OPIOID PEPTIDE LEVELS IN THE BRAIN AND BLOOD OF RATS WITH IMMOBILIZATION STRESS

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The brain of vertebrates, including man, contains an extensive medial antinociceptive system, extending from the dorsomedial and ventromedial hypothalamic nuclei through the nuclei of the periaqueductal gray matter to the nuclei raphe in the medulla, axons of which are widely represented in the brain stem and spinal cord. Local analgesic systems also are found in the brain stem and spinal cord [1, 2]. Activation of these systems during exposure to stress leads to strong inhibition of nociceptive transmission at all levels, which lies at the basis of the phenomenon of stress-induced analgesia. Participation of these systems in modulation of the emotional, behavioral, and hemodynamic components of stress also is postulated [3]. The neurotransmitters and neuromodulators of the antinociceptive systems are monoamines, enkephalins, and endorphins, changes in whose blood and brain tissue levels are linked with the functional state of these systems [2]. Meanwhile data on the character of changes in the blood and nerve tissue levels of enkephalins and endorphins during exposure to stress are not sufficiently definite [3, 9], and this makes analysis of the role of the brain opiate systems in the mechanisms of realization of stress and of adaptation to it more difficult.

For this reason, in the investigation described below, the concentrations of enkephalins and of β-endorphin (β-E) in the blood and brain tissues of rats were studied after single and repeated exposures to immobilization stress.

EXPERIMENTAL METHOD

Immobilization stress was induced in male Wistar rats weighing 250-300 g by rigid fixation of the animals to stands by Selye's method [8]. Single immobilization (IM) was carried out for 30 and 150 min. Repeated IM was carried out for 150 min daily for 7 and 40 days. The rats were decapitated and concentrations of metenkephalin (ME), leu-enkephalin (LE), and β-E were investigated in the hypothalamus, midbrain, medulla, and pituitary gland, and the enkephalin concentration was determined in the corpus striatum. These parts of the brain were isolated by the method in [11]. The β-E concentration in the animals' blood plasma was determined. Concentrations of opioid peptides in tissues and blood plasma were determined by radioimmunoassay using commercial kits from "Immuno Nuclear Corporation," (USA). Preparation of the tissues for analysis included extraction in 1M acetic acid (2 ml per part of the brain) for 15 min on a boiling water bath followed by homogenization of the cold sample in acid and centrifugation of the homogenate at 6000 g for 15 min at 6°C. The residue was rehomogenized in 2 ml of 1 M acetic acid and centrifuged under the same conditions; the two supernatants were pooled and neutralized with NaOH. Aliquots of solution, each 0.5 ml, were taken for determination of enkephalins and kept at −20°C; the remainder was lyophilized.

To separate β-E from β-lipotrophic hormone the lyophilized extracts were dissolved in 0.7 ml of 0.1 M back buffer, containing 0.1% bovine serum albumin (pH 4), and after centrifugation at 6000g for 5 min, the samples were subjected to gel filtration on a column measuring 0.9 × 25 cm packed with Sephadex G-50, in the same buffer at 4°C. Fractions containing β-E activity were pooled and used for analysis. Blood plasma for β-E assay were obtained by the standard method with heparin. To prevent enzymic proteolysis of β-E, 850 μg/ml of bacitracin was added to the plastic test tubes. An aliquot of plasma (0.5 ml) was subjected to gel fil-

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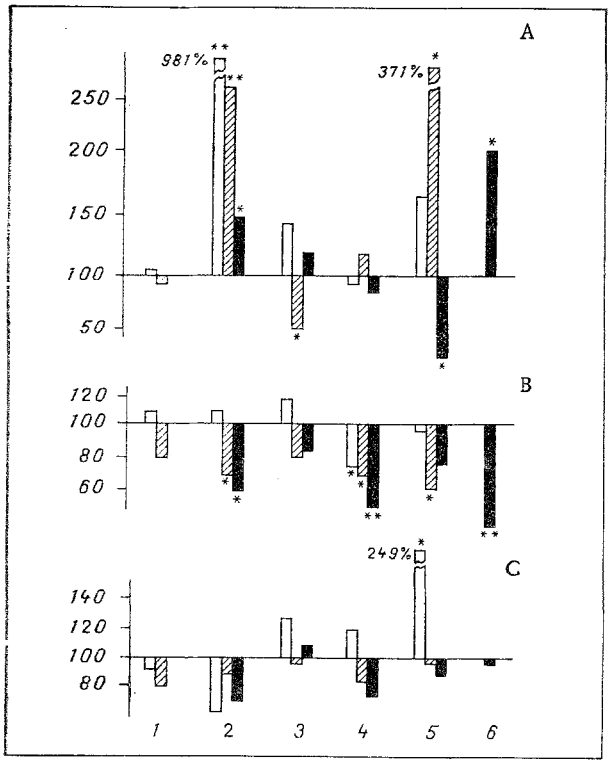

