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Effect of Enkephalins on the Hydroionic Exchange in Rat Ontogenesis

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The accumulated data on the diverse effects of opioid peptides on the functioning of many, if not all, organs and systems of the human and animal organism [14] underscore the need to study their influence on one of the most important systems involved in the maintenance of homeostasis, namely the regulatory system of hydroionic balance, with the kidney being the central organ in this system. Taking into account the age-dependent changes occurring in this system [1,2,4-7,10], we thought it interesting to compare the effect of opioid peptides on hydroionic exchange in different age groups of animals. Since the specific share of enkephalins and endorphins manifesting their modulating effect on the organism's functions [13] increases under stress conditions [3,9,12-14], it is reasonable to assume that under conditions of hyperhydration, administration of enkephalins will promote a more rational, economical, and optimal return of the organism to the initial state of hydroionic equilibrium.

The objective of the present study was to study renal function in rats of three age groups under the action of an arginine-containing synthetic analog of Leu-enkephalin (SAE).

MATERIALS AND METHODS

The experiments were performed on albino Wistar rats of three age groups: group 1) 25-30-day-old rats (n=102); group 2) 45-50-day-old rats (n=62), and group 3) adult rats (older than 180 days, n=115). After preliminary weighing, the experimental animals were placed in special cages for 1-2 h for the collection of background samples of urine. Subsequently, their stomachs were loaded with water (5 ml/100 g body weight) via a polyethylene probe. At the same time, the animals received an intraperitoneal injection of a synthetic analog of Leu-enkephalin in a dose of 100 μg/kg and a volume of 0.5 ml/100 g. Samples of urine and plasma were collected 1, 3, and 6 h after

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peptide injection. At the end of the experiment the animals were decapitated. The control animals received a single intraperitoneal injection of physiological buffer in the same volume against a background of 5% water loading. The urine and plasma samples collected were used for determination of the sodium and potassium content by flame photometry, the creatine level was established by a standard method in a DA-240-4 autoanalyzer (Finland) and osmolarity was measured with a Digimatic osmometer (USA). The data obtained were subjected to variational and differential statistical analysis using Student's t test [8]. The following renal functions were assessed by conventional methods [11]: diuresis - urination rate (ml/100 g/h); sodium and potassium cation excretion (mmole/100 g/h); the rate of glomerular filtration (RGF) (ml/100 g/h); the excreted fraction of sodium and potassium cations (%), and relative liquid reabsorption (%).

RESULTS

Analysis of the findings showed that the dynamics of hydroionic balance restoration in the control animals of three age groups was the same (Fig. 1). The maximal deviations from the background values were observed 1 h after the water loading (diuresis increased 5-6 times, sodium- and potassiumuresis 2-5 times). After three hours all the indexes decreased in value and after 6 hours they dropped to their baseline values or even lower.

In the experimental animals of different age certain differences in comparison with the control were revealed. In the first group Leu-enkephalin inhibited the diuretic reaction. The effect of the peptide on sodium and potassium excretion with the urine was analogous (Fig. 1). The reaction of young rats of the second group to administration of Leu-enkephalin had the opposite character: diuresis and excretion of sodium and potassium were stimulated, and the maximal excess of ionouresis over the control was registered 3 h after peptide administration. By the 6th hour of observation, excretion of water and electrolytes had dropped below than that in the control animals so that for the whole period of observation no reliable differences were recorded (Table 1).

Thus, in 45-50-day-old rats administration of SAE affected the dynamics of the animals' reaction to water loading, stimulating diuresis and ionouresis during the first three hours after injection but inhibiting them later.

In the adult rats of the experimental groups the changes in the excretion of liquid and electrolytes were of the opposite nature.

