricular epicardial electrograms, His-bundle electrogram and the early activity of the sinoatrial node were recorded. In addition action potentials were recorded from the left auricle and right ventricle of the dog hearts. Local application around the sinoatrial and atrio-ventricular nodes of EDTA and EGTA caused sinus bradycardia and later sinus arrest within 1 min after introduction of the drugs.

For substitution of the sinus rhythm junctional (seldom lower atrial) escape rhythm developed. One to two minutes later it was followed by atrial premature beats and even later atrial flutter and fibrillation could be recorded.

In one third of the cases atrial fibrillation was followed by secondary ventricular fibrillation. Atrio-ventricular impulse conduction was prolonged in the av node by EDTA and EGTA.

Atrial action potentials and the slow (Ca dependent) ventricular action potentials were depressed by EDTA and EGTA.

Introduction

Calcium (Ca^{2+}) influx, the "slow calcium channels", play a decisive role in the depolarization of the cells of the sinoatrial and atrio-ventricular (av) nodes.

Thus, the inhibition of Ca^{2+}-influx can considerably influence the pacemaker activity of the sinoatrial node and impulse conduction of the av node. The Ca^{2+}-antagonist verapamil and similar compounds significantly inhibit the activity of the sinoatrial node and the impulse conduction of the av node if they are applied in high concentration to the respective areas. Adenosine derivatives inhibit sinoatrial node activity by blocking the Ca^{2+}-channels [19].

It seemed therefore interesting to study the effect of other Ca^{2+}-antagonists (the Ca^{2+}-chelator EDTA and EGTA) on cardiac pacemaker activity, impulse conduction and irritability.

Materials and methods

Electrophysiological–pharmacological experiments

The experiments were carried out on 22 anaesthetized (30 mg/kg pentobarbital i.v.), artificially ventillated open-chest dogs. The initial and middle portions of the right coronary artery were isolated. Two muscle branches, supplying the right ventricle, originate from the artery immediately before the origin of the artery, supplying the sinoatrial node. Thin rubber bands were placed underneath the origin of this artery and the right coronary artery distal to the former. A thin catheter was placed into the right coronary artery proximal to the origin of the sinoatrial node artery. The other and of the catheter was introduced into the right common carotid and the right coronary artery was perfused at constant 10—15 ml/min rate by a peristaltic pump. By intermittent stretching of the rubber loops, isolated perfusion of the sinoatrial node artery, the proximal portion of the right coronary artery or the muscle branches of the right ventricle could be performed. By injecting Evans-blue into the sinoatrial node region, part of the right atrium and auricle and the av-node region were stained. The experimental model is illustrated in Fig. 1.

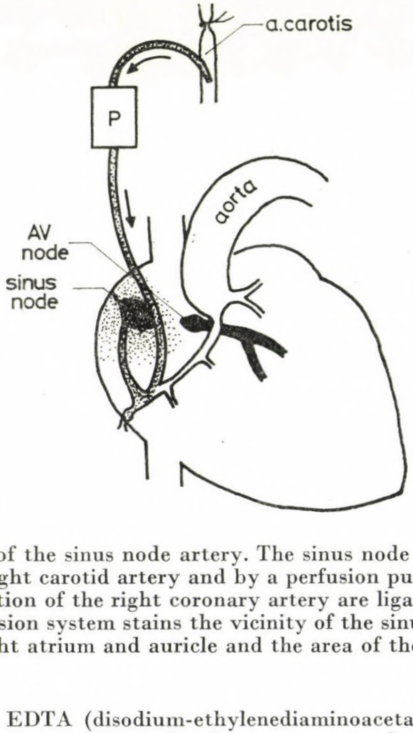

Fig. 1. Isolated perfusion of the sinus node artery. The sinus node artery is perfused through a catheter fixed into the right carotid artery and by a perfusion pump. Muscle branches originating from the initial portion of the right coronary artery are ligated by a rubber band. The dye injected into the perfusion system stains the vicinity of the sinus node, the adjacent parts of the right atrium and auricle and the area of the av-node

The Ca^{2+}-chelators EDTA (disodium-ethylenediaminoacetate) and EGTA (ethyleneglycolacetate) were injected into the isolated coronary circulation in the dose-range of 0.01—0.04 mM (30—120 μg).

ECG recordings served for the analysis of pacemaker activity and impulse conduction before and after drug application. Beside the usual extremital bipolar ECG recordings epicardial atrial and ventricular electrocardiograms were performed by placing unipolar electrodes on the right and left atria and on the right and left ventricles.

For better analysis of atrio-ventricular impulse conduction, His-bundle ECGs were recorded by placing a 6-pole electrode in the vicinity of the av-node and His-bundle through the right subclavian vein. A 15—100 Hz frequency-range preamplifier was used for recording of the His-bundle electrograms.

Unipolar electrodes were fixed in the vicinity of the sinoatrial node for detailed analysis of the pacemaker activity of the sinoatrial node. In part of the experiments the electrical signal of sinoatrial node activity, the small wave before the right atrial wave, was recorded by a 15 Hz frequency amplifier.

Cellular microelectrophysiological studies were also performed in 12 dogs by action potential recording. The chest was opened in sodium-pentobarbital anaesthesia and after ligation of the large vessels the heart was excised. Action potentials were recorded in tissue pieces removed from the left auricle and from the trabecular musculature of the right ventricle.

A capillary glass microelectrode filled with 3 M KCl was used for recording of the action potentials. The preparations were superfused by Tyrode solution bubbled with 95% O_2 and 5% CO_2 mixture at 37 °C. Square-wave impulses of 0.5 msec duration and 1 Hz frequency were used for electrical stimulation of the muscle preparations. Membrane potential and its first derivative were simultaneously recorded on an oscilloscope [12].

Results

Administration of EDTA or EGTA into the artery of the sinoatrial node caused first bradycardia and later a transient cessation (sinus arrest) of pacemaker activity. Bradycardia as well as asystole were observed. Afterwards the sinus rhythm was replaced by a supplementary rhythm; more frequently junctional rhythm and seldom lower atrial-coronary sinus rhythm developed. Atrial irritability increased significantly 2 to 3 min after injection of drugs: multifocal atrial premature beats and transient atrial flutter were observed. The frequent and variable atrial arrhythmia corresponded to the clinically well known chaotic atrial arrhythmia. After a few minutes the pacemaker activity of the sinoatrial node was resumed but in some cases a "moving" of the sinoatrial pacemaker activity (wandering P-wave) could be observed. Under the effect of either EDTA or EGTA atrial fibrillation developed in all cases. In the majority of cases (66%), however, atrial fibrillation was transient. Fibrillation often developed in one atrium only. Ventricular fibrillation occurred in 40% of the cases. It was always secondary, i.e. atrial fibrillation was followed by frequent ventricular tachycardia and later by ventricular fibrillation. Ventricular fibrillation never developed if the drugs were applied directly through the coronary branches supplying the right ventricle.

Severe arrhythmia (sinus arrest, atrial chaotic arrhythmia, atrial and ventricular fibrillation) never occurred if EDTA or EGTA were administered intravenously (30 mg/kg by slow i.v. infusion). Only a slight bradycardia accompanied by simple atrial premature beats were observed in these cases.

The change of impulse conduction was characteristic after EDTA or EGTA injection. Atrio-ventricular impulse conduction was prolonged and the PQ interval lengthened after injection of EDTA or EGTA into the sinoatrial-node artery.

The protraction of impulse conduction occurred in the av node. These disturbances of pacemaker activity and impulse conduction were characteristic and constant and they could be reproduced in the same animal; the acute effect lasted only 10 min. ECG changes following EDTA or EGTA injection are illustrated in Tables I and II.

Table I

Effect of EDTA on cardiac pacemaker activity and impulse conduction (14 dogs)

Type of arrhythmia	Number of cases
Sinus bradycardia	12
Sinus arrest	12
Junctional escape rhythm	11
Lower atrial escape rhythm	2
"Moving" P-wave	4
Frequent atrial premature beats	14
Chaotic atrial rhythm	11
Atrial fibrillation transient	10
permanent	4
Ventricular secondary fibrillation	5
PQ (A—H) prolongation	12

Table II

Effect of EGTA on cardiac pacemaker activity and impulse conduction (10 dogs)

Type of arrhythmia	Number of cases
Sinus bradycardia	9
Sinus arrest	9
Junctional escape rhythm	7
Lower atrial escape rhythm	2
Wandering P-wave	3
Frequent atrial premature beats	10
Chaotic atrial rhythm	9
Atrial fibrillation transient	7
permanent	3
Ventricular secondary fibrillation	4
PQ (A—H) prolongation	8

The rhythmic disturbances and changes of impulse conduction can not always be analysed by usual ECG recordings. Epicardial atrial and ventricular ECGs as well as the His-bundle ECG and the recording of sinus node activity were of great help in the recognition of junctional rhythm, sinus arrest or the impulse conduction disturbances of the av-node. The EDTA- and EGTA-induced characteristic arrhythmogenic effects are demonstrated in Figs 2 to 5.

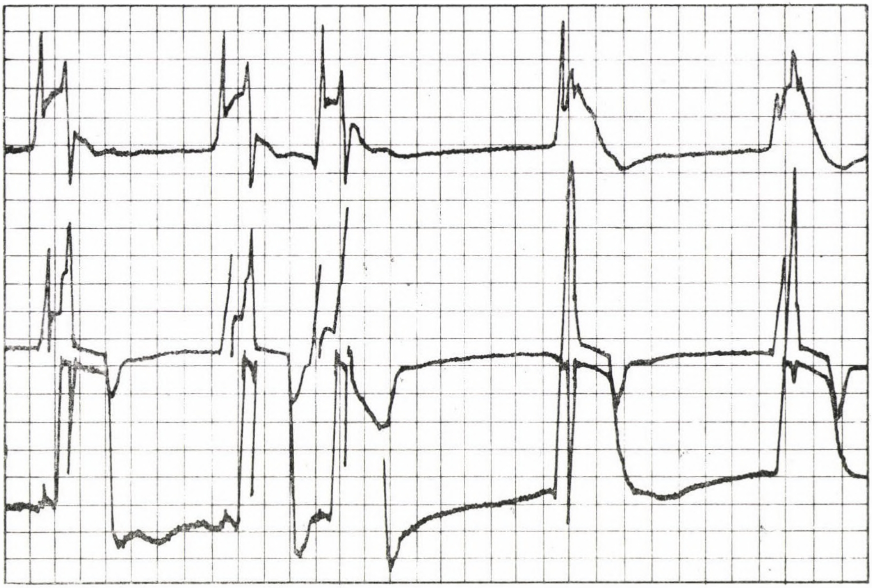

Fig. 2. Early effects of EDTA on sinus node activity and on atrial (heart) irritability. Recording of right atrial (upper tracing) left atrial (middle tracing) and left ventricular epicardial electrograms (lower tracing) at 50 mm/sec paper speed. EDTA decreases sinus pacemaker activity which is followed by complete sinus arrest. Junctional escape rhythm occurs, but atrial premature beats can also be observed

Fig. 3. Atrial fibrillation after EGTA treatment. Right atrial (upper tracing), left atrial (middle tracing) and right ventricular (lower tracing) ECG recorded at 25 mm/sec paper speed. On the left side of the figure normal sinus rhythm can be seen 30 sec after EGTA injection, while the right side demonstrates the occurrence of atrial fibrillation 90 sec after EGTA injection (low-amplitude irregular atrial fibrillation waves on the right atrial ECG, the left atrial ECG while in more regular flutter at a lower frequency can be observed)

Fig. 4. Chaotic arrhythmia after EGTA treatment. Right atrial ECG (upper tracing) His-bundle ECG (middle tracing) and left ventricular ECG (lower tracing); recordings at 50 mm/sec paper speed 60 sec after EGTA administration. Following EGTA treatment frequent, multifocal atrial premature beats can be observed, which is followed by the occurrence of chaotic atrial arrhythmia

Fig. 5. Atrial and secondary ventricular fibrillation after EDTA administration. Right atrial (upper tracing), left atrial (middle tracing) and left ventricular epicardial ECG (lower tracing); recorded at 25 mm/sec paper speed. Atrial premature beats and later atrial fibrillation developed 90 sec after EDTA injection. They were followed by the occurrence of tachycardia and ventricular fibrillation

The effects of EDTA or EGTA on atrial and ventricular action potentials are also characteristic. The atrial action potential was significantly depressed after the addition to the solution of $2-4\times10^{-5}$ M EDTA or EGTA. Testing potential and depolarization velocity were decreased (the rate of rise of phase O) and the plateau was shortened. EDTA or EGTA even in higher dose had no effect on papillary muscle action potentials. The action potentials of papillary muscle depolarized by 25 mM K^+-Tyrode's solution (inhibition of the Na^+-channel) and then stimulated by isuprel (depolarization due to Ca^{2+}-transport) were almost totally depressed by EDTA or EGTA.

Discussion

Ca^{2+}-transport i.e. Ca^{2+}-influx into the cell through the "slow" calcium channel — seems to be of primary importance in the pacemaker activity of the sinus node.

Verapamil, the blocker of slow Ca^{2+}-channel, induces pronounced bradycardia and even sinus arrest [2, 14, 24, 25]. As a consequence of sinus arrest following verapamil administration, junctional subsidiary rhythm may develop [4, 5, 19].

Beside the activity of the sinus node, verapamil and its derivatives inhibit atrio-ventricular impulse conduction as well, and impulse conduction is protracted in the av node [1, 10, 18).

Verapamil and related Ca-antagonistic compounds block the slow Ca^{2+}-channel inhibiting thereby the slow Ca^{2+}-entry without influencing the rapid Na^+-entry [9, 16].

Injection of adenosine and adenosine derivatives such as adenosine triphosphate into the sinus node artery causes bradycardia and a consequent escape rhythm [7, 19]. Our previous studies indicate that adenosine acts on the activity of the sinoatrial and av nodes by the inhibition of Ca^{2+}-channels and so the effect is closely similar to that of verapamil [19].

The Ca^{2+}-chelator EDTA was originally used as an antiarrhythmogenic agent in the treatment of digitalis intoxications, in digitalis induced arrhythmia. Since Ca^{2+} play an important role in the action mechanism of digitalis, the application of EDTA was effective in the treatment of digitalis arrhythmia [13, 20, 22, 23]. Otherwise neither EDTA nor EGTA have antiarrhythmogenic effects. According to JAMES [7] administration of EDTA into the sinus node artery is followed by bradycardia after a transient tachycardia and atrial premature beats also occur. Calcium EDTA was ineffective.

According to our investigations EDTA or EGTA injected into the area of the sinoatrial node or av-node causes a decrease of pacemaker activity of the sinus node: bradycardia and sinus arrest develop. For substitution of sinoatrial node pacemaker activity an escape rhythm can be observed, usually a junctional and rarely a lower atrial rhythm.

These effects of the Ca^{2+}-chelator EDTA or EGTA are similar to those of the Ca^{2+}-channel blockers verapamil or adenosine [19]. Late effects of EDTA occurring 1 to 2 minutes after its injection into the sinus node artery are different. They are characterized by increased irritability of the heart, first of all of the atria: frequent multifocal atrial premature beats, atrial flutter and fibrillation, often localized to one atrium only, are detected. Chaotic atrial rhythm develops often. Ventricular irritability (arrhythmogenic ability) is not increased; there occurred, no ECG changes if EDTA or EGTA were injected into the right ventricular myocardium.

The effect of EDTA and EGTA on cardiac action potentials is very characteristic. Action potentials of ventricular trabecular or Purkinje-fibre preparations are not influenced by the Ca^{2+}-chelators. In auricular preparations, however, they depress the action potential, since that of the auricles and atria are dependent not only on Na^{+}-entry, but slow Ca^{2+}-entry also contributes to their development.

Their effect on the plateau phase (2nd phase) is of utmost importance; the amplitude of the plateau depends on the slow Ca^{2+}-entry. In contrast to the data of CRANEFIELD [3], we observed a significant shortening of the length of the plateau in the presence EDTA or EGTA. High external potassium concentration depolarizes the ventricular muscle cells and sodium transport is totally inhibited. Catecholamines (noradrenaline) activate the action potential in depolarized preparations which are Ca^{2+}-dependent [17, 21]. EDTA or EGTA totally abolished the action potentials in these preparations. Both the electrophysiological studies following sinus node artery perfusion and the cellular microelectrophysiological observations indicate that EDTA and EGTA influence the pacemaker activity of the sinus node and the impulse conduction of the av-node by a Ca-antagonistic effect (Ca^{2+}-transport blockade). The exact cause of this is not known.

It is suggested that the Ca^{2+}-chelators have a dual action on cells of the sinus and av-nodes. After binding outside the cell membrane they penetrate into the cells. After a certain time the bound Ca^{2+} is released and the intracellular Ca^{2+} concentration increases.

An other mechanism may also play a role in the mentioned arrhythmogenic effect of the Ca^{2+}-chelators: the tight couplings between the cells are disturbed [11, 15] and this leads to a disturbance of impulse conduction between the sinus node and the atria, thus the development of the reentry-type arrhythmia is assumed.

The changes in cardiac pacemaker activity, impulse conduction and irritability after EDTA or EGTA administration indicate that complex Ca^{2+}-binding may play some role in the pathomechanism of some arrhythmias.

Although the available clinical data are not sufficient, the development of chaotic atrial arrhythmia and atrial fibriallation after EDTA or EGTA administration may support this hypothesis.

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COMPARATIVE STUDIES WITH LINEAR AND CYCLIC SOMATOSTATIN ON THE SELF-STIMULATION OF RATS

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In the present study the dose-related effects of linear and cyclic somatostatin were compared on the self-stimulation rate of rats. Twenty μg of linear somatostatin administered intracerebroventricularly (icv.) markedly decreased the self-stimulation rate, while 5 μg and 10 μg was ineffective. Cyclic somatostation in a dose of 1 μg caused a transitory but not significant increase in the self-stimulation rate, which later returned to the control level. Five μg and 10 μg of the peptide decreased the self-stimulation rate.

These results indicate that the cyclic somatostatin is more effective in inhibiting the self-stimulation rate than the linear one.

The effects of somatostatin in the different behavioral tests are controversial. The reason for this controversy might be the difference in the structure of the various preparations. For example, cyclic somatostatin increases locomotor activity [5], while the same dose of linear somatostatin decreases activity to the point of catalepsy [3].

Some reports have suggested that excitatory or inhibitory action of the hormone depends the employed dose [1, 3, 6, 7, 8, 9]. The administration of lower doses induces primarily behavioral activation and excitation, while larger doses produce only transient excitatory behavioural effects followed by marked restriction of movements [10].

Previously we have reported that cyclic somatostatin significantly decreases the self-stimulation rate, however a transitory, non-significant increase was found with lower doses [14]. In the study the effect of linear and cyclic somatostatin was compared on the self-stimulation behaviour using different doses in rats.

Materials and methods

Adult male Wistar rats weighing 200—250 g at the time of the operation were used for the experiments. Under anaesthesia (sodium hexobarbital), one pair of enamel-insulated steel electrodes was implanted stereotaxically in the right lateral hypothalamus [4, 11] for intracranial stimulation, and sealable cannula was inserted in the left lateral brain ventricle. One week was allowed for recovery following the operation.

By pressing a lever in the experimental cage, the rats could activate an electric stimulator to apply trains of 50—90 square pulses through the connected electrodes (frequency, 125 Hz; pulse width, 0.1—0.2 msec; amplitude, 5.6—6.8 V). The current was continuously monitored on an oscilloscope by measuring the voltage drop across a 100 ohm resistor in series with the rat.

The rat was connected to the stimulation circuit by a swivel commutator in order to prevent twisting of the leads. Lever operations per minute were recorded by an electric counter. When the performance of each rat reached a stable level, drug treatment was started [11].

After the first 15 min of the experimental session, linear somatostatin (5 μg, 10 μg, 20 μg) and cyclic somatostatin (1 μg, 5 μg, 10 μg) dissolved in saline were injected icv. through the ventricle cannula without handling of the animal (liquid volume 5 μl, injection time 60 sec). The rats were free for self-stimulation for 60 min following application. Normal saline solution was given under the same conditions in control sessions.

Placement of the electrodes was examined histologically. The animals were killed with an overdose of hexobarbital and the brains were perfused with 10% formaldehyde solution. The localization of the electrodes was undertaken in 100 μ frozen sections.

Somatostatin (linear and cyclic) (Serono, Italy) was used in our experiments. Mann—Whitney's non-parametric test was used for statistical analysis.

Results

The icv. administration of 5 μg linear somatostatin had no influence on self-stimulation, while 10 μg was able to produce a slight decrease. Twelve min after the administration of 20 μg, the peptide produced a deep depression of self-stimulation ($p < 0.01$) which lasted throughout the whole experimental session (30th min, $p < 0.05$; 57th minute, $p < 0.05$) and returned to the control level only by the 60th minute (Fig. 1/A).

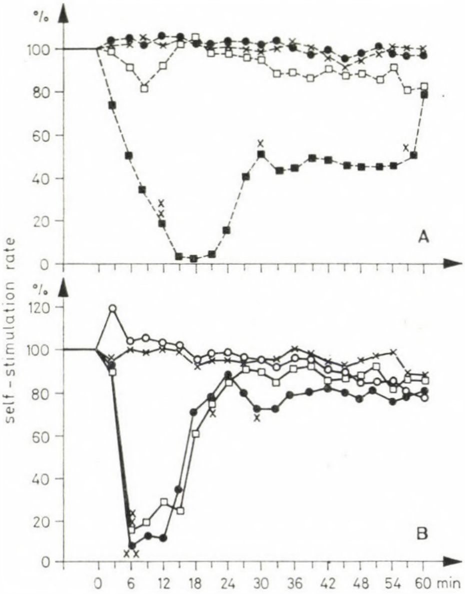

Fig. 1. The dose-related effect of linear and cyclic somatostatin on the self-stimulation behaviour of rats. A (linear somatostatin): ×- - - -× saline (14); ●- - - -● 5 μg somatostatin (7); □- - - -□ 10 μg somatostatin (8); ■- - - -■ 20 μg somatostatin (4). B (cyclic somatostatin): ×——× saline (18); ○——○ 1 μg somatostatin (12); ●——● 5 μg somatostatin (8); □——□ 10 μg somatostatin (8). Number of animals are given in parantheses. * $P < 0.05$; ** $P < 0.01$; NS = $P > 0.05$

The icv. administration of 1 μg cyclic somatostatin increased self-stimulation activity in the first 3 min after the application, however this change was not significant. Five μg somatostatin markedly decreased ($p < 0.01$) self-stimulation 6 min after the administration. Twenty-four min after the administration the level of the self-stimulation activity returned to the control level, thereafter again showing a tendency to decrease. In the 30th minute the self-stimulation activity became significantly lower compared to the control ($p < 0.05$). The administration of 10 μg peptide induced similar changes. The self-stimulation rate decreased between the 6th ($p < 0.01$) and 21st minute ($p < 0.05$) (Fig. 1/B).

Discussion

These observations have demonstrated that both linear and cyclic somatostatin can influence hypothalamic self-stimulation activity. Immediately after the administration of high doses of the peptide (linear, 20 μg; cyclic, 5 μg and 10 μg) grooming, washing, head shaking and left or right turning could be observed in most of the cases. In one case (cyclic somatostatin) barrel rotation could be seen which had been reported also by others [3].

While cyclic somatostatin markedly decreased the self-stimulation rate in a dose of 5 μg, the linear somatostatin was less effective and decreased the self-stimulation only in a dose of 20 μg. These results show that both linear and cyclic somatostatin are able to decrease the hypothalamic self-stimulation activity, however, the cyclic is more potent.

In another biological test (inhibition of growth hormone secretion) linear and cyclic somatostatin have the same potency [13].

Somatostatin stimulates both the synthesis and utilization of dopamine and noradrenaline in the brain [5], while substances that release catecholamines rapidly from functional stores facilitate self-stimulation [12]. The transitory, however not significant increase in the activity of self-stimulation after the administration of low concentrations of cyclic somatostatin might be related to the increased activation of the catecholaminergic system.

From earlier investigations it seems that at higher concentrations somatostatin might act, at least in part, through cholinergic mechanisms [3]. Pharmacological activation of the acetylcholine system usually supresses self-stimulation and pharmacological blockade often facilitates the self-stimulation behaviour [12]. It is possible that the decrease in the level of self-stimulation at higher concentration of the peptide is due to activation of the cholinergic system. However, since somatostatin is present in the brain [2], it may influence the self-stimulation behaviour directly as well. Whether the action reported here is a direct one, or it is mediated by altered transmitter activates, remain to be seen.

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Recensiones

H. Autrum (ed.)

Handbook of Sensory Physiology

Vol. VII. (6) C, Comparative Physiology and Evolution of Vision in Invertebrates. Part C: Invertebrate Visual Centers and Behavior II

Springer Verlag, Berlin—Heidelberg—New York 1981, 663 pages with 216 figures, Price DM 290,—; approx. US $ 131.90

The series Handbook of Sensory Physiology comes to its conclusion by this Volume VII/6 on the comparative Physiology and Evolution of Vision in Invertebrates. This book, the third part of Volume VII/6 appeared with the title Invertebrate Visual Centers and Behavior II. In the first one H. Autrum (München) gives a meticulous survey on the adaptation processes in invertebrates, providing the most up-to-date electrophysiological, behavioral and membrane aspects of adaptation. The next chapter is "Comparative Physiology of Vision in Molluscs" by J. B. Messenger (Sheffield). Like their body constitution the visual organs of the molluscs also reveal an extraodinarily variability. The chapter describes the characteristics occurring in the seven molluscean classes. The third chapter "Organization and Physiology of the Insect Dorsal Ocellar System" by L. J. Goodman (London) deals with the ocellar system which is a peculiar visual organ situated between the two eyes of the insects. Finally the fourth chapter "Spatial Vision in Arthropods" by R. Wehnar (Zürich) offers a critical review of the immense amount of data obtained in this field. In summary, the reader finds an excellent summary of the topic at a very high scientific level. As part of the Handbook of Sensory Physiology series the volume merits to be found on the shelves of every bioscience library. It is of interest for all the physiologists, zoologists and morphologists working in visual physiology and related fields.

F. Obál

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The Oculomotor System of the Rabbit and its Plasticity

Springer Verlag, Berlin—Heidelberg—New York 1981, 240 pages with 128 figures, Price DM 79,—; approx. US $ 36,—

The volume is part of the series Studies on Brain Function. The author gives a survey of his own studies concerning the oculomotor system of the rabbit and expresses his opinions on the functioning of this intriguing mechanism. The six chapters of the volume deal with spontaneous eye movements, eye movements occurring during passive oscillation, processing the signals obtained during eye movement as well as with the adaptation of the oculomotor system. It adds significantly to the value of the book, that a chapter on "Investigations on Optokinetic Nystagmus" by J. W. G. Ter. Braak was included in the volume as an appendix. This paper appeared in German in 1936. J. Collewijn's translation makes it available also for the English readers. The volume offers a valuable reading for all the scientists and clinicians interested in the oculomotor system and related fields.

F. Obál

D. Garlick (ed.)

Festschrift for F. C. Courtice

(The School of Physiology and Pharmacology. The University of New South Wales, P.O. Box 1, Kensington, N.S.W. Australia 2033: 1981, 252 pages, with 101 illustrations.)

This book is dedicated to professor Frederick Colin Courtice, a prominent Australian scientist in the field of physiology and experimental pathology, by colleagues and former students on the occasion of his seventieth birthday.

The volume covers 23 exciting topics focusing largely on the physiopathology of arterial hypertension, microcirculation and lymphatic physiology, written by 38 contributors from Australia (30), Japan (1), New Zealand (4) and the USA (3): some of the titles are "The causes of hypertension" by P. B. Korner, "Touch and Txture" by Jan Darian-Smith, "The role of biliary phospholipids in fat absorption" by W. J. Simmonds, "Steroid and steroid conjugate excretion" by W. Steinbeck, "The evolution of experimental approaches to studying aspects of lymphatic physiology", by Bede Morris, "Arterioles, capillaries and blood-tissue exchange" by G. Outteridge and D. Garlick etc.

The vitality and fruitfulness of this Australian medical research school founded by F. C. Courtice is reflected by the high scientific standard of the comprehensive chapters too. It is worth mentioning that many of the authors contribute also to the Organisation of the 29th International Congress of Physiology to be held in Sydney (28th August—3rd September, 1983), including F. C. Courtice, who is acting as the treasurer of the Congress.

E. Monos

Lawrence I. Gilbert and Earl Frieden (eds.)

Metamorphosis

A problem in developmental biology. 2nd edition 578 pages. Plenum Press, New York and London 1981

Metamorphosis, as pointed out by Wald in this volume, is a misnomer. Actually, not only and not mainly the change of the form is concerned but biochemical, adaptation, nutritional, etc., alterations are involved which basically alter the living organism. Metamorphosis as a subject of research provides partly a phytogenetic and partly an ontogenetic model for the biologist. It was probably the first target of studies in developmental biology, and observations in this field have allowed to construct the modern basis of contemporary developmental biology.

Metamorphosis is observed in several species such as worms, insects, echinodermata, vertebrates, etc. It often occurs with different characteristics even in closely related species but always produces basic alterations. Metamorphosis is regulated by metamorphosis which act at the gene level by rescheduling repression, depression and in some instances the same programme is reversed during the life cycle (secondary metamorphosis). The hormones generally appear in sets of two, and the prevalence of one of them determines the direction of changes.

The hormones of metamorphosis and their actions are now the subject of active investigation. The results are promising but the field is not yet fully explored. Earlier the thyroid hormones (T_4 and T_3) were thought to be the sole essential factors in tadpole metamorphosis. Now we know that their cooperation with prolactin is indispensable. The tadpole kept in the larval state by prolactin is transformed and becomes a land animal under the effect of thyroxine, that goes into the water to lay eggs under the influence of prolactin. In response to a thyroxine injection it is reoriented towards the land from the water.

Thus, metamorphosis in every animal species is associated with marked changes which can be studied by morphological and biochemical methods from various aspects. The 23 contributors to this volume are from among those who have probably dealt the most with these problems and they cover both the basis of the subject and the 13-year progress that has been achieved since publication of the first edition by Etkin and Gilbert.