Fig. 1. Concentrations of opioid peptides (in % of control) in part of brain and in blood of rats with immobilization stress. A) Single IM for 150 min; B) adapted control; C) repeated IM for 150 min. 1) Corpus striatum; 2) hypothalamus; 3) midbrain; 4) medulla; 5) pituitary; 6) blood plasma. Unshaded columns — ME, black column — β-E, obliquely shaded — LE. *P < 0.05, **P < 0.01 compared with intact animals. Results of six experiments given.

tration under conditions similar to those described above for β-E from brain tissue. The statistical significance of the data was estimated by Student's t test.

EXPERIMENTAL RESULTS

Values of the parameters studied in the control were as follows: The concentration of ME in the corpus striatum, hypothalamus, midbrain, medulla, and pituitary was 1.02 ± 0.15, 0.750 ± 0.048, 0.233 ± 0.021, 0.330 ± 0.023, and 0.696 ± 0.057 pmole/mg; the LE concentration was 0.453 ± 0.045, 0.342 ± 0.036, 0.088 ± 0.009, 0.101 ± 0.011, and 0.365 ± 0.049 pmole/mg, respectively; the β-E concentration in the hypothalamus, midbrain, medulla and pituitary was 0.163 ± 0.022, 0.014 ± 0.001, 0.018 ± 0.003, and 8.95 ± 1.06 pmoles/mg, and in the blood plasma 121.8 ± 18.1 fmoles/ml. IM of the rats for 30 min led to a marked decrease in the ME and LE concentrations in the hypothalamus (47 and 75% of the control, respectively; P < 0.05). Longer (150 min) IM caused a marked increase in concentration of the opioid peptides in the hypothalamus and of enkephalins in the pituitary (Fig. 1A). Meanwhile the β-E concentration in the pituitary fell, but its blood level rose, suggesting an increase in its release into the systemic circulation. The concentrations of opioid peptides thus depended on the duration of IM, i.e., on the intensity of stress. The results are evidence of definite changes in the pattern of synthesis, release, or utilization of these peptides with an increase in the duration of stress. A fall in the enkephalin level in the hypothalamus after 30 min of IM agreed with data of other workers who used different types of short-term stress [6, 10, 12], and was probably caused by their more intensive release from storage sites or outflow into the neurohypophysis and lateral septum, which participates in the formation of pain sensations [13]. The increase in the concentrations of the opioid after IM for 150 min was evidently the result of an adaptive increase in their synthesis at this time. Retrograde transport of β-E from the pituitary into the hypothalamus also is possible [7] and could be an important factor in the development of stress-induced analgesia.

Repeated IM of the rat for 7 days caused the LE level to rise in the striatum and midbrain (176 and 150% of the control, respectively; P < 0.001). Meanwhile changes in the opioid

concentrations in the hypothalamus and pituitary absorbed after single IM disappeared, indicating the development of adaptive processes in these parts of the brain. This result is in harmony with the disappearance of analgesia during repeated exposures to the same type of stress [3, 4].

Animals exposed to IM for 39 days were divided into two groups on the 40th day: 1) without IM on the day of sacrifice (adapted control), 2) IM for 150 min. Concentrations of LE and β-E in the hypothalamus, medulla, and midbrain, and of LE in the pituitary and β-E in the blood plasma of animals of the adapted control group were significantly lower than in intact rats; the ME level was depressed only in the medulla (Fig. 1B). This different character of response of LE and ME to stress may perhaps be associated with the different functional roles played by these peitides in the body. LE, with higher affinity for δ-receptors, is more closely linked with the regulation of the emotional state and behavior [1, 5]. After regular IM the opioid concentrations in the parts of the brain and in the blood of these rats rose to the control levels (Fig. 1C).

The stage of adaptation to repeated IM is thus characterized as a whole by a decrease in LE and β-E concentrations in most structures tested, but this does not prove exhaustion of the opiate systems of the brain, for regular IM led to a rise in the opioid levels. Nevertheless, we know that chronic intensive stress may lead to disturbance of the activity of many systems of the body and, in particular, to an increase in sensitivity to pain [3]. The decrease in the concentrations of opioid peptides discovered in these experiments may be one cause of these disturbances, but the mechanism of the lowering of the opioid levels calls for further investigation.

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