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BLOOD LEVELS OF MET-ENKEPHALIN AND BETA-ENDORPHIN IN THE EARLY PERIOD AFTER ACUTE BLOOD LOSS IN RATS

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KEY WORDS: Met-enkephalin; beta-endorphin; acute blood loss

Data on increased secretion of beta-endorphin (BE) and enkephalins in various types of shock have been published in recent years [12-14]. A broad spectrum of physiological effects, namely a marked antistressor action [8], ability to inhibit secretion [16] and peripheral effects [11] of catecholamines, and the character of the effect on the hemodynamics and metabolism [2, 6, 13], – suggests the possibility that enkephalins and BE may be involved in the development of shock. Nevertheless, information in the literature on this subject [13, 14] is quite contradictory, due perhaps to the different conditions of the investigations and differences in the pathogenesis of the various types of shock. The aim of this investigation was to study blood levels of Met-enkephalin (ME) and BE in rats in the early period after acute blood loss and to analyze dependence of the character of the course of the pathological process on the blood opioid levels.

EXPERIMENTAL METHOD

Experiments were carried out on 174 Wistar rats weighing 330-350 g and 110 CBWH albino mice weighing 22-25 g. A model of acute blood loss was created by bleeding from the right common carotid artery in a volume corresponding to 3% of body weight. The carotid artery was catheterized under superficial ether anesthesia. BP in the carotid artery was recorded by the direct method on a "Thomson" polygraph (France). Blood samples were taken immediately before hemorrhage (background values) and 1 and 30 min after bleeding. These time intervals were chosen allowing for the known phasic nature of changes in hemodynamic parameters in the early period after acute blood loss [3]. Intact rats served as the control group.

Concentrations of BE and ME in samples of blood plasma were determined by radioimmunoassay using standard kits from "Immuno Nuclear Corporation" (USA). Preliminary treatment of the blood plasma for investigation of ME including extraction with methanol, whereas BE were isolated by chromatography using reagents supplied with the kits. Radioactivity was counted on a "Tracor" gamma-spectrometer (USA). The blood lactate concentration was determined by an enzymic method using kits from "Boehringer" (West Germany). Optical density was recorded on a "Specord M-40" spectrophotometer (East Germany). Hypoxic hypoxia was induced in the animals by placing them in an airtight chamber with a capacity of 75 ml for mice and 1400 ml for rats, carbon dioxide being absorbed by soda lime, and the duration of survival of the mice was recorded by determining cessation of respiratory movements [1]. The delta-receptor agonist DADLE, the mu-receptor agonist DAGO (obtained at the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR) and

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TABLE 1. Mean BP (in mm Hg), and Blood BE (in pmoles/liter), ME (in pg/ml) and Lactate (mmoles/liter) Levels in Rats Surviving and Dying in the Course of 24 h of Observation after Acute Blood Loss ($M \pm m$)

Parameter	Group of animals						
	control	1-			2-		
		background	1st minute	30th minute	background	1st minute	30th minute
BE	5,2±1,1	15,6±3,4	13,8±3,3	26,8±6,8	17,1±4,6	21,8±5,4	5,3±2,1*
p		<0,05	<0,05	<0,01	<0,05	<0,05	>0,10
ME	32,8±8,4	57,3±13,6	151,4±39,5	34,4±12,2	51,4±14,1	47,2±14,4*	50,3±21,8
p		>0,05	<0,05	>0,10	>0,05	>0,10	>0,10
Lactate	1,34±0,12	1,46±0,12	2,48±0,12	4,74±0,51	1,50±0,11	3,22±0,26*	7,53±1,12*
p		>0,10	<0,01	<0,01	>0,10	<0,01	<0,01
BP	117±8	—	46±3	118±5	—	38±4	101±6
p			<0,01	>0,10		<0,01	>0,05

Legend. *$p < 0.05$ compared with corresponding value in rats of group 1 at same times of observation.

natural BE and ME (from "Serva") were injected intraperitoneally into mice and into the femoral vein of rats in a dose of 0.1 mg/kg. The results were subjected to statistical analysis by Student's test. The degree of association between the parameters studied was determined by correlation analysis with calculation of coefficients of correlation (r).