The contribution of senior investigators along with those of the younger generation proved to be fruitful since in that way the wisdom of the elders in intermingled with the molecular biology oriented dynamic knowledge of the young. The volume actually encompasses everything on metamorphosis which is worth knowing at the postgraduate level. Moreover, it also provides much new information to experts especially interested in metamorphosis. One might criticize the number of references which is not proportionate at the end of the chapters, but this has no influence on the value of the book.

The volume will be of interest not only to experts engaged in metamorphosis studies but also to those who are interested in developmental biology.

GY. CSABA

D. T. KRIEGER

Cushing's Syndrome

Monographs on Endocrinology

Springer Verlag, Berlin—Heidelberg—New York 1982, 142 pages, 27 figures and 24 tables,
Price DM 88,—; approx. US $ 41.00

The most important advantage of the monograph is that it summarizes not only the topic given in the title but also the most recent results of physiological and pathophysiological studies of the CRF-ACTH-adrenocortical system. In doing so the monograph provides important informations for the reader about ACTH and related peptides.

The first introductory chapter states that Cushing disease is of hypophyseal origin while the Cushing syndrome is a primary adrenal disease. The second chapter deals with the regulation of ACTH-release and discusses its biochemistry. The characteristics of the precursor peptide are described and it also deals with the other peptides: ACTH, MSH, LPH and endorfins. This chapter is followed by a short description of the biological aspects of ACTH-release and a review of its physiology. The discussions about CRF in the last decade are reviewed including vasopression in this connection. The report on present knowledge ends with the results of VALE et al. (1981) concerning the peptide structure of CRF. The next pages deal with the neurotransmitter regulation of ACTH-release and a schematic diagram illustrates the inhibiting and releasing properties of various neurotransmitter substances. The next subchapters summarize the neuro-anatomy of ACTH-release, the various modes of feed-back regulation and the diurnal periodicity of the release. Similar problems of LPH and other related peptide hormones are also discussed.

The next chapter deals with the actions of ACTH describing and explaining the effects of various fragments and the action of ACTH on adrenal morphology and steroid genesis. A short subchapter describes the extraadrenal actions of ACTH. The next chapter discusses the biological activity of adrenocortical hormones and their synthesis, but it does not contain too much new informations.

The description of the clinical picture of Cushing disease and syndrome is the next topic involving the characteristics of the familiar diseases, the connection with pregnancy and the clinical problems of the Nelson syndrome. As a supplement to this chapter the author summarizes the experimental data obtained in two animal species with a condition corresponding to Cushing disease. After a description of the pathology of micronodular adrenocortical hyperplasia, adrenocortical hyperplasia and adrenocortical tumours, the problems of pituitary tumors consisting of ACTH-releasing cells and the "silent" pituitary tumours are discussed. This in followed by a long chapter on actopic ACTH release.

The next large chapter deals with the laboratory diagnostics of Cushing disease and syndrome. The description of routine tests is at the textbook level. Interesting subchapters deal with the diagnostic importance of the plasma ACTH level and that of other related peptides. This part of the monograph describes the latest diagnostically and physiologically important but somewhat controversial results concerning abnormal TRH and GnRH reactions, the somatostatin and STH reponses and the prolactin metabolism of patients with Cushing disease.

The chapter about differential diagnostics summarizes the hormonal tests, the modern X-ray techniques of visualizing the pituitary and adrenal gland, and the computer tomographic ultrasound, radioscanning and angiographic methods.

The therapeutical chapter describes the possible drug treatments, the methods and results of adrenal and pituitary surgery, the effect of pituitary irradiation and the drug therapy influencing the neurotransmitter system. The clinical effects of these methods is somewhat overestimated as far as the present situation is concerned.

I. HOLLÓ

V. N. NAJJAR, Martinus NIJHOFF (eds.)

The Wondrous Career of an Amino Acid Developments in Molecular and Cellular Biochemistry

Vol. 1: The Biological Effects of Glutamic Acid and Its Derivatives

Dr. W. Junk Publishers, The Hague—Boston—London 1981, 405 pages. ISBN 90-6193-841-4. Reprinted from Molecular and Cellular Biochemistry Vols. 38 and 39. 1981, Price Dfl. 195,—; approx. US $ 85,00

During the past one and a half decades glutamate has made a fantastic career throwing its amino acid companions into the shade. Practically two major functions had formerly been ascribed to glutamate. First, it occurs in abundance in some proteins and beside aspartate it is one of the amino acids determining the number of negative charges in the polypeptide chain. Secondly, glutamate is a metabolite establishing a link between amino acid and carbohydrate metabolisms through the tricarboxylic acid cycle. Further, it had also been known since long that its nitrogen accumulating function is performed by the action of transaminases.

It has since been clarified that, whether glutamate functions as a free amino acid or it is built in the polypeptide chain, its function is not restricted to such everyday duties. The formation of free glutamate by the action of glutamate dehydrogenase protects the central nervous system against the toxic effect of ammonia on the one hand and, on the other, it is a precursor of γ-aminobutyric acid (GABA) which is involved in neurotransmission. The γ-carboxyglutamyl side chains, derivatives of glutamate formed in proteins by the action of vitamin K, ensure that blood clotting is restricted to the immediate vicinity of the injury, and clots are not normally formed elsewhere in the circulation. It seems probable that γ-carboxyglutamyl side chains are also involved in other processes related to Ca-binding, such as the building of the inorganic components of bones. The study of the function of γ-glutamyl transpeptidase looks back only on a short history. The function of this enzyme is to supply the cells with glutamate by means of an appropriate transport mechanism, and to maintain redox equilibrium by means of glutathione synthesis. It is a relatively recent finding that folic acid occurs and functions in the form of polyglutamate both in procaryotic and mammalian organisms.

This volume is composed of a series of papers. It contains 24 papers mostly of the mini-review type which have originally been published as Vols. 38 and 39 of Molecular and Cellular Biochemistry (1981). The book obviously provides new information in abundance for the researcher of this field, and it may as well be very useful, if not even more useful, for researchers of bordering fields, and finally it may be of particular importance for those who "ex officio" are supposed to be well-informed, and for those who are engaged in teaching biochemistry at the universities.

It is said in the Foreword that this volume comprises the first one of a series. The second one will deal with immunologically active peptides. The material of the second volume has already been published. It certainly will soon appear, if it has not appeared yet, in the form of a separate volume of this series.

Since this volume is representational in its contents, the editor could have arranged the papers according to their topics. It would have probably served the reader if the papers on glutamate and GABA, i.e. the antagonistic action of the two compounds in the nervous system, would have been arranged close after each other. Similarly, the papers on γ-carboxyglutamate and those on the uterin derivatives etc. could have been grouped together, helping there with the reader to find a logical order in the selection of publications. Despite this negligible drawback, which makes orientation in the volume merely inconvenient, this collection of papers certainly provides much valuable new information for those concerned with research in this field.

P. ELŐDI

J. T. Wilke

A Neuropsychological Model of Knowing

University Press of America, Washington, 1981, 77 pages

The book is a review of the data dealing with physiological mechanisms of human cognitive processes. Emphasis is placed upon the fundamental nature of the role of the limbic system and reticular formation in the acquisition, recognition, and knowledge. The implications at the cognitive level for such issues as tacit knowledge, the nature of meaning, and the modes of knowing of each of the two cerebral hemispheres are presented.

The book will be of use to neurophysiologists and psychologists.

T. Kukorelli

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РЕЗЮМЕ

КАРДИОВАСКУЛЯРНЫЕ РЕАКЦИИ У КРЫС ПРИ САМОРАЗДРАЖЕНИИ ГИПОТАЛАМУСА

Е. А. ЮМАТОВ и Е. А. КИТЯТКИН

Мы регистрировали артериальное давление и частоту сердцебиений у крыс во время самораздражения латерального гипоталамуса, при оптимальных параметрах раздражения. При изолированном самораздражении отмечались гипер-гипотензивные, гипертензивные и гипотензивные реакции, чаще других типов встречался двухфазный тип реакции. При одноцикловом самораздражении наблюдались гипергипотензивные и гипертензивные реакции с различной частотой сердцебиений, 75% всех реакций были двухфазного типа, а гипертензивный тип реакций наблюдался только в 25% всех исследованных случаев. Главным типом (92) реакций артериального кровяного давления в периоды беспрерывного 60—140-минутного раздражения являлось постепенное повышение уровня среднего давления на 15—40 мм рт. ст. выше исходного уровня ($p > 0{,}001$), величина которого зависела от продолжительности раздражения. Частота сердцебиений изменялась разнообразнее: тахикардия и брадикардия встречались почти с одинаковой частотой. Сравнительный анализ кардиоваскулярных реакций, возникающих во время гипоталамического самораздражения, и *escape*- реакций, вызываемых раздражением вентро-медиального гипоталамуса, показал своеобразие автономных явлений во время положительных и отрицательных эмоциональных реакций. Результаты сравнительного анализа поднимают вопрос относительно стрессовых реакций поведения при самораздражении.

БИОЛОГИЧЕКОЕ, ХИМИЧЕСКОЕ И ФИЗИКО-ХИМИЧЕСКОЕ ИЗУЧЕНИЕ КОМПОНЕНТОВ БИОЛОГИЧЕСКИХ ЖИДКОСТЕЙ, ИМЕЮЩИХ МОЛЕКУЛЯРНУЮ МАССУ ОКОЛО 500—5000 ДАЛЬТОН, У БОЛЬНЫХ ШИЗОФРЕНИЕЙ

М. ИДЕИ, Й. ГРОФ, Я. МЕНЬХАРТ и А. ФАЙОР

1. Нативная кровяная плазма и сыворотка больных, страдающих шизофренией, содержат вещества, которые, по-видимому, являются специфическими для этого нейропсихиатрического заболевания, и которые, в условиях *in vitro*, ингибируют реактивность *vas defens* к электрическому раздражению.
2. За биологическое действие этих жидкостей ответственна гетерогенная фракция, состоящая из компонентов с молекулярной массой от 500 до 5000 дальтон, ралагаемая на 4 подфракции хроматографией SG-25, абсорбируемая при длине волны 254 нм, содержащая главным образом нингидриновые и *Lowry* — положительные вещества и углеводы, термостабильная, не перевариваемая трипсином, но гидролизируемая соляной кислотой.

КАЧЕСТВЕННЫЕ И КОЛИЧЕСТВЕННЫЕ ИЗМЕНЕНИЯ КОМПОНЕНТОВ КРОВЯНОЙ СЫВОРОТКИ, ИМЕЮЩИХ ОЛИГОПЕПТИДНУЮ ПРИРОДУ (МОЛ. МАССА = 500—5000 ДАЛЬТОН), У БОЛЬНЫХ С ЗЛОКАЧЕСТВЕННЫМИ ОПУХОЛЯМИ

Ж. РИБАИ, К. ВАЛЛЕНТ, Й. ГРОФ, Я. МЕНЬХАРТ и М. ИДЕИ

Авторы провели сравнительное исследование воднорастворимой фракции, экстрагированной кислым этанолом, и кровяной сыворотки больных с злокачественными опухолями, с подобной же фракцией, полученной от людей, не страдающих злокачественными новообразованиями. Были получены следующие результаты:

1. обнаружено характерное химическое количественное изменение. Это изменение проявляется в увеличении содержания фракций $S_\alpha aN$ и $F_\alpha aN$

2. анализы, выполненные хроматографией на геле, ультрафильтрацией и изотахофорезом, или комбинацией этих методов, показали, что:

а) количество фракции, имеющей гетерогенный состав и обозначенной нами буквой В (B_1 и B_2), которая распределяется (Каv = 0,77) в эффективном проникающем объеме (V_{eff}) геля SG-25 и абсорбируется при 206 нм, возрастает;

б) в основе этого увеличения — по крайней мере, отчасти, — лежит увеличение количества одного из компонентов (1-я фракция) с молекулярной массой 500 – 5000 дальтон, элюируемой при Кav = 0,67 (фракция B_1) и ведушей себя как анион в нашей изотахофоретичекой системе, или же появление характерных для этой болезни двух «новых» компонентов (фракции А и В).

ИССЛЕДОВАНИЕ ВЕЩЕСТВ С СРЕДНЕЙ МОЛЕКУЛЯРНОЙ МАССОЙ (MS = 300—500 0 Д) В СЫВОРОТКЕ КРОВИ БОЛЬНЫХ С ПСОРИАЗОМ

И. ФЕЛМЕРАН, Й. ГРОФ, М. ИДЕИ и Я. МЕНЬХАРТ

В сыворотке крови больных с псориазом и лиц, у которых псориаз не был подтвержден клинически, мы определяли качественные и количественные отношения фракци, содержащей вещества с молекулярной массой 300 – 5000 дальтон, пользуясь комбинацией методов фракционной преципитации и хроматографии на геле (SG 25). Исследовалась также химическая *Lowry*, содержание свободного и связанного альфааминоазота и углевода).

Были получены следующие результаты: 1. в одной группе больных с псориазом количество абсорбирующих при 206 нм составляющих, распределяющихся (молекулярная масса = 300 – 5000 Д) в эффективном проникающем объеме (*Veff*) геля Сефадекс G 25 – по сравнению с контрольной группой лиц, не страдающих псориазом, — увеличивается в значительной степени; 2. на заднем плане этого увеличения количества стоит возрастание содержания компонентов, содержащих положительный компонент *Lowry* ($\triangle$ % = 78), свободный альфа-аминоазот ($\triangle$ % = 44) и углевод ($\triangle$ % = 86).

Эти результаты, повидимому, подтверждают гипотезу, согласно которой, у одной части больных с псориазом, в возникновении и/или поддержании клинической симптоматики принимают участие также вещества, пенетрирующие через применяемые в клинике диализирующие мембраны. Химически эти вещества относятся к олигопептидам (мол. масса < 5000 Д).

О ЦИТОПРОТЕКТИВНОМ ДЕЙСТВИИ СОЕДИНЕНИЙ, СОДЕРЖАЩИХ СУЛЬФГИДРИЛЬНУЮ ГРУППУ

Г. А. БАЛИНТ и В. ВАРРО

Авторы показали, что соединения (цистеин, глютатион, цистеамин и димеркапрол (BAL)), содержащие сульфгидрильную группу, оказывают выраженное защитное (действие на язву желудка, вызванную индометацином и в то же-время обладают выраженным ульцерогенным действием в случае язвы, вызванной стрессом.

На основании результатов исследований авторы поднимают вопрос о необходимости различать «истинные» и «не-истинные» цитопротекционные средства.

ВЛИЯНИЕ КОЛХИЦИНА НАЦИТОПРОТЕКЦИОННЫЙ ЭФФЕКТ, ОКАЗЫВАЕМЫЙ ПРОСТАЦИКЛИНОМ НА ЭПИТЕЛИАЛЬНЫЕ КЛЕТКИ ЖЕЛУДКА

Г. КАРАЧОНЬ, Г. А. БАЛИНТ, ДЬ. ЛУКАЧ и В. ВАРРО

В опытах на крысах линии Вистар с экспериментальной язвой, вызванной стрессом (иммобилизация), авторы исследовали влияние простагландина I_2 и колхицна на слизистую оболочку желудка, пользуясь гистохимическими методами. Язвенный индекс снижался в ответ на воздействие простагландина I_2, введение колхицина до некоторой степени предотвращало уменьшение индекса. Слизистое содержимое под воздействием стресса уменьшилось, введение простагландина I_2 предотвращало этот эффект. В свою очередь, введение колхицина подавляло эту защитную реакцию. Объем ядра эпителиальных клеток при иммобилизации достоверно увеличивался, при введении же колхицина достоверно уменьшался. Простагландин I_2 не оказывал влияния на эти явления.

Результаты настоящего исследования показывают, что цитопротективное дйствие простагландина I_2 не сопровождается изменения ядерного объема.

СПРОВОЦИРОВАННОЕ ИНДОМЕТАЦИОНОМ ОБРАЗОВАНИЕ ЯЗВЫ ЖЕЛУДКА. СВЯЗЬ МЕЖДУ ТОРМОЖЕНИЕМ ОБРАЗОВАНИЯ ЯЗВЫ (ЦИМЕТИДИНОМ И ПРОСТАЦИКЛИНОМ) И СИСТЕМОЙ ЦИКЛИЧЕСКОГО АДЕНОЗИН-МОНОФОСФАТА (cAMP) В СЛИЗИСТОЙ ОБОЛОЧКЕ ЖЕЛ1ДКА

Ф. МОРОН, Т. ЯВОР, М. БАТА, М. ФИГЛЕР и Д. МОЖИК

Введением индометациана крысам вызывали у них образование язвы желудка. Индометацин вводился подкожно, в дозе 2 мг/кг. Авторы изучали защитное действие, оказываемое простациклином и циметидином на язву, а также определяли уровень cAMP в слизистой желудка во время образования язвы и при задерживании язвообразования.

Простациклин (125, 250, 500 мкг/кг) и циметидин (2, 5, 50 мг/кг) вводили животным одновременно с индометацином. Обработку животных — после применения провоцирующего агента — производили через 4 часа. Определяли количество язв и степень их тяжести, а также уровень cAMP в слизистой оболочке желудка.

Авторы показали, что:

1. Во время образования язв, вызванных введением индометацина, уровень cAMP в слизистой оболочке желудка не изменяется; 2. циметидин и простациклин дозозависимо уменьшают число язв и степень их тяжести; 3. во время действия циметидина и простациклина, тормозящих образование язвы, дозозависимо снижается уровень cAMP в слизистой желудка, но это изменение уровня cAMP не связано с степенью противоязвенного эффекта.

Авторы пришли к следующим выводам:

1. образование язвы желудка под влиянием воздействия индометацина не зависит от системы ATP — аденилциклаза — cAMP — аденилциклаза — cAMP слизистой желудка;

2. противоязвенное действие простациклина и циметидина в условиях данной модели язвы не зависит от системы тканевого cAMP.

ВЛИЯНИЕ СВЯЗЫВАЮЩИХ Ca^{++}-КОМПЛЕКС EDTA И EGTA НА ДЕЯТЕЛЬНОСТЬ СИНУСНОГО УЗЛА И АРИТМИЧЕСКУЮ ГОТОВНОСТЬ СЕРДЦА

Ф. ШОЛТИ, Ш. ЮХАС-НАДЬ, В. КЕЧКЕМЕТИ, Э. ЧАКО, В. НЕМЕ и В. КЕКЕШИ

В экспериментах на собаках, методом перфузии артерии инусного узла, авторы изучали влияние EDTA и EGTA, связывающих $Ca^{+}{}_{+}$-комплекс, на деятельность синусного узла и аритмическую готовность сердца. Кроме стандартных ЭКГ производили эпикардиальные электрограммы правого и левого предсердий, правого и левого желудочков, пучка Гиса и регистрировали раннюю активность синусного узла. Отводили также потенциал действия из левого ушка и правого предсердия. Применение EDTA и EGTA в районе синусного и атрио-вентрикулярного узлов вызывало синусную брадикаддию, после которой — в течение минуты после введения — наступала сотановка деятельности синусного узла. В таких случаях синусный ритм заменялся юнкциональным (иногда нижне-предсердным) ритмом . Вслед за этим (через 1—2 мин) возникали предсердные экстрасистолы и трепетание предсредний, после чего наступала фибрилляция предсердий (поздний эффект).

В одной трети случаев фибрилляция предсердий сопровождалаь вторичной фибрилляцией желудочков. Проведение возбуждения между предсердиями и желудочками после введения EDTA и EGTA замедлялось в атроио-вентрикулярном узле. При регистрации потенциала действия отмечалась депрессия потенциалов действия предсердия и медленных (Ca -зависимых) потенциалов действия желудочков в ответ на введение EDTA или EGTA.

СРАВНИТЕЛЬНЫЕ ПОВЕДЕНЧЕСКИЕ ИССЛЕДОВАНИЯ ВЛИЯНИЯ ЛИНЕЙНОГО И ЦИКЛИЧЕСКОГО СОМАТОСТАТИНА НА САМОРАЗДРАЖЕНИЕ У КРЫС

Л. ВЕЧЕИ, Х. ШВАРЦБЕРГ и Д. ТЕЛЕГДИ

В настоящих экспериментах на крысах проводили сравнительные исследования зависимости доза-эффект, касающиеся влияния линейного и циклического соматостатина на поведенческие реакции животных (самораздражение). Вслед за введением 20 мкг линейного соматостатина в боковые желудочки мозга, частота самораздражений сильно снижалась, меньшие же дозы — 5 и 10 мкг — оказались неэффективными.

Введение I мкг циклического соматостатина вызвало краткосрочное увеличение частоты самораздражений (различие статистически недостоверно). После введения 5 и 10 мкг пептида в мозговые желудочки интенсивность самораздражения уменьшилась.

Результаты насоящих экспериментов показывают, что циклический соматостатин эффективнее тормозит реакцию самораздражения, чем гормон, имеющий линейную структуру.

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Physiologia — Pathophysiologia

BIOCHEMICAL COMPARTMENTATION OF FISH TISSUES. HEXOKINASE IN THE BRAIN

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Differential distribution of gross, particulate and soluble forms of hexokinase in the cerebrum, cerebellum, pituitary, hypothalamus and thalamus and medulla oblongata was studied in 9 inland teleosts of different phylogenetic age to clarify the compartmentation of glucose metabolism. Enzyme activity (gross) was maximum in the cerebrum and minimum in the medulla oblongata, suggesting that glucose metabolism is more intensive in the former structure. The activity in the particulate fraction was highest in the cerebellum followed by the cerebrum, midbrain and medulla oblongata. Gross, particulate and soluble fraction of the enzyme was higher in major carp species than in cat fish or snake head species. Total particular and soluble fraction was highest in the cerebrum, cerebellum and midbrain. The distributional trend of the enzyme was similar in the 9 fishes.

Functional heterogeneity and lack of uniformity in energy metabolism is a unique property of the brain. Different regions of the brain vary in their metabolic properties due to metabolic compartmentation. Several workers have studied in various animal groups the biochemical compartmentation and intracellular heterogeneity of the brain [1, 2, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 22].

Glucose is the main source of energy available to the brain tissue because glycolysis is the major pathway in the central nervous system. Therefore, studies of hexokinase (ATP: D-glucose-6-phosphotransferase EC) 2.7. (1.1) one of the key enzymes of glycolysis in different regions of the brain may be of considerable importance to understand the role of the enzyme. Though several reports have appeared on the distribution of hexokinase in various types of cells and different layers of specific brain areas, a general conclusion has not been reached concerning the distributional pattern of hexokinase in the cerebrum, cerebellum, midbrain and medulla [5]. Besides, all these reports were related to higher vertebrates.

In fishes from tropical environment the question does not seem to have been investigated. Therefore, in the present work a study was made of the differential distribution of total, particulate and soluble forms of hexokinase in 4 regions of the brain in 9 fresh water teleosts belonging to various phylogenetic levels. The fishes under study were *Catla catla* (Ham), *Labeo rohita* (Ham), *Cirrhina mrigala* (Ham), *Heteropneustes fossilis* (Bloch), *Clarias* *batrachus* (Linn), *Mystus seenghala* (Sykes), *Channa striatus* (Bloch), *Channa punctatus* (Bloch) and *Channa marulius* (Ham).

Material and methods

Healthy, mature and well fed fish of the above 9 species, ranging in size from 18 to 20 cm, were obtained locally and kept in the laboratory on their natural diet [14] for 6 days before starting the experiment. Ten fish of each species were decapitated and the cerebrum, cerebellum, pituitary, hypothalamus and thalamus and medulla oblongata were removed.

Homogenate preparation

Tissue homogenates (10%) were prepared in the Potter–Elvehjem homogenizer in ice cold buffer solution pH 7.3 containing 0.02 M triethanol amine, 0.25 M sucrose and 0.01 mM dithiothreitol. About 2 ml of each homogenate was dialysed against the same medium at 4 °C, for 1h. The dialysed extracts were centrifuged (K-70 Refrigerated Centrifuge with eight column disks) at 14 000 rpm for 20 min to obtain soluble (supernatant) and particulate (pellet) fractions. The pellets were suspended in the above medium to the original concentration of the homogenate. The pellet suspension and whole homogenates were treated with 0.2% Triton X-100 (final concentration) and kept at 4 °C for 30 min before use to release the enzyme.

Enzyme assay

The enzyme assay mixture contained in a final volume of 1.25 ml, 0.1 M Tris-HCl buffer pH 7.5: 16 mM $MgCl_2$: 16 mM ATP/Mg^{2+} complex pH 7.0: 0.37 mM NADP, 5 mM glucose and one unit of glucose-6-phosphate dehydrogenase. The reaction was started by the addition of 0.01 ml of the tissue extract. A Zeiss spectrophotometer was used for following the rate of reduction of NADP at 340 μm for 6 min. A unit of the enzyme activity was defined as μmol of NADPH formed per g fresh tissue weight at 25 °C [4, 15].

Results

It is evident from Table I to III that regional differences were recorded in the hexokinase activity in whole, particulate and soluble homogenate fractions in four different compartments of 9 teleosts. Whole homogenate (gross) hexokinase activity was maximum in the cerebrum and minimum in the medulla oblangata. The distributional profile of enzyme activity as noticed in the whole homogenate of the 4 brain regions was not reflected in the particulate and soluble fractions. Particulate hexokinase activity was highest in the cerebellum, followed by the cerebrum, midbrain and medulla oblongata. The optimum of soluble enzyme activity was found in the midbrain region. The total, particulate and soluble fractions were highest in the cerebrum, cerebellum and midbrain, respectively. The distributional trend was the same in the four brain regions of the 9 fishes.

Among the fishes, the soft, herbivorous and major carp species (*C. catla*, *L. rohita* and *C. mrigala*) contained the highest total, particulate and soluble forms of hexokinase (Table I) then the catfish (*H. fossilis*, *C. punctatus* and *C. marulius*) (Table III).

Table I

Hexokinase in various regions of the brain–major carps

Regions of brain	Fish	Hexokinase (unit/mg protein) activity		
		Whole homogenate	Particulate	Soluble
(a) Cerebrum	*C. catla*	0.39 ±0.05	0.20 ±0.02	0.08 ±0.006
(b) Cerebellum		0.24 ±0.04	0.32^{a*} ±0.03	0.06 ±0.005
(c) Pituitary thalamus and hypothalamus		0.20^{a*} ±0.03	0.16^{c*} ±0.01	0.10 ±0.003
(d) Medulla oblongata		0.17^{a*} ±0.03	$0.14^{a*,b**}$ ±0.01	0.05 ±0.002
Whole brain		0.47 ±0.10	0.38 ±0.09	0.12 ±0.03
(a) Cerebrum	*L. rohita*	0.30 ±0.03	0.18 ±0.04	0.06 ±0.002
(b) Cerebellum		0.21^{a*} ±0.02	0.26 ±0.03	0.05 ±0.001
(c) Pituitary thalamus and hypothalamus		0.18^{a*} ±0.02	0.13^{b**} ±0.01	0.07 ±0.003
(d) Medulla oblongata		0.14^{a**} ±0.03	0.12^{b**} ±0.01	0.04 ±0.001
Whole brain		0.41 ±0.08	0.32 ±0.08	0.10 ±0.02
(a) Cerebrum	*C. mrigala*	0.25 ±0.03	0.15 ±0.02	0.05 ±0.001
(b) Cerebellum		0.19 ±0.02	0.22 ±0.02	0.04 ±0.001
(c) Pituitary thalamus and hypothalamus		0.15 ±0.02	0.12 ±0.02	0.09 ±0.003
(d) Medulla oblongata		0.12 ±0.02	0.10 ±0.02	0.03 ±0.002
Whole brain		0.37 ±0.08	0.28 ±0.07	0.12 ±0.04

Values (Unit/mg protein) are mean ±SD of 6 estimations. The data were subjected to analysis of variance and "F"

$P < 0.05*$, $P < 0.01**$

Discussion

The activity of a glycolytic enzyme in a particulate compartment of the brain indicates its capacity for glycolysis in the region [20]. If the hexokinase activity is less in white fibre tracts of the brain, it indicates a lower capacity of energy production via glucose metabolism [21]. The maximum

Table II

Hexokinase in various regions of the brain–catfish

Regions of brain	Fish	Hexokinase (Units/mg protein) activity		
		Whole homogenate	Particulate	Soluble
(a) Cerebrum	*H. fossilis*	0.23 ±0.03	0.13 ±0.002	0.04 ±0.002
(b) Cerebellum		0.18 ±0.02	0.20^{a*} ±0.02	0.03 ±0.001
(c) Pituitary thalamus and hypothalamus		$0.13^{a*,b*}$ ±0.02	0.10^{b**} ±0.02	0.07 ±0.002
(d) Medulla oblongata		0.11^{a**} ±0.02	0.09^{b**} ±0.01	0.03 ±0.001
Whole brain		0.29 ±0.06	0.24 ±0.05	0.06 ±0.001
(a) Cerebrum	*C. batrachus*	0.20 ±0.03	0.11 ±0.01	0.05 ±0.001
(b) Cerebellum		0.15 ±0.01	0.09^{a*} ±0.02	0.02 ±0.001
(c) Pituitary thalamus and hypothalamus		0.12^{a*} ±0.01	0.10 ±0.01	0.06 ±0.002
(d) Medulla oblongata		0.09^{a*} ±0.01	0.07^{a*} ±0.01	0.02 ±0.005
Whole brain		0.24 ±0.07	0.19 ±0.07	0.07 ±0.02
(a) Cerebrum	*M. seenghala*	0.16 ±0.02	0.09 ±0.01	0.04 ±0.007
(b) Cerebellum		0.11 ±0.02	0.16^{a*} ±0.03	0.02 ±0.001
(c) Pituitary thalamus and hypothalamus		0.09^{a*} ±0.01	0.07^{b*} ±0.02	0.04 ±0.001
(d) Medulla oblongata		0.07^{a**} ±0.02	0.06^{b*} ±0.03	0.01 ±0.001
Whole brain		0.19 ±0.04	0.15 ±0.06	0.06 ±0.01

Values (Unit/mg protein) are mean ±SD of 6 estimations. The data were subjected to analysis of variance and "F" test

$P < 0.05$*, $P < 0.01$**

and minimum levels of hexokinase in the whole homogenate of the cerebrum and medulla oblongata proves that the rate of glucose metabolism is higher in the cerebrum than in the medulla.

