EFFECT OF ENKEPHALINS ON THE VASOPRESSIN AND ALDOSTERONE LEVELS IN ACUTE EXPERIMENTAL MYOCARDIAL ISCHEMIA

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Under the influence of extremal factors acting on the living organism the blood level of hormones regulating water and salt metabolism (vasopressin and aldosterone) rises [1, 4]. Besides their well-studied homeostatic influence, these hormones also have a marked action on the cardiovascular system, and in the case of a hyperergic response their adaptive action may be converted into pathogenetic. For instance, the onset of myocardial necrosis in response to injection of large doses of mineralocorticoids has been described [3]. Vasopressin can induce coronary spasm sufficiently severe to lead to the appearance of areas of necrosis, and can depress myocardial contractility [4]. The writers showed previously that under the influence of enkephalins several metabolic parameters in rats with acute myocardial necrosis are restored to normal [2].

The object of this investigation was to study the effect of a stable enkephalin analog on the level of hormones controlling water and salt metabolism (vasopressin and aldosterone) in rats with acute myocardial ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 128 noninbred male albino rats weighing 160-180 g. Acute myocardial ischemia was reproduced experimentally by electrical coagulation of the left coronary artery [6], after which half of the experimental animals received an intraperitoneal injection of the stable arginine-containing hexapeptide analog of Leu-enkephalin (D-Ala^2-Leu^5-Arg^6-enkephalin), in a dose of 1.25 nmole/kg body weight (the preparation was synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Director, Dr. Chem. Sci. M. I. Titov). This dose was chosen as being optimal. The remaining experimental animals were given an injection of physiological saline. In some experiments, to block opiate receptors, naloxone (Koch Light Endolaboratories, USA) was used in a dose of 0.5 mg/kg, subcutaneously, 15 min before injection of the enkephalin analog. To collect urine, the rats were kept in individual metabolism cages. Blood was taken after decapitation under superficial ether anesthesia. Concentrations of sodium and potassium in the urine were determined by flame photometry (Flapho-4 photometer, East Germany). The antidiuretic activity (ADA) of the blood plasma was determined by a biological method on rats, using arginine-vasopressin (Serva, West Germany) as the standard. ADA was expressed in microunits vasopressin/ml blood plasma. Aldosterone in blood plasma was determined by radioimmunoassay, using kits from CEA Sorin (France), on a Gamma-counter (Tracor, USA). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that 24 h after the production of acute myocardial ischemia in the rats, marked oliguria was present. The volume of urine was reduced by more than 60%. Sodium excretion was reduced by 84% and potassium excretion with the urine by 16%. The sodium/potassium ratio in the urine was reduced by 5.5 times, indirect evidence of activation of the mineralocorticoid function of the adrenals. The same conclusion could be drawn from the rise of 100% in the plasma immunoreactive aldosterone level.

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TABLE 1. Effect of Enkephalin Analog on Diuresis and Mineralocorticoid Function of Adrenals in Rats with Acute Myocardial Ischemia (M ± m)

Experimental conditions	Number of observations	Diuresis, ml/24 h	Sodium excretion, μmoles/24 h	Potassium excretion, μmoles/24 h	Sodium/potassium ratio	Aldosterone, ng/ml
Intact rats	17	7,6±0,4	1620,0±49,1	468,0±19,1	3,46±0,21	443,9±36,7
P_2		<0,01	<0,001	<0,05	<0,01	<0,05
Acute myocardial ischemia + physiological saline	19	2,9±0,5	253,4±11,2	401,3±14,5	0,63±0,06	877,7±61,3
P_1		<0,01	<0,001	<0,05	<0,01	<0,05
Acute myocardial ischemia + enkephalin analog	13	5,35±0,3	920,5±21,5	407,8±13,8	2,26±0,28	462,4±116,7
P_1		<0,01	<0,001	<0,05	<0,05	>0,05
P_2		<0,02	<0,001	>0,05	<0,01	<0,05

Legend. P_1) Index of significance compared with intact animals; P_2) index of significance relative to group of animals with acute myocardial ischemia, receiving physiological saline.

