ENKEPHALINS AND THE STATE OF THE SYMPATHICOADRENAL SYSTEM IN ACUTE MYOCARDIAL ISCHEMIA

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Massive catecholamine release in extremal states can exert a marked histotoxic action, including on the myocardium [5]. Hypercatecholaminemia plays a special pathogenetic role in acute coronary pathology when it contributes to the further spread of necrosis of the heart muscle [4]. This is also shown by the close correlation found between adrenalin (A) and noradrenalin (NA) excretion and the severity of the condition of patients with myocardial infarction [1]. There is reason to suppose that endogenous opioid peptides can modulate the intensity of adrenergic processes in the body. This is confirmed by data showing that under the influence of neuropeptides NA release from sympathetic terminals is reduced [13], and morphine, antagonist of opiate receptors, inhibits activity of the adrenal medulla [10, 14] and NA uptake by the tissues [11].

The effect of peptides of enkephalin type on activity of the sympathicoadrenal system (SAS) in acute myocardial ischemia has not been studied, and this was the reason for the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male albino rats weighing 180-200 g. After adaptation of the animals for 5 days to the conditions of life in an individual metabolism cage, their 24-hourly urine was collected and the samples suitably acidified and cooled. To determine the catecholamine levels the original data were calculated as the mean value between two portions obtained over a period of 2 days. In view of the high individual variability of the original values in different animals, the dynamics of the parameters for each rat was estimated during the subsequent period of observation. After initial data had been obtained for all the animals acute myocardial ischemia was produced by diathermy coagulation of the left descending coronary artery by the method of Staab et al. All animals which survived after the operation were divided arbitrarily into three groups. Animals of group 1 received physiological saline in a volume of 200 μl, animals of group 2 received physiological saline together with an intraperitoneal injection of D-Ala^2-D-Leu^5-D-Arg^6-enkephalin in a dose of 1.25 nmole/100 g body weight (in a final volume of 200 μl), and animals of group 3 received an injection of D-Ala^2-Leu^5-Arg^6-enkephalin under similar conditions. The Leu-enkephalin analogs used have alanine in position 2, which makes them much more resistant to enzymic degradation [12]. During the 24 h after production of experimental myocardial ischemia in the rats, the urine was again collected. Activity of SAS was estimated on the basis of urinary excretion of A, NA, dopa, and dopamine (DA) concentrations of which in the urine were determined fluorometrically, fluoroescence being recorded on a Hitachi 650-60 spectrofluorometer (Japan). The results were subjected to statistical analysis: the confidence intervals calculated from Strelkov's tables [6], and the significance of changes in the parameters studied was determined by the Wilcoxon–Mann–Whitney nonparametric (Pu) test.

EXPERIMENTAL RESULTS

As Table 1 shows, acute myocardial ischemia (AMI) was accompanied by marked activation of the SAS, which is characteristic of extremal situations [2]. Excretion of A and NA, for instance, 24 h after cauterization of the coronary artery, was more than doubled. The urinary excretion of dopa was increased by 48% and of DA by 86%. The very small decrease in the A/NA ratios is evidence of the stronger effect of the pathological factor on the transmitter stage of the SAS. Calculation of ratios between end products of synthesis of

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TABLE 1. Effect of Enkephalins on Excretion of Catecholamines (in μg/24 h) by Rats with AMI

Group animals	Number of observations	A	NA	dopa	DA	A/NA	(A + NA + DA)/dopa	(A+NA)/DA
1: Initial data for AMI (24 h) + physiological saline	20	13,1±4,0	29,62±7,07	49,33±7,68	79,5±12,4	0,442	2,48	0,537
	20	28,6±5,87	78,51±25,5	73,09±15,96	147,9±23,9	0,364	3,49	0,724
		Pu_1<0,01	Pu_1<0,05	Pu_1<0,05	Pu_1<0,001	—	—	
2: Initial data for AMI (24 h) + D-Leu^5-D-Arg^6	20	16,5±6,21	32,17±13,3	49,24±6,79	75,28±11,6	0,514	2,52	0,647
	20	19,78±10,0	21,86±5,8	38,24±5,15	47,96±17,8	0,904	2,34	0,868
		Pu_1>0,05	Pu_1<0,01	Pu_1<0,01	Pu_1<0,05	—	—	—
		Pu_2<0,05	Pu_2<0,01	Pu_2<0,05	Pu_2=0,01	—	—	—
3: Initial data for AMI (24 h) + D-Ala^2-Leu^5-Arg^6	20	15,32±1,22	29,23±3,5	49,39±8,3	84,8±17,5	0,524	2,62	0,525
	20	5,99±1,09	16,9±2,84	34,85±4,48	68,06±8,9	0,354	2,61	0,336
		Pu_1<0,001	Pu_1<0,01	Pu_1>0,05	Pu_1>0,05	—	—	—
		Pu_2<0,001	Pu_2<0,01	Pu_2<0,01	Pu_2<0,01	—	—	—

Legend. Pu_1) Significance relative to initial data; Pu_2) significance relative to corresponding period of AMI but without enkephalin.

sympathomimetic amines and their precursor, dopa, showed an increase in this parameter of 41%, evidently attributable to intensive production of A, NA, and DA, accompanied by exhaustion of the dopa reserves. Stimulation of the next stage of catecholamine synthesis was shown by an increase in the ratio of the total A + NA to DA excretion, evidence of more rapid hydroxylation of DA.