On the other hand, the major component of the medulla is made up of white fibra tracts and white matter, while the cerebrum and cerebellum are rich in grey matter. Even the glucose utilization rate, oxygen consumption

Table III

Hexokinase in various regions of the brain–murrel

Regions of brain	Fish	Hexokinase (Units/mg protein) activity		
		Whole homogenate	Particulate	Soluble
(a) Cerebrum	*C. striatus*	0.14 ±0.01	0.08 ±0.02	0.02 ±0.003
(b) Cerebellum		0.10 ±0.02	0.11 ±0.01	0.02 ±0.004
(c) Pituitary thalamus and hypothalamus		0.08^{a*} ±0.01	0.06^{c**} ±0.02	0.04 ±0.002
(d) Medulla oblongata		0.07^{a*} ±0.02	0.04^{b**} ±0.01	0.01 ±0.001
Whole brain		0.16 ±0.02	0.13 ±0.05	0.04 ±0.001
(a) Cerebrum	*C. punctatus*	0.10 ±0.02	0.07 ±0.02	0.02 ±0.001
(b) Cerebellum		0.08 ±0.01	0.09 ±0.02	0.02 ±0.001
(c) Pituitary thalamus and hypothalamus		0.07 ±0.01	0.05 ±0.01	0.04 ±0.001
(d) Medulla oblongata		0.05^{a*} ±0.02	0.03^{b*} ±0.01	0.01 ±0.001
Whole brain		0.12 ±0.03	0.09 ±0.03	0.03 ±0.001
(a) Cerebrum	*C. marulius*	0.08 ±0.02	0.05 ±0.01	0.01 ±0.001
(b) Cerebellum		0.06 ±0.01	0.07 ±0.02	0.01 ±0.001
(c) Pituitary thalamus and hypothalamus		0.05 ±0.02	0.06 ±0.01	0.04 ±0.002
(z) Medulla oblongata		0.04 ±0.02	0.02^{b-c*} ±0.001	0.01 ±0.001
Whole brain		0.10 ±0.03	0.07 ±0.02	0.04 ±0.01

Values (Unit/mg protein) are mean ±SD of 6 estimations. The data were subjected to analysis of variance and "F" test

$P < 0.05^{*}$, $P < 0.001^{**}$

and local cerebral blood flow are higher in the grey than in the white matter [18, 19]. It is further known from many *in vitro* studies that the metabolic rate in the grey matter is higher than in the white matter [9]. From all this it may be concluded that in the cerebrum and cerebellum, regions rich in grey matter, the metabolic rate is higher while in the medulla, rich in white matter, it is lower than in any of the other regions.

The regional variations observed between particulate and soluble fractions might be explained by the distribution of neurons and glia in different regions of the brain, as the cytoplasmic enzyme is located in the glia and particulate hexokinase in neurons. It is assumed that the brain compartments are different in neuronal and glial composition which difference may manifest itself with variations of the ratio of particulate versus soluble fractions [3, 7].

Among the three groups of fishes, optimum hexokinase in total, particulate and soluble homogenate fractions was recorded in the major carp species. This indicates that the phylogenetically older major carps have more grey matter than the other two groups of fish.

An indirect relationship has been observed between the course of evolution and the fall in the total, particulate and soluble fractions of hexokinase. Further, the phylogenetically younger parts of the brain (cerebrum) contain more hexokinase than the older parts. The regional variations might be related to the diet and its involvement in metabolism. This means that phylogeny has some effect on the animal in general and its organs in particular as regards biochemical compartmentation [12, 13, 14, 15, 16, 17].

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CHANGES OF WATER AND ELECTROLYTE METABOLISM IN RUMINANTS AFTER WATER DEPRIVATION AND NaCl LOAD

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The regulation of Na^+ and water metabolism was studied in sheep with rumen fistula after water deprivation for 48 hours. Na^+ and K^+ concentrations were determined in blood plasma, blood, urine and saliva, with or without NaCl load.

The results showed that the water content of the ruminal fluid plays an important role in the control of electrolyte and water metabolism. The absorption of water and Na^+ from the rumen favours the maintenance of isosmolality and isovolaemia until the concentration of Na^+ in the ruminal fluid has exceeded that in blood plasma. Thereafter the balance of water movement between rumen and extravascular space is shifted towards the rumen inhibiting thereby a further increase of Na^+ concentration and ensuring the life conditions for the ruminal microbial population.

During water deprivation not only water is lost but as a result of Na^+ excretion relative hyponatraemia also ensues as can readily be assessed from the ratio of salivary Na^+ and K^+ concentrations. Upon NaCl load the salivary Na^+/K^+ ratio was increased indicating the extent of Na^+ supply.

With regard to the productiveness of ruminants the importance of the NaCl supply and of the regulation of water balance is widely accepted. A deficient supply in sodium results in reproductive anomalies as described by Ahlswede [1], Lotthammer and Ahlswede [5], Moustafa et al. [6]. According to Fredrik et al. [3] in cattle there is no such correlation between the serum Na^+ and K^+ levels and the Na^+ and K^+ supply which might be used for diagnostic purposes. This explains the importance of the investigations of Skydsgaard [8], Kemp and Hartmans [4] and of Reichel et al. [7], describing the correlation between salivary Na^+ and K^+ concentrations and sodium supply. We have studied how the salivary Na^+ and K^+ concentrations and water metabolism are influenced by the regulatory mechanisms in sheep after transient water deprivation and under a consecutive NaCl load.

In large-scale animal breeding it may namely occur that, due to some failure, no drinking water is available for 1 or 2 days. The fodder is supplemented with NaCl as generally prescribed and not according to a chemical analysis, thus the addition to the fodder of NaCl in excess may often result in a NaCl load.

Methods

Eight Hungarian merino sheep weighing 47–48 kg were used after water deprivation for 48 hours. The composition of the fodder which was offered even during the water deprivation period was starch, 56.2%: protein 14.1%: water content 15.1%: NaCl-content 0.65%.

Before the studies a rumen fistula was created and catheters were inserted into the parotid duct, ureter and jugular vein for collecting samples. Extracellular fluid volume was estimated using ^{24}Na isotope. The water content of the ruminal fluid was determined by polyethylene glycol. The dose of NaCl was 12.8 mmol/kg body mass dissolved in 300 ml water and given into the rumen via the fistula. The solution also contained 7.5 MBq $^{24}Na^+$. Data are given in means ±S.D.

Results and discussion

Water deprivation for 48 hours was found to influence the salt and water metabolism of sheep. Water content of the ruminal fluid and the serum Na^+ concentration were decreased significantly. Na^+ concentration in saliva also diminished whereas that of K^+ increased. Extracellular fluid volume did not change significantly (Table I). The reduction of serum Na^+ concentration appeared to be related to the low Na/K ratio in the saliva; this interpretation however, requires futher studies.

The enhanced NaCl concentration of the ruminal fluid induced by the intraruminal load decreased markedly after 1 hour, but increased again be-

Table I

Changes in parameters after 48 hour water deprivation in sheep

	Before water deprivation	After water deprivation	P
Ruminal fluid			
Na^+ mmol/l	101 ± 6.2	83 ± 49	>0.05
K^+ mmol/l	34.2 ± 11.2	21.8 ± 10.5	>0.05
Water content ml/body mass (kg)	70.7 ± 10.7	39.0 ± 10.3	<0.05
Serum			
Na^+ mmol/l	153.7 ± 2.8	145.8 ± 5.7	<0.05
K^+ mmol/l	4.10 ± 0.45	4.15 ± 0.25	>0.05
Saliva			
Na^+ mmol/l	181.2 ± 4.2	156.8 ± 9.0	<0.05
K^+ mmol/l	5.08 ± 0.72	15.37 ± 6.93	<0.05
Na^+/K^+ mmol/l	35.6	10.2	
Urine			
ml/min	2.99 ± 1.70	0.43 ± 0.15	<0.05
Na^+ mmol/l	39.3 ± 35.6	15.0 ± 11.0	<0.05
K^+ mmol/l	109.5 ± 134.1	294.0 ± 69.0	<0.05
K^+/Na^+ mmol/l	2.78	19.60	
Extracellular fluid volume			
ml/kg body mass	186.0 ± 2.6	181.0 ± 11	>0.05

tween 90–150 min in association with a reduction of K^+ concentration. Three hours post-loading a value below 160 mmol/l was noted (Fig. 1). In another series the water volume of the ruminal fluid was found to decrease until 120 min, thereafter it increased transiently but significantly while the Na^+ concentration fell below 150 mmol/l (Fig. 2). Serum Na^+ concentration was augmented after the load and decreased steadily until 180 min and increased thereafter transiently. The alterations of the serum Na^+ concentration were associated with unchanged serum K^+ concentration (Fig. 3).

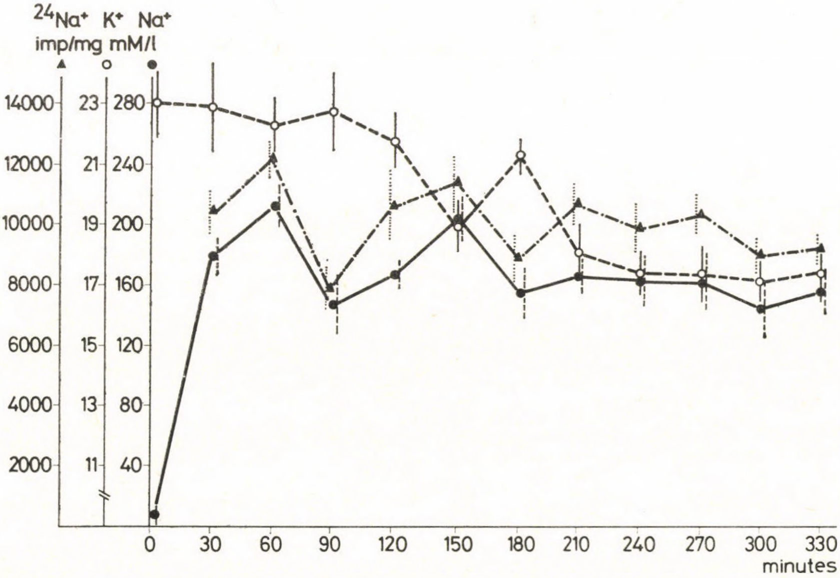

Fig. 1. Changes in ruminal fluid Na^+ and K^+ concentrations and in ^{24}Na specific activity after NaCl load

The NaCl load exerted an effect on the concentration of Na^+ in the saliva too, and raised it to a value over 180 mmol/l, whereas K^+ dropped below 5 mmol/l. The Na^+/K^+ ratio dropped from 35 (before water deprivation) to 10 (after water deprivation). In response to the load it increased continuously, to 20 after 50 min, to 35 after 90 min, and even higher thereafter (Fig. 4).

Under the NaCl load the haematocrit dropped from 33 to 27. The extracellular fluid volume between 180–240 min was 18% less than the initial value (Fig. 5).

These data unequivocally indicate that the changes in electrolyte and water metabolism were due to the water deprivation for 48 hours. No significant alterations were seen in extracellular fluid volume or the haematocrit. During water deprivation the fluid requirement of the animals was replenished from the water content of the ruminal fluid in association with a reduced

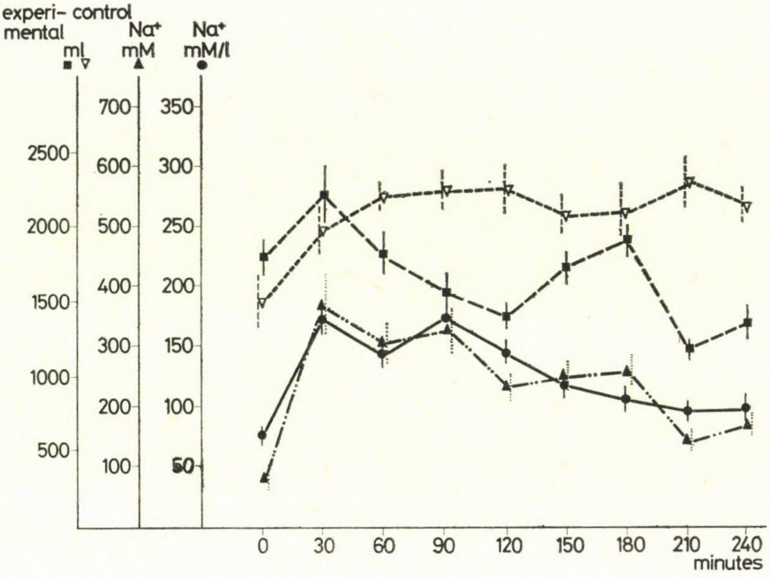

Fig. 2. Changes in ruminal fluid volume after NaCl (experimental) and water (control) loads

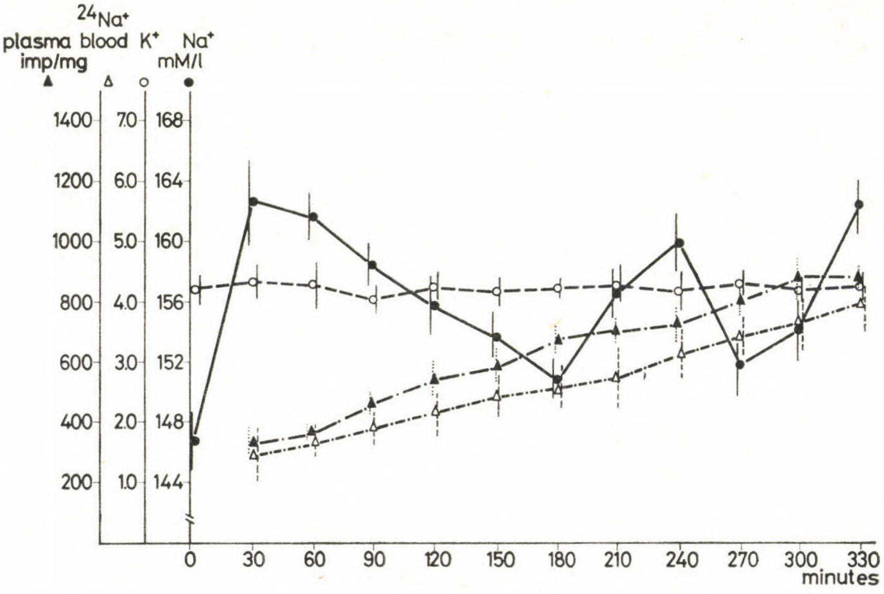

Fig. 3. Changes in serum Na^+ and K^+ concentrations and in blood ^{24}Na specific activity after NaCl load

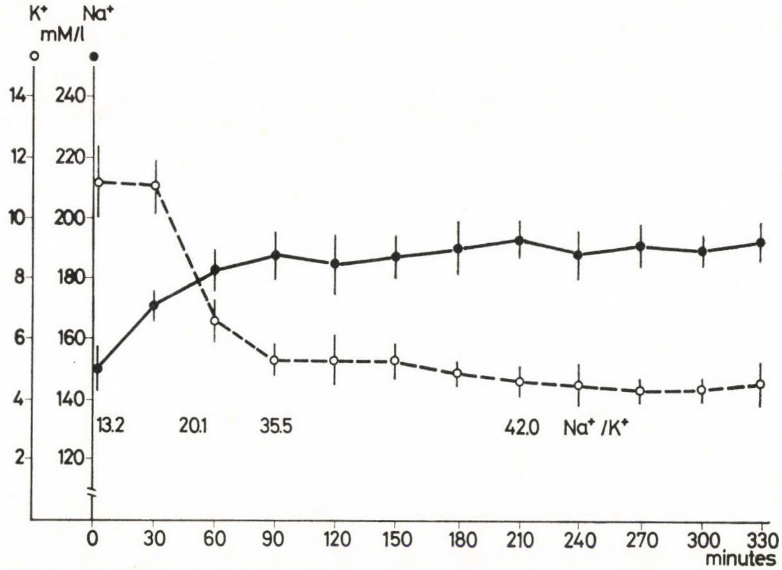

Fig. 4. Changes in salivary Na^+ and K^+ concentrations after NaCl load

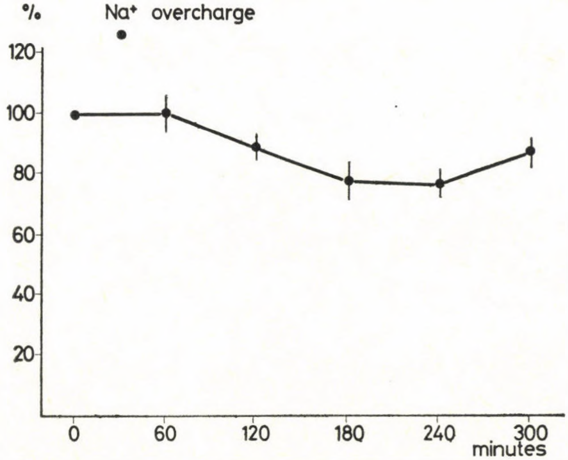

Fig. 5. Changes in extracellular fluid volume after NaCl load

diuresis. The water content of the ruminal fluid decreased significantly. The water absorbed from the rumen is capable of compensating for the transient water loss of ruminants. Urinary output of Na^+ was depressed whereas that of K^+ was enhanced: the K^+/Na^+ ratio was elevated from 2.8 to 19.6. All these appear to indicate a Na^+ retention and a relative Na^+ deficiency. The relative hyponatraemia was also reflected by the salivary Na^+/K^+ ratio which decreased from 35 to 10. The Na^+ concentration of the ruminal fluid is low therefore it could not compensate for the urinary loss of Na^+.

Ruminal fluid is known to play an important role in the water metabolism of sheep. According to ENGELHARDT [2], 7–12 l of saliva is swallowed into the rumen during 24 h and the same volume passes into the omasum, abomasum and intenstinal canal. On the other hand, across the wall of the rumen 50 l water passes daily in both directions.

The intraruminal administration of NaCl raised the Na^+ concentration and water content of the ruminal fluid. The absorption of Na^+ from the rumen gradually increased the specific activity of ^{24}Na in the serum. The high Na^+ concentration in the serum that developed during the first half hour postload decreased gradually while the ruminal fluid lost water, too. The reduction of ruminal fluid volume resulted in an enhancement of its Na^+ concentration and the reversed Na^+ gradient augmented the reexcretion of water into the rumen in association with a repeated increase in serum Na^+ and a reduction in the ruminal fluid Na^+ concentration. This mechanism prevents the prolonged increase of osmotic pressure in the rumen after transient water deprivation which would otherwise destroy the ruminal microorganisms that are essential for ruminal digestion. According to the results the salivary Na^+/K^+ ratio reliably reflects the hypo- and hypernatraemic states produced by water deprivation and NaCl load.

In agreement with other data we may conclude that the saliva test is one of the most suitable diagnostic methods available to control the salt supply of ruminants.

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COMPUTER SIMULATION FOR STUDYING CALCIUM DEPENDENT ABNORMALITIES IN FIRING MECHANISM OF MOLLUSCAN NEURONES

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Computer modelling technique is proposed to assist in physiological research on invertebrate neuronal membranes. The firing mechanism of a single patch of invertebrate neuronal membrane has been studied in dependence on maximum Ca^{++} conductance. The calculations are based on modification of Hodgkin–Huxley's data completed by a straight line approximation between experimental points of the kinetic parameters of Ca^{++} current and early transient potassium current. The time course of conductance changes is assumed to be proportional to m^2h for Ca^{++} current. Three distinct potassium currents are involved into the model, viz. transient potassium current, delayed potassium current and Ca^{++}-dependent potassium current. The modified Euler method run on a digital computer has been used for numerical integration of kinetic equations.

Significant effects of Ca^{++} conductance on spike broadening, plateau development and spike afterhyperpolarization are represented. In the range of small Ca^{++} conductance an infinite spontaneous activity can be triggered by a short (suprathreshold) current pulse which may be considered a model of pacemaker activity. Plateau development resulting from potassium blocking or decreasing potassium equilibrium is facilitated by Ca^{++} conductance in the range of greater Ca^{++} conductance. The effects of voltage sensitivity of the coupling coefficient describing the current of Ca^{++}-dependent K^+ channels were studied and compared to the voltage independent case. The coupling coefficient seems to be a crucial factor in broadening the range of Ca^{++} conductance responsible for pacemaker activity. For greater values of Ca^{++} conductance, a decrease of the coupling coefficient leads to a transition from prolonged bursting to interruption of burst activity by burst-afterhyperpolarization.

The blocking effect of 4-aminopyridine on fast outward current has been studied by the model which has a practical significance considering that aminopyridine is known as a convulsive agent. We suppose that it is reasonable to study the convulsive effects of aminopyridine by the model based on the kinetics of the isolated neuronal membrane. The model may help in understanding the ionic background underlying abnormal network activity during epileptic discharges of mammalian neurones.

The primary purpose of this paper was to develop a computer modelling technique for the analysis of modifying effects of the Ca^{++} system in molluscan neurones. Recent results of voltage-clamp measurements describing the characteristics of the transmembrane ionic currents in isolated neurones of the ganglia of molluscs allowed to summarize the available data by means of a digital computer and perform different model experiments.

The kinetics of inward currents were investigated by means of microelectrodes and intracellular dialysis of nerve cells [4, 5, 6, 14, 15, 22, 23]. Sodium and calcium currents underlying the inward current of the membrane

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were successfully separated and the activation and inactivation kinetics of the Ca^{++} current have been clarified [1, 14, 15, 16, 17]. The general form of inward current containing a small Ca^{++} current in molluscs is similar to that described for the squid axon [12] but its time course is considerably slower [4, 5, 6, 22, 23]. Potassium currents were intensively studied under voltage-clamp conditions and separated into three distinct components [26] viz. transient potassium current I_A which is blocked by externally applied 4-aminopyridine [4-Ap]; delayed outward current I_K which is not affected by 4-Ap and Ca^{++}-dependent potassium current $I_{K,Ca}$. A good correlation has been found between the change in intracellular Ca^{++} concentration and $I_{K,Ca}$ [8] suggesting that $I_{K,Ca}$ is not voltage dependent. In contrast, the results of GORMAN and THOMAS [9] have shown that the activation of K^+ conductance underlying $I_{K,Ca}$ also depends on voltage.

The participation of ionic conductances in development different firing patterns of the membrane has been studied during the normal repetitive state [4, 5, 6]; pacemaker activity [2, 3, 7]; plateau development resulting from K^+ channel blocking [20, 28, 29]; prolonged spike or burst-afterhyperpolarization of the membrane potential [18]; and depolarizing shift activity coinciding with burst development [11, 27]. Our model experiments proved the significant role of Ca^{++} conductance and Ca^{++}-mediated K^+ conductance in developing or influencing the previously listed activity patterns. A modification of the coupling coefficient between Ca^{++} and K^+ channels may lead to the inhibition of pacemaker bursting by evoking prolonged burst-afterhyperpolarization. We present also the results as regards 4-Ap effects which show similarities with bursting activities of units observed in 3-Ap-treated mammalian cortex [25].

Model equations and numerical procedure

The governing relationship for membrane current per unit area is expressed by

$$I(t) = C_m \cdot \frac{dV}{dt} + g_{Na} \cdot (V - E_{Na}) + g_{Ca} \cdot (V - E_{Ca}) + g_A \cdot (V - E_A) + \\ + g_K \cdot (V - E_K) + g_C \cdot (V - E_C) + g_L \cdot (V - E_L) + c_I \tag{1}$$

The symbols for (1) and other expressions are listed in Table I. The time and voltage-dependent ion conductances are expressed by

$$\begin{aligned} g_{Na} &= \bar{g}_{Na} \cdot m_{Na}^3 \cdot h_{Na}; \quad g_{Ca} = \bar{g}_{Ca} \cdot m_{Ca}^2 \cdot h_{Ca}; \\ g_A &= \bar{g}_A \cdot m_A^4 \cdot h_A; \quad g_K = \bar{g}_K \cdot n_K^2; \quad g_C = g_{Ca} \cdot c_{K,Ca} \end{aligned} \tag{2}$$

where m_{Na}, h_{Na} denote the H–H variables with decreased rate functions; m_{Ca} and h_{Ca} represent the activation and inactivation kinetics of Ca^{++} current; m_A, h_A denote the activation and inactivation parameters of the early potassium channel; n_K is the H–H variable of the delayed potassium channel including decreased rate functions and $c_{K,Ca}$ is the coupling coefficient

Table I

Data employed in calculations

Constants

C_m	membrane capacity	6.64	$\mu F/cm^2$
E_{Na}	sodium equilibrium potential	100.0	mV
$E_K = E_A = E_C$	potassium equilibrium potential	- 20.0	mV
E_{Ca}	calcium equilibrium potential	190.0	mV
E_L	leakage equilibrium potential calculated to give a balance of inward and outward currents for resting membrane potential		
(All potentials are referred to resting potential of 0 mV)			
$\bar{g}_{Na,n}$	peak value of sodium conductance	11.0	$m\Omega^{-1}/cm^2$
(Suffix n denotes the normal value; without note always $\bar{g}_i = \bar{g}_{i,n}$ where i = Na, Ca, A, K or L.)			
$\bar{g}_{Ca,n}$	peak value of calcium conductance	$0 \div 2.5$	$m\Omega^{-1}/cm^2$
$\bar{g}_{A,n}$	peak value of early potassium conductance	2.6	$m\Omega^{-1}/cm^2$
$\bar{g}_{K,n}$	peak value of delayed potassium conductance	1.3	$m\Omega^{-1}/cm^2$
$\bar{g}_{L,n}$	leakage conductance		
$c_{K,Ca}$	K^+–Ca^{++} coupling coefficient	0.083	
c_I	steady-state or pulse current input in $\mu A/cm^2$		
F	Faraday constant	96 493	C/mole
z	charge of calcium ion	2	
R	gas constant	8.314	J/K°
T	absolute temperature	293	°K
δ	electrical distance of Ca^{++} binding site through the membrane from the inside	0.49	
τm_A	time constant of the activation of g_A	18.0	ms
τh_A	time constant of the inactivation of g_A	250.0	ms
$a m_{Na}$	multiplicative constants modifying	0.248	
$b m_{Na}$	the rate functions of Na^+ activation	0.248	
$a h_{Na}$	multiplicative constants modifying	0.252	
$b h_{Na}$	the rate functions of Na^+ inactivation	0.252	
$a n_K$	multiplicative constants modifying	0.080	
$b n_K$	the rate functions of K^+ activation	0.080	

Functions

V	membrane potential in mV, a function of time
g_{Na}	sodium conductance in $m\Omega^{-1}/cm^2$
g_{Ca}, g_A, g_K, g_C	similar to g_{Na} for calcium, early potassium, delayed potassium and Ca^{++}-sensitive potassium channels, respectively
αm_{Na}	forward rate function in ms^{-1} for state variable m_{Na} $a_{m_{Na}} \cdot 0.1 \exp(-V + 25)/(\exp((-V + 25)/10) - 1)$

Table I. continued

βm_{Na}	backward rate function for m_{Na} $bm_{Na} \cdot 4 \exp(-V/18)$
αh_{Na}	forward rate function for h_{Na} $ah_{Na} \cdot 0.07 \exp(-V/20)$
βh_{Na}	backward rate function for h_{Na} $bh_{Na}/(\exp((-V+30)/10)+1)$
$m_{Ca}(\infty)$	steady-state value of m_{Ca} calculated by straight line approximations between the experimental points given in Table III of AKAIKE et al. [1]
τm_{Ca}	time constant of m_{Ca} in msec estimated similarly to $m_{Ca}(\infty)$
$m_{Ca}(\infty)$	steady-state value of m_{Ca} calculated by straight line approximation of the experimental data given in Fig. 3C of THOMPSON [26] 0 $V \leq -60$ $4\sqrt{V/50+1.2}$ $-60 < V < -10$ 1 $V \geq -10$
$h_A(\infty)$	steady-state value of h_A calculated similarly to $m_A(\infty)$ 1 $V \leq -90$ $-V/40 - 1.25$ $-90 < V < -50$ 0 $V \geq -50$
αn_K	forward rate function for n_K $an_K \cdot 0.01 \cdot (-V+10) \cdot (\exp((-V+10)/10)-1)^{-1}$
βn_K	backward rate function for n_K $bn_K \cdot 0.125 \exp(-V/80)$
τm_{Na}	voltage-dependent time constant of m_{Na} $1/(\alpha m_{Na} + \beta m_{Na})$
$m_{Na}(\infty)$	steady-state value of m_{Na} $\alpha m_{Na}/(\alpha m_{Na} + \beta m_{Na})$
τh_{Na}, τn_K	similar to τm_{Na} for h_{Na} and n_K, respectively
$h_{Na}(\infty)$, $n_K(\infty)$	similar to $m_{Na}(\infty)$ for h_{Na} and n_K, respectively
m_{Na}, h_{Na}	H–H-like state variables for sodium conductance, described by (3)
n_K	H–H-like state variable for potassium conductance, described by (3)
$c_{K,Ca}(E)$	voltage-dependent coupling coefficient (dimensionless) between calcium and potassium current if the absolute value of resting potential is $E = V-40$ (mV) $c_{K,Ca} \cdot \exp(zF\delta E \cdot 10^{-3}/RT)$

denoting the ratio of the momentary Ca^{++} conductance and the third component of potassium conductance. Each kinetical variable is described by simple first-order kinetics:

$$\tau_x \cdot \frac{dx}{dt} = -x + x(\infty) \tag{3}$$

where τ_x is the voltage-dependent time constant and $x(\infty)$ the steady-state value of state variables ($x = m_{Na}, h_{Na}, m_{Ca}, h_{Ca}, m_A, h_A$ or n_K). The constants $am_{Na}, bm_{Na}, ah_{Na}, bh_{Na}, ah_K$ and bh_K used for multiplying the H–H rate functions to give a slower time course are evaluated by means of experimental data given in the literature [14, 26]. Using the experimental results of THOMPSON [26], the functions $m_A^4(\infty), h_A(\infty)$ are approximated by straight lines as it is given in Table I. Both the voltage independent and voltage dependent calculations of $c_{K, Ca}$ are taken into consideration as has been done for the results of GORMAN and THOMAS [9]. The peak values of conductance representing inward current or outward potassium currents are calculated on the basis of experiments of CONNOR and STEVENS [4, 5, 6]. Comparison of the peak values of Na^+ and Ca^{++} conductances was summarized by KOSTYUK and KRISHTAL [14] where the m^2h law was proposed for calcium current.