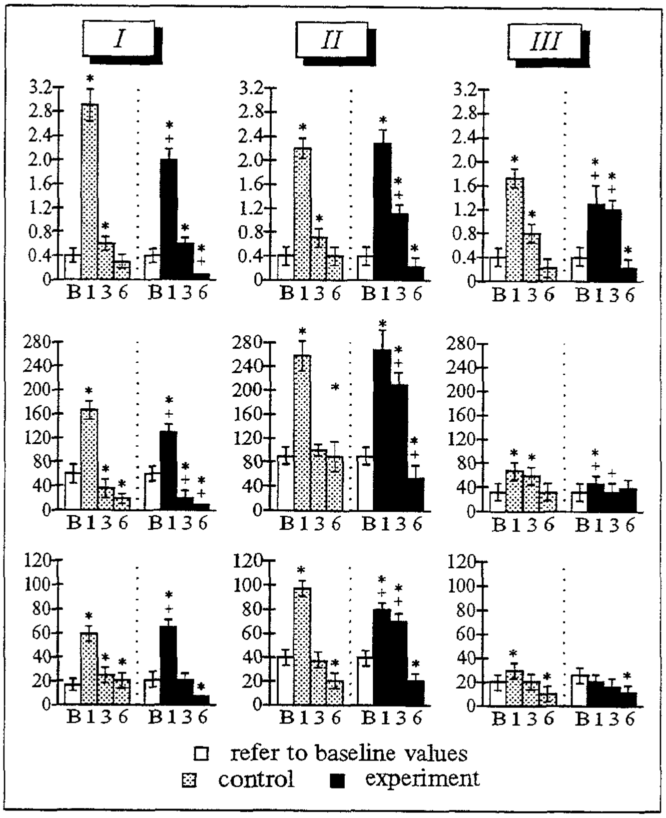

Fig. 1. Effect of SAE on diuresis, sodiumuresis, and potassiumuresis in rats of different age against the background of 5% hyperhydration. *I*) group 1 (25–30–day–old rats); *II*) group 2 (45–50–day–old rats); *III*) group 3 (adult rats). Here and in Figs. 2 and 3 an asterisk denotes significant differences with respect to the background, a plus, significant differences with respect to the control.

The diuresis dynamics also changed under the influence of enkephalin but, unlike in young rats of groups 1 and 2, it acquired a more regular character, becoming more stretched out in time (Fig. 1). During the first hour of observation the diuresis was reliably lower compared to the control, while 3 h after injection it significantly increased. As a result, on average, no differences in the volume of excreted liquid were revealed in comparison with the control (Table 1).

However, this conclusion is not valid for the composition of the excreted liquid. Under conditions of hyperhydration Leu-enkephalin impeded sodium excretion in adult rats but had practically no influence on the excretion of potassium, although it altered its dynamics.

In order to understand the mechanisms inducing these changes, we analyzed the data on the rate of glomerular filtration and relative liquid reabsorption ($\%R_{H_2O}$, Fig. 2).

Administration of SAE hampered glomerular filtration in groups 1 and 3 but stimulated it in rats of group 2. Liquid reabsorption after water loading decreased in the control animals of all

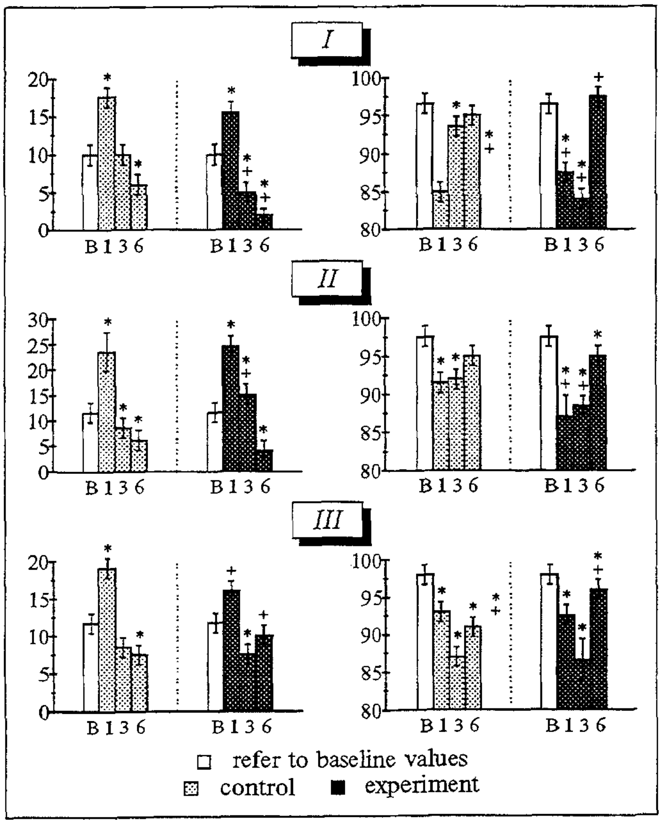

Fig. 2. Rate of glomerular filtration (RGF) and relative liquid reabsorption ($\%R_{H_2O}$) under the influence of SAE against the background of 5% water loading in rats of different age.

three age groups. After injection of Leu-enkephalin in the 25-30-day-old rats, this index dropped to a lower level and in its dynamics was similar to the reaction of adult rats, with minimal deviations from the background values being observed 3 h after loading.

Since the process of liquid reabsorption in the 25-30-day-old rats is not the prevailing one in the regulation of the hydroionic exchange [1] and changes in the control groups of rats occurred due to the involvement of a more ancient and less economical mechanism of filtration, activation of the nephron tubule apparatus may be explained as a consequence of the positive effect of Leu-enkephalin on the regulation of hydroionic exchange in the young rats of the first group.

