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MODULATING EFFECT OF ENKEPHALINS ON HEMATOPOIESIS DURING STRESS

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KEY WORDS: stress; bone marrow; enkephalins

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-

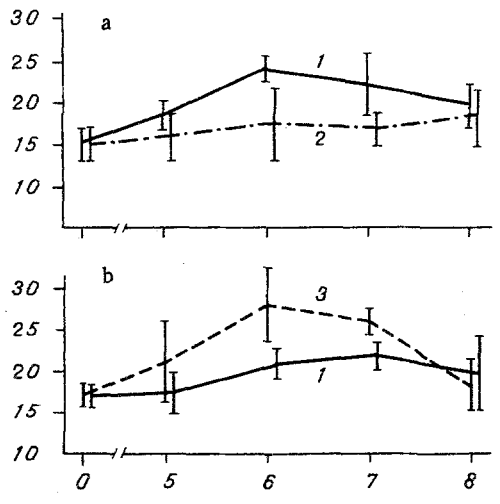

Fig. 1. Time course of TNMK in mice exposed to immobilization for 6 h (a) and 3 h (b), and receiving injection of physiological saline (1), LE (2), and ME (3). Abscissa, time after beginning of immobilization (in days); ordinate, number of cells ($\times 10^6$). Confidence limits at $p = 0.05$ level.

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TABLE 1. Effect of Enkephalin on Time Course of Absolute Number ($\times 10^6$ per femur) of Immature Neutrophils (numerator) and Erythroid Cells (denominator) in Bone Narrow of Mice Subjected to Immobilization (X ± m)

Time of investigation, days	Immobilization for 6 h		Immobilization for 3 h	
	control (physiological saline)	LE	control (physiological saline)	ME
Before immobilization	2,8±0,2 / 2,2±0,3	3,8±0,2 / 2,2±0,3	4,5±0,3 / 2,5±0,2	4,5±0,3 / 2,5±0,2
5	5,0±0,4* / 2,7±0,5	3,9±0,3** / 2,1±0,5	4,3±0,3 / 2,9±0,2	5,5±0,6 / 3,3±0,5
6	5,5±0,4* / 3,9±0,6*	3,0±0,3** / 2,0±0,5**	5,8±0,2* / 3,7±0,3*	8,9±0,9** / 4,4±0,2*
7	5,0±0,4* / 3,6±0,7	4,1±0,2** / 1,9±0,2**	4,9±0,2 / 3,4±0,3*	5,4±0,2* / 4,8±0,2**
8	5,6±0,6* / 2,9±0,6	4,5±0,2 / 2,4±0,6	4,2±0,5 / 3,2±0,2*	3,8±0,2 / 3,5±0,3*

Legend. *p < 0.05 compared with intact animals (before immobilization); **p < 0.05 compared with stress control.

location. The total number of myelokaryocytes (TNMK) in the femur was counted. The myelogram was determined by examination of bone marrow films.

EXPERIMENTAL RESULTS

Marked hyperplasia of the bone marrow developed on the 6th-7th day after immobilization for 6 h in animals of the control group (Fig. 1). In this case TNMK reached maximal values on the 6th day of the experiment (147 ± 3.0% of the background value). Analysis of the myelograms showed that the increase in TNMK was due to stimulation of both granulopoiesis and erythropoiesis (Table 1). LE had a modulating effect on medullary hematopoiesis during stress. Hyperplasia of the bone marrow did not develop in mice receiving this opioid peptide. TNMK and the number of cells of the various branches of hematopoiesis varied as a rule within the original normal limits (before immobilization). LE thus can normalize hematopoiesis during a stress reaction. Meanwhile ME, under conditions of immobilization stress, had the opposite action on hematopoiesis (Fig. 1, Table 1). This peptide stimulated medullary hematopoiesis. For instance, whereas in the control mice the development of only moderate hyperplasia of the bone marrow was observed after immobilization for 3 h, in animals receiving two injections ME caused marked stimulation of hematopoiesis (Fig. 1). In this case TNMK on the 6th day after the beginning of immobilization reached 164.7 ± 11.2 and 125.9 ± 4.1% of the initial value respectively in animals of the experimental and control groups. Counting the absolute numbers of cells of the different branches of medullary hematopoiesis led to the conclusion that ME has a stimulating effect on erythropoiesis and granulopoiesis (Table 1).

Moderate stimulation of hematopoiesis in mice immobilized for 3 h also was observed after a single injection of ME. Under these conditions TNMK rose to 135.3 ± 5.1% of its initial value on the 7th day after the beginning of immobilization (up to 120.3 ± 4.8% in the control).

The results are thus evidence that, in principle, hematopoiesis can be modulated during stress by enkephalins. The suppressive and stimulating action of LE and ME respectively on hematopoiesis was demonstrated. When the possible mechanisms of the regulating action of peptides on myelopoiesis are discussed, the results of previous experiments must be taken into account, in which the role of hormones of the pituitary-adrenal system in the mechanisms of stimulation of hematopoiesis during stress was demonstrated [2]. It was shown, in particular, that intensification of hematopoiesis under conditions of immobilization stress is due to the stimulating effect of glucocorticoids on proliferation and differentiation of committed cells: precursors of erythropoiesis and granulo-monocytopoiesis [1]. Meanwhile, there is evidence of activation and depression of glucocorticoid production in stress by ME [7, 8] and LE [3,

4], respectively. Assumig an indirect action (through the pituitary-adrenal system) of these opioid peptides on hematopoiesis, we selected immobilization for different periods of time as an adequate model. We expected to find that LE would have a suppressive effect on hematopoiesis (normalization of glucocorticoid production), and that ME would have a stimulating action (due to additional activation of glucocorticoid production).

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COMPARISON OF THE EFFECTS OF ANXIOLYTICS AND MORPHINE ON NOCICEPTIVE RESPONSES IN RATS

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There is much evidence, although some is contradictory, that anxiolytics of the benzodiazepine series possess analgesic properties [1]. It has been shown that benzodiazepines can lengthen the latent period of motor responses during exposure to pain [3]. Most workers attribute this elevation of the thresholds of nociceptive motor responses to the ataxic and sedative effect possessed by these preparations [1, 3].

Buspirone, an anxiolytic of the azaspirodecanedione series, which has recently been synthesized, is very similar to the benzodiazepines in its pharmacological properties in behavioral models of anxiety and aggression [4], and also in the clinical treatment of anxiety states [2]. Meanwhile, unlike the benzodiazepines, buspirone has no inhibitory action on muscle tone or movement coordination and does not possess sedative properties [4, 6]. For these reasons it is possible to study whether buspirone gives rise to any antinociceptive effects, and the investigation described below was carried out for this purpose. We also studied the effect of buspirone and diazepam on the depression of nociceptive responses produced by morphine.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 180-220 g, kept eight to 10 to a cage, and receiving water and food ad lib. Nociceptive sensitivity was assessed by the latent period (LP) of the hind limb licking response (LLR) and the tail withdrawal reflex (TWR). To measure LP of LLR the animal was placed in a Plexiglas cage measuring 30 × 30 × 30 cm, the floor of which consisted of a metal plate, kept at a constant temperature of 55°C, and the time interval before the animal licked one of its hind limbs was

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