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EMITIC AND ANTIEMETIC PROPERTIES OF SOME REGULATORY PEPTIDES

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KEY WORDS: peptides; vomiting; μ- and δ-opioid receptors; chemoreceptive trigger zone; vomiting center

Information on the emitic action of certain regulatory peptides (RP), mainly of enkephalins and β-endorphin, can be found in the literature. For instance, in 1977 it was shown [9] that Met-enkephalin (ME), when injected into the stomach, induces vomiting in cats. The emetic effects of enkephalins and β-endorphin in cats and dogs were later observed by other workers [1, 5, 8]. However, no special investigation of the vomiting action of the various RP has yet been undertaken. In addition, the possible antiemetic effects of endogenous peptides have virtually not been studied. The existence of these latter properties of the peptides would provide a basis for their prospective use in the prevention and treatment of vomiting, which is a characteristic symptom of many pathological processes and, in particular, of motion sickness and radiation sickness.

The aim of the present investigation was accordingly to compare the emetic and antiemetic properties of certain RP: enkephalins, endorphins, β-lipotropin, ACTH, and substance P (SP).

EXPERIMENTAL METHOD

Experiments were carried out on 30 unanesthetized, unrestrained cats weighing 2.2-4.5 kg. Under general anesthesia (pentobarbital sodium, 30-40 mg/kg, intraperitoneally) cannulas were introduced into the fourth ventricle of the brain at coordinates P = 11, L = 0, H = −4.6, from the atlas [13], 3-5 days before the beginning of the experiment. The position of the cannula in the fourth ventricle was verified later with the aid of Evans' blue dye at autopsy on the animals. In the course of the experiment the ECG and respiration were recorded on an RM-150 polygraph (Nikon Kohden, Japan), and later the heart rate (HR) and respiration rate (RR) per minute were calculated from these parameters.

Morphine (an agonist of μ-opioid receptors), ME and Leu-enkephalin (LE), D-Ala -D-Leu - enkephalin (DDLE) — an agonist predominantly of δ-opioid receptors, and β-, γ-, and des-Tyr-γ-endorphins, obtained from Professor M. I. Titov, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, porcine β-lipotropin and $ACTH_{1-39}$, provided by Corresponding Member of the Academy of Medical Sciences of the USSR, Yu. A. Nankov, of the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR), and also SP (provided by Professor K. Hecht, East Germany), were dissolved in doses of 10-500 μg in sterile isotonic sodium chloride solution, and injected into the fourth ventricle from a microsyringe (Hamilton, Great Britain) in a volume of 50-100 μl. Opioid receptors were blocked by means of naloxone (Endo Laboratories, USA), a specific antagonist of opiates and opioids, and ICI 154, 129, a selective antagonist of δ-opioid receptors (provided by Dr. Med. Sci. O. S. Medvedev), were used.

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TABLE 1. Effect of Opioid Peptides and Morphine on HR and RR in Cats during and after Vomiting (M ± m)

Preparation and its dose, μg	Number of expts.	LP, sec	Background	1 min after injection of preparations	During vomiting (2-6 min)	Time after vomiting, min: 1	3	5	10	15	20	25
Morphine (10—200)	13	206±27	142±12	141±11	154±11	151±10	133±10	128±10	123±8	117±8	120±9	119±9
			41±5	45±6		48±6	64±14	45±5	63±16	65±17	56±14	52±10
ME (10—300)	6	147±29	165±17	155±15	148±18	165±14	155±17	146±14	158±14	148±13	152±18	158±24
			36±3	34±3		37±5	38±7	34±7	35±6	36±6	34±5	31±4
LE (10—300)	6	124±24	153±4	16±6	134±10	176±4***	143±9	132±12	119±12*	117±14*	117±13*	120±9**
			41±7	45±6		45±8	60±17	52±12	70±14	57±11	51±9	59±10
β-endorphin (10—100)	5	181±47	165±14	160±17	139±14	174±15	147±11	143±11	152±13	146±16	151±11	164±17
			35±9	32±7		36±7	46±21	44±21	45±23	36±6	36±4	33±4
DDLE (100—500)	6	126±27	146±12	148±11	122±7	182±14	169±25	170±25	174±16	166±19	159±17	162±18
			53±9	58±8		73±13	71±13	65±12	69±16	65±16	52±9	56±10

Legend. *p < 0.05, **p < 0.02, ***p < 0.01 compared with background (Student's test). Here and in Table 3 above the line — HR, beats/min; below the line — RR, cycles/min.