In Fig. 1 the simplified flowchart of the computer program run on a digital computer can be seen. The new values of state variables are estimated at the next time step by the modified EULER method [19]. If $x(t)$ denotes one of the state variables at the present time step, $x(t + \Delta t)$ is calculated by

$$x(t + \Delta t) = x(t) + \Delta t \cdot \left[- (\alpha_x + \beta_x) \cdot \left(x(t) + 0.5 \cdot \Delta t \cdot \left(- (\alpha_x + \beta_x) \cdot x(t) + \alpha_x\right)\right) + \alpha_x\right] \quad (4)$$

where α_x, β_x are the forward and backward rate functions, respectively. In cases, where no explicit function is available for the rate functions currents A and Ca^{++} they are estimated by means of τ_x and $x(\infty)$, supposing a two-state kinetical scheme. To obtain a reasonably accurate result Δt must be kept small. The error due to a finite value of Δt is estimated by calculating the firing frequency for different values of Δt, accepting that the "true" value of the frequency results at Δt which approaches zero. We suggest $\Delta t = 0.2$ msec to be sufficiently small because in this case the error in frequency is $\leq 3\%$.

Results

First, the pure Na^+ spike is demonstrated by the model (Fig. 2). Applying a current stimulus of short duration, the mixed Na^+–Ca^{++} spike shows a small broadening on the falling phase and a prolonged afterhyperpolarization depending on $\bar{g}_{Ca}$ (Fig. 3*a*). A slight depression of excitability results from an increase of leakage conductance (Fig. 3*b*). The picture of stimulus-evoked firing with constant $c_{K, Ca}$ is given in Fig. 4. There is a narrow range of $\bar{g}_{Ca}$ near 1.0 $m\Omega^{-1}/cm^2$ which determines the state of membrane developing an infinite pacemaker activity evoked by a suprathreshold current pulse. The only condition to evoke this state of the membrane which is characterized by a constant firing frequency for long periods is the suprathreshold excitation and, independently of its amplitude and duration, a single pulse starts the activity. This result supports the view that Ca^{++} has a significant role in pacemaker activity. A high Ca^{++} content of membrane conductance enhances plateau development and increases the afterhyperpolarization which seems to be fixed, at least in the model time, at a steady-state level of hyperpolarization (Fig. 4). Constant depolarizing current input also evokes irreversible afterhyperpolarization (Fig. 5).

Modifying effects of potassium channels

An increase of the normal value of potassium conductances ($\bar{g}_{A, n}$, $\bar{g}_{K, n}$) depresses excitability of the membrane (Fig. 6*b*). The decrease of potassium

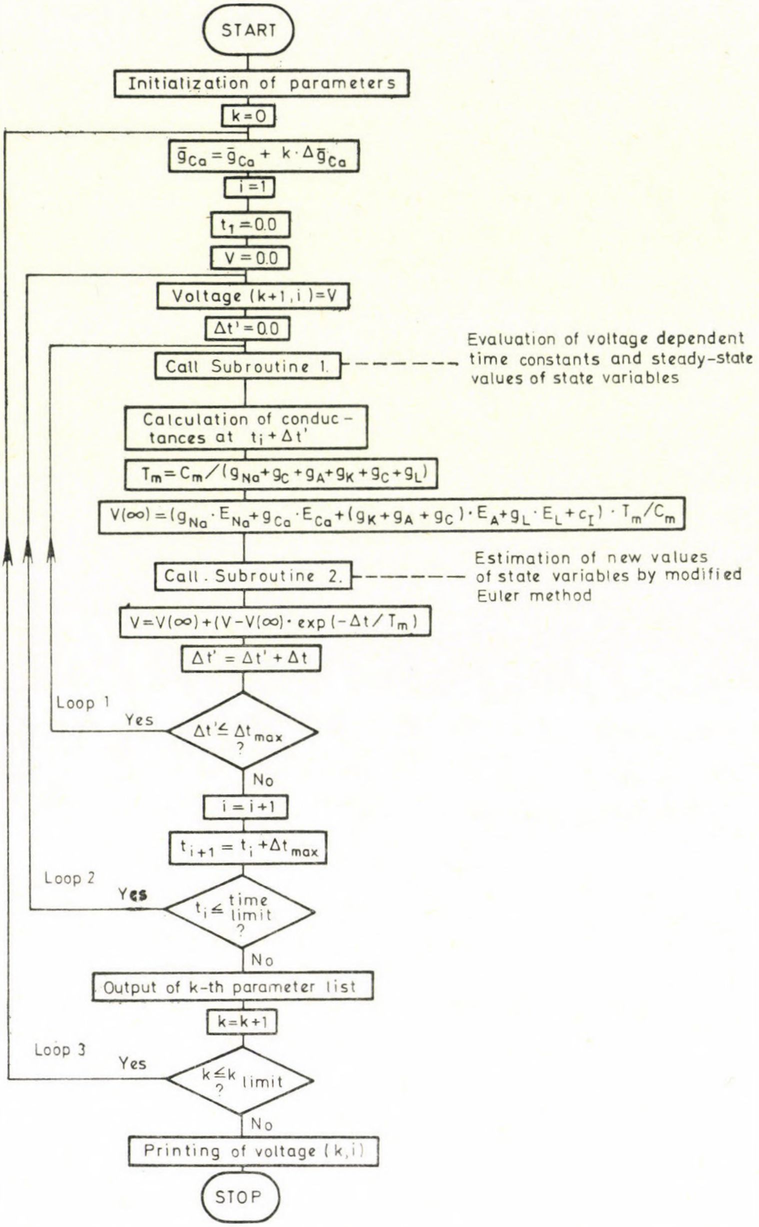

Fig. 1. **Flowchart of the program in FORTRAN IV which simulates Ca^{++}-dependent firing of neuronal membrane. T_m denotes the time constant of membrane and $V(\infty)$ is the steady-state value of potential calculated at the beginning of each time step. The time interval of integration is Δt (= 0.2 msec) and the scale factor is Δt_{max} which defines resolution of the output. The matrix Voltage $(k+1, i)$ stores the voltage distribution for the peak Ca^{++} conductance $\bar{g}_{Ca} + k \cdot \Delta\bar{g}_{Ca}$. Loop 1 calculates the voltage at the next time step. Loops 2 and 3 evaluate the voltage for the whole time period and whole range of $\bar{g}_{Ca}$, respectively**

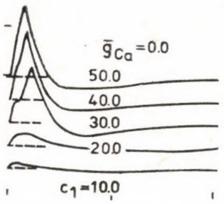

Fig. 2. **Sodium spikes induced by current stimuli of different intensities (in μA/cm^2) and 4 msec. Time bars every 100 msec. Voltage bars here and in other figures every 50 mV**

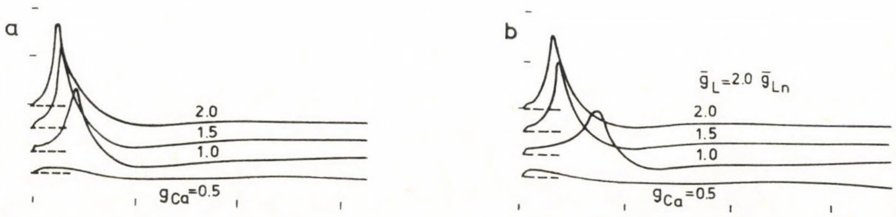

Fig. 3. **Sodium-calcium spikes as a function of $\bar{g}_{\mathrm{Ca}}$ with normal (*a*) and enhanced (*b*) leakage conductance. Note prolonged afterhyperpolarization resulting from an increase of $\bar{g}_{\mathrm{Ca}_2}$. The amplitude of the input stimulus is 10 μA/cm^2 and its duration, 4 msec. The excitability of the system considerably increases as compared to the case with $\bar{g}_{\mathrm{Ca}} = 0.0$ (Fig. 2). Time bars every 100 msec**

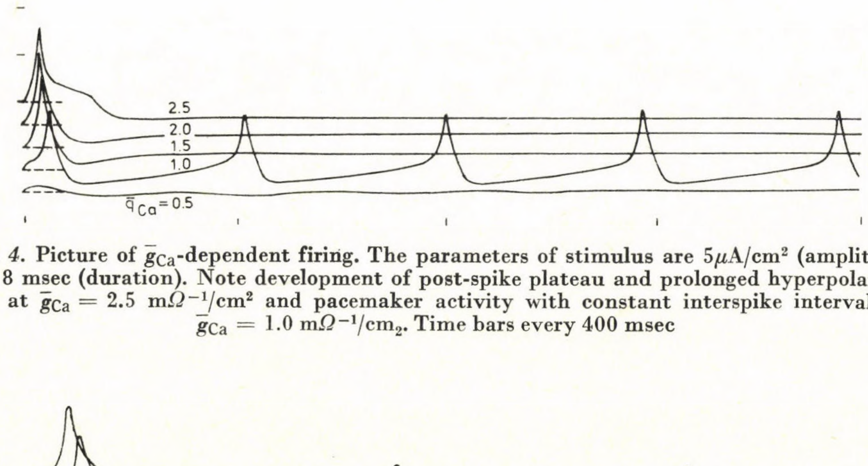

Fig. 4. **Picture of $\bar{g}_{\mathrm{Ca}}$-dependent firing. The parameters of stimulus are 5μA/cm^2 (amplitude) and 8 msec (duration). Note development of post-spike plateau and prolonged hyperpolarization at $\bar{g}_{\mathrm{Ca}} = 2.5$ mΩ^{-1}/cm^2 and pacemaker activity with constant interspike intervals at $\bar{g}_{\mathrm{Ca}} = 1.0$ mΩ^{-1}/cm$_2$. Time bars every 400 msec**

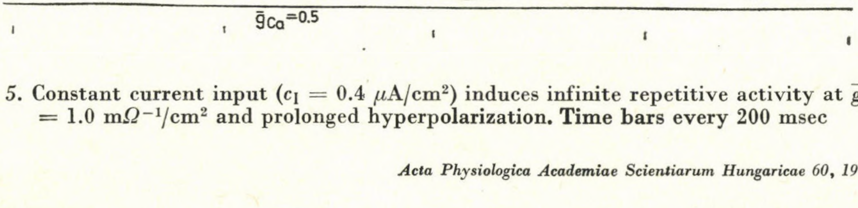

Fig. 5. **Constant current input ($c_{\mathrm{I}} = 0.4$ μA/cm^2) induces infinite repetitive activity at $\bar{g}_{\mathrm{Ca}} = 1.0$ mΩ^{-1}/cm^2 and prolonged hyperpolarization. Time bars every 200 msec**

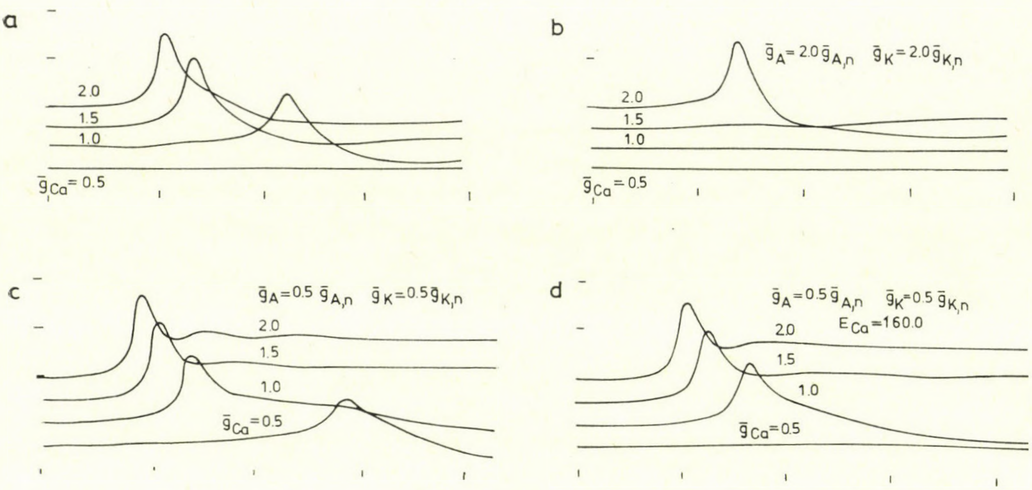

Fig. 6a–d. **Effects of increase and decrease of potassium conductances and E_{Ca} on firing mechanism of the model under constant stimulus condition ($c_I = 0.3\ \mu A/cm^2$). Time bars every 50 msec**

conductances leads to plateau development at greater $\bar{g}_{Ca}$ and duration of the finite plateau is reduced by a decrease of E_{Ca} (Fig. 6*c*, *d*). Figure 7*a*, *b* shows the effects of the decrease and increase of $\bar{g}_{A,n}$ and $\bar{g}_{K,n}$ for a longer period of model time. The range of $\bar{g}_{Ca}$ responsible for infinite repetitive firing is displaced by the change of potassium conductances in contrast to the pattern seen in Fig. 5. The finite depolarizing shift results from the summed effects of a decrease of potassium equilibrium and an increase of $\bar{g}_{Ca}$ (Fig. 7*c*). A suprathreshold current pulse of short duration also evokes depolarizing shifts if the equilibrium potential of the K^+ ion decreases (Fig. 8*a*, *b*).

Changes in firing mechanism as a result of involvement of voltage-dependent K^+–Ca^{++} coupling coefficient into the model

Modification of $c_{K,Ca}$ by a multiplicative factor $c_{K,Ca}(E)$ leads to some interesting changes in firing mechanism. No significant effects were observed at greater $\bar{g}_{Ca}$ range in the steady-state level of afterhyperpolarization contrast to the voltage independent case and in both cases afterhyperpolarization practically did not depend on $c_{K,Ca}$ (Fig. 9). The range near $\bar{g}_{Ca} = 1.0$ $m\Omega^{-1}/cm^2$ was investigated using small steps in $\bar{g}_{Ca}$ and the results are presented in Figs 10 and 11. Figure 10 shows the dependence of pacemaker activity on the changes of potassium and calcium equilibrium potentials. There is a proportionality between $c_{K,Ca}$ and the width of the range of $\bar{g}_{Ca}$ which is favour-

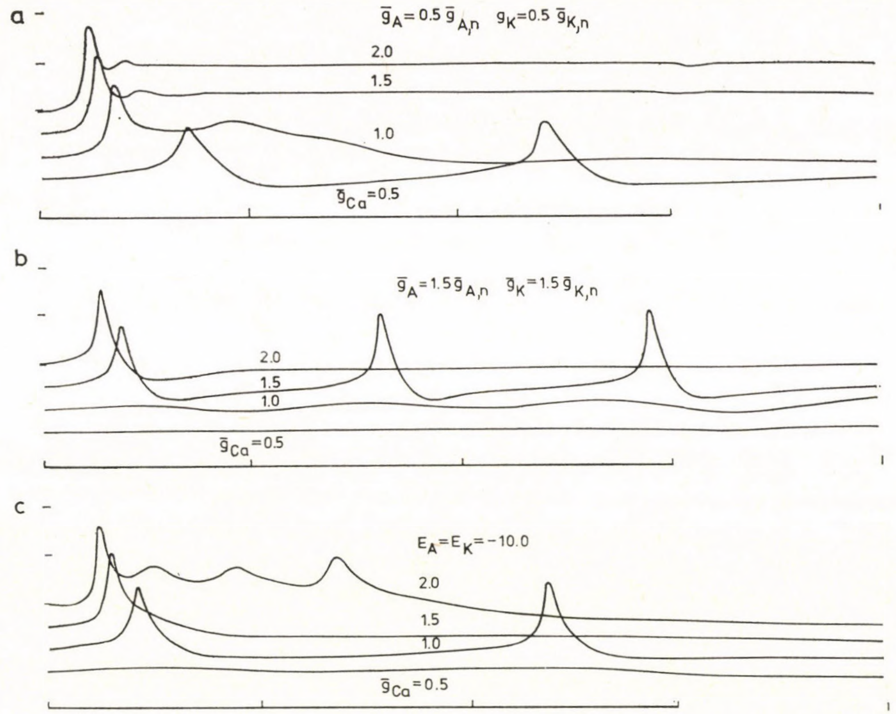

Fig. 7a. Infinite plateau develops at greater $\bar{g}_{Ca}$, as a result of the blockage of potassium conductances. *b* Increase of $\bar{g}_A$ and $\bar{g}_K$ influences the $\bar{g}_{Ca}$-dependence of infinite repetitive activity. *c* Finite depolarizing shift develops on decreasing the equilibrium potential of potassium ions ($c_I = 0.4\ \mu A/cm^2$). Time bars every 200 msec

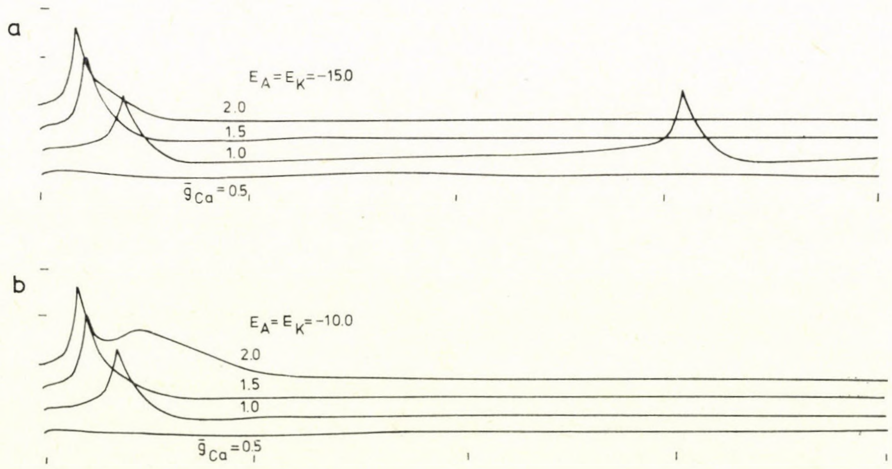

Fig. 8a, b. Decrease of potassium equilibrium potential modifies the firing patterns when a single current shock is applied ($c_I = 5\ \mu A/cm^2$, duration 4 msec). Time bars every 200 msec

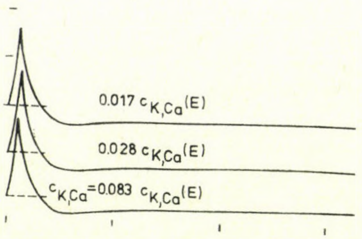

Fig. 9. **Decrease of voltage-dependent K^+–Ca^{++} coupling coefficient has no significant effect on afterhyperpolarization ($\bar{g}_{Ca} = 2.0$ mΩ^{-1}/cm^2). Time bars every 200 msec, $c_I = 10$ μA/cm^2 duration 8 msec**

able for starting infinite pacemaker activity by a suprathreshold pulse (Fig. 11). On applying steady-state current input an infinite plateau develops for higher $\bar{g}_{Ca}$ after decreasing the potassium equilibrium potential (Fig. 12*a*). Spontaneous abnormal bursting coinciding with depolarizing shifts can be evoked by a single stimulus if the potassium equilibrium potential decreases (Fig. 12*b*). There is a distinct range of $c_{K,Ca}(E)$ where prolonged, spontaneous bursting is disrupted by burst-afterhyperpolarization (Fig. 13).

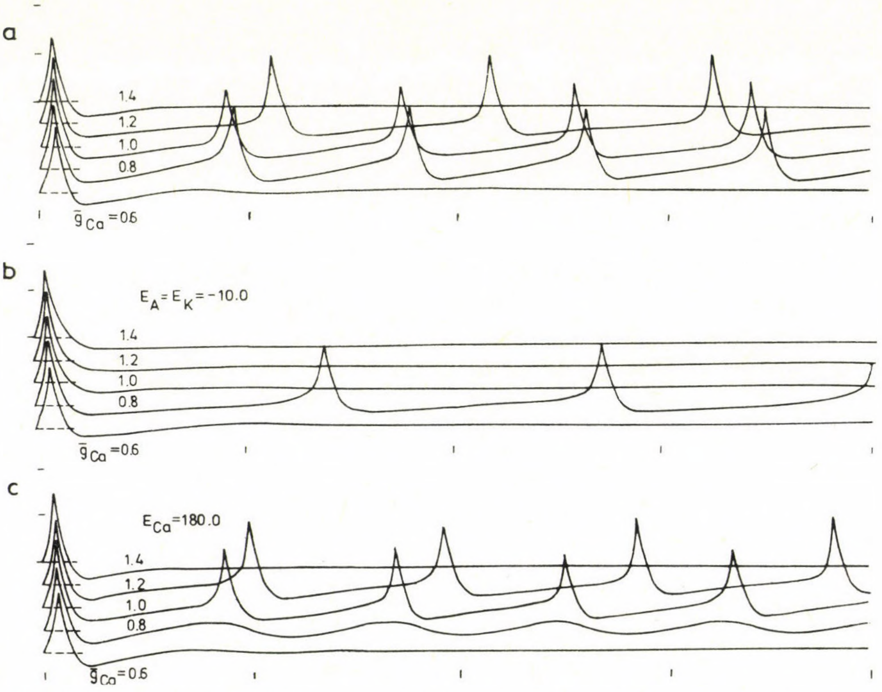

Fig. 10a, b. **Decrease of equilibrium potential of potassium ions influences the $\bar{g}_{Ca}$-dependence of pacemaker activity in contrast to the normal state. The model involves the voltage-dependent K^+–Ca^{++} coupling coefficient. *c* Slight decrease of E_{Ca} produces some changes in firing. Stimulus parameters: $c_I = 10$ μA/cm^2; duration 8 msec. Time bars every 400 msec**

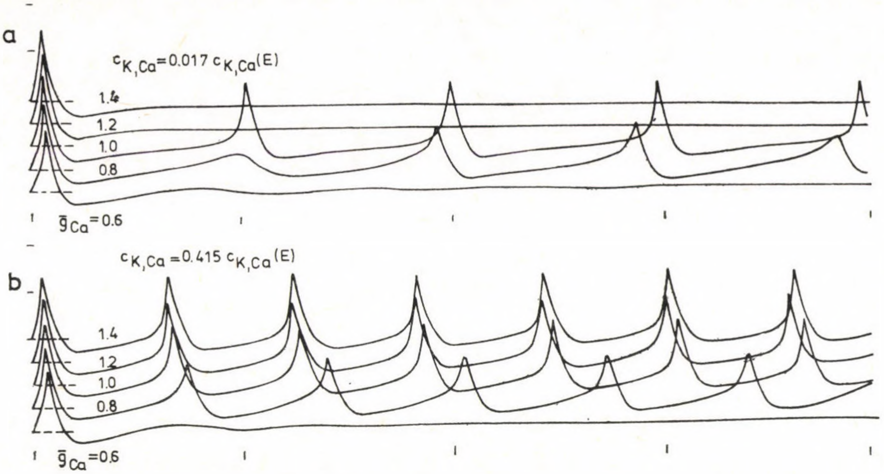

Fig. 11a, b. Changes of coupling coefficient between K^+ and Ca^{++} currents influence the $\bar{g}_{Ca_2}$-dependence of pacemaker activity ($c_I = 10\ \mu A/cm^2$, duration 8 msec). Time bars every 400 msec

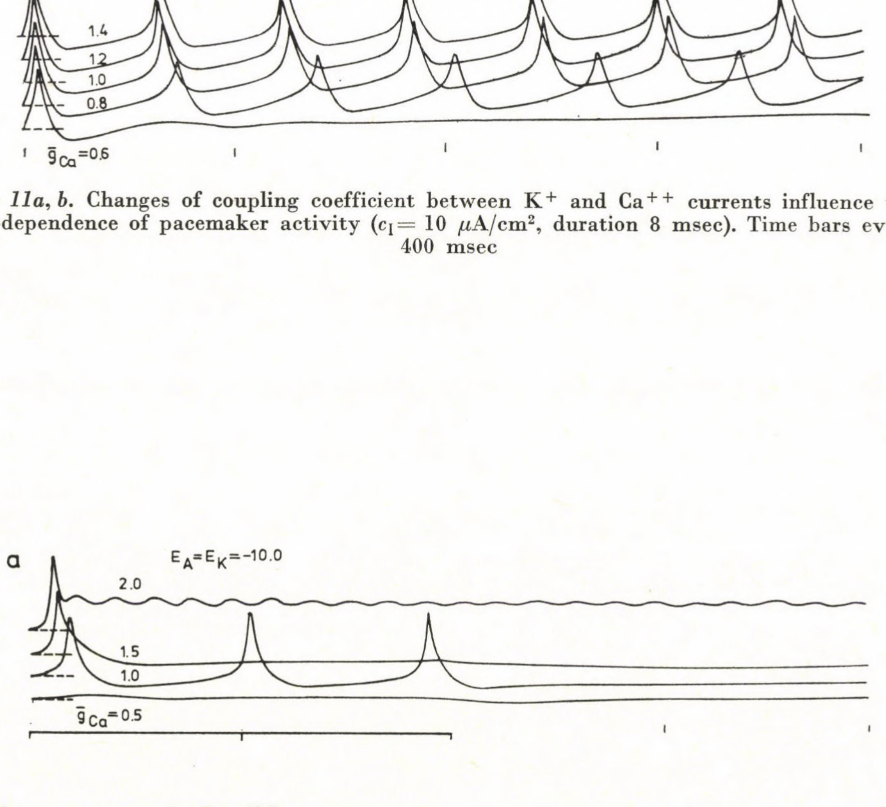

Fig. 12. Effects of decrease of potassium equilibrium potential. *a* Infinite plateau with small oscillations develops when steady-state current input ($c_I = 0.4\ \mu A/cm^2$, 800 msec duration) is applied, if $\bar{g}_{Ca} = 2.0\ m\Omega^{-1}/cm^2$. At $\bar{g}_{Ca} = 1.0\ m\Omega^{-1}/cm^2$ the repetitive activity ceases after following the end of the stimulus which results from displacement of the range of pacemaker activity. *b* Single stimulus evokes prolonged or pacemaker bursting at $\bar{g}_{Ca} = 2.0\ m\Omega^{-1}/cm^2$ ($c_I = 10\ \mu A/cm^2$, 8 msec duration). Time bars every 400 msec

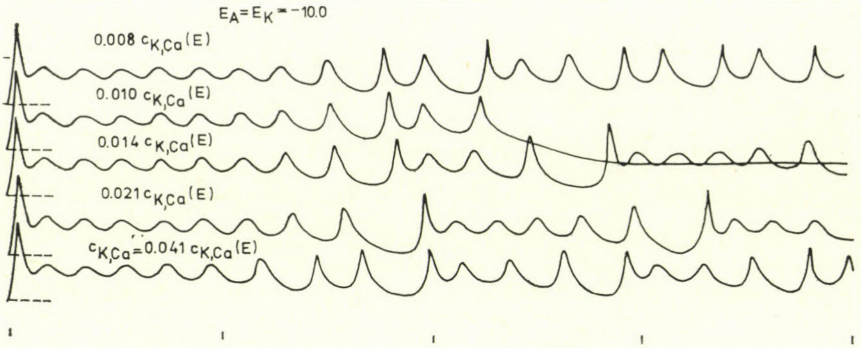

Fig. 13. Decrease of coupling coefficient leads to a change in the structure of prolonged burst activity. There is a narrow range of $c_{K,\,Ca}$ where burst activity is disrupted by steady-state afterhyperpolarization ($\bar{g}_{Ca} = 2.0$ mΩ^{-1}/cm^2, $c_I = 10$ μA/cm^2, 8 msec duration). Time bars every 400 msec

Modelling aminopyridine effects

4-Ap influences the peak value of g_A which decreases to $\bar{g}_A \approx 0.52$ mΩ^{-1}/cm^2 and τ_{h_A} which increases to about 1250 msec, according to the data of Thompson [26]. Under normal conditions the previous modifications of the kinetic parameters of current A have no significant effect on the $\bar{g}_{Ca}$-dependent firing pattern (Fig. 14*a*). To evoke a prolonged bursting with depolarizing

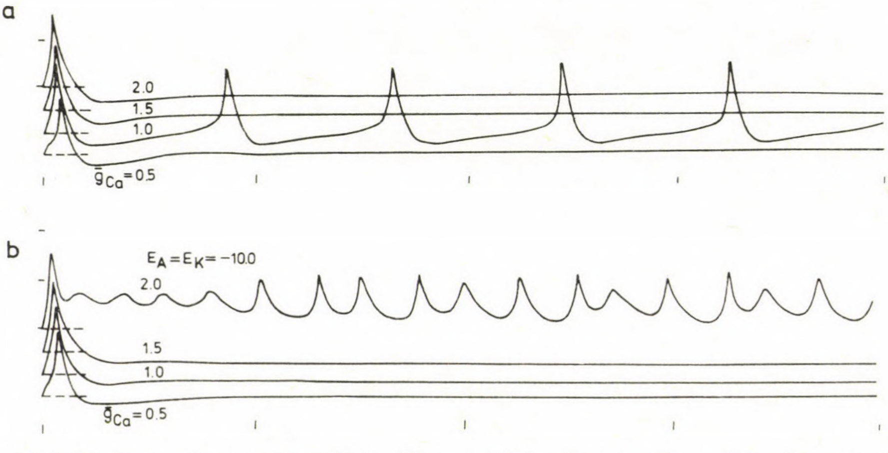

Fig. 14. Modelling aminopyridine effects. The model involves a voltage-dependent coupling coefficient between K$^+$ and Ca^{++} ions. *a* No significant change can be observed at a normal value of the potassium equilibrium potential. *b* Decrease of potassium equilibrium potential evokes prolonged bursting at greater $\bar{g}_{Ca}$. For $\bar{g}_{Ca} = 1.0 + 1.5$ mΩ^{-1}/cm^2 steady-state afterhyperpolarization develops ($c_I = 10$ μA/cm^2, 8 msec duration). Time bars every 400 msec

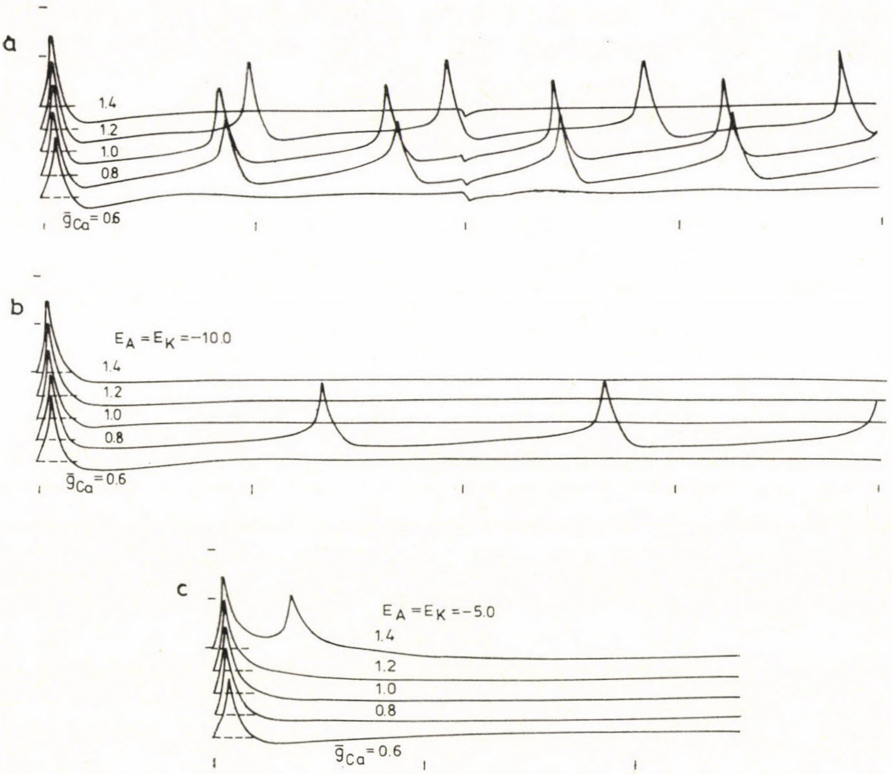

Fig. 15. Modelling aminopyridine effects using $c_{K,\,Ca}(E)$. *a* $\bar{g}_{Ca}$-dependence of firing pattern at pacemaker state remains essentially unchanged as compared to the pattern without 4-Ap effect (Fig. 10a). Hyperpolarizing current shock of –5 μA/cm² amplitude and 8 msec duration has no significant influence. *b* Similarly the state with no 4-Ap effect (Fig. 10b) the decrease of potassium equilibrium potential tightens the range of pacemaker activity. *c* Additional decrease of E_A and E_K leads to complete abolishment of pacemaker activity in a small $\bar{g}_{Ca}$ range and a spike doublet develops ($c_I = 10$ μA/cm², 8 msec duration). Time bars every 400 msec

shift activities it is necessary to reduce the equilibrium potential of potassium ions to –10 mV (Fig. 14*b*). Detailed analysis of firing in the range of $\bar{g}_{Ca}$ responsible for pacemaker activity shows strong influences of the reduction of E_A and E_K (Fig. 15).