EXPERIMENTAL RESULTS

The BE concentration in the blood of rats surviving the first day of observation (group 1) was found to be raised in response to the operative trauma alone (background value immediately before bleeding), whereas the blood ME level during this period did not change statistically significantly (Table 1). These results are in full agreement with those obtained by other workers, who found similar changes in blood BE and ME levels in man in response to operative trauma [15]. At the first minute of the posthemorrhagic period the raised BE level did not change significantly, but remained considerably higher than in intact rats, but by the 30th minute a distinct tendency was observed for it to rise even more. The blood ME concentration in the animals of group 1 rose sharply by the first minute, and fell to the control level by the 30th minute after bleeding (Table 1). The increase in BE concentration in the blood of animals in response to operative trauma was evidently due to a stress reaction, just as in other extremal states [8, 15], whereas elevation of the ME level was evidently more closely linked with pathological changes in the body caused by hypovoletnia, reduction of the cardiac output, and circulatory hypoxia. The role of hypoxia as a factor initiating increased activity of the enkephalinergic component of the opioid system also is confirmed by the results of experiments on a model of normobaric hypoxic hypoxia in rats. A marked increase was found (up to 77.4 ± 12.1 pg/ml) in the ME concentration in the animals' blood ($p < 0.05$) relative to corresponding values in rats of the control group, against the background of marked hyperlactatemia (3.93 ± 0.41 mmoles/liter, $p < 0.01$ compared with the control).

In the animals dying in the course of 24 h of observation (group 2, mean survival 6.0 ± 2.18) changes in the blood opioid peptide levels were observed in response to operative trauma, similar to those in the rats of group 1. However, no statistically significant changes in the blood ME level were found in the rats of group 2 after blood loss compared either with the control animals or with background values; the BE level remained raised at the first minute and fell to the control value by the 30th minute of the posthemorrhagic period (Table 1).

These differences in the time course of the blood opioid peptide level in the animals dying in the course of or surviving for the first day suggests that after acute blood loss the increase in activity of the endogenous opioid system is adaptive in character. This also is confirmed by the results of experiments in which exogenous opioids were given to rats with acute blood loss. Administration of enkephalin analogs (the preparations were injected 10 min after bleeding) reduced the mortality rate among the animals in the posthemorrhagic period. Moreover, whereas the delta-receptor agonist DADLE reduced mortality of the rats only a little (from 62 to 41%), the mu-receptor agonist DAGO reduced it to 32%.

To analyze the mechanisms of the effect of opioid peptides on the course of acute blood loss we compared the blood BE and ME levels with values of BP in the early posthetnorrhagic period. As the data in Table 1 show, similar changes in mean BP were observed in the dying and surviving animals. Not until the 30th minute after hemorrhage were values of BP in the rats of group 1 somewhat higher than in rats of group 2. No correlation could be observed between values of blood opioid peptide concentrations and mean BP. The coefficients of correlation between ME and BP and between BE and BP were −0.21 and −0.14

respectively (in both cases $p < 0.05$). These results are in agreement with those of another investigation [13] in which no correlation could be found between the times of the peak levels of opioid peptides in the blood and BP values in hemorrhagic shock. The favorable influence of opioid peptides on the course of the posthemorrhagic period in rats is evidently unconnected with their action on the hemodynamics, as is confirmed by the results of experiments with exogenous opioids. In particular, injection of DAGO, despite the hypotensive action and negative chrono- and inotropic effect typical of mu-agonists [6, 14], reduced the mortality of the animals with acute blood loss.

A key role in the pathogenesis of the early posthemorrhagic period belongs to circulatory hypoxia [4, 5]. In this connection, there are some interesting data on the ability of morphine and certain enkephalin analogs to increase the resistance of animals to hypoxia, as was shown in experiments on a model of normobaric hypoxic hypoxia [1]. Our own investigations showed that injection of ME increased the duration of survival of mice with hypoxic hypoxia from 9.7 ± 0.5 to 11.9 ± 0.7 min ($p < 0.05$), whereas injection of BE increased their survival from 9.6 ± 0.6 to 12.2 ± 0.6 min ($p < 0.05$). This effect of natural opioids is evidently due to their interaction with mu-receptors, because DAGO had a stronger action than BE or ME, increasing the duration of survival of mice from 10.1 ± 0.4 to 14.7 ± 0.5 min ($p < 0.01$), whereas DADLE had no significant effect on this parameter compared with the control (10.1 ± 0.4 and 11.2 ± 0.4 min respectively, $p > 0.05$).