EXPERIMENTAL RESULTS

Morphine, LE, ME, DDLE, and β-endorphin, when injected into the fourth ventricle, induced single or multiple dose-dependent episodes of vomiting in all the animals (after control injections of 100 μl of sterile isotonic sodium chloride solution no emetic effect was oberved). Data showing the effect of these preparations on HR and RR of the cats during and after vomiting, and also the latest periods (LP) of the vomiting response for each of the substances tested are given in Table 1. Opioid peptides, in doses of 10-300 μg, caused no statistically significant changes in RR of the animals. HR proved to be a more informative parameter than RR. For instance, when LE was injected tachycardia was observed immediately after vomiting, and it subsequently was replaced by bradycardia.

Naloxone, a specific blocker of opioid receptors, when injected into the fourth ventricle in doses of 10 to 105 μg, 1-5 min before administration of the opioids and morphine, completely prevented the emetic effect of the preparations in 18 of 20 cases (Table 2). As a result of the action of naloxone no statistically significant changes were found in HR after injection of LE. The results are evident that the vomiting reaction induced by endogenous opioids and morphine is due to stimulation of opioid receptors of the chemoreceptive trigger zone of the vomiting center, the presence of which has been established previously [4, 5, 11]. Considering the heterogeneity of the opioid receptor population, we decided to carry out experiments with ICI 154, 129, a selective δ-antagonist. Experiments showed that this compound blocked the vomiting action of the δ-agonist DDLE statistically significantly, but did not prevent the emetic effect of the μ-agonist morphine (Table 2). Consequently, the chemoreceptive trigger zone of the vomiting center in cats evidently contains different types of opioid

TABLE 2. Blocking of the Emetic Effect of Opioid Peptides and Morphine by Naloxone and ICI 154, 129, a Selective Antagonist of δ-Opioid Receptors

Preparation and its dose, μg	Morphine	ME (10—500)	LE (10—500)	β-endorphin	DDLE
Naloxone (10—105)	5*/0	5*/1	5*/0	5*/1	4*/0
ICI 154, 129	4*/4	—	—	—	5*/1

Legend. Numerator gives number of animals; denominator — number of animals with vomiting. *p < 0.025 compared with control (Fisher's extract method).

TABLE 3. Effect of Regulatory Peptides without Vomiting Action on HR and RR in Cats (M ± m)

Preparation and its dose, μg	Number of experiments	Background	Time after injection of preparation, min						
			1	3	5	10	15	20	25
SP (50—100)	5	133±17 32±5	134±22 33±5	132±23 30±6	129±20 28±6	123±16 27±5	128±19 22±4	120±16 28±10	107±25 21±2
ACTH (10—200)	6	109±9 39±5	125±11 42±5	114±12 44±5	108±11 40±5	107±7 28±6	105±10 28±4	98±10 30±7	100±10 28±6
β-Lipotropin (10—200)	6	119±9 40±4	124±6 40±8	105±9 32±7	103±8 32±5	106±8 31±5	102±7 31±4	101±9 28±4	111±12 34±6
γ-endorphin (10—100)	5	120±12 40±6	124±15 45±5	109±14 32±8	104±12 26±4	94±12 23±3*	93±10 26±3	92±5 32±7	95±10 23±3*
des-Try-γ-endorphin (100)	5	137±11 39±9	129±14 46±11	127±14 41±9	117±11 40±8	108±13 30±5	103±13 30±3	102±10* 24±3	105±11 23±3

Legend. *$p < 0.05$ compared with background (Student's test).

receptors (at least μ- and δ-opioid receptors, as the results of our experiments show).

Unlike the RP described above, ACTH, SP, β-lipotropin, and γ- and des-Try-γ-endorphins, when injected into the fourth ventricle in doses of 50 to 200 μ, did not cause vomiting. After injection of the preparations bradycardia (or a tendency toward it) was observed, and RR fell (Table 3). Additionally, in two experiments (with γ-endorphin and SP) the animals developed a sleep-like state.