TABLE 2. Effect of Various Procedures on Plasma ADA Level in Rats (M ± m)

Experimental conditions	Plasma ADA, microunits vasopressin/ml	P
Intact rats	4,75±0,10	—
Myocardial ischemia:		
after 1 h	9,55±0,26	<0,05
after 6 h	17,19±0,67	<0,001
after 24 h	15,06±0,65	<0,001
Myocardial ischemia + enkephalin analog		
after 1 h	2,56±0,14	<0,05
after 6 h	4,93±0,17	>0,05
after 24 h	5,64±0,24	>0,05
Myocardial ischemia + naloxone + enkephalin		
after 1 h	9,39±0,24	<0,05
after 6 h	18,78±0,80	<0,001
after 24 h	15,32±0,66	<0,001

Legend. Intact group consisted of 25 animals, nine rats used in the remaining experiments.

ADA 1 h after production of myocardial ischemia was doubled, after 6 h it was increased by 3.6 times, and after 24 h by 3.2 times (Table 2).

Injection of the enkephalin analog into rats with myocardial ischemia was followed by partial normalization of diuresis. Although the volume of diuresis remained below its initial level, it was appreciably higher than that in rats with myocardial ischemia receiving physiological saline. Similar changes affected sodium excretion, which was 3.6 times higher than its level in the control animals. The sodium/potassium ratio in the urine also rose. The immunoreactive aldosterone concentration was within the limits observed in intact animals. Injection of the enkephalin analog prevented the rise in ADA in rats with acute myocardial ischemia. During the first hour after the beginning of ischemia ADA actually was observed to fall compared with its level in intact rats. This phenomenon requires further study, because its nature is not clear, considering that in intact rats injection of the enkephalin analog did not cause ADA to fall until 3 h later (Table 2). The mortality among the animals with acute myocardial ischemia also was reduced.

Blocking of opiate receptors by naloxone completely abolished the effects of the enkephalin analog on the changes in ADA both in intact rats and in animals with acute myocardial ischemia (Table 2).

Stress, in the form of acute myocardial ischemia, thus stimulates the mineralocorticoid function of the adrenals and vasopressin secretion, as has previously been described in the literature [1, 4]. The data published in this paper are evidence that enkephalins can prevent stress-induced stimulation of the mineralocorticoid function of the adrenals and vasopressin

secretion by the posterior lobe of the pituitary. Renal function is thus partially corrected, and the degree of the damaging effect of excess of aldosterone and vasopressin on the myocardium is probably reduced. Elevation of the neuropeptide concentration in the blood and cerebrospinal fluid in various types of stress is probably aimed in this same direction [5]. The inhibitory effect of enkephalins on secretion of these hormones is specific and is mediated through opiate receptors, since blocking of these receptors by naloxone abolishes their effect.

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CHANGES IN HOMEOSTATIC BALANCE OF PROSTACYCLINE-THROMBOXANE GENERATING SYSTEMS IN ZOOSOCIAL STRESS

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Blood vessel walls have the property of forming a powerful antiaggregating substance, namely prostacycline (PGI_2), which has a vasodilator action [7, 8], from arachidonic acid and its unstable metabolites, prostaglandins (PG) H_2 and G_2. The antiaggregating activity of the wall of the abdominal aorta can be judged from the degree of inhibition of ADP-induced platelet aggregation on incubation of healthy rat plasma with pieces (9 mg) of the aorta of experimental animals [5, 9]. Incubation of platelets with an aggregating agent, especially thrombin, leads to a considerable increase in the malonic dialdehyde (MDA) level, and the thromboxane concentration can be judged by the MDA level [5].

The functional state of the prostacycline-thromboxane system in stress, especially in its zoosocial version, has been inadequately studied. To shed light on this problem the writers studied the antiaggregating activity of the aortic wall (AAAW) and the thromboxane concentration in zoosocial stress (ZS), which is classed as a negative-emotional type of the general adaptation syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 250-300 g. Chronic ZS was stimulated by prolonged social isolation. The rats were kept in single iron cages for 16 weeks, which induced negative emotion, leading to stress [3]. Under pentobarbital anesthesia (1 ml of 1% solution/200 g body weight) the abdominal aorta of the animals was excised, rinsed in 50 mM Tris-HCl buffer, pH 7.5, and the prostacycline activity of the aortic wall was determined by the method described previously [9]. To obtain platelet-rich plasma, blood was taken from the abdominal aorta of healthy animals, stabilized with 3.14% sodium citrate solution (9:1), and subjected to differential centrifugation in order to obtain platelet-deprived and platelet-enriched plasma. Platelet aggregation was induced with the disodium salt of ADP in a final

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