In the rats of the second group liquid reabsorption also decreased under the influence of SAE. Enhanced diuresis in this age group may be attributed to activation of the processes within the nephron glomerular zone (Table 1) and domination of filtration over resorption. Thus, the effect of Leu-enkephalin on the hydroionic exchange in young rats induced a fundamentally different reaction.

As follows from the calculation presented in Fig. 3, the share of the excreted fraction of sodium ions (EF_{Na}) in the first and third age groups decreased under the action of SAE and remained almost the same in the medium-aged rats of the second group (Table 1). The share of the excre-ted fraction of potassium ions (EF_K) in the rats of the first and third groups showed a tendency to decrease, whereas in the second group it increased 2-fold.

Thus, SAE enhanced sodium reabsorption in the young and adult groups and altered the dynamics of this process in the middle age group. Reabsorption of potassium ions under the influence of Leu-enkephalin did not change in the adult and 25-30-day-old rats but decreased 2-fold (or its secretion increased) in the 45-50-day-old rats.

The changes in the ionic composition of urine in the experimental animals correlated with the changes in plasma composition (Table 1).

The sodium concentration after injection of SAE increased in young and adult rats, while potassium ions remained at the same level. Plasma osmolarity decreased in the first group and increased in the third group.

In the middle age group SAE injection induced a significant shift in the sodium and potassium concentration to a higher level, whereas in the control animals we observed the opposite reaction. Plasma osmolarity in the experimental animals increased (Table 1).

Thus, enkephalin influenced the processes maintaining the hydroionic equilibrium in the rat organism, but the strength of its effect varied and was dependent on age and on the functional maturity of the mechanisms of osmotic homeostasis regulation.

In the young animals under the influence of SAE after a 5% liquid loading the efficiency of the polyuretic and ionouretic reaction declined. If we assume that these processes are responsible for maintaining the ionic equilibrium, then the effect of Leu-enkephalin on the hydroionic exchange in the 25-30-day-old rats is of a positive character. It implies that SAE favors a timely functional switching on of the mechanisms stabilizing the water-salt balance when the internal medium constants significantly deviate from the normal values. According to published data [1,4-7], these mechanisms are already formed but remain functionally immature and inert in rats of this age group.

In the middle age group the positive effect of SAE is rather doubtful: although Leu-enkephalin certainly activates all the processes regulating the hydroionic equilibrium, the subsequent changes have a very high amplitude and cannot be regarded as useful, since in excreting the excess water the organism loses osmotically active substances, which disturbs the ionic equilibrium.

The most pronounced positive effect of SAE was observed in the adult rats. Injection of Leu-enkephalin induced a smooth and prolonged reaction to hyperhydration in comparison with the control. Such a reaction made it possible to excrete the excess water without losing osmotically active substances, thus providing for a renal reaction more adequate to the situation. Prolongation of the diuretic reaction after injection of SAE during the first three hours of observation, unlike the peaklike intensification of diuresis after 1 h in the control, testifies to activation of the processes in the nephron tubule apparatus which is a sign of a positive effect of SAE on the hydroionic exchange. It should be pointed out that domination of reabsorption over filtration is the direction taken during functional maturation of the mechanisms regulating ionoosmotic equilibrium in ontogenesis [2]. The decrease of diuresis after injection of SAE against a background of 5% hyperhydration in comparison with the control should also be regarded as positive, since the ability to maintain the liquid volume despite disturbances in the organism's internal medium constants is an advantage of the mature organism over the infantile one [4].