Discussion

Our aim was to present a modelling method by means of which the firing mechanism of neuronal membrane of invertebrates can be interpreted to an extent allowed by the present experimental results. The model includes the calcium system and the calcium-sensitive potassium system which seems to be of great importance in developing spike-afterhyperpolarization, pacemaker activity, prolonged bursting, slow depolarizing potentials, finite and infinite plateaus of membrane potential. We suggest that the described computer model is suitable for a detailed analysis of the membrane mechanisms listed previously. Our purpose was not the application of the method for the analysis of the characteristics of conductance and current underlying the voltage changes, nevertheless the method is suitable for visualizing the changes of ionic currents and conductances too. In practice the model is useful for studying the abnormal firing and ionic background of convulsive unit activity. The use of aminopyridine as a convulsive agent [24, 25] and the information available concerning K^+ channel blocking effects [26, 30] facilitates the understanding of the ionic control of epileptic activity. Using mathematical tools, trials have been carried out to realize the functional role of ionic conductances during epileptic activity in the mammalian nervous system [21].

From the results of the modelling procedure it is concluded that the involvement of Ca^{++} current in the kinetic description of the membrane significantly modifies the findings of the original investigations including the Na^+–K^+ mechanism [12, 13]. The calcium system produces a specific instability in the Na^+–K^+ system pacemaker spiking and bursting and beside the resting potential as a stable level of membrane potential, there appear the prolonged spike and burst after hyperpolarization and finite and infinite plateaus as new stable levels of the membrane potential. A detailed analysis of the stability and oscillatory characteristics of this complex system becomes possible by studying the limit cycle behaviour in phase-plane as a function of peak calcium conductance. The limit cycles have already been applied for the investigation of stimulus control of repetitive firing in squid axon membrane [10].

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STERIC MAPPING OF SOME PROSTAGLANDIN RECEPTOR SITES

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The Minimal Steric (Topologic) Difference method was applied to map some prostaglandin receptors. Experimental data concerning luteinizing activity of corpora lutea, and pressor respectively depressor activity on sheep blood were considered. The linear correlation coefficients obtained were $r = 0.927$ ($N = 12$) for luteinizing activity, $r = 0.943$ ($N = 15$) for depressor activity, and $r = 0.943$ ($N = 12$) for pressor activity. Similarities and differences between these three receptors are discussed.

Quantitative Structure Activity Relations (QSAR) do not seem to have been extensively used in prostaglandin research. As ubiquitus local hormones, prostaglandins take part in several biological mechanisms [1, 2]. The corresponding PG-receptors are most probably different for each kind of biological activity. At least, prostaglandins have a large variation of activity for relatively small changes in steric structure and an adequate account of the steric factors seems to be some sort of bottleneck in most QSAR-procedures. Here we want to demonstrate the possibility of obtaining QSAR's for prostaglandins by use of the MTD procedure.

In the MTD-procedure [3, 4] one constructs a topological network, the hypermolecule ($\widehat{\mathrm{H}}$) by an atom per atom superposition of the studied molecules (H atoms are neglected). The steric structure of an "i" molecule is described by a set of M binary x_{ij} variables, with $x_{ij} = 1$ if vertex "j" is occupied by the molecule "i" and $x_{ij} = 0$ if it is not. The M vertices j of the hypermolecule are attributed by an optimization procedure either to the receptor cavity ("good" vertices, $\varepsilon_j = -1$), to the receptor walls ("bad"-vertices, $\varepsilon_j = +1$) or to a steric irrelevant region ($\varepsilon_j = 0$). The regressional equation for this method and the minimal steric difference (MTD) are given by the equations,

$$\hat{\mathrm{A}} = \alpha - \beta \mathrm{MTD}_i \tag{1}$$

$$\mathrm{MTD}_i = \mathrm{s} + \sum_{j=1}^{\mathrm{M}} \varepsilon_j x_{ij} \tag{2}$$

where s is the number of cavity ($\varepsilon_j = -1$) vertices, α and β are usual regressional coefficients. Other structural parameters may also be included in the regressional equation (1).

Method and results

The series of compounds used for QSAR and the data for PG-binding to ovine corpora lutea, for depressor and pressor activity for sheep blood are taken from a review by Jones [1]. The compounds are listed in Tables I, II and III. In these tables A_L is the logarithm of the inverse relative association constant of the PG's from ovine corpora lutea ($A_L = 0$ for $PGF_{2\alpha}$); A_D and A_P are the logarithms of the inverse relative PG-concentrations producing depressor or pressor activity in sheep blood ($A_D = 0$ for PGE_2, $A_P = 0$ for $PGD_{2\alpha}$). The plasma of sheep contains little or no prostaglandin isomerase [1] and therefore the primary depressor effect following intraaortic injection of prostaglandin in sheep is considered a valid measure of the potency at the receptor site.

The hypermolecule $\hat{H}$ which results from the atom per atom superposition of these molecules is shown in Fig. 1. The pentacycle and most of the 1–7 and 13–20 chain atoms fit in $\hat{H}$, constituting vertices common for all compounds. Only those vertices are numbered (j from 1 to 17) which are not occupied in all compounds. As example, in PGE_2 the two chains occupy vertices $j = 1,2$ and 17, the ketonic $O_{(9)}$ $j = 6$, the $OH_{(4\alpha)}$-group $j = 4$, the $OH_{(15\alpha)}$-group $j = 5$, the carbonylic O atom of the COOH group $j = 12$. PGF_2 has instead of the ketonic $O_{(9)}$ and $OH_{(9\alpha)}$ group at $j = 3$. C-atoms with sp^2-hybridization are considered to produce only minor modifications in the stereochemistry of the pentacycle. The 1–7 chain in PGB's occupies vertices $j = 1.15$ (instead of 1.2) and vertices $j = 10.11$ in 8-iso PGE_1. The 13–20 chain of PGB's and PGC's occupies vertex $j = 16$ (instead of $j = 17$). For the C_5–C_6 and C_{13}–C_{14} hydrogenated compounds we assume conformations similar to the cis and trans configurations of the parent unsaturated prostaglandins.

Regarding the optimisation procedure, as the number of molecules in the studied series and the number of vertices is small, the molecules were arranged in decreasing order of activity, and several ε_j-attributions tried, until the best possible antiparallelism between the activities A and the MTD-values is obtained. Then the receptor map so obtained is tested for optimality conditions [4].

Concerning the binding to the receptor from ovine corpora lutea (Table I), the optimized receptor map obtained by the MTD procedure is

$$S_L\left\{\begin{array}{llll} j(\varepsilon = -1): & 1,2,4,12,15 & & 3,4,12 \\ j(\varepsilon = \ \ 0): & 3,5,9\text{–}11,16,17 & \text{or } (S_L'): & 1,2,5,9,15\text{–}17 \\ j(\varepsilon = +1): & 6,7,8 & & 6,7,8,10,11. \end{array}\right.$$

Table I

Experimental and calculated luteinizing activity of prostaglandins

i	Structure	A_L	MTD_L	$\hat{A}_L(3)$	$j(x_{ij} = 1)$
1	$PGF_{2\alpha}$	0.00	1	–0.54	1–5, 12, 17
2	15-methyl-$PGF_{2\alpha}$	–0.15	1	–0.54	1–5, 9, 12, 17
3	13, 14-dihydro-$PGF_{2\alpha}$	–0.36	1	–0.54	1–5, 12, 17
4	$PGF_{1\alpha}$	–1.30	1	–0.54	1–5, 12, 17
5	PGE_2	–1.43	2	–1.99	1, 2, 4–6, 12, 17
6	$PGF_{2\beta}$	–1.86	2	–1.99	1, 2, 4, 5, 7, 12, 17
7	13, 14-dihydro-15-oxo-$F_{2\alpha}$	–2.04	2	–1.99	1–4, 8, 12, 17
8	PGE_1	–2.53	2	–1.99	1, 2, 4–6, 12, 17
9	1-hydroxymethyl-$PGF_{2\alpha}$	–2.58	2	–1.99	1–5, 17
10	8-iso-PGE_1	–3.41	3	–3.45	3–5, 10–12, 17
11	PGA_2	–3.41	3	–3.45	1, 2, 5, 6, 12, 17
12	PGB_2	–3.41	3	–3.45	1, 5, 6, 12, 15, 16

Legend: see section Method and Results: $j(x_{ij} = 1)$ indicates the vertices of $\hat{H}$, occupied by each molecule.

Table II

Experimental and calculated depressor activity of prostaglandins

i	Structure	$\hat{A}_D$	MTD_D	$\hat{A}_D(6)$	$\hat{A}_P(7)$	$j(x_{ij} = 1)$
1	PGE_2	0.00	0	−0.15	−2.17	1, 2, 4–6, 12, 17
2	15-methyl-PGE_2	−0.04	1	+0.09	−3.32	1, 2, 4–6, 9, 12, 17
3	PGE_1	−0.11	0	−0.15	−3.30	1, 2, 4–6, 12, 17
4	PGE_0	−0.38	0	−0.15	−4.43	1,2, 4–6, 12, 17
5	PGC_2	−0.89	1	−1.33	−2.17	1, 2, 5, 6, 12, 16
6	PGC_1	−0.97	1	−1.33	−3.30	1, 2, 5, 6, 12, 16
7	11-desoxy-PGE_1	−1.11	1	−1.33	−2.17	1, 2, 5, 6, 12, 17
8	11-desoxy-PGE_0	−1.32	1	−1.33	−3.30	1, 2, 5, 6, 12, 17
9	11-desoxy-PGE_2	−1.36	1	−1.33	−4.43	1, 2, 5, 6, 12, 17
10	PGA_0	−1.62	1	−1.77	−4.43	1, 2, 5, 6, 12, 17
11	PGA_1	−1.76	1	−1.77	−3.30	1, 2, 5, 6, 12, 17
12	PGA_2	−1.91	1	−1.77	−2.17	1, 2, 5, 6, 12, 17
13	15-methody-PGE_2	−2.00	1	−1.32	−3.15	1, 2, 4–6, 12, 13, 17
14	15-oxo-PGE_2	−2.50	2	−2.51	−2.17	1, 2, 4, 6, 8, 12, 17
15	R-15-hydroxy-PGE_2	−2.74	2	−2.51	−3.15	1, 2, 4, 6, 9, 12, 17

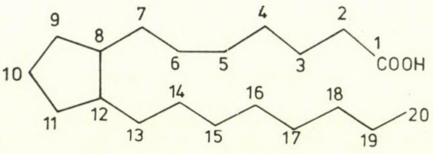
Legend: see Table I. $\hat{A}_P(7)$ are pressor activities calculated with MTD_P values for S_P and eq. (7).

Table III

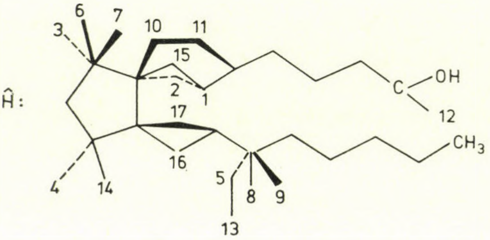
Experimental and calculated pressor activity of prostaglandins

i	Structure	A_P	MTD_P	$\hat{A}_P(7)$	$\hat{A}_D(6)$	$j(x_{ij} = 1)$
1	PGD_2	0.00	0	−0.18	−3.69	1–3, 5, 12, 14, 17
2	PGD_1	−1.02	0	−1.31	−3.69	1–3, 5, 12, 14, 17
3	15-oxo-$PGF_{2\alpha}$	−1.12	1	−1.18	−3.69	1–4, 8, 12, 17
4	R-15-hydroxy-$PGF_{2\alpha}$	−1.76	2	−2.14	−3.69	1–4, 9, 12, 17
5	$PGF_{2\alpha}$	−1.84	1	−1.16	−1.33	1–5, 12, 17
6	15-oxo-$PGF_{1\alpha}$	−2.15	1	−2.29	−3.69	1–4, 8, 12, 17
7	15-methoxy-$PGF_{2\alpha}$	−2.22	2	−2.14	−2.51	1–5, 12, 13, 17
8	15-methyl-$PGF_{2\alpha}$	−2.31	2	−2.31	−2.07	1–5, 9, 12, 17
9	$PGF_{1\alpha}$	−2.51	1	−2.29	−1.33	1–5, 12, 17
10	PGD_0	−2.87	0	−2.44	−3.69	1–3, 5, 12, 14, 17
11	15-oxo-$PGF_{0\alpha}$	−3.00	1	−3.42	−3.69	1–4, 8, 12, 17
12	15-oxo-$PGF_{2\beta}$	−3.23	3	−3.12	−2.51	1, 2, 4, 7, 8, 12, 17

Legend: see Table I. $\hat{A}_D(6)$ are depressor activities calculated with MTD_D values for S_D (with $\varepsilon_3 = \varepsilon_{14} = +1$) and eq. (6).



1a.



1b.

Fig. 1a. Prostaglandin skeleton with numbered atoms
Fig. 1b. Prostaglandin hypermolecule "H" with numbered vertices $j = 1,2, \ldots, 17$, where only vertices not occupied in all molecules are numbered

The two receptor maps are equivalent, i.e. they produce the same order of MTD-values for all molecules of the studied set. Thus, either $j = 1,2,15$ are cavity ($\varepsilon_j = -1$) vertices and $j = 10,11$ irrelevant ($\varepsilon_j = 0$), or $j = 1,2,15$ are irrelevant and $j = 10,11$ are wall ($\varepsilon_j = +1$) vertices. The regressional equation is

$$\hat{A}_L = 0.91 - 1.45\ \mathrm{MTD}_L;\ r = 0.927;\ \mathrm{EV} = 88\%\ (\mathrm{N} = 12) \tag{3}$$

For the receptor for depressor activity in sheep blood:

$$S_D \begin{cases} j(\varepsilon = -1): & 4,5 \\ j(\varepsilon = 0): & 16,17 \\ j(\varepsilon = +1): & 8,9,13 \end{cases}$$

$$\hat{A}_4 = -0.14 - 1.18\ \mathrm{MTD}_D;\ r = 0.810;\ (\mathrm{N} = 15) \tag{4}$$

while for the receptor for pressor activity, if one restrains to the "2"-class prostaglandin

$$S_P \begin{cases} j(\varepsilon = -1): & 3,14 \\ j(\varepsilon = 0): & 4,5,8 \\ j(\varepsilon = +1): & 7,9,13 \end{cases}$$

$$\hat{A}_5 = -0.21 - 0.98\ \mathrm{MTD}_P;\ r = 0.945;\ (\mathrm{N} = 7) \tag{5}$$

The regressional equations (4) and (5) can be improved by introducing two indicator variables; δ and Δ (or Δ'). For the presence of an O atom in position 15α ($j = 5$), $\delta = 0$, while for the presence of a CH_3 group, $\delta = 1$. For depressor activity ($\Delta = 1$ if a cyclic $\Delta_{10,\,11}$ double bond is present ($\Delta = 0$ if absent), while for pressor activity $\Delta' = 0.1$ or 2 as there is none, a $\Delta_{13,\,14}$ or a $\Delta_{13,\,14}$ and a $\Delta_{5,6}$ double bond are present. The corresponding regressional equations (see also Tables II and III) are,

$$\hat{A}_D = -0.15 - 1.18\ \mathrm{MTD}_D + 1.42\delta - 0.44\Delta\ ;\ r = 0.943;\ \mathrm{EV} = 89\%\ (\mathrm{N} = 15) \qquad (6)$$

$$\hat{A}_P = -2.44 - 0.98\ \mathrm{MTD}_P - 0.17\delta + 1.13\Delta';\ r = 0.943;\ \mathrm{EV} = 89\%\ (\mathrm{N} = 12) \qquad (7)$$

The MTD values calculated with the optimized maps S_L, S_D and S_P are also listed in Tables I to III. We remind that for the empty or occupied vertices j in all molecules of a given series, nothing can be inferred by the MTD-procedure; therefore, attributions for such vertices are not listed in the optimized receptor maps (S_L, S_D and S_P).

Discussion and conclusions

According to the optimized receptor map S_L (Fig. 2) the structural requirements for the luteinizing activity of PG's are,

- the C_9-atom with α configuration of the OH-group ($\varepsilon_3 = 0$ for 9α–OH, $\varepsilon_6 = +1$ for a 9-keto group, $\varepsilon_7 = +1$ for a 9β-OH group)
- C_{11} with α configuration for the OH-group ($\varepsilon_{11} = -1$)
- C_8 not β-substituted, which means α-configuration or sp^2-hybridization ($\varepsilon_1 = \varepsilon_2 = \varepsilon_{15} = -1$, but $\varepsilon_{10} = \varepsilon_{11} = 0$)
- presence of a carboxyl group at C_1($\varepsilon_{12} = -1$).

Thus, the pentacycle of the PGF molecule should fit optimally into the receptor cavity, as well as the C_7 and C_6 atoms of the C_1—C_7 side chain, if C_8 is not β-substituted. The requirement of a C_1-carboxyl group (ionized at pH 7) is probably electrostatic and not steric in nature. For the C_{13}—C_{20} side chain, steric requirements seem less severe ($\varepsilon_5 = \varepsilon_9 = \varepsilon_{16} = \varepsilon_{17} = 0$; $\varepsilon_8 = +1$).

The receptors for depressor and pressor activity (S_P and S_D, Fig. 2) put somewhat different requirements on the prostaglandin molecules. The resulting common feautures are,

- the C_9 and C_{15} atoms with α-OH groups as substituents ($\varepsilon_3 = 0$, $\varepsilon_5 = -1$, in S_D, $\varepsilon_3 = -1$, $\varepsilon_5 = 0$ in S_P)
- substituents at C_{15}, larger than the OH-group or not in α-configuration, penetrate the receptor walls ($\varepsilon_7 = \varepsilon_8 = \varepsilon_9 = \varepsilon_{13} = +1$ in S_D and S_P).

Distinct requirements for depressor activity (see S_D and eq. (6))
- αOH-group at C_{11} ($\varepsilon_4 = -1$)
- αCH_3 instead of αOH at C_{15} strongly enhances depressor activity (S_D may have a small rigid hydrophobic region at the $j = 5$ vertex),
- a cyclic $\Delta_{10,11}$ double bond decreases depressor activity.

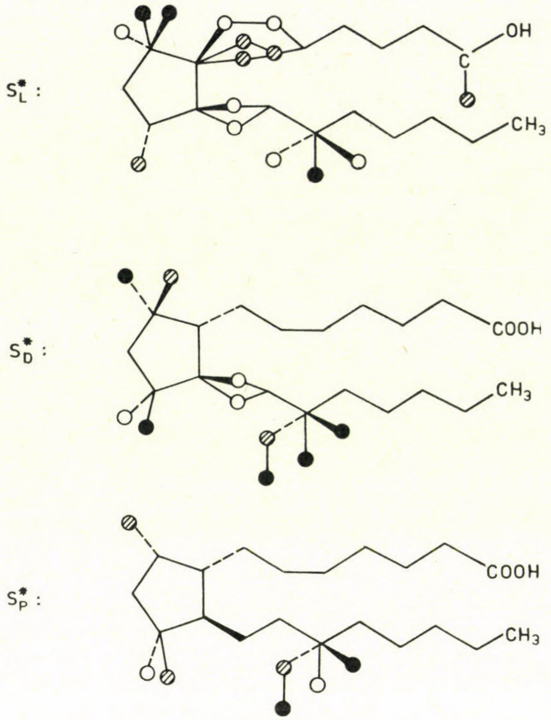


Fig. 2. The most active prostaglandin in the studied series with respect to the indicated receptor is given in parantheses

Distinct requirements for pressor activity (see S_P and eq. (7)),

- keto group at C_{11} ($\varepsilon_{14} = -1$, $\varepsilon_4 = 0$)
- $\Delta_{5,6}$ and $\Delta_{13,14}$-double bonds enhance pressor activity (probably steric fit requirements for the cis configuration of the C_1—C_7 side chain and for the trans configuration of the C_{13}—C_{20} chain).

The unfavourable effect upon depressor activity of the cyclic $\Delta_{10,11}$ double bond cannot be explained in the framework of the MTD procedure; it could be due to some metabolic inactivation process.

Pressor and depressor activities are complementary, therefore pressor substances should become depressors only at sensibly higher concentrations than those at which their pressor effect is already exerted and the reverse should also be true. A detailed review of the complex interactions between arachidonic acid metabolites and the renin angiotensin system was published recently [5]. This can be used to test the real predictability of the receptor maps S_{D_1} and S_P and the regressional equations $\hat{A}_D$ and $\hat{A}_P$. Unfortunately, Jones lists only molar potencies relative to PGE for depressor, and to $PGD_{2\alpha}$ for pressor activity. Also, vertices $j = 3$ and 14 are inoccupied in all depressor PG's of Table II they will be considered as "bad" vertices, $\varepsilon_3 = \varepsilon_{14} = +1$ for S_D in calculating depressor activities for the pressor PG's of Table III. The two activity "scales" can be compared for $PGF_{0\alpha}$ suggested [1] to give a biphasic response: the calculated activities for $PGF_{0\alpha}$ ($MTD_D = 3$, $MTD_P = 1$) are rather similar $\hat{A}_D = -3.69$; $\hat{A}_P = -3.42$.

The calculated pressor activities of depressor PG's (using S_P and eq. (7)) are listed in Table II; the calculated depressor activities for pressor PG's (with S_D and eq. (6)), in Table III. Only one of the 15 depressor PG's of Table III have a calculated $\hat{A}_D$ higher than $\hat{A}_P$.

The MTD procedure thus yields information about the structural characteristics of prostaglandin molecules, required for a certain biological activity. This information is contained in the optimized receptor maps, which give information about the shape of receptor cavities corresponding to the physiologically active state (i.e. of the receptor combined with the corresponding prostaglandin effector).

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EFFECT OF VIBRATION ON THE ACTIVITY OF THE PLASMA KALLIKREIN-KININ SYSTEM IN THE RAT

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Rats were exposed to whole body vibration horizontally for four hours at 5 Hz frequency and 2 cm amplitude. Of the components of the plasma kallikrein-kinin system the free (spontaneous) and kaolin-activated kallikrein (prekallikrein) activities, the concentration of bradykinin, the bradykinin splitting total kininase activity, total kallikrein-inhibitor activity and the concentration of α_2-macroglobulin, a major plasma kallikrein inhibitor, were estimated.

Results showed that in response to acute vibration plasma free kallikrein activity was increased significantly in association with a significant reduction of prekallikrein concentration. The concentrations of bradykininogen and total kininase activity were significantly elevated, too. Neither total kallikrein-inhibitor activity nor the concentration of alpha$_2$-macroglobulin were changed indicating that the plasma kallikrein inhibitors did not play a role in the alterations of plasma free kallikrein and prekallikrein activities.

During acute vibration the plasma kallikrein-kinin system was activated probably by the enhanced catecholamine secretion. We suggest that the biological importance of this phenomenon is in the defense against the impaired microcirculation caused by catecholamines.

Several experimental findings [8, 9, 10] and clinical observations [5] prove that noxious vibration plays a role in the genesis of various civilization diseases such as peptic ulcer, hypertension and myocardial infarction. As a harmful byproduct of urbanization, intensified industrial development and extensive urban and highway transport a novel civilization disease has been recognized as vibration disease [14]. Both the civilization diseases and the vibration damage are characterized by microcirculatory changes in some organs and organ systems [4, 13].

In addition to the vegetative mediators (norepinephrine, acetylcholine), the renin-angiotensin-aldosterone system, prostaglandins, serotonin and histamine, the kallikrein-kinin system is known to play an important role in the control of local microcirculation [17].

In order to elucidate the mechanism of action of the vibration damage, the changes in the components in the kallikrenin-kinin system were studied in rats exposed to acute vibration. No such data are available in the literature.

Methods

Male rats of the CFY strain weighing from 120 to 150 g were used after a 16-hour starvation period. The animals were divided into two groups, one control and one experimental with a single exposure to a 4-hour vibration. Parameters for vibration: 5 Hz frequency, 2 cm amplitude, horizontal. During exposure the rats could move freely in their vibration chamber.

Immediately after the termination of the vibration exposure the rats were exsanguinated via the femoral artery. Haematocrit was also estimated. The blood was collected in two polyethylene tubes at 4 °C with or without isosmotic sodium citrate as anticoagulant. The components of the kallikrein-kinin system were determined in the citrate plasma, whereas the alpha$_2$-macroglobulin in the sera.

Plasma-free (spontaneous) kallikrein, kaolin activated kallikrein (prekallikrein) activities and total kallikrein-inhibitor activity were estimated according to Colman et al. [3] with the modification of Gomozkov et al. [11]. The principle of the assay: the inactive prekallikrein in the plasma is activated by kaolin. The activated kallikrein splits the benzoyl-arginine-ethylester (BAEE) substrate and the aethyl group thus released forms a green product with 2-hydrazino-2,3-dihydro-3-methyl-benzothiazol-HCl (MBTH). This can be measured by photometry. Into tubes I and II 0.48 ml, tubes III and IV 0.46 ml Tris buffer (100 mmol/l, pH 8.0) and 0.5 ml BAEE solution (20 mmol/l) were pipetted. To tube I (blank) 0.2 ml 10% $ZnSO_4$ and 20 μl non-activated plasma were added. Into tube II 20 μl non-activated plasma was pipetted.

Meanwhile 0.3 ml plasma was incubated with 0.3 ml kaolin suspension (10 mg kaolin was suspended in 10 ml physiologic saline-phosphate buffer 100 mmol/l, pH 7.6) in a water bath at 25 °C. After a 1-min incubation 40 μl was transferred into tube III, and after 3 min incubation the same volume was transferred into tube IV. All four tubes were then incubated at 37 °C for 30 min; the reaction was stopped in tubes II + III and IV by 10% $ZnSO_4$ and the precipitate was centrifuged (7000 rpm). From the resultant supernatant fluid 0.4 ml aliquots were transferred into four other test tubes and to these subsequently 0.2 ml distilled water, 0.2 ml 1% $KMnO_4$ dissolved in 10% $KHSO_4$ and five minutes later 0.2 ml of 1% NH_4OH were added. After the solution had been bleached to the tubes 1.5 ml of 1% MBTH were added, 20 min later 1.5 ml 2% $NH_4Fe(SO_4)_2 \cdot 12\,H_2O$ and 60 min later the optical density of the solutions was measured in tubes II, III and IV at 635 nm against tube I (blank). Since the solution in tube I was generally not quite colourless we also used an absolute blank (0.5 ml phosphate buffer — 100 mmol/l pH 7.6 — and 0.1 ml 10% $ZnSO_4$) and the absorbant of tube I was read against this blank. The optical density thus obtained was subtracted from the values for tubes II, III and IV. Concentrations were read using calibration curves constructed using ethanol and kallikrein activites and were expressed in μmol ethanol/ml plasma/h. The free (spontaneous), kallikrein activity was obtained from tube II, whereas the concentration of prekallikrein was calculated from the difference between values for tubes II and III. The total kallikrein-inhibitor activity was expressed on the basis of the percent reduction of kallikrein activity between tubes III and IV.

Alpha$_2$-macroglobulin was estimated by the method of Veremeyenko and Volohonskaya [19]. The alpha$_2$-macroglobulin forms a complex with the trypsin added to the serum and this complex releases nitroaniline from N-alpha-benzoyl-DL arginine-4-nitroanilide hydrochloride (BAPNA) which can be estimated by photometry. The amount of the nitroaniline formed and the amount of alpha$_2$-macroglobulin present are proportional.

The serum was diluted 20-fold by phosphate buffer (50 mmol/l, pH 7.6). To 0.3 ml of diluted serum 0.3 ml 20 mg% trypsin were added dissolved in phosphate buffer 50 mmol/l, pH 7.6 then 0.2 ml of 50% soy-bean trypsin inhibitor (REANAL) was added to neutralize the excess trypsin. The alpha$_2$-macroglobulin-trypsin complex is not sensitive to the inhibitor. After 15 min the volume was brought to 1 ml and incubated for 2 min at 20 °C in a water bath. Then 3.2 ml of 10 mmol/l BAPNA (50 mmol/l dissolved in phosphate buffer pH 7.6) were added and the incubation was continued for a further 30 min period. The reaction was stopped by 1.4 ml 5% phosphotungstenic acid. The precipitate formed was removed by centrifugation and the absorbance of the supernate was read at 385 nm against blank treated alike, except that trypsin was added after the addition of the phosphotungstenic acid. The concentration of the alpha$_2$-macroglobulin was expressed in g/l.

