FUNCTIONAL STATE OF THE ENDOGENOUS OPIOID PEPTIDE SYSTEM IN RATS WITH TRAUMATIC SHOCK

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The discovery of a new class of biologically active substances, namely opioid peptides, has involved the elucidation of their role in the pathogenesis of critical states [13-15]. Nevertheless, many aspects of this problem, including the role of endogenous opioids in traumatic shock, have not yet been adequately studied. Data in the literature on the effect of agonists and antagonists of opiate receptors on the course of traumatic shock [5, 12] do not enable the role of enkephalins and endorphins in the pathogenesis of shock to be assessed objectively.

The aim of this investigation was to study the functional state of the endogenous opioid peptide system in traumatic shock.

EXPERIMENTAL METHOD

Experiments were carried out on 120 Wistar rats weighing 180-200 g and on 546 CBWH mice weighing 22-25 g. Traumatic shock was produced in the rats and mice by applying forceps of a special design on both hind limbs for 3 and 6 h [4]. The blood pressure (BP) in the carotid artery was measured on a "Thomson" polygraph (France). The rats were killed by decapitation in groups immediately after the end of 3 h of compression and 24 and 48 h after the end of 6 h of compression of the soft tissues of the hind limbs. Intact animals served as the control group. All operations and painful manipulations were carried out under ether anesthesia. Immediately after the end of compression of the soft tissues of the limbs, the mice were given intraperitoneal injections of the following preparations: beta-endorphin (BE) from "Sigma," gamma-endorphin (GE), the carboxyl terminal fragment (18-31) of BE (C18-31-peptide), enkephalin analogs – DAGO and DADLE (obtained at the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR). Mortality of the mice was estimated after 16, 24, and 48 h of the post-traumatic period.

Concentrations of BE, Met-enkephalin (ME), and Leu-enkephalin (LE) in tissues of the midbrain and adrenals and in the blood were determined by radioimmunoassay, using kits from "Immuno Nuclear Corporation" (USA). Preliminary treatment of the blood plasma for ME assay included extraction with methanol, and chromatographic isolation of BE. Preparation of the tissues included heat treatment and extraction with hydrochloric acid [11]. The blood lactate concentration was determined with the aid of kits from "Boehringer," and urea with kits of reagents from "La Chema." Radioactivity of the samples was counted on a "Tracor" gamma-spectrometer (USA) and optical density was recorded on a "Specord M-40" spectrophotometer.

The results were subjected to statistical analysis by Student's test.

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TABLE 1. Time Course of Changes in Mean BP, Blood Lactate and Urea Concentrations, BE Content in Adrenals, and BE, ME, and LE Concentrations in Midbrain of Rats with Compression Trauma ($\bar{X} \pm m$)

Test object	Groups of experimental animals			
	control group	rats with traumatic shock		
		3 h	24 h	48 h
Mean BP, mm Hg	114±6	101±8	87±5	73±6
p		>0.05	<0.01	<0.01
Blood: lactate, mmoles/liter	1.24±0.11	2.47±0.16	3.01±0.22	2.93±0.22
p		<0.01	<0.01	<0.01
Urea, mmoles/liter	6.9±0.4	13.6±1.1	16.3±2.7	17.2±3.4
p		<0.01	<0.01	<0.05
BE, pmoles/liter	5.32±1.17	16.61±2.68	13.93±2.73	14.67±1.12
p		<0.01	<0.05	<0.01
ME, μg/ml	38.7±6.4	56.9±4.3	45.9±5.9	82.1±7.9
p		<0.05	<0.05	<0.05
Brain: BE, pmoles/g	0.88±0.10	1.23±0.07	0.10±0.09	1.20±0.05
p		<0.05	>0.05	<0.05
ME, ng/g	16.8±1.6	23.2±2.2	12.1±1.1	18.1±2.7
p		<0.05	<0.05	>0.05
LE, ng/g	6.1±0.6	5.7±0.6	8.2±0.8	4.2±0.5
p		>0.05	<0.05	<0.05
Adrenal: ME, ng/g	6.4±0.6	5.7±0.6	6.6±0.8	4.2±0.8
p		>0.05	>0.05	<0.05
LE, ng/g	3.7±0.4	6.2±0.7	7.9±1.1	2.0±0.3
p		<0.05	<0.05	<0.05

EXPERIMENTAL RESULTS

As the data in Table 1 show, a progressive decrease in values of the mean BP and an increase in blood lactate and urea levels were observed in the animals with compression trauma, reflecting the time course of development of shock.

A threefold increase in the blood BE concentration took place as early as 3 h after the beginning of compression and it remained high throughout the period of observation. The blood ME concentration rose but not significantly after 3 h, then fell a little, and 24 h after trauma it did not differ statistically significantly from values in rats of the control group; by the end of the 2nd day of the postcompression period a twofold increase in the blood ME concentration was noted. In the adrenals the ME level showed no significant change, whereas the LE concentration rose during the first 24 h of observation. Concentrations of enkephalins in the adrenals 48 h after trauma were below the corresponding values in intact rats. Brain levels of ME and BE in the rats rose 3 h after the beginning of compression, but the LE level was unchanged. By the end of the 1st day after trauma the BE concentration in the brain had fallen to the control value, the ME level was below it, and the LE level rose. The BE concentration in the brain 48 h after trauma was increased, ME was unchanged, and LE was below the corresponding values in intact rats (Table 1).