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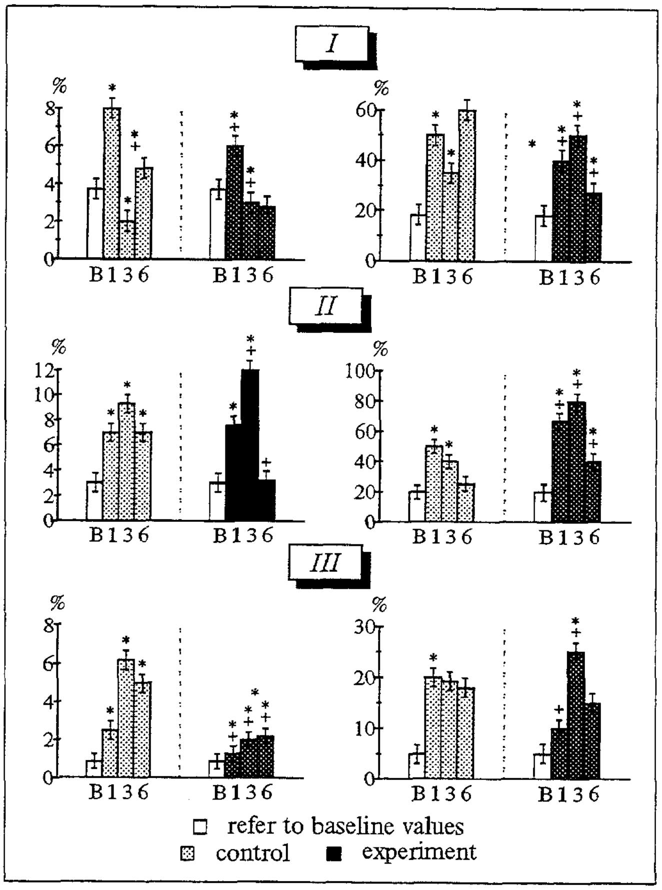

Fig. 3. Excreted fractions of sodium (EF_{Na}) and potassium (EF_K) ions after injection of SAE against the background of 5% water loading in rats of different age.

TABLE 1. Mean Values of Partial Functions of the Kidney in Rats of Different Age during 6 hours after Injection of SAE against a Background of 5% Hyperhydration

Index	Group of animals					
	1st		2nd		3rd	
	control *n*=41	experiment *n*=39	control *n*=25	experiment *n*=28	control *n*=30	experiment *n*=31
Diuresis (V), ml/100 g/h	1.2±0.8	0.8±0.05*	1.0±0.07	1.1±0.2	0.9±0.07	0.9±0.1
Sodiumuresis (U_{Na}×V), mmole/100 g/h	73.5±5.9	49.1±4.2*	148.8±25.9	167.9±26.3*	44.1±2.8	31.3±3.7*
Potassiumuresis (U_K×V), mmole/100 g/h	33.5±11.5	22.0±12.3*	44.5±3.2	53.7±4.8*	14.2±2.0	14.7±0.9
Rate of glomerular filtration (RGF), ml/100 g/h	10.4±3.7	7.6±4.8*	12.5±5.5	14.5±6.2	11.2±3.8	10.7±2.9
Water reabsorption (R_{H_2O}), %	90.7±3.3	89.1±4.0	92.3±1.7	90.7±3.2	96.1±1.9	91.1±3.3
Excreted fraction of sodium (EF_{Na}), %	4.7±2.1	3.8±1.0	7.9±0.7	7.4±2.9	4.9±1.0	2.0±0.2*
Excreted fraction of potassium (EF_K), %	47.0±7.9	38.1±7.3	39.1±8.7	63.4±13.5*	20.5±0.5	19.5±4.5
Sodium concentration in plasma (P_{Na}), mmole/liter	139.3±0.7	143.6±0.5*	141.9±2.0	143.1±0.9	141.3±0.8	146.9±0.7*
Potassium concentration in plasma (P_K), mmole/liter	6.5±0.1	6.5±0.03	5.0±0.07	6.2±0.06*	6.4±0.7	6.6±0.1*
Plasma osmolarity (P_{osm}), mmole/liter	306.0−8.4	296.4−4.5	307.4−3.3	312.3−5.8	315.3−2.9	321.1−3.2*

Note. *n* = number of animals in a given experimental series; asterisk = significant differences with respect to control, $p<0.05$

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Changes in Blood Serum Neuroreactivity in Coronary Parients

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Isolated mollusk neurons have been used as a test object to assess blood serum neuroreactivity. Such neurons are devoid of a glial sheath, this making their membrane readily accessible to serum substances. Coronary patients' blood sera have been tested over a course of intravenous laser therapy. Such therapy is widely practiced in the treatment of cardiology patients, but the mechanisms of the therapeutic effect of light are not clear and there are no definite criteria for assessment of the therapeutic effect [2,3].

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MATERIALS AND METHODS

Blood was collected before therapy and after a course of intravenous laser therapy (IVLT) consisting of six sessions [4]. Blood sera were frozen and kept before testing at -80°C. They were defrosted directly before the experiments. Sera of 13 patients were tested. Neurons of *Lumnaea stagnalis* aged 0.5 to 2 years were used. After 40 min incubation of nervous tissue in 0.3% enzyme solution (pronase, Serva, Germany) the neurons (without glial cells) were removed from the peripharyngeal ganglia and placed on the glass bottom plate of the chamber. Physiologycal saline of the following composition was used: 55 mM NaCl, 1.6 mM KCl, 4 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5 mM Tris, pH 7.8. The

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