The bradykininogen (kallikrein substrate) assay was performed according to Diniz et al. [6]. Trypsin splits the bradykininogen and releases the free bradykinin; this was estimated and from its amount the bradykininogen concentration could be calculated.

Citrate plasma, 0.1 ml and 1.0 ml of 0.2% acetic acid were incubated for 30 min in a boiling water bath; after cooling, the solution was neutralized with NaOH (0.5 mol/l). One ml Tris-buffer (100 mmol/l, pH 7.5) and 0.2 ml 100 mg% trypsin solution dissolved in Tris buffer were then added. The incubation lasted for 30 min at 37 °C. The reaction was terminated by the addition of 0.2 ml of 200 mg% SBTI. The amount of bradykinin released was estimated using a rat-uterus bioassay system. The uterus from rats in oestrus was suspended in organ bath containing 10 ml de Jalon solution bubbled with oxygen and kept at a constant temperature by an ultrathermostat. The bradykinin-induced uterine contractions were transformed to electric signals and recorded using a RADELKIS recorder. The amount of bradykinin was calculated from calibration curves constructed from responses to known amounts of bradykinin. Results were expressed as the amount of bradykinin released from 1 ml of plasma, as ng/ml.

The bradykinin splitting total kininase activity was estimated in a bioassay system according to Erdős [7], modified by Veremeyenko [18].

To 50 μl plasma, 1.75 ml TRIS–HCl buffer (100 mmol/l, pH 7.2) and 0.2 ml bradykinin solution (4000 ng) were added and incubated at 37 °C. The enzyme reaction was stopped by boiling. After the incubation the remaining bradykinin was bioassayed in 0.2 ml aliquots using the aforementioned *in vitro* uterus preparation. Total kininase activity was expressed as the amount of bradykinin split by 1 ml plasma in 1 min, as ng/ml/min.

Statistical analysis of the data was performed by Student's independent *t* test.

Results

Results are illustrated in Figs 1, 2 and 3. Figure 1 shows the changes in plasma spontaneous kallikrein and prekallikrein activities. Plasma free kallikrein activity was increased significantly ($p < 0.05$) by about 50% to acute vibration exposure.

The kaolin-activated kallikrein (prekallikrein) activity showed opposing changes being significantly ($p < 0.05$) decreased by about 30%. Figure 2 demonstrates alterations in the concentration of bradykininogen and the total kininase activity in rats after vibration.

As is seen the level of kallikrein substrate was increased fourfold ($p < 0.05$) in response to a single exposure to vibration. The total kininase activity was markedly augmented ($p < 0.05$). Figure 3 shows the plasma total kallikrein-inhibitor activity and the concentration of alpha$_2$-macroglobulin, a major plasma kallikrein inhibitor. It appears that neither total kallikrein inhibitor activity nor alpha$_2$-macroglobulin concentration were changed by vibration.

Discussion

Results of this paper show that the applied acute vibration exposure induced changes in the kallikrein-kinin system. In response to a single four-hour vibration the free plasma kallikrein activity was significantly reduced. These findings seem to suggest that under the effect of acute exposure to vibration the conditions favour the activation of kallikrein (factor III activation, tissue hypoxia and acidosis). The simultaneous increase in the concentration of kallikrein substrate indicates and enhanced mobilization of bradykininogen from the liver. The elevation of total kininase activity was probably

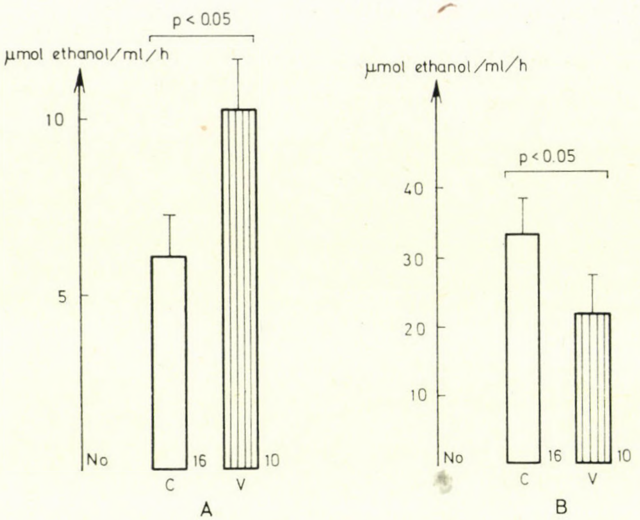

Fig. 1. Changes in plasma free (A) and kaolin activated (B) kallikrein activities in response to acute vibration. C = control, V = vibration, Means ±S.E.

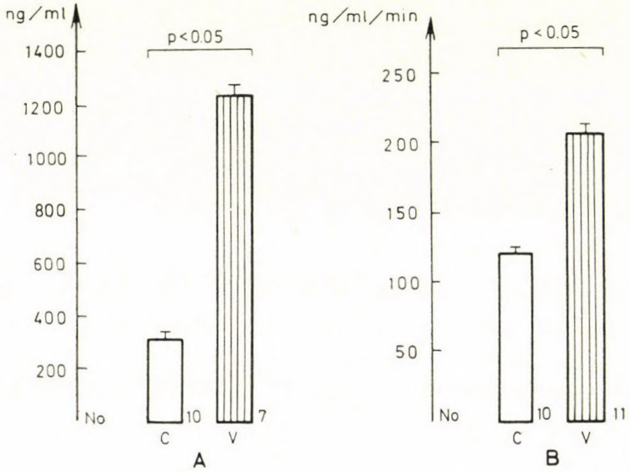

Fig. 2. Changes in plasma concentrations of bradykininogen (A) and total kininase activity (B) in response to acute vibration. C = control, V = vibration, Means ±S.E.

a regulatory response against the predominance of bradykinin. Thus, under the effect of acute vibration the activity of the kallikrein-kinin system was enhanced or shifted towards an increased bradykinin production. The kallikrein inhibitors did not appear to be involved in these changes.

In our oppinion the vibration-induced alterations in the kallikrein-kinin system occurred due to the hormonal imbalance accompanying vibra-

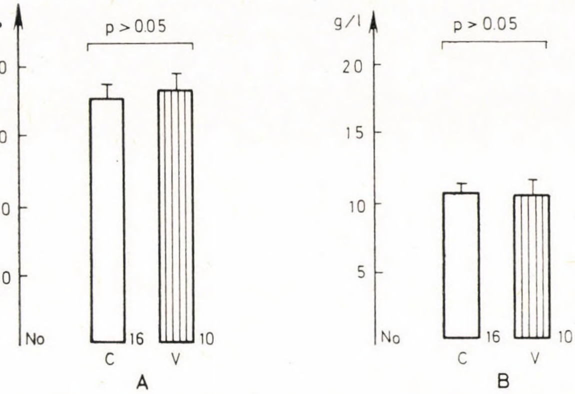

Fig. 3. Changes in plasma total kallikrein-inhibitor activity (A) and serum concentrations of alpha$_2$-macroglobulin (B) in response to acute vibration. C = control, V = vibration, Means ±S.E.

tion. It is known that vibration markedly increases the blood levels of norepinephrine and glycocorticoids [1]. Changes of the former predominate after acute vibration, whereas longlasting vibration produces more marked elevation of the concentrations of glycocorticoids. Rotehild et al. [15] have shown that epinephrine increases the conversion of prekallikrein to kallikrein and the release of bradykinin from the liver which is a direct consequence of the epinephrine-induced tissue hypoxia and acidosis as suggested by Dietzel et al. [5]. The glycocorticoids exert an opposing action on the activity of the kinin system. Cline and Melmon [2] have found that glycocorticoids inhibit the activation of prekallikrein, whereas Lancberg et al. [12] have reported on enhanced kinin splitting kininase activity.

Since the release of catecholamines occurs faster, their prevalance during acute vibration is highly probable.

In summary, we suggest that the kallikrein-kinin system plays an important role in the development of the microcirculatory alterations induced by vibration. The biological importance of these phenomena is probably the defense against tissue hypoxia produced by the increased release of catecholamines.

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EFFECTS OF DRUGS ON THE PRODUCTION OF STRESS ULCER IN THE RAT

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The authors studied the effect of drugs with different mechanisms of action on the prevention of stress ulcer production in the rat. Stress ulcer was induced by a method developed by the authors: intact, starved rats were swimmed in water at 23 °C for 5 hours.

1. Atropine (0.1–0.5 and 1.0 mg/kg i.m.), cimetidine (1.0–5.0 and 25 mg/kg i.p.), prostacyclin (PGI_2) (5.0–25.0 and 100 μg/kg i.p.) and phentolamine (0.35–1.75–3.5 and 7.0 mg/kg i.m.) were shown to decrease the production of stress ulcers significantly, in a dose-dependent fashion.

2. Propranolol (0.35–1.75–3.5 and 7.0 mg/kg i.m.) did not influence the production of stress ulcers. The finding that drugs with different actions could considerably reduce or prevent the production of stress ulcer appears to indicate the complexity of the neural, hormonal and biochemical processes involved in the pathogenesis.

On the basis of the present results the authors suggest the use of a preventive therapeutic regimen in clinical practice with an appropriate combination of drugs.

Factors such as gastrotoxic (ulcerogenic) drugs, some severe diseases (acute pulmonary or cardiac insufficiency, sepsis, acute renal or hepatic failure, complex burn injuries, long-lasting surgery) acute intracranial pressure elevations (brain surgery and injury, stroke), etc. are known to be responsible for the production of *acute* erosions of the gastric and duodenal mucosa. These are called *stress ulcers* irrespective of the morphology and pathogenesis.

There are several methods known for inducing experimental stress ulcers [1, 3, 4, 18, 19] which are suitable for the study of the pathogenetic factors. As for the underlying pathogenetic events, opinions vary widely, and thus no uniform standpoint has been adopted to date with regard to drug prevention and therapy. The objective of the present paper was

1. to study the effects of drugs with different mechanisms of action on ulcer production in a stress ulcer model developed in our laboratory;
2. to establish the role of neural and humoral factors in the pathogenesis of stress ulcer produced in swimmed rats.

Methods

Experimental animals

A total of 166 Wistar rats of either sex weighing 210 to 250 g were used. Animals were starved for 24 hours while free access to water was allowed. Rats were forced to swim continuously for a period of 5 hours in a deep concrete tub with a water temperature of 23 °C. No

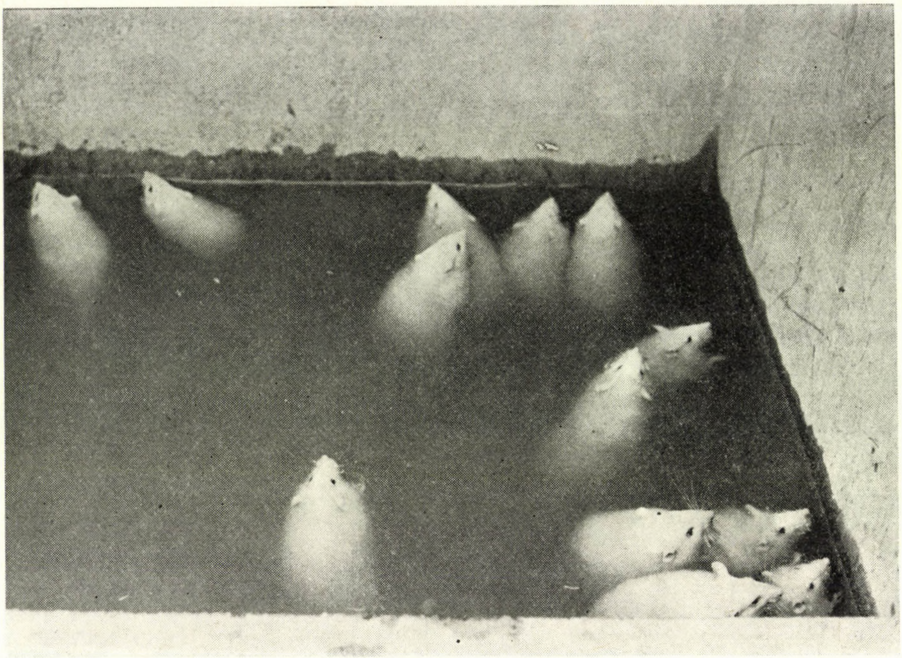

Fig. 1. The stress-ulcer model: rats with intact stomach were forced to swim for 5 hours in water at 23 °C

extra weights were applied. The pylorus had not been ligated (Fig. 1). Swimming in water below body temperature resulted in the production of acute haemorrhagic lesions (principally erosions) in the glandular part (area of parietal cells) of the stomach in all the rats. Only a few rats succumbed.

Drugs

Conscious rats were pretreated immediately before swimming. Drugs were dissolved in physiologic saline (4.0 ml/kg).

1. *Cimetidine* (Histodil®, Gedeon Richter Pharmaceutical Works, Budapest) 1.0–5.0–25.0 mg/kg i.p.
2. *Atropine* (Atropinum sulfuricum ®, EGYT, Budapest) 0.1–0.5–1.0 mg/kg i.m.
3. *Prostacyclin* (PGI_2) (Chinoin, Budapest) immediately before study freshly dissolved and diluted as prescribed 5.0–25.0–100 μg/kg i.p.
4. *Propranolol* (Inderal® ICI, Cheshire, England) 0.35–1.75–3.50–7.0 mg/kg i.m.
5. *Phentolamine* (Regitine Ciba-Geigy, Basel, Switzerland) 0.35–1.75–3.50–7.0 mg i.m.

All groups had their matching controls injected with physiologic saline i.p. or i.m.

Examination of the gastric mucosa

After termination swimming the animals were killed by aether. The stomach was removed and opened along the greater curvature and the mucosa rinsed carefully with tap water and examined using a 10× magnification lens. The following characteristics were recorded:

1. *Average count of lesions*
2. *Severity index* established according to a score described elsewhere [15, 16]:
   Grade 0, intact stomach
   Grade 1, diameter of the erosion less than 1 mm
   Grade 2, diameter of the erosion from 1 to 2 mm
   Grade 3, diameter of the erosion from 2 to 4 mm
   Grade 4, diameter of the erosion more than 4 mm

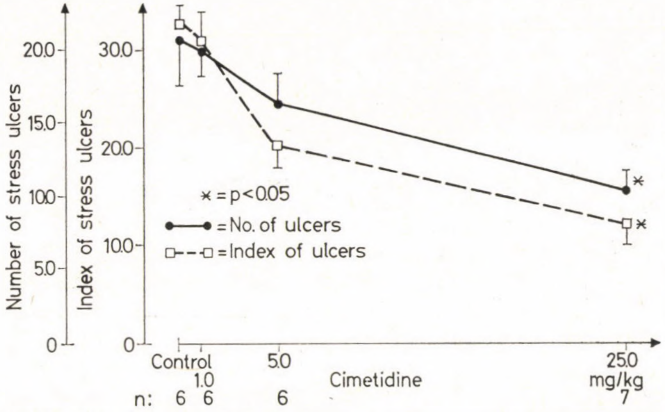

Fig. 2. Effect of various doses of cimetidine on the production of stress ulcer. n = number of animals in one experimental group. Points indicate means, vertical lines are S.E.M.

The severity index was calculated by summing up the scores for the individual erosions. The antiulcerogenic effect of drugs was calculated according to the formula:

$$\text{Antiulcerogenic effect (\%)} = \frac{\text{ulcer index}_{\text{control}} - \text{ulcer index}_{\text{treated}}}{\text{ulcer index}_{\text{control}}}$$

Statistical evaluation

Data are means ±S.E.M. Data were analysed for statistical significance by Student's unpaired *t* test (average count of lesions) and by the Mann–Whitney test (severity index), significance $p < 0.05$.

Results

The 5-hour swimming produced stress ulcer in all of the rats without pylorus ligation after fasting for 24 hours. Acute lesions (principally oval-linear haemorrhagic erosions) were observed in the glandular part, i.e. parietal cell zone of the rat stomach. The rumen and the antrum were found intact by gross examination.

1. *Effect of cimetidine*

The H_2 receptor inhibitor cimetidine prevented the acute stomach lesions (ulcer score and severity index) in a dose-dependent fashion. At a dose of 25 mg/kg the antiulcerogenic effect was significant ($p < 0.05$) (Fig. 2).

2. *Effect of PGI_2 (prostacyclin)*

PGI_2 also exerted a dose-related protection tested on the basis of both ulcer parameters. The dose of 100 μg/kg had a strongly significant antiulcero-

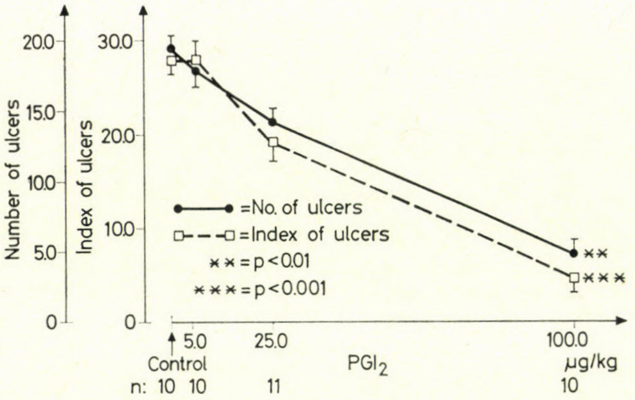

Fig. 3. **Effect of prostacyclin (PGI$_2$) on the production of stress ulcer in rats forced to swim. For symbols, see legend to Fig. 2**

genic action as shown by both the ulcer score ($p < 0.01$) and ulcer index ($p < 0.0001$) (Fig. 3).

3. Effect of atropine

Atropine provided a protective effect on the gastric mucosa of the rats. A dose of 0.5 mg/kg already afforded an almost 100% protection ($p < 0.001$) (Fig. 4).

4. Effect of propranolol

The beta blocker propranolol had no appreciable effect on the stress ulcer production in the rats (Fig. 5).

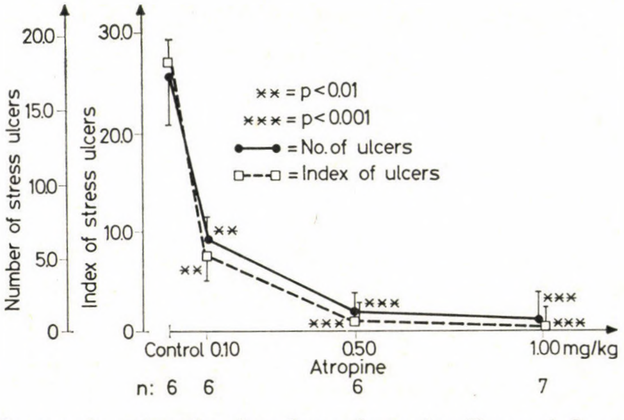

Fig. 4. **The antiulcerogenic action of various doses of atropine. For symbols, see legend to Fig. 2**

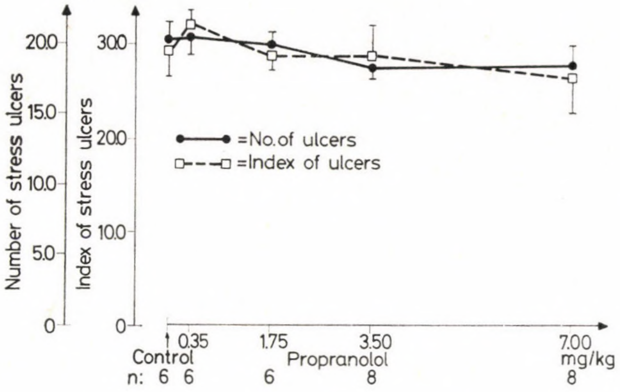

Fig. 5. **Effect of propranolol on the production of stress ulcer**

5. *Effect of phentolamine*

The alpha receptor blocker phentolamine was potent in reducing the score and severity index of the stress ulcers at doses of 3.5 and 7.0 mg/kg (Fig. 6).

The antiulcerogenic actions of the various drugs tested are illustrated in Table I.

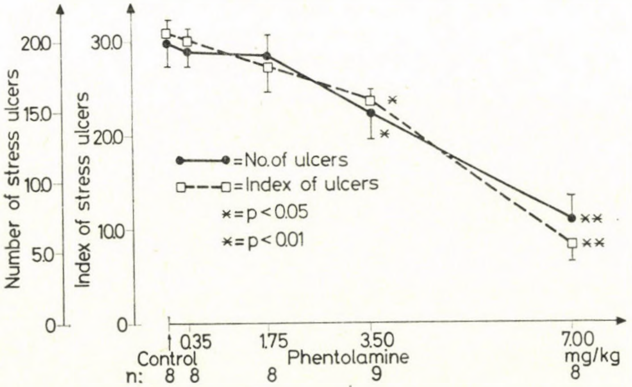

Fig. 6. **Effect of phentolamine on the production of stress ulcer in rats forced to swim. For symbols, see legend to Fig. 2**

Table I

Inhibition by various drugs of the production of stress ulcer in rats forced to swim

Drug	Dose	Antiulcerogenic action	Significance
Atropine	1.0 mg/kg i.m.	98%	$p < 0.001$
Cimetidine	25.0 mg/kg i.p.	44%	$p < 0.05$
PGI_2	100.0 μg/kg i.p.	75%	$p < 0.01$
Phentolamine	7.0 mg/kg i.m.	56%	$p < 0.01$
Propranolol	7.0 mg/kg i.m.	0.6%	$p > 0.05$

Discussion

There are several pathogenetic factors considered to be of importance in the genesis of experimental stress ulcer, e.g. the degranulation of mast cells in the gastric mucosa [7], the elevation of tissue histidine decarboxylase activity [9], the change in the secretion of the protective mucus [8], and various biochemical alterations [4, 11].

The same holds true for the pathogenesis of human stress ulcer. Table II contains the factors that might play a role in the pathogenesis of the human stress ulcer based on the literature.

Data by others and our own observations suggest that the incidence of gastrointestinal ulcers is currently on the increase. In our intensive care unit the incidence reaches 20%. It is often serious, resulting in the deterioration of the patients' conditions in many cases leading to death (intractable bleeding, perforation).

Table II

I. *Gastric hypersecretion* (Cushing ulcer)

II. *Reduction of mucosal resistance*
  1. Disturbance in blood supply
    a) Cardiorespiratory insufficiency
    b) Sympathetic activity
    c) Microthrombi (DIC)
      Sepsis
      Burn (Curling ulcer)

  2. Disturbance of cellular metabolism

  3. Altered mucus secretion
      Quantitative
      Qualitative

III. *H^+–rediffusion*
  Bile acids, lysolecithin
  Drugs

Thus the prevention and therapy of stress ulcer is a common problem in clinical practice. On the other hand, there is no satisfactorily applied and uniform regimen for the therapy or prevention. A wide variety of somatic and emotional stress might produce acute ulceration both in man and animals. In our opinion this does not necessarily mean that the pathologic processes involved in the production of lesions are essentially different. There is general agreement among investigators that for the production of stress ulcer the simultaneous action of several noxious factors is absolutely necessary. The assessment of these factors is of paramount importance for effective ulcer prevention and therapy. The many confliciting data and problems not fully understood justify the attempts for further exploration of this problem.

We have tested the antiulcerogenic effects of drugs using a readily reproducible stress ulcer model. Results obtained by drugs with different mechanisms of action shed light on the importance of neural and humora factors involved in the pathogenesis of stress ulcer.

Atropine, cimetidine, PGI_2 and phentolamine, with some differences in their potencies, exerted a significant antiulcerogenic action on the production of stress ulcer in rats forced to swim for 5 hours.

Atropine, cimetidine and prostacyclin might act principally by suppressing acid output. It is known that during the development of stress ulcer the gastric acid secretion is reduced [2, 12], although the presence of hydrochloric acid still plays an important role in the onset of mucosal lesions. Further suppression of gastric acid secretion or the neutralization of free hydrochloric acid in the stomach by various antacids prevent the development of stress ulcer.

Prostaglandins [5, 10, 14] and atropine [13] are known to exert a favourable effect on the gastric mucosa independently of the action on acid secretion. This is the so-called cytoprotective action which results in enhanced resistance of the mucosal cells to a wide variety of noxious effects. This cytoprotection might have also occurred in our experiments, increasing the defense of the mucosa against the humoral and metabolic changes elicited by the stressors.

Catecholamines and adrenergic activity also play an important role in the production of stress ulcers [1, 3, 4, 6]. Adrenaline and noraderenaline treatment caused acute haemorrhagic erosions in the glandular part of the stomach in pyloric ligated rats [16, 16] which were similar in appearance to stress ulcer. These lesions could be prevented by phentolamine.

In the present study also the alpha blocker phentolamine reduced the incidence of stress ulcer in rats, whereas propranolol afforded no protection compared to the controls. Other investigators using the restraint-ulcer model report on a preventive action principally of beta blockers [17].

The results suggest that the production of stress ulcer in the rats forced to swim depended on complex neural and humoral factors; among them the importance of the parasympathetic and sympathetic alpha adrenergic activity is obvious.

The favourable effect of drugs suppressing hydrochloric acid output underlines the importance of the aggressive action of acid in the stomach.

Among the pathologic processes involved in the genesis of stress ulcer no hierarchy can be established since the suppression or abolishing of any of them will result in the reduction or prevention of stress ulceration.

On the basis of the present results a reasonable combination of drugs might be promising in the prevention of stress ulceration. This requires further studies in this field.

Acknowledgement

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EFFECT OF LASER IRRADIATION AND PROSTAGLANDIN $F_{2\alpha}$ ON THE PRODUCTION OF GASTRIC MUCUS IN HUMAN PATIENTS

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An increase in gastric mucus production was observed after $PGF_{2\alpha}$ administration and low dose argon-laser irradiation in human patients. The effect of $PGF_{2\alpha}$ on functioning of the gastric mucosa is opposite to that of the PGE group. The observed increase in mucus production is a new aspect of the bio-stimulatory effect of laser irradiation.

One of the main functions of the gastric mucosa besides the secretion of hydrochloric acid and pepsinogens is the production of mucus [3]. New data have been published recently which help to understand the physiological role of the gastric mucosal lining. According to recent results [10], the speed of diffusion of hydrogen ions through a mucus layer is one-fourth of that observed through a water layer of the same thickness. This slowing down of diffusion is accounted for by the water-coat covering the mucus and also by the water drops adhering to the interstitium of the mucus because of their inaccessibility to diffusion. Such physicochemical phenomena explain the protection provided by the gastric mucus against the harmful effects of hydrochloric acid [7]. The acid diffusing into the mucosa in spite of this protection is neutralized by bicarbonate ions produced there.

The effect of prostaglandins have mostly been studied on the female genital apparatus. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has proved to be useful in inducing abortion and labour. Few data are available about the effects of $PGF_{2\alpha}$ on the gastric mucosa.

In the field of medical applications of laser the phenomenon of biostimulation has gained prominence [9]. This effect can be observed with low dose laser; in the case of argon-laser this corresponds to 300–500 mW. No data could be found about the effect of argon-laser on functioning of the gastric mucosa. The aim of the present study was therefore to investigate the effect of $PGF_{2\alpha}$ and argon-laser irradiation on the production of gastric mucus in human patients.

Methods

Patients of either sex 30 to 50 years were selected for the investigation, in whom primary chronic superficial gastritis was previously diagnosed on the basis of histologic examination of samples obtained by endoscopy. Determination of mucus content was carried out by the method of Hamerman and Schuster [6]. Following the introduction of a rubber stomach tube the gastric juice was removed and its volume measured. While leaving the tube in place, either an injection of $PGF_{2\alpha}$ or laser irradiation was done. One hour later the gastric juice was removed again, its volume measured and the concentration of mucus determined. The dose of $PGF_{2\alpha}$ (Chinoin) injected subcutaneously was 1 mg.

Laser irradiation was carried out by using an Argon-Laser-Photocoagulator (Spectra Physics Type 770). Argon-laser can be directed via a flexible fibre-optic system made of quartz, similarly as the Nd YAG laser [1]. This characteristic of the argon-laser allows the irradiation of various parts of the gastrointestinal tract through a flexible endoscope. The argon-leser operates at 0.45 and 0.51 $m\mu$ wavelengths, in the blue-green range of the visible spectrum. The antrum was irradiated with 500 mW for 200 s, the cumulative dose being 100 J. Since the irradiated surface area of the gastric mucosa was 50 cm^2, the energy density of the irradiation amounted to 2 $J \cdot cm^{-2}$.

Results

The fasting concentration of gastric mucus was in the normal range. One hour after the injection of $PGF_{2\alpha}$ the concentration increased markedly, while the volume of gastric fluid was unchanged (Fig. 1).

One hour after laser irradiation a significant augmentation of gastric mucus production was measured (Fig. 2).

Discussion

The observed effect of PGF_2s on function of the gastric mucosa was the opposite of that of PGE_1. Gastric juice production was found to be reduced

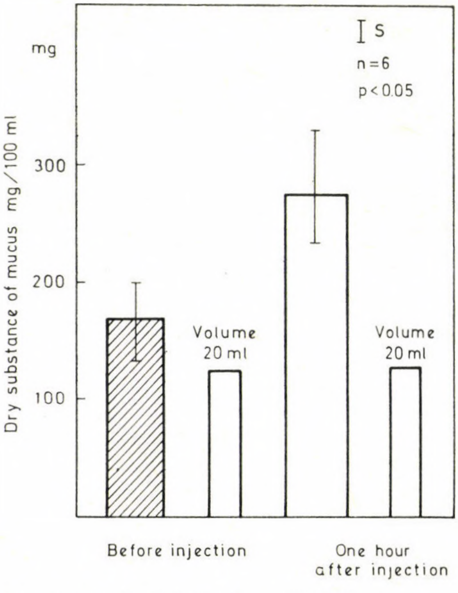

Fig. 1. Effect of a subcutaneous (s.c.) injection of $PGF_{2\alpha}$ on gastric mucus production

by PGE_1, while after $PGF_{2\alpha}$ treatment both total acidity and pepsinogen concentration were augmented [5]. In the present study the stimulatory effect of $PGF_{2\alpha}$ on a third parameter of gastric function, mucin production, has been observed.