Injection of D-Ala^2-D-Leu^5-D-Arg^6-enkephalin into rats with AMI prevented stimulation of the SAS. For instance, the 24-hourly A excretion in rats of this group did not differ significantly from the initial level, but was 31% less than in animals with myocardial ischemia and receiving physiological saline instead of neuropeptides. NA excretion with the urine not only was not increased, but was actually reduced by 32% compared with the initial values. Excretion of dopa (by 22%) and DA (by 36%) in the urine also was reduced. The decrease in NA excretion in rats of this group, noted above, was responsible for an increase of 76% in the A/NA ratio. Unlike in rats of the control group with AMI, in the group of animals treated with enkephalin the parameter obtained by dividing the total excreted A + NA + DA by the quantity of dopa excreted with the urine in 24 h, was not increased, but was actually somewhat reduced. This fact may probably be interpreted as indicating preservation of the reserve capacity of the SAS on injection of D-Ala^2-D-Leu^5-D-Arg^6-enkephalin into rats with AMI. It is quite possible that the point of application for the inhibitory action of the peptide is the enzyme dopa-decarboxylase, which prevents a decrease in the concentrations of all the subsequent components of the chain of formation of sympathetic nervous system mediators in the urine (and, consequently, in the blood also). No significant difference was observed in the character of the change in the ratio (A + NA)/DA compared with the group of rats which did not receive enkephalin after production of myocardial ischemia.

Injection of another Leu-enkephalin analog, in whose molecule the D-amino acids in positions 5 and 6 were replaced by their L-isomers, had an even stronger action on activity of the SAS.

Introduction of levorotatory leucine and arginine into the structure of enkephalin evidently increased the specificity of binding of the agonist with opiate receptors, in much the same way as differences in substrate–receptor interrelations existing for levorphanol and dextrophan (L- and D-isomers of morphine) [8].

For instance, injection of D-Ala^2-Leu^5-Arg^6-enkephalin into rats with AMI led to a highly significant fall in the 24-hourly excretion, not only of NA by 42%, as in rats of the previous group, but also of A by 61%. The urinary excretion of dopa and DA in animals of this group did not differ significantly from the initial values, but was significantly lower than for rats with myocardial ischemia not treated with enkephalins. The decrease in the A/NA ratio in this case indicated predominant inhibition of activity of the humoral component of SAS, i.e., activity of the adrenal medulla. The action of D-Ala^2-Leu^5-Arg^6-enkephalin on rats with AMI prevented an increase in the ratio of the total end products of catecholamine synthesis to dopa, which, as in rats of the previous group, could indicate preservation of the reserve capacity of the SAS due to the action of the peptide, on account of its inhibitory effect on dopa-decarboxylase. The increase in the ratio obtained by dividing the total A + NA excreted with the urine by the quantity of DA excreted suggests that D-Ala^2-Leu^5-Arg^6-enkephalin may not only act on dopa-decarboxylase activity, but may also partially inhibit DA-β-hydroxylase, which limits NA and A formation from DA, also. Enkephalins may evidently exert their action on the enzyme of adrenal synthesis through opiate receptors, found in the adrenal medulla on membranes of chromaffin vesicles [10].

Peptide ligands of opiate receptors can thus exert an inhibitory action on activity of the SAS. Considering data showing elevation of the opioid peptide level in the body in extremal situations [8], it can be postulated that, through depression of function of the SAS, they can limit the pathogenic action of stress developing under conditions of hyperergia. Further arguments in support of this hypothesis may be given by the existence of opiate receptors in the adrenals [10] and inhibition of NA release from sympathetic neurons under the influence of opioid neuropeptides [7].

Normalization of certain constants of homeostatis in rats with AMI following their injection with enkephalins may partially explain the weakening of the cardiotoxic action of an excess of adrenomimetics, which the writers demonstrated previously [3].

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STRUCTURAL CHANGES IN THE GASTRIC MUCOSA IN EXPERIMENTAL HYPERTHYROIDISM

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The secretory function of the stomach is known to depend on the functional state of the endocrine glands [1, 4, 11]. Data on the character of the digestive disorders arising in patients with thyrotoxicosis, obtained by different investigators, are highly contradictory. Some workers have observed reduced proteolytic activity of the gastric juice in patients with toxic goiter [10, 12], whereas others found increased activity [13].

The essentially contradictory nature of different items of information about disturbances of the structure of the gastric mucosa in thyrotoxicosis, which cannot yet be explained, has led to a search for objective criteria for evaluating the morphological changes in this disease.

The aim of the present investigation was accordingly to study the morphological substrate lying at the basis of the disturbed pepsin-forming function of the stomach in rats with experimental hyperthyroidism.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 180-200 g. Thyroxine (from Reanal, Hungary) was injected intraperitoneally into the animals in doses of 0.1 mg/kg (small) and 2.5 mg/kg (large)

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