The increase in the duration of survival of the mice with normobaric hypoxic hypoxia characterizes the central action of opioid peptides, and is the result of increased resistance of the brain to hypoxia [1]. This factor may be important for the realization of the favorable effect of opioids on the course of acute blood loss, for primary hemorrhagic collapse develops [10] in the early period after hemorrhage, and during its course hypoxia arises not only in the peripheral organs, but also in the brain [4, 9], and this often serves as the principal cause of death in the posthemorrhagic period [4].

Meanwhile opioid peptides are evidently able to "protect" not only the brain against hypoxia, but also other organs and tissues. For instance, addition of BE to the perfusion solution increases the resistance of the isolated heart to hypoxia [7]. We showed previously that injection of enkephalin analogs into animals with acute blood loss reduces the severity of hyperlactatemia [2], and this reflects the severity of circulatory hypoxia in different types of shock [4,5]. This weakening of the manifestations of circulatory hypoxia by opioids is confirmed by the results of the present investigation. In the rats of group 1, against the background of a higher blood opioid peptide level, there was a less marked increase in the blood lactate concentration than in the animals of group 2 during corresponding periods of activation (Table 1). Thus opioid peptides can increase the resistance of the animal to hypoxia, including under conditions of acute posthemorrhagic circulatory failure, which is evidently one of the principal mechanisms of the favorable action of opioids on the course of acute blood loss.

On the basis of these results we can agree with the opinion of those investigators [13, 14] who consider that opioid peptides play a different role in the pathogenesis of types of shock which differ in their etiology. In particular, the increase in the blood levels of BE and ME observed in the present experiments in the early period after single blood loss is of adaptive importance, due both to the character of the physiological effects of the opioid peptides and also to the particular features of the pathogenesis of the posthemorrhagic period after acute blood loss.

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EFFECT OF AN ANTIOXIDANT OF THE 3-HYDROXYPYRIDINE CLASS ON MICROCIRCULATORY DISTURBANCES ASSOCIATED WITH EXPERIMENTAL DYSLIPOPROTEINEMIA AND ITS ALIMENTARY CORRECTION

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The important role of dyslipoproteinemia (DLP) in microcirculatory disturbances (MCD) has now been established. The response of the microcirculatory system to DLP is generalized in character and is accompanied by the development of pathological changes in the target organs [9, 4, 10]. During prolonged spontaneous regression of experimental atherosclerosis complete restoration of lipid homeostasis and of MCD is not observed [5]. In clinical investigations MCD have been found in association with acute disturbance of the coronary circulation and an atypical course of myocardial infarction [2, 12]. It has been shown that during remission of coronary heart disease changes in the terminal vascular bed undergo virtually no degree of regression, and this promotes progression of the disease [11]. An important stage of atherogenesis and, in particular, of membrane pathology in DLP, is activation of lipid peroxidation (LPO). Recent investigations [1, 6-8, 9] have demonstrated the vasoconstrictor properties of LPO products and their ability to accelerate aging of erythrocytes and to slow the blood flow at the level of small and medium-sized vessels. This accounts for the importance of elucidating the role of LPO in the MCD associated with DLP.

In the investigation described below the effect of the antioxidant mexidol was studied on parameters of lipid metabolism, LPO, and the state of the microcirculatory system in the early stages of atherogenesis and during alimentary correction of DLP.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male chinchilla rabbits weighing 2-2.5 kg. The animals of group 1 received cholesterol in a dose of 0.3 g/kg body weight with vegetables for 2 months. Animals of group 2, also on an atherogenic diet (AGD) additionally received the antioxidant (AO) mexidol from the second month in a dose of 30 mg (for 1 month). It was shown previously that a marked rise of the level of atherogenic lipoproteins (ALP), MCD, and initial atherosclerotic changes in the aorta are observed in animals kept on an AGD for 1 month [4, 5]. After 2 months on the AGD, animals of groups 1 and 2 were transferred to a standard diet (SD) for 9 months (alimentary correction of DLP). Intact animals receiving SD throughout the same period served as the control. The animals were withdrawn from the experiment by air embolism. The index of atherosclerotic damage of the aorta (IDA) was determined by Avtandilov's method. The state of the microcirculatory bed was studied in total film preparations of the mesentery of the small intestine (Kupriyanov's method). The area of cross section of the microvessels was determined with the aid of a semiautomatic image analysis system (Leitz "ASM," West Germany) and their adrenergic innervation was studied by the method of Falck and Ovman. The intensity of specific fluorescence of catecholamines in the

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