Laser irradiation is now applied extensively in medicine in view of its special advantages. Its main use in gastroenterology is primarily connected with the thermic effect causing photocoagulation. This effect is best utilized to stop bleedings which need [1, 2, 4, 8] high energy irradiation.

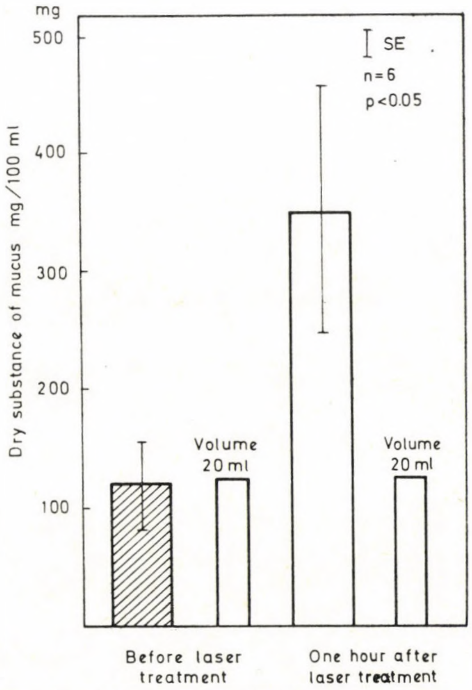

Fig. 2. Effect of argon-laser on gastric mucus production

The non-thermal effect of low energy laser irradiation is connected with its polarized light characteristic [9]. This bio-stimulatory action is ascribed by some authors to enzyme activation. The present experiments have furnished additional evidence of this bio-stimulatory effect.

The stimulatory effect of $PGF_{2\alpha}$ and laser irradiation on the gastric mucosa may be explained either by their influence on the mucosal barrier or else by changes of mucosal serotonin function.

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RELATIONSHIP BETWEEN THE TISSUE REDOX STATE POTENTIAL AND $\mathrm{d}a_{k}^{\circ}/\mathrm{d}t$ CHANGES OF $[\mathrm{K}^{+}]_0$ ACTIVITY DURING k-STROPHANTOSIDE OR ACETYLCHOLINE INDUCED CONTRACTURES

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It was established that oxidosis evoked by methylene blue during k-strophantoside and acetylcholine contractures in skeletal muscle was accompanied by an increase in the changes of $[\mathrm{K}^{+}]_0$ activity, while redosis evoked by ascorbate under the same conditions was accompanied by a decrease in the changes of $[\mathrm{K}^{+}]_0$ activity. These changes in $[\mathrm{K}^{+}]_0$ activity parallelled the alterations of contractures caused by oxidosis or redosis suggesting that changes in the $[\mathrm{K}^{+}]_0$ transient play an important role in these phenomena.

In the past few years increasing attention has been paid to the questions of redox regulation in excitatory and muscle contraction processes, as it was reviewed by us earlier [5]. It has become clear that in excitable systems there exists — beyond those already known — a fairly complicated regulatory mechanism which consists of (*i*) redox state potential (E_0')variations of exogenous or metabolical origin and (*ii*) tissue sites which are sensitive to these variations.

While studying the redox regulation of excitatory processes it was observed in our laboratory that k-strophantoside (STR)-induced contractures of the frog rectus abdominis muscles were increased by an exogenous oxidant (methylene blue) but they were decreased by reductants (ascorbate and dithiothreitol) [1]. It was also established that methylene blue increased (47%) while ascorbate decreased (65%) the acetylcholine-induced contractures [7]; (on the other hand, the changes of $[\mathrm{K}^{+}]_0$ activity were increased in the former and decreased in the latter study (Puppi, unpublished results).

The aim of the present study was to evaluate the possible role of $[\mathrm{K}^{+}]_0$ in the variation of ion fluxes caused by oxidants and reductants.

Materials and methods

Experiments were performed on the isolated rectus abdominis muscle of the frog (*Rana esculenta*) at 23 °C. An OP–K-711 type K^{+} sensitive electrode (measuring range: pX mol/dm^3 0. . . . . .6 10°. . . . . .10^{6}, reproducing capability: ±1.5 mV) was used around the membrane to which a 1 mm thick rubber ring was fixed. The muscles were also fastened to the electrode shell drum. As a result of this procedure a chamber of 25 mm^3 capacity was formed between the surface of the muscle and the ion-selective membrane of the electrode. This limitation of the extracellular space allowed for near optimal application of the electrode and at the same time insured the standardization of the extracellular space. Because of the drum-like fixation, the muscle contractures were isometrical and did not influence the volume of the chamber. The electrode with the muscle was submerged into Ringer solution, which was in contact with a KCl reservoir through an agar bridge. The reference electrode in this reservoir was an Ag/AgCl one. Chemical agents were added to the Ringer solution bathing the muscle. The measure of the increase ($+\,da_k^o/dt$) or decrease ($-da_k^o/dt$) of the activity of extracellular K^+ (a_k^o) was determined at 1 min intervals and expressed in per cent of the respective control values.

Generally the K^+ selective membrane of the electrode was facing the distal side of the muscle. Since in some preliminary experiments it was found that the results were the same when the electrode was facing the proximal side of the muscle, the possiblity of measuring an oriented transport from one side of the muscle to the other could be excluded.

The kinetics of the potential changes measured by the electrode was monitored partly on a potentiometer of high input resistance (OP-205) and partly on a potentiometric recorder (OH-814) connected to the former. The solution was continuously aerated. Prior to treatments the calibrated concentration of $[K^+]_0$ in the chamber varied between 2.6–3.3 mM. For each muscle variation in the da_k^o/dt values of $[K^+]_0$ activity was expressed as the per cent change of the da_k^o/dt values of $[K^+]_0$ activity before treatment.

The following chemicals were used (in final concentrations): acetylcholine hydrochloride (ACh), 10 μM; methylene blue — as oxidant — (MB), 0.1 mM; ascorbate — as reductant — (ASC), 0.1 mM; k-strophantoside (STR), 0.1 mM. All these solutions were buffered to pH 7.4 before use with TRIS–HCl. This agent — per se — did not influence the electrode potentials.

According to our earlier observations, 3 min after their application, MB (0.1 mM) increased the E_0' value by 16 mV and ASC (0.1 mM) decreased this parameter by 25 mV in the frog rectus abdominis muscle [7].

Results and discussion

Application of MB or ASC per se did not evoke significant changes in the da_k^o/dt values of $[K^+]_0$ activity. Three min after the addition of STR the da_k^o/dt of activity of $[K^+]_0$ was increased by 20%, with a contracture of 25% of the maximum. Upon simultaneous application of STR and MB, the da_k^o/dt of $[K^+]_0$ activity rose by 66% and the contracture attained 45% of the maximum. A possible explanation for this finding is that both oxidation [6, 3, 4, 2] and STR inhibit ($Na^+ + K^+$) ATP-ase in an additive manner.

Simultaneous application of STR + ASC (reductant) resulted in a 45% decrease in the da_k^o/dt of the activity of $[K^+]_0$ and only 10% of the maximal contracture was attained. One of the reasons for this may be an increase in active $[K^+]_0$ influx, since reduction is known to increase active transport [6, 3, 4, 2]. Three min after the application of ACh a 52% increase in the da_k^o/dt of $[K^+]_0$ activity was observed along with a 60% contracture. This effect could have resulted from the action of ACh both on the active and passive movements of K^+.

Simultaneous application of ACh and STR produced an additive effect both in the increase in the da_k^o/dt of $[K^+]_0$ activity (75%) and contracture (85%). Simultaneous addition of STR + MB + ACh caused some increase in both the contracture (82%) and the da_k^o/dt value of $[K^+]_0$ activity (95%).

Following the simultaneous application of STR + ASC + ACh, i.e. by making the milieu more reducing, there was a 18% increase in the $da\ /dt$ of $[K^+]_0$ activity, which was equal to the net effect of the individual actions of

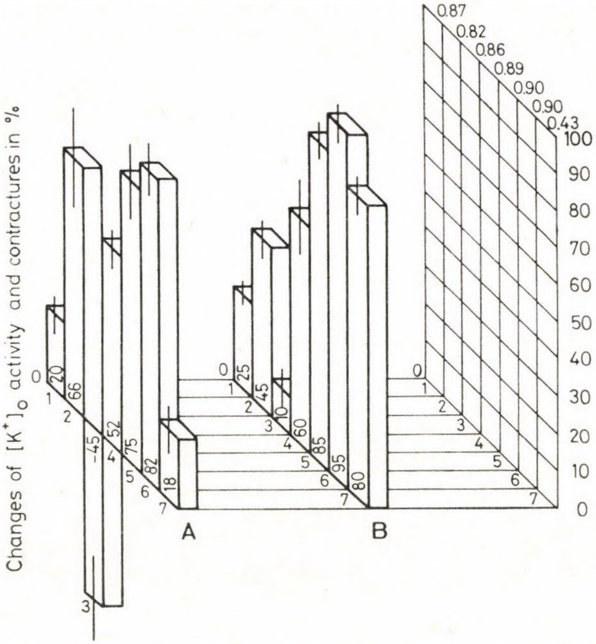

Fig. 1. Changes in the $\mathrm{d}a_k/\mathrm{d}t$ value of $[K^+]_0$ activity (A) and amplitude of contractures (B) in per cent of maximum following k-strophantoside (blocks 1), methylene blue + k-strophantoside (blocks 2), ascorbate + k-strophantoside (blocks 3), acetylcholine (blocks 4), acetylcholine + k-strophantoside (blocks 5), acetylcholine + methylene blue + k-strophantoside (blocks 6), and acetylcholine + ascorbate + k-strophantoside (blocks 7) treatments during contractures of the frog rectus abdominis muscle caused by k-strophantoside and acetylcholine 3 min after the application of the agents. Numbers at the bottom of the blocks designate per cent changes, while those above the ordinate represent correlation coefficients between the $\mathrm{d}a_k^{\circ}/\mathrm{d}t$ values of $[K^+]_0$ activity and contractures. Vertical bars: ±S.E. In the case of only methylene-blue and ascorbate treatments neither contractures nor changes in $[K^+]_0$ activities were observed

STR, STR + ASC and ACh, both STR and ACh increasing this parameter, while ASC decreasing it. The degree of contracture, however, did not exhibit this additivity. To elucidate this single case when the correlation between the $\mathrm{d}a_k^{\circ}/\mathrm{d}t$ of $[K^+]_0$ activity and the contracture level was low, the following considerations might be of value. In the case of simultaneous application of STR+ASC, the former, by inhibiting the $(Na^+ + K^+)$ ATPase promotes a depolarizing tendency, while ASC — as a reductant — stimulates the $(Na^+ + K^+)$ ATPase [6, 3, 4, 2]. In the case of simultaneous use of STR + ACh + ASC, both STR and especially ACh promote depolarization, and it appears that the hyperpolarizing action of ASC is already not capable to compensate for the effects of two depolarizing agents. Though this action is not reflected in the changes of the $\mathrm{d}a_k^{\circ}/\mathrm{d}t$ values of $[K^+]_0$ nevertheless it might be assumed that there were changes in the movement of the Na^+ and/or Ca^{2+} ions playing no lesser role in the excitation processes than K$^+$. Experiments are in progress to test this assumption.

In conclusion, there is a very high positive correlation between the level of contractures and da_k°/dt when an oxidation or reduction modifies the STR, ACh contractures. In view of this the importance of K$^+$ transient as an effector in the response to alterations of redox state potential on the mechanical activity of skeletal muscles seems to be beyond doubt.

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EFFECTS OF CHOLECYSTOKININ-RELATED PEPTIDES ON RETENTION OF PASSIVE AVOIDANCE BEHAVIOUR

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Effects of intraperitoneal administration of cholecystokinin (CCK)-related peptides were studied on retention of single-trial learning passive avoidance behaviour. The COOH-terminal octapeptide of CCK (CCK-1–8-SE), the unsulfated octapeptide (CCK-1–8-NS), as well as the COOH-terminal tetrapeptide of CCK (CCK-5–8), administered immediately after the learning trial, facilitated passive avoidance behaviour. The data indicate that these peptides may influence memory consolidation processes.

In recent years the efforts of endocrinologists, neurochemists and biochemists have revealed that the mammalian brain contains a number of neuronally localized peptides. Several peptides have been recognized as occurring in the gastrointestinal endocrine cells and in the central and peripheral nervous system [see reviews: 17, 21]. Gastrin-like immunoreactivity was originally demonstrated in the central nervous system by Vanderhaeghen et al. [30], however, it seems to be clear by now that the peptide which reacted with gastrin antiserum was in fact the cholecystokinin octapeptide (CCK-8) [3, 24, 27, 29]. Chromatographic separation of porcine cortical peptides revealed that CCK-8 is the principal CCK-like peptide, and smaller amounts of CCK-33, CCK-12 and CCK-4 are also present [28]. The brain contains only trace amounts of true gastrin although appreciable amounts are found in the pituitary gland of the pig [27]. The small amounts of true gastrin in the mammalian brain probably originate from the vagal nuclei where true gastrin has been found [27]. This gastrin has been shown to be transported down the cervical vagus nerve [16] to the gut where it presumably contributes to the vagal reflex. Golterman et al. [14] showed that CCK is synthesized in the brain, mostly in the cells of the ventral tegmental area (A 10 group) and transported intra-axonally to the terminal areas of this group [4, 17].

The physiological role of "brain-born" CCK and their fragments has not yet been properly elucidated. Earlier it was shown that peripheral administration of CCK-8 sulfate ester (CCK-1–8-SE) and unsulfated CCK-8 (CCK-1–8-NS) increased the step-through latency of single-trial learning passive avoidance behaviour [7], the most effective dose being 400 nmol/kg intraperitoneally. It has also been demonstrated that CCK-1–8-SE and fragments of this peptide containing tyrosine-sulfate-methionine residue are able to modulate the monoamine contents of different brain areas, as well as the plasma corticosterone level [6, 12, 13], however CCK-1–8-NS is practically without effect on the monoamine content of the discrete brain areas or adrenocortical activity [6, 8].

The present study was carried out in order to measure the activity of a number of cholecystokinin fragments in a dose of 400 nmol/kg on the step-through latency of single-trial learning passive avoidance response.

Materials and methods

Animals

Male CFY rats of an inbred strain weighing 150–180 g were used. The animals were housed 5–6 per cage at room temperature (20–21 °C). All animals had access to commercial food and tap water *ad libitum* and were kept on a controlled illumination schedule (lights on between 6 a.m. and 6 p.m.). Experiments were carried out daily between 6 a.m. and noon.

Passive avoidance behaviour

Animals were trained in a step-through-type one-trial learning passive avoidance test, as described previously [1, 7]. Animals were treated 30 s after the learning trial and they were tested 24 h later.

Treatment

CCK-1–8-SE (Asp–Tyr/SO_3H/–Met–Gly–Trp–Met–Asp–Phe–NH_2), CCK-1–8-NS (Asp–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2), CCK-2–8-SE (Tyr/SO_3H/–Met–Gly–Trp–Met–Asp–Phe–NH_2), CCK-3–8 (Met–Gly–Trp–Met–Asp–Phe–NH_2), CCK-4–8 (Gly–Trp–Met–Asp–Phe–NH_2), CCK-5–8 (Trp–Met–Asp–Phe–NH_2), CCK-6–8 (Met–Asp–Phe–NH_2), CCK-7–8 (Asp–Phe–NH_2), CCK-1–4-NS (Asp–Tyr–Met–Gly–NH_2) and CCK-2–4-SE (Tyr/SO_3H/–Met–Gly–NH_2) were dissolved in physiological saline and given intraperitoneally in 400 nmol/kg dose in a volume of 1.0 ml/kg 30 sec after the learning trial. Control animals received the physiological saline solution in a volume of 1.0 ml/kg.

The peptides were synthetized by one of us (Dr. B. PENKE), the method, yield, purity have been published elsewhere [6, 25, 26].

Statistical analysis

Statistical analysis of the results was performed by analysis of variance.

Results

The effects of CCK-1–8-SE, CCK-1–8-NS and their fragments on avoidance latency of one-trial learning passive avoidance behavior are shown in Fig. 1. CCK-5–8, CCK-4–8, CCK-3–8, CCK-2–8-SE, CCK-1–8-SE and CCK-1–8-NS significantly facilitated passive avoidance behaviour, while fragments without the COOH-terminal tetrapeptide residue of CCK-8 did not change avoidance latency.

Discussion

Although CCK has been demonstrated to be present in high concentrations in the brain [2, 20, 24, 27], little is known about its effects on the central nervous system. CCK is a putative short-term satiety hormone which may

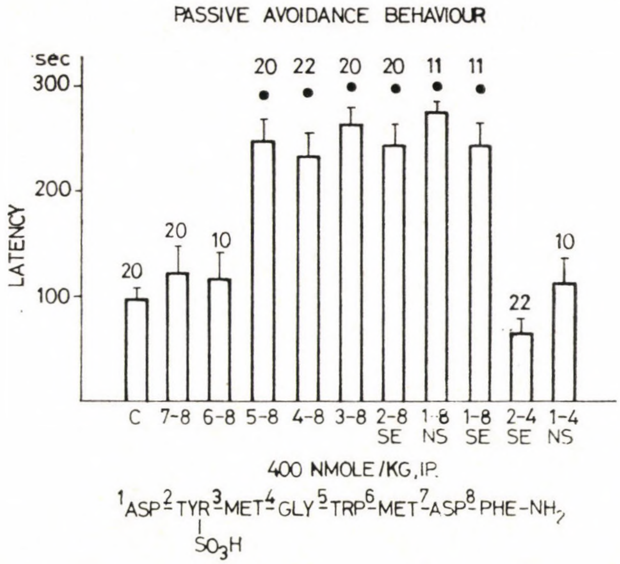

Fig. 1. **Effect of cholecystokinin-related peptides on avoidance latency of single-trial learning passive avoidance behaviour. Data are means ±S.E.M. ● = $p < 0.05$ vs. control; C = control**

regulate meal size and the intermeal interval [22, 23]. It has also been suggested that this peptide might play a role in pain perception [18], furthermore ZETLER [31] showed that high doses of peripheral administered CCK produced analgesia in mice. In our previous reports it was shown that both peripherally and centrally administered CCK-1–8-SE or CCK-1–8-NS impaired acquisition and facilitated extinction of conditioned feeding behaviour and bench-jumping active avoidance behaviour [5, 11]. In these test situations, the similarity between the effect of peripherally and centrally administered CCK-1–8-SE and CCK-1–8-NS suggests that these peptides interact with central nervous mechanisms rather than peripheral ones. Earlier it was also shown that CCK-1–8-SE, CCK-1–8-NS, CCK-5–8, CCK-4–8, CCK-3–8, CCK-2–8-SE following intracerebroventricular administration facilitated passive avoidance behaviour [10]. In the present study we showed that peripheral administration of the same CCK-related peptides increased the latency of single-trial learning passive avoidance response, showing that these peptides affect the central nervous system independently of the route of administration, furthermore that the effects in one paradigm can be generalized to other behaviour. Nevertheless, following intracerebroventricular administration 0.001 times the dose of these CCK-related peptides is needed to cause the same effect on avoidance latency as compared to peripheral (intraperitonel) administration of the peptides. It is noteworthy that with other neuropeptides (ACTH-like peptides, vasopressin,

β-lipotropin, etc.) this ratio is 1:100 [15]. It seems that the absorption, metabolism and passage through the blood-brain barrier of these CCK-related peptides are different.

The present data, together with previous findings [10] clearly indicate that the COOH-terminal tetrapeptide of CCK-8 is the shortest sequence which affects passive avoidance behaviour of rats, while the NH_2-terminal part of this molecule (Tyr/SO_3H/-Met) is responsible for changes in brain monoamine metabolism and pituitary-adrenocortical activity [6].

In this behavioural paradigm sedative effects of CCK-8 can be ruled out, since in our previous study there were no differences in any of the parameters of the open-field activity following intraventricular or intraperitoneal administration [11, 19]; on the other hand, in this passive avoidance test situation animals were tested 24 h after peptide treatment and it is unlikely that the sedative effect of CCK-related peptides would last so long [32, 33]. It is possible that the facilitating effect of these peptides is based on interference with memory consolidation processes; data following intracerebroventricular administration of CCK-antiserum strengthen this assumption [9], although further analysis is needed to show the mechanism of action of CCK-related peptides in this behavioural paradigm.

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EFFECTS OF INTRACEREBROVENTRICULARLY ADMINISTERED CHOLECYSTOKININ ANTISERA ON ACTIVE AVOIDANCE AND CONDITIONED FEEDING BEHAVIOUR OF RATS

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The effects of intracerebroventricular administration of two different cholecystokinin antisera were tested on acquisition and extinction of active avoidance behaviour as well as on acquisition, maintenance and extinction of conditioned feeding behaviour of rats. Both antisera delayed the extinction of active avoidance and conditioned feeding response, while they were ineffective on acquisition and maintenance. These data suggest that the endogenous CCK of the brain might be a physiological modulator of extinction processes.

There are abundant data on the relationship between the COOH-terminal octapeptide of cholecystokinin (CCK-8) and food intake [e.g. 15, 23, 25], however, relatively little attention has been payed to other behaviours. In a series of experiments with mice, Zetler [38, 39, 40, 41] found that CCK-8 had analgesic, sedative, anticonvulsant and neuroleptic-like properties. Recently, Crawley et al. [2] have observed that CCK-8 reduces exploration and social interactions in mice and rats. In our earlier investigations it was shown that both peripheral and intracerebroventricular administration of cholecystokinin octapeptide sulfate ester (CCK-8-SE) or unsulfated cholecystokinin octapeptide (CCK-8-NS) impaired acquisition of active avoidance and conditioned feeding behaviour, while these peptides caused a facilitated extinction of active avoidance and conditioned feeding behaviour of rats [7, 8, 13]. On the other hand, it was also shown that these peptides increased the step-through latency of single-trial learning passive avoidance behaviour of rats, indicating that CCK interfered with retrieval and/or consolidation processes [10].

If endogenous CCK has an important role as regards the active avoidance and conditioned feeding behaviour, then administration of the specific antiserum into the lateral cerebral ventricle of rats might neutralize the endogenously produced and released CCK, in this way causing a deficit of CCK in the central nervous system, with opposite effects as compared to CCK-8. This approach has been successfully used earlier by us, demonstrating that the actions of CCK-8 on brain monoamine content as well as on passive avoidance behaviour could be reversed by CCK-antiserum [9, 12, 20], furthermore by Della-Fera et al. [5] showing that CCK antibody injected in cerebral ventricles stimulated feeding in sheep.

The present experiments were designed to clarify the role of endogenous CCK on active avoidance and conditioned feeding behaviour of rats, by means of the intracerebroventricular administration of two different CCK antisera.

Materials and methods

Animals

CFY adult male rats, weighing 150–180 g at the beginning of the investigation, were used. The animals were housed 5–6 per cage with free access to tap water and housed at room temperature (20–21 °C). The animals were kept on an artificial light schedule (12 h light, 12 h dark), the light period starting at 6 a.m. The training and experimental sessions were carried out daily between 6 a.m. and noon.

Surgery

For the intracerebroventricular administration of CCK antisera a cannula was implanted into the lateral cerebral ventricle under pentobarbital (35 mg/kg, i.p.) anaesthesia as described earlier [14]. The rats were allowed to recover for 7 days. The correct positioning of the cannula was checked individually by injection of methylene blue after the experiments had been completed.

Behavioural procedures

Active avoidance behaviour. Acquisition and extinction of active avoidance behaviour were studied in a bench-jumping situation; the experimental apparatus and procedure have been described previously [13, 34]. These animals had free access to commercial food *ad libitum.* One group of animals was treated 12 h prior to the first acquisition session and they were tested on day 1–6 of acquisition. The total number of conditioned avoidance responses scored by each animal during the first three and second three sessions of 10 trials served as the index of avoidance behaviour. The other group of animals was treated 12 h after having reached the criterion of learning (i.e. 80% or more conditioned avoidance responses during three consecutive days) and they were subjected to extinction of active avoidance behaviour for 6 days. The total number of conditioned avoidance responses scored by each animals during the first three and second three sessions of 10 trials served as the index of extinction of avoidance behaviour.

Conditioned feeding behavior. Acquisition, maintenance and extinction of conditioned feeding behaviour were studied in a special conditioning (Skinner-type) box. The experimental apparatus and whole procedure have been described previously [7, 13]. In this experiment the rats were deprived of food for three days before training and for 18 h before each session. After each experimental day the animals were placed into their own cage to be fed *ad libitum* (6 h).

One group of animals was treated 12 h prior to the first acquisition session of conditioned feeding paradigm and they were tested on day 1–6 of acquisition. The total number of conditioned feeding responses scored by each animal during the first three and second three sessions of 10 trials of acquisition served as the index of acquisition of conditioned feeding behavior. The second group of animals was treated 12 h after having reached the criterion of learning (i.e. 70% or more conditioned feeding responses during three consecutive days) and they were subjected to maintenance of conditioned feeding behavior for 6 days. During maintenance the conditioned stimulus was applied together with reinforcement. The total number of conditioned feeding responses scored by each animal during the first three and second three sessions of 10 trials of maintenance of conditioned feeding behavior served as the index of maintenance of conditioned feeding behaviour. The third group of animals was treated 12 h after having reached the criterion of learning (i.e. 70% or more conditioned feeding responses during three consecutive days) and they were subjected to extinction of conditioned feeding behaviour for 6 days. During extinction the conditioned stimulus was not followed by reinforcement. The total number of conditioned feeding responses scored by each animal during the first three and second three sessions of 10 trials of extinction of conditioned feeding behavior was considered as the index of extinction.

Treatment

Two kinds of CCK antisera were used. One of these (CCK-4 antiserum) was obtained from Dr. J. F. Rehfeld (Institute of Medical Biochemistry, University of Aarhus, Aarhus, Denmark); it had been raised in rabbits against synthetic human gastrin-17 as described by Rehfeld et al. [29]. This antiserum (No. 4562) is specific for the four COOH-terminal amino acids (Trp–Met–Asp–Phe–NH_2), which are identical in gastrin and CCK [28]. The other CCK antiserum (CCK-33 antiserum) was obtained from Dr. J. C. Thompson (The University of Texas Medical Branch, Galveston, Texas, U.S.A.); this had been raised in rabbits against 16% pure CCK-33. The antibody is primarily directed against the NH_2-terminal portion of the CCK molecule. The method of preparation and the specificity of the CCK antiserum were described earlier [22, 26]. The liophylized antisera were dissolved in 0.9% saline, and 1:10 and 1:2 dilutions in 5 μl were injected through the cannula of freely moving, conscious animals. The control animals received normal rabbit serum in the same dilutions and volume.

Statistical analysis

Kruskal–Wallis' and Mann–Whitney's non-parametric ranking tests [36] were used for statistical analysis of the data. A probability level of 0.05 or less was accepted as a significant difference.

Results

As can be seen from Table I, intracerebroventricular treatment 12 h prior to the first acquisition session with CCK-4 or CCK-33 antisera did not change acquisition of the bench-jumping avoidance response either on days 1–3 or on days 4–6. On the other hand, the same antisera injected 12 h prior to the first extinction session, both in 1:10 and 1:2 dilutions, delayed the extinction of the bench-jumping active avoidance response, as compared with that of the control animals treated with normal rabbit serum.

Administration of CCK-4 or CCK-33 antiserum 12 h prior to the first acquisition session or 12 h before the first maintenance session of conditioned feeding paradigm was ineffective on the response rate of conditioned feeding behaviour (Table II). Postlearning treatment with these CCK antisera 12 h prior to the first extinction session significantly increased the conditioned avoidance responses (i.e. delayed extinction of this behaviour) both in 1:10 and 1:2 dilutions, and both on days 1–3 and on days 4–6.

Discussion

Cholecystokinin (CCK) is a peptide hormone originally identified in the gut and recently found in the neurons of the central nervous system. CCK has been localized in the cerebral cortex of several mammalian species as well as in various extracortical areas including the striatum, hippocampus, hypothalamus, olfactory bulb and pituitary [1, 6, 18, 24, 27, 33, 35]. CCK-containing cell bodies and fibres have been found in the A10 dopamine cell group of the ventral tegmental area [1, 35, 37], and it has been shown that CCK and dopamine may coexist in a subpopulation of these mesolimbic neurons [19]. This finding is of special interest since the mesolimbic dopamine pathway

Table I

Effects of CCK antisera in two different dilutions on the acquisition and extinction of active avoidance behaviour of rats

Treatment	Dilution	Acquisition			Extinction		
		N	Day 1-3	Day 4-6	N	Day 1-3	Day 4-6
Normal rabbit serum	1:10	8	8.4±1.5*	18.0±1.5	8	19.3±1.4	5.8±1.0
CCK-4	1:10	8	6.5±1.4	16.5±1.9	8	24.4±1.2^{a}	10.9±1.1^{a}
CCK-33 antiserum	1:10	8	7.7±1.5	21.9±1.3	8	24.8±1.1^{a}	11.8±1.2^{a}
Normal rabbit serum	1:2	8	8.0±1.4	18.9±1.3	8	20.0±1.3	6.9±1.0
CCK-4 antiserum	1:2	8	7.6±1.7	19.4±1.9	8	26.6±0.8^{a}	16.3±1.0^{a}
CCK-33 antiserum	1:2	8	9.0±2.0	19.4±2.2	8	27.4±0.7^{a}	17.0±1.0^{a}

* = Mean ±S.E.M.
^{a} = $p < 0.05$ vs. control

Table II

Effects of CCK antisera in two different dilutions on the acquisition, maintenance and extinction of conditioned feeding behaviour of rats

Treatment	Dilution	Acquisition			Maintenance			Extinction		
		N	Day 1-3	Day 4-6	N	Day 1-3	Day 4-6	N	Day 1-3	Day 4-6
Normal rabbit serum	1:10	8	6.9±1.3*	16.1±2.0	6	24.4±1.3	23.4±1.1	6	13.7±1.4	11.0±0.9
CCK-4 antiserum	1:10	8	6.4±1.3	17.0±1.9	6	23.8±1.7	22.2±1.5	6	18.5±1.6^{a}	14.3±1.0^{a}
CCK-33 antiserum	1:10	8	6.9±1.4	17.3±2.0	6	24.0±1.3	21.3±0.9	6	19.5±1.2^{a}	13.7±1.2^{a}
Normal rabbit serum	1:2	8	7.5±1.3	15.8±2.0	6	24.0±1.3	24.7±1.0	6	13.5±1.5	9.8±1.1
CCK-4 antiserum	1:2	8	8.3±1.3	16.9±1.9	6	22.0±1.1	22.0±1.4	6	20.8±1.3^{a}	16.7±0.7^{a}
CCK-33 antiserum	1:2	8	6.6±1.2	18.0±1.9	6	23.8±1.5	22.5±1.5	6	21.3±1.2^{a}	17.2±0.7^{a}

* = Mean ±S.E.M.
^{a} = $p < 0.05$ vs. control

has been implicated in the regulation of behaviour, including schizophrenia [3, 31, 32]. As a result it has been suggested that CCK may play a role in this disorder, perhaps modulating the release of dopamine [11, 18, 19, 31].