In the next series of experiments the possible antiemetic properties of those RP which did not induce vomiting in cats were investigated. It was found that β-lipotropin and ACTH have a weak antiemetic action. For instance, in doses of 10 to 1000 μg the peptides blocked morphine-induced vomiting in only three of 10 cases ($p > 0.05$, Fisher's exact method). Conversely, SP and γ- and des-Tyr-γ-endorphins had a marked antiemetic effect. In 14 of 15 experiments these preparations completely prevented the vomiting response due to injection of β-endorphin ($p < 0.025$, Fisher's exact method). This positive effect of these RP against vomiting was evidently connected with their neuroleptic properties, which are characteristic features of γ- and des-Tyr-γ-endorphins [2, 14, 15], and with possible antagonism of SP and endogenous opiods in the CNS [10, 12].

The results of the present investigation are thus evidence of an emetic action of β-endorphin and blocking of this effect by a physiologically active fragment of this RP, namely γ-endorphin, into which it can be converted during proteolysis in the brain [6, 7]. The writers showed previously that β-endorphin plays a direct part in the genesis of vestibulo-vegetative disorders (nausea, vomiting, and so on) arising in motion sickness [3]. It can accordingly be postulated that activation of the endogenous opioid system in response to vestibular stimulation (which is probably a protective response) leads to the formation of various opioid peptides in the body, which can affect the central mechanisms of regulation of a particular autonomic function in opposite directions and, in particular, they may modulate the vomiting response in man and animals in motion sickness.

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MODULATING EFFECT OF ENKEPHALINS ON HEMATOPOIESIS DURING STRESS

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Increasing attention has been paid in recent years to the study of the role of neuropeptides in the regulation of many different bodily functions [5]. In particular, data have been obtained to show that ligands of opiate receptors participate in the formation of adaptive reactions during exposure of the body to extremal influences [6, 9]. Antistressor effects have been discovered in endogenous enkephalins and their synthetic analogs [3, 4]. It has accordingly become logical to suggest that ligands of opiate receptors may be able to regulate hematopoiesis during stress. However, the role of neuropeptides in regulation of the blood system has until now remained virtually unstudied.

The aim of this investigation was to study the effect of enkephalins on medullary hematopoiesis in stress.

EXPERIMENTAL METHOD

Experiments were carried out on 400 noninbred male mice weighing 18-20 g. The animals were immobilized for 3-6 h in recumbency in the supine position. Mice exposed to immobilization for 3 h were given one (3 h after the beginning of immobilization) or two (3 and 6 h after the beginning of immobilization) intraperitoneal injections of Met-enkephalin (ME, from Fluka, USA) in a dose of 100 μg/kg, whereas animals immobilized for 6 were given a single (6 h after the beginning of immobilization) intraperitoneal injection of Leu-enkephalin (LE) in a dose of 100 μg/kg (LE was obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Dr. Chem. Sci. M. I. Titov). Animals of the corresponding control groups received an injection of physiological saline in the same volume (0.2 ml) at the corresponding times after the beginning of immobilization. On the 5th-8th days after immunization the mice were killed by cervical dis-

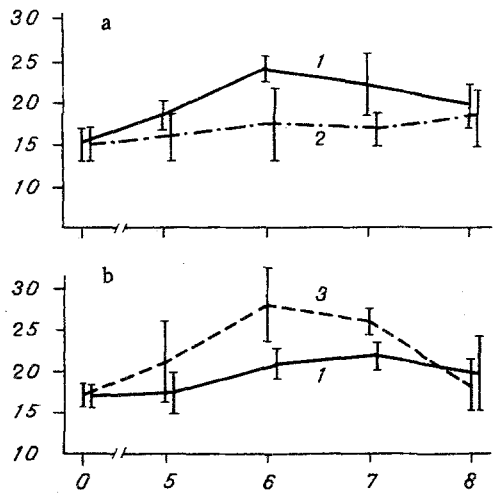

Fig. 1. Time course of TNMK in mice exposed to immobilization for 6 h (a) and 3 h (b), and receiving injection of physiological saline (1), LE (2), and ME (3). Abscissa, time after beginning of immobilization (in days); ordinate, number of cells ($\times 10^6$). Confidence limits at $p = 0.05$ level.

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