The results are evidence that function of the opioid system is on the whole increased in rats in the post-traumatic period, and its activity reaches a maximum in the early stages after trauma. The phasic nature of the subsequent changes in blood and tissue opioid levels is evidently attributable to a gradual lowering of the reserve capacity of the opioid system during aggravation of the shock process. This conclusion is confirmed by lowering of the enkephalin levels in the brain and the marked decrease in their concentration in the adrenals towards the end of the 2nd day of observation, indicating a significant degree of retardation of synthetic processes after secretion of opioid peptides [11].

To examine the effects of enkephalins and endorphins on the course of traumatic shock experiments were carried out on mice with exogenous opioid administration.

Administration of BE in doses of 0.1 and 1.0 mg/kg lowered the survival rate of the mice with shock, but not significantly. The aminoterminal fragment of BE, namely GE, increased the mortality among the animals even more. Preliminary injection of C18-31-peptide did not change the effect of GE on the course of the post-traumatic period in mice (Table 2). Weaker activity of BE than of GE was evidently associated, not with differences in the character of action of its individual fragments, but the particular features of its stereospecific structure and its affinity for

TABLE 2. Survival Rate of Mice with Traumatic Shock after Injection of Physiological Saline (control) and of Opioid Peptides

Preparation administered	Dose, mg/kg	Number of mice	Survival rate, %		
			16 h	24 h	48 h
Control		29	41	34	10
BE	0,1	29	38	26	7
BE	1,0	29	41	26	7
Control		27	41	30	11
GE	0,1	27	37	26	7
GE	1,0	27	33	22	4
C18-31-peptide	0,1	27	44	30	11
C18-31-peptide	1,0	27	44	33	7
Control		29	41	34	10
GE together with C18-31-peptide	0,1	29	37	29	7
GE together with C18-31-peptide	1,0	29	33	26	4
Control		40	38	30	7
DAGO	0,1	40	43	33	20
DAGO	1,0	40	48	38	26
Control		38	39	32	13
DADLE	0,1	40	33	23	10
DADLE	1,0	39	33	18	8

particular types of opiate receptors. BE interacts equally with mu- and delta-receptors, whereas its aminoterminal fragment exhibits more the properties of a delta-agonist [10]. These data suggest that mu- and delta-receptors play different roles in the realization of the effect of opioid peptides on the course of shock.

Injection of the selective delta-receptor agonist DADLE led to a dose-dependent decrease, whereas that of the mu-agonist DAGO led to a corresponding increase in the survival rate of the animals with shock (Table 2). The intact mechanisms of involvement of enkephalins and endorphins in the pathogenesis of shock call for further study. Nevertheless, it can be concluded from the results that interaction of opioid peptides with mu- and delta-receptors mediates processes with different effects on the course of traumatic shock.

The writers showed previously that administration of the enkephalin analog dalargin immediately after soft tissue decompression leads to a decrease in the mortality of rats, whereas if the preparation is given 48 h after trauma, BP falls rapidly and the animals die [1]. An increase in activity of the opioid system can evidently play a different pathogenetic role in the early and late stages after trauma, depending on the phase of the shock process.

Elevation of the blood and brain enkepnalin ana enaorpnin levels in the early post-traumatic period may have an adaptive role, thanks to their analgesic [13], antistressor [8, 9], and antiischemic [6] action and their ability to increase the energy potential of the tissues [3]. In the late postcompression period (the late torpid, terminal phase of shock) opioids exhibit antagonism toward endogenous pressor hormones (catecholamines, vasopressin [1, 7, 9]), and may aggravate disturbances of the systemic and regional hemodynamics and worsen the course of shock.

The data described above point to significant changes in function of the opioid system during the course of the post-traumatic period, and this may be an important factor in the pathogenesis of traumatic shock.

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EFFECT OF EXPERIMENTAL UREMIA AND INJECTION OF EXOGENOUS PARATHYROID HORMONE ON AXOSPINOUS SYNAPSES IN HIPPOCAMPAL AREA CA_3

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The encephalopathy which patients with chronic renal failure (CRF) develop is due to a combination of pathogenetic factors which exert a toxic influence on the nervous system. A leading role in this situation is ascribed to parathyroid hormone (PTH), although the mechanism of its toxic action is not yet clear [11]. Elevation of the blood PTH level is accompanied by an increase in the calcium concentration in the brain, its accumulation being greatest in the neocortex and hypothalamus [10], and characteristic changes also are found in the EEG [11]. Experiments have shown that PTH is present in the cerebrospinal fluid, where its concentration is equal to one-third of that in the blood serum [6].

The aim of this investigation was to study ultrastructural morphometric parameters of axospinous synapses in area CA_3 of the hippocampus (HC), analysis of which enables changes in the efficiency of the axospinous synapses in this part of the brain to be characterized in response to injection of exogenous PTH and during the development of experimental uremia, when the conditions are created for accumulation of endogenous PTH in the blood [10]. The reason why area CA_3 of HC was chosen was not only the absence of experimental data enabling the efficiency of synapses in this part of the brain to be assessed in uremia, but also the ability of area CA_3 of HC to exert modulating influences on activity of hypothalamic nuclei involved in the regulation of water and electrolyte metabolism [2].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200 g. To create experimental uremia a model of subtotal nephrectomy was used [12]; the development of uremia was monitored by biochemical tests of the blood and urine, using standardized techniques. Exogenous PTH was administered by intraperitoneal injections in a dose of 50

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