The present study indicates that endogenous CCK of the brain may be involved in the processes of extinction of bench-jumping active avoidance or conditioned feeding behaviour. Both CCK antisera delayed extinction of these behavioural responses. This effect is opposite to that induced by the intracerebroventricular administration of sulfated or unsulfated CCK-8 [13]. It is noteworthy that intracerebroventricular administration of the same CCK antisera attenuates the passive avoidance response of rats, while intracerebroventricular injection of CCK-8-SE and CCK-8-NS facilitate it [10, 12]. In the present study we failed to find any significant effect of the intracerebroventricularly injected CCK antisera on acquisition and maintenance, although CCK-8-SE and CCK-8-NS impaired the acquisition of both active avoidance and conditioned feeding behaviour of rats [7, 13]. It is possible that in these test situations the demonstration of facilitation is a more difficult task. Another explanation could be that the effect of CCK-8 peptides on acquisition processes is not a specific one. It is also possible that CCK-8-SE and CCK-8-NS are able to attenuate the attention, motivation or to modify the arousal of animals. It seems unlikely that these peptides have sedative effects, since following intracerebroventricular administration of CCK-8-SE and CCK-8-NS we failed to find any effect of these peptides on open-field activity [13, 21]. Furthermore it is not possible to establish whether the CCK-8 induced disruption of avoidance responding and of conditioned feeding responding reflected general sedative or neuroleptic-like properties of CCK-8 since sedatives and neuroleptics both exert similar effects on avoidance [16]. The reported analgesic properties of CCK-8 [38] were probably not responsible for the disruption of avoidance since low to moderate doses of morphine enhance avoidance performance [4, 17]. On the other hand, CCK-8 facilitated the extinction of avoidance when shock was not present (extinction and conditioned feeding paradigm).

The present experiments suggest that endogenous brain CCK may, under physiological conditions, modulate the extinction of active avoidance and conditioned feeding behaviour, however, further experiments are needed to unravel the attenuating influence of CCK-8-SE and CCK-8-NS on active avoidance and conditioned feeding paradigms.

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Pharmacologia

RELAXATION BY PROSTACYCLIN (PGI_2) AND 7-OXO-PGI_2 OF ISOLATED CEREBRAL, CORONARY AND MESENTERIC ARTERIES

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The relaxant effects of prostacyclin (PGI_2) and its stable 7-oxo-analogue, a product of Chinoin Pharmaceutical Works, were compared on isolated canine cerebral, bovine coronary, rabbit coeliac and human mesenteric arterial strips. The resting tone of the coronary, cerebral and human mesenteric arteries as well as the stimulation- or noradrenaline-evoked tone of coeliac vessels were reduced by both prostanoids. The relaxant action of 7-oxo-PGI_2 was slow in onset and lasted until it was washed out. The IC_{50} values were 4 to 40×10^{-9} mol/l for PGI_2 and 1 to 6×10^{-7} mol/l for 7-oxo-PGI_2. Compared to PGI_2, 7-oxo-PGI_2 was more potent in relaxing coronary arteries than the other vessels studied. Isolated tracheae from guinea-pigs were contracted by PGI_2 while relaxed by 7-oxo-PGI_2.

Since the discovery and chemical identification of prostacyclin a number of analogues have been synthesised. Some of them were reported to have biological properties similar to those of PGI_2 [1, 4–7, 9–10, 12–17]. Here we describe the actions of a stable analogue of PGI_2 (7-oxo-PGI_2) on the tone of smooth muscle of isolated cerebral, coronary and mesenteric arteries. 7-oxo-PGI_2 was synthesised in the Chinoin Pharmaceutical Works, Budapest; a report on the haemodynamic and antiaggregatory effects of the substance has been published by Kovács et al. [10].

Methods

Isolated arteries

Helically cut strips of canine basilar, bovine coronary, rabbit coeliac and human mesenteric arteries were suspended in muscle chambers containing 5 ml Krebs' solution at 37 °C. The human arteries were obtained from operated patients. Basilar, coeliac and human mesenteric arteries were stretched to a baseline load of 5 mN and the initial tension of coronary strips was adjusted to 20 mN. Changes in vessel tone were monitored by means of force-displacement transducers and recorded on potentiometric recorders (Radelkis OH-814). The effects of PGI_2 and 7-oxo-PGI_2 were studied either on the resting tone (cerebral, coronary and human mesenteric) or on increased tone produced by noradrenaline or electrical stimulation. For transmural nerve stimulation rectangular pulses (0.5 ms in duration, 120 V), were delivered by a Grass S 48 stimulator via a pair of platinum electrodes placed on the top and bottom of the organ chamber. The frequency and duration of stimulation were varied according to the responsiveness of the tissue. The substances tested were injected into the organ bath in volumes not exceeding 0.1 ml. The solvents used at this volume did not affect either resting or high tone of the vessel strips. IC_{50} values (concentrations that produced 50% reduction of spontaneous or enhanced tone) were calculated from dose-response curves and the potencies of PGI_2 and 7-oxo-PGI_2 were compared.

Isolated tracheal spirals

Spirally cut tracheae [3] from guinea-pigs were equilibrated under an initial tension of 5 mN for 150 min in Krebs' solution at 37 °C bubbled through with 5% CO_2 in oxygen. Changes in tension were measured isometrically as described above for isolated arteries. PGI_2 and 7-oxo-PGI_2 were dissolved in Tris buffer (pH 8.2) and added to the bath at small volumes (up to 0.1 ml) in concentrations that were equipotent for relaxing bovine coronary arteries.

Results

Isolated arteries

Both PGI_2 and 7-oxo-PGI_2 reduced the tone of the artery strips in a concentration dependent manner. Figure 1 shows the effects of these two substances on the spontaneous tone of a bovine coronary artery. The high concentration of prostacyclin (1.4 μmol/l) was added in order to establish full relaxation. The relaxant action of the 7-oxo-analogue developed somewhat slower than that of PGI_2. This can be seen mostly at higher concentrations. The duration of action of these two prostanoids in vitro was also studied in three experiments. The effect of the 7-oxo-analogue lasted until it was washed out whereas PGI_2, due to its unstable character, gradually lost relaxant activity (see also Fig. 2).

The two prostanoids also relaxed canine isolated cerebral arteries which had been previously contracted by 1 μmol/l $PGF_{2\alpha}$ (Fig. 2). This tracing shows that (*i*) indomethacin potentiates the contractile responses to $PGF_{2\alpha}$; (*ii*) PGI_2 and its 7-oxo-derivative reduce the $PGF_{2\alpha}$-induced tone; (*iii*) the relaxant

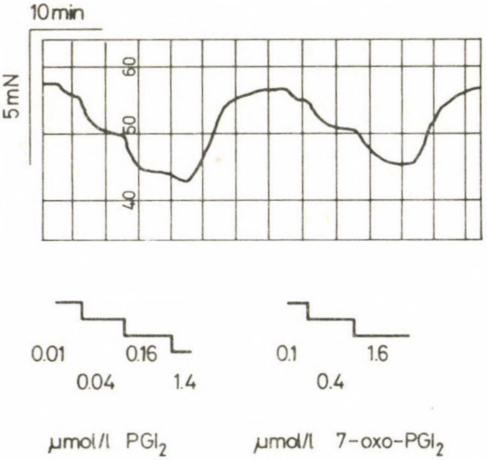

Fig. 1. The relaxant effect of PGI_2 and its stable analogue 7-oxo-PGI_2 on the tone of a bovine coronary artery strip in the presence of 3 μmol/l indomethacin

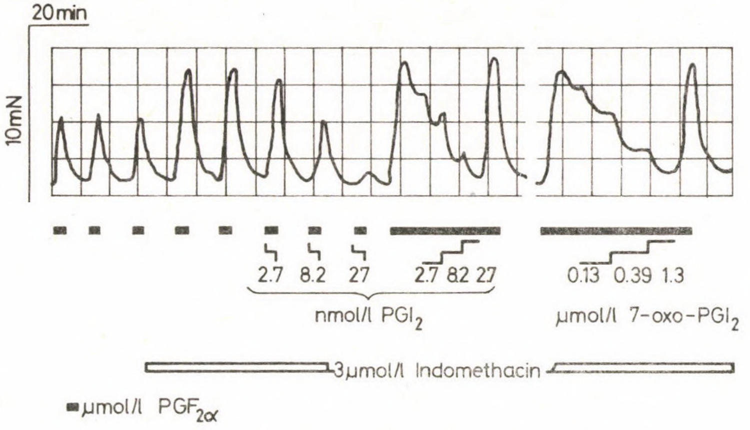

Fig. 2. **The contraction-potentiating effect of indomethacin and the inhibitory effect of PGI_2 and 7-oxo-PGI_2 on phasic and tonic contraction induced by $PGF_{2\alpha}$ (10^{-6} mol/l)**

action of PGI_2 develops faster than that of 7-oxo-PGI_2; (*iv*) PGI_2 gradually loses its relaxant effect whereas 7-oxo-PGI_2 does not, when keeping these substances in the bath for 12 to 15 min.

The electrically or noradrenaline-evoked contractions of rabbit isolated coeliac artery strips were markedly reduced by PGI_2 and its analogue. Figure 3 shows the inhibitory action of the two prostanoids on contractions produced by electrical stimulation. 7-oxo-PGI_2 was added to the bath 15 min prior to stimulation, while PGI_2 2 min prior to it.

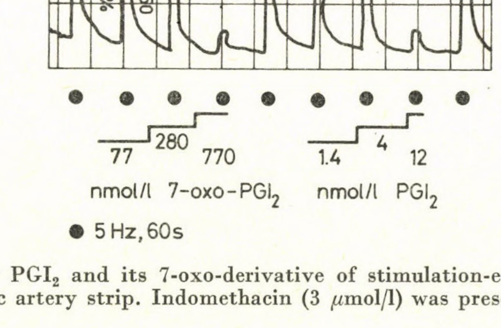

Fig. 3. **Inhibition by PGI_2 and its 7-oxo-derivative of stimulation-evoked contractions of a rabbit coeliac artery strip. Indomethacin (3 μmol/l) was present throughout**

Table I

Relaxant effect of PGI_2 and 7-oxo-PGI_2 on the smooth muscle cells of isolated artery strips. Indomethacin (3 μmol/1) was present throughout the experiments

Type of artery	Tone induced by	IC_{50} (μmol/l)		Relative potency	n
		PGI_2	7-oxo-PGI_2		
Bovine coronary	—	0.04 (0.025–0.055)	0.4 (0.25–0.55)	0.1	6
Dog cerebral	—	0.015 (0.01 –0.02)	0.6 (0.4 –0.8)	0.025	5
Rabbit caeliac	noradrenaline (0.3 μmol/1)	0.01 (0.007–0.013)	0.5 (0.4 –0.6)	0.02	6
	electr. stim. (5 Hz for 2 min)	0.005 (0.003–0.007)	0.23 (0.18–0.28)	0.022	5
Human mesenteric	—	0.004 (0.003–0.005)	0.12 (0.08–0.16)	0.03	5

IC_{50}-values with their 95% confidence limit for PGI_2 and its 7-oxo-derivative are given in μmol/l. Relative potency: ratio of IC_{50} for PGI_2 and 7-oxo-PGI_2. *n*: number of experiments

Like animal tissues, strips of human mesenteric arteries were also relaxed by PGI_2 and its 7-oxo-analogue. The smooth muscle cells of this human vessel appears to be at least as sensitive as those of rabbit coeliac artery.

The summary of the results is presented in Table I. When comparing the IC_{50} values, both prostanoids were more potent in relaxing mesenteric vessels than coronary or cerebral arteries. The relative potency (a ratio of IC_{50} for PGI_2 and 7-oxo-PGI_2) was higher in coronary arteries than in other vessels studied.

Isolated tracheal spirals

The effect of the two prostanoids on the resting tone of isolated tracheal strips of guinea-pigs was studied in 5 preparations. Since 7-oxo-PGI_2 was 10 times less potent in relaxing coronary arteries, its concentrations used in the study on tracheal smooth muscle were 10 times higher than those of PGI_2. In other words, as regards their coronary relaxing action, they were used in equipotent concentrations. Interestingly, 7-oxo-PGI_2 produced concentration-related relaxation whereas PGI_2 caused concentration-dependent contraction, a well-known effect of prostacyclin [2, 8, 11]. Table II summarizes the results of these experiments.

Discussion

7-oxo-PGI_2 appears to be very similar to prostacyclin with respect to actions on the tone of isolated coronary, cerebral and mesenteric arteries. Its potency is about one order of magnitude lower than that of PGI_2. This is in

Table II

Contraction by PGI_2 and relaxation by 7-oxo-PGI_2 of tracheal smooth muscle of guinea-pigs

Contraction (mN±S.E.M.) by PGI_2 (µmol/l) (5)			Relaxation (mN±S.E.M.) by 7-oxo-PGI_2 (µmol/l) (5)		
0.08	0.26	0.80	0.8	2.6	8.0
0.44±0.1	1.22±0.5	1.60±0.6	0.37±0.09	0.79±0.14	1.45±0.47

In brackets: number of experiments

line with the findings of Kovács et al. [10] who reported that 7-oxo-PGI_2 was 15 and 50 times less potent in inhibiting platelet aggregation and lowering blood pressure of dogs, respectively. Also, the 7-oxo-derivative of prostacyclin was found to be 25 times weaker than the parent compound in causing hypotension in anaesthetized rats (Hadházy et al., unpublished data).

All these results suggest that prostacyclin and its 7-oxo-analogue might have the same profile of action. However, the opposite effects of the two prostanoids on the tone of isolated tracheae of guinea-pigs represent a substantial difference between their action on smooth muscle cells. This interesting and important finding shows that there is a chance of synthesizing selective prostacyclin analogues.

The effect of PGI_2 — owing to its chemical instability — gradually declined whereas the action of its 7-oxo-analogue lasted until it was removed from the organ bath. Kovács et al. [10] determined the stability of 7-oxo-PGI_2 by using UV spectroscopy and they did not see any change after 26 days at room temperature in buffers of pH 6.7 to 10.7. Thus, this substance appears to be quite resistant to hydrolysis. The duration of vasodepressor action of 7-oxo-PGI_2 in dogs [10] and rats [Hadházy et al. unpublished data] was, however, not longer than that of the parent compound. This indicates that the 7-oxo-analogue undergoes enzymatic transformation in the body.

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ТОМ 60 — ВЫП. 4

РЕЗЮМЕ

БИОХИМИЧЕСКАЯ КОМПАРТМЕНТАЦИЯ ТКАНЕЙ РЫБ. СООБЩЕНИЕ VI. ГЕКСОКИНЕЗА В ГОЛОВНОМ МОЗГУ

С. А. ШАФФИ

Автор изучал распределение активности гексокиназы в головном мозге, мозжечке, гипофизе, гипоталамусе, таламусе и продолговатом мозге, в грубом гомогенизате клеток, частицах клеток и в растворимой фракции, у 9 видов костистых рыб разного филогенетического возраста. Цель исследования заключалась в определении обмена глюкозы в отдельных частях нервной системы. Активность энзима грубой фракции была самой высокой в головном мозге, самой низкой — в продолговатом мозге, по-видимому потому, что в головном мозге обмен глюкозы более интенсивен. Активность энзима клеточных частиц была выше всего в мозжечке, затем в голвном мозге, диэнцефалоне и продолговатом мозге. Активность цельных частиц и растворимой фракции была наиболее высокой в головном мозгу, мозжечке и диэнцефалоне. Распределение энзима было сходным у всех исследованных 9 видов рыб.

ИЗМЕНЕНИЕ ВОДНО-ЭЛЕКТРОЛИТНОГО ОБМЕНА У ЖВАЧНЫХ В УСЛОВИЯХ ЛИШЕНИЯ ВОДЫ И НАГРУЗКИ ХЛОРИСТЫМ НАТРИЕМ

Б. СЕГЕДИ, Б. ЮХАС и М. КЕРЕСТЕШ

Авторы изучали изменения регуляции обмена электролитов и воды у жвачных с фистулой рубца, в условиях 48-часового лишения воды и нагрузки хлористым натрием. В экспериментах определяли состав содержимого рубца животных, концентрацию натрия и калия в плазме, крови и выделениях слюнных желез, а также изменения этих показателей под влиянием нагрузки хлористым натрием.

Результаты проведенных экспериментов показали, что, в регулировании водно-электролитного обмена, существенную роль играет содержание воды в жидкости рубца. Всасывание воды и натрия из рубца оказывает компенсирующее влияние на поддержание изоосмотических и изоволемических отношений до тех пор, пока концентрация натрия в жидкости рубца не превысит концентрацию натрия в плазме крови. После этого нарушенное равновесие водного обмена между рубцом и внутрисосудистым пространством смещается в сторону рубца и задерживает дальнейшее увеличение концентрации натрия, благодаря чему обеспечиваются подходящие условия жизнедеятельности для микрофлоры рубца.

Под влиянием лишения воды животное не только теряет воду, но, вследствие выведения натрия, устанавливается состояние относительной гипонатремии, что хорошо показывает соотношение концентраций натрия и калия в слюне. После нагрузки хлористым натрием, пропорция натрия/калий в слюне увеличивается, показывая степень обеспечения ионами натрия.

ПРИМЕНЕНИЕ ВЫЧИСЛИТЕЛЬНО-МАШИННОЙ СИМУЛЯЦИИ ДЛЯ ИЗУЧЕНИЯ Ca-ЗАВИСИМЫХ ОТКЛОНЕНИЙ, НАБЛЮДАЕМЫХ В РАЗРЯДАХ НЕЙРОНОВ УЛИТКИ

Ф. ПОНГРАЦ и М. СЕНТЕ

Для дополнения физиологических исследований нервных мембран мягкотелых авторы рекомендуют применять ЭВМ-моделирование. Механизм залпов с небольшого участка мембраны они изучали в зависимости от максимальной Ca^{++}-кондуктанции. Вычисления основываются на модификации данных *Hodgkin—Huxley*, будучи дополнены прямой аппроксимацией экспериментальных кинетических параметров, полученных на течение Ca^{++} и переходного течения калия. Авторы полагают, что изменение кондуктации во время тока Ca^{++} пропорционально m^2h. Модель учитывает три отдельных калиевых тока: транзитного, замедленного и Ca^{++}-зависимого калия. Номерное |интегрирование кинетических уравнений производилось модифицированным методом Эйлера на дигитальной вычислительной машине.

Результаты демонстируют значительное влияние кондуктанции Ca^{++} на расширение спайка, формирование плато испайковую дополнительную гиперполяризацию. В маленькой области кальциевой кондуктанции пульсом короткого (надпорогового) тока можно вызвать бесконечную спонтанную активность; это явление можно принять в качестве модели пейсмекерной активности. Образование плато, вызванное блокированием калия или уменьшенным равновесием калия, кондуктанция Ca^{++} повышала в области большей кондуктанции Ca^{++}. Изучали влияние чувствительности напряжения связывающего коэффициента, описывающего Ca^{++}-зависимый калиевый ток и сравнивали с независимым случаем напряжения. Связывающий коэффициент кажется существенным фактором в увеличении области кондуктанции Ca^{++}, ответственной за пейсмекерную активность. При больших значениях кондуктации кальция уменьшение связывающего коэффициента приводит к прерыванию затянутой взрывной активности *(Burst)*, происходящей с взрывной дополнительной гиперполяризацией.

На этой модели изучали также блокирующее действие 4-аминопиридина на быстрый вытекающий ток. Это исследование имеет практическое значение, принимая во внимание известное судорожное действие аминопиридина и новые объяснения, касающиеся ионного фона этого явления. Авторы предполагают, что возможно изучение судорожного эффекта аминопироидина с помощью модели, основанной на кинетике изолированной нервной мембраны. Модель может оказать помощь в понимании ионного фона, регулирующего эпилептические разряды нейронов млекопитающих.

ПРИМЕНЕНИЕ МЕТОДА MTD ДЛЯ КАРТОГРАФИРОВАНИЯ ПРОСТАГЛАНДИНОВЫХ РЕЦЕПТОРОВ

З. ШИМОН и Ф. КЕРЕК

Мы применили метод минимальной пространственной разницы (MTD) для «картографирования» простагландинов. Расчетные данные были взяты из результатов экспериметальных измерений лютеинизирующего и изменяющего кровяное давление (у овец) действия разных простагландиновых производных. Линейная корреляция рассчитанных МТД и измеренных результатов состовляла в случае лютеинизрующего эффекта: $r=0{,}927$ ($N = 12$), в случае понижающего кровяное давление действия: $r = 0{,}943$ ($N = 15$), в случае повышающего: $r = 0{,}945$ ($N = 15$). Этим методом удалось обрисовать одинаковые или различающиеся особенности трех типов простагландиновых рецепторов.

ОСТРОЕ ВЛИЯНИЕ ВИБРАЦИИ НА АКТИВНОСТЬ КАЛЛИКРЕИНКИНИНОВОЙ СИСТЕМЫ

Т. ГАТИ, И. БУДАВАРИ, Д. СОМБАТ и Дь. ЛОШОНЦИ

Авторы изучали влияние вибрации, действующей в течение 4 ч на все тело, на основании определения следующих показателей: среди компонентов плазменной калликреин-кининовой системы концентрации и активности свободного (спонтанного) и активируемого каолином калликреина (прекалликреина) активности брадикининогена и расщепляющей брадикинин суммарной кининазы, далее активности суммарного калликреинингибитора в плазме, а также концентрации одного из главных ингибиторов калликреина — альфа$_2$-макроглобулина. Параметры вибрации были следующими: частота 5 герц, амплитуда 2 см, направление — горизонатльное.

Авторы показали, что, под влиянием вибрации, активность свободного калликреина плазмы достоверно повышается, концентрация же плазменного прекалликреина параллельно понижается. Концентрация брадикининогена достоверно увеличивается, также как и суммарная активность киназы. Ни суммарная активность ингибитора калликреина, ни концентрация альфа$_2$-макроглобулина не изменяются, указывая на то, что, в изменении активности свободного калликреина и прекалликреина в плазме, плазменные ингибиторы калликреина роли не играют. Согласно полученным результатам, во время острой вибрации калликреин-кининовая система плазмы активизируется, что вызывается, вероятно, повышенной секрецией катехоламинов, и биологическое значение этого заключается в уравновешивании неблагоприятного действия катехоламинов на микроциркуляцию.

ВЛИЯНИЕ ЛЕКАРСТВ НА ОБРАЗОВАНИЕ СТРЕССОРНОЙ ЯЗВЫ У КРЫС

Л. НАДЬ, Д. МОЖИК, М. ФИГЛЕР, Э. ГЕРБЕР, Т. ПАШТ, Ж. ВЕЗЕКЕНИ и Т. ЯВОР

В экспериментах на крысах авторы исследовали эффект различных фармакологических средств на предупреждение возникновения у животных стрессорной язвы. Появление стрессорной язвы авторы вызывали с помощью разработанного ими метода: крыс с интактным желудком, после голодания, заставляли плавать в воде (23°C) в течение 5 часов.

Результаты экспериментов показали, что

1. *атропин* (0,1 – 0,5 и 1,0 мг/кг внутримышечно),
   циметидин (1,0 – 5,0 и 25 мг/кг внутрибрюшинно),
   простациклин (ПГI$_2$) (5,0 – 25,0 и 100,0 мкг/кг внутрибрюшинно) и
   фентоламин (0,35—1,75—3,50 и 7,00 мг/кг внутримышечно) дозозависимо и достоверно уменьшали вероятность возникновения стрессорной язвы.
2. *Пропранолол* (0,35 – 1,75 – 3,50 и 7,00 мг/кг внутрибрющинно) не оказывал влияния на образование стрессорной язвы.

Тот факт, что различные фармакологические препараты способны значительно уменьшить или предотвратить возникновение язвы в ответ на стрессорное воздействие, указывает на многообразие нервных, гормональных и биохимических процессов, играющих роль в патогенезе язвы.

На основании результатов исследований, авторы обращают внимание на возможности превентивной медикаментозной терапии, используемой в рациональных комбинациях.

ВЛИЯНИЕ ПРОСТАГЛАНДИНА И ЛАЗЕРНОГО ЛУЧА НА ВЫРАБОТКУ СЛИЗИ В ЖЕЛУДКЕ ЧЕКОВЕКА

И. СЛАМКА, Л. КОВАЧ и А. ТЁРЁК

Авторы отмечали увеличение выработки желудочной слизи под влиянием простагландина 2-альфа. Малые дозы аргонового лазерного облучения повышали продукцию слизи в желудке. Простагландин 2-альфа (PGF) действует противоположным образом на деятельность слизистой оболочки желудка, нежели простагландины группы E (PGF). В области выработки желудочной слизи получены новые данные относительно биостимулирующего действия лазерного луча.

СВЯЗЬ МЕЖДУ ТКАНЕВЫМ РЕДОКС-ПОТЕНЦИАЛОМ И ИЗМЕНЕНИЯМИ da_k^o/dt АКТИВНОСТИ K^+_0 ПРИ ВВЕДЕНИИ К-СТРОФАНТОЗИДА ИЛИ АЦЕТИЛХОЛИНА, ВЫЗЫВАЮЩИХ СОКРАЩЕНИЯ

Я. ВИТТМАНН, А. ПУППИ и М. ДЕЛИ

Показали, что значения актиности $[K^+]_0$, определяемые параллельно с контрактурами, вызванными к-строфантозидом и ацетилхолином, увеличиваются в случае оксидоза, вызванного метиленовым синим, и уменьшаются в случае редоза, вызванного аскорбатом. Поскольку между упомянутыми изменениями активности $[K^+]_0$ и связанными с контрактурой изменениями выявляется тесная корреляция, то есть основание предполагать, что одним из эффекторных факторов регулирования редокса мышечных контрактур является преходящее, соответственно направление изменение K^+.

ВЛИЯНИЕ ПЕПТИДОВ ХОЛЕЦИСТОКИНИНОВОГО ПРОИСХОЖДЕНИЯ НА ПАССИВНОЕ ОБОРОНИТЕЛЬНОЕ ПОВЕДЕНИЕ

МАТЬЯШ ФЕКЕТЕ, БОТОНД ПЕНКЕ и ДЮЛА ТЕЛЕГДИ

Нами изучалось влияние пептидов, имеющих холецистокининовое (ССК) происхождение, на ретенцию однократно сочетанного пассивно-оборонительного поведения, после введения их внутрибрюшинно. Будучи введены сразу после *learning trial*, СООН-терминальный окстапептид ССК (ССК-1-8-SE), ССК октапептид без сульфатной группы и СООН-терминальный тетрапептид (ССК-5-8), стимулировали пассивно-оборонительное поведение. Эти данные позволяют предполагать, что указанные пептиды могут оказывать влияние на процессы консолидации памяти.

ВЛИЯНИЕ ХОЛЕЦИСТОКИНИНОВОЙ АНТИСЫВОРОТКИ, ВВЕДЕННОЙ В БОКОВОЙ ЖЕЛУДОЧЕК МОЗГА, НА АКТИВНОЕ И КОНДИЦИОНИРОВАННОЕ ПИЩЕВОЕ ПОВЕДЕНИЕ КРЫС

МАТЬАШ ФЕКЕТЕ, МАРИЯ БАЛАЖ и ДЮЛА ТЕЛЕГДИ

Нами изучалось воздействие введенных в боковой желудочек мозга двух различных антисывороток холецистокинина на обучение и угасание активного оборонительного поведения, а также на процессы обучения, поддержания и угасания кондиционированного пищевого поведения. Обе антисыворотки замедляли процесс угасания активного оборонительного и условного пищевого ответов, но не оказывали влияния на процессы обучения и поддержания этих рефлексов. Результаты настоящих экспериментов показывают, что холецистокинин может играть роль в физиологической регуляции процессов экстинции.

РЕЛАКСАЦИОННОЕ ДЕЙСТВИЕ ПРОСТАЦИКЛИНА (PGI_2) И 7-ОКСО—PGI_2 НА ИЗОЛИРОВАННЫЕ МОЗГОВЫЕ, КОРОНАРНЫЕ И БРЫЖЕЕЧНЫЕ СОСУДЫ

Б. МАЛОМВЁЛЬДИ, П. ХАДХАЗИ, К. МАДЬЯР, К. КАНАИ и В. ШИМОНИДЕС

На препаратах изолированных артерий разных видов животных и трахее морской свинки авторы изучали понижающее тонус гладкой мускулатуры действие нового препарата — стойкого аналога простациклина (7-оксо-PGI_2), — разработанного на фармацевтическом заводе *Chinoin*, а также PGI_2.

Спирально иссеченные полоски сосудов и трахею укрепляли (подвешивали) в растворе Кребса, определяя также изометрически изменения тонуса. Из пропорции вызывающих 50% расслабление или торможение концентраций (EC_{50}), высчитанных из уравнений регрессионных прямых кривой доза-эффект, определяли относительную эффективность в отношении аналога простациклина.

Как PGI_2 так и аналог простациклина (7-оксо – PGI_2) одинаково уменьшали базальный тонус венечных сосудов теленка, сосудов мозга собаки и мезентериальных сосудов человека, а также тормозили сокращения отпрепарированной у кролика чревной артерии (полосок), вызванных электрическим раздражением и норадреналином. В зависимости от сосудистого препарата и вызывающего тонус раздражителя высчитанные значения EC_{50} равнялись: PGI_2: $5-40\times10^{-9}$ и 7-оксо-PGI_2: $1-6\times10^{-7}$ моль/литр. Снижающая сосудистый тонус и тормозящая сокращение эффективность у 7-оксо-аналога была на один порядок меньше, чем у PGI_2. Препарат трахеи морской свинки PGI_2 сокращал, тогда как 7-оксо-аналог — даже и в стократной концентрации — оказывал на препарат релаксационное действие.

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