It will also be noted that after chromatographic fractionation of the peptide extracts of the brain, ACTH-IP were found in many regions of the chromatogram, in agreement with data in the literature [5]. To elucidate the causes of this phenomenon, the isolated fractions must be subjected to further experimental fractionation and analysis.

Thus the ACTH-IP spectrum in the brain and the qualitative character of changes in their concentration after exposure to emotional stress are both genetically dependent, and this is an important basis for future research to study the functional role of ACTH-IP in the formation of hereditary features of emotional behavior.

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EFFECT OF MYELOPEPTIDES ON PHYSIOLOGICAL AND PATHOLOGICAL PAIN

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Intact human and animal bone marrow cells produce low-molecular-weight peptides with various bioregulatory properties (myelopeptides) [8]. Peptides with mol. wt. of 1.3-2 kilodaltons cause a two-threefold increase in antibody formation at the peak of the immune response [4, 5]. Myelopepties have not only immunostimulating activity, but also analgesic properties. It has been shown that injection of myelopeptides into animals causes changes in bioelectrical responses to nociceptive stimulation characteristic of analgesics of the morphine and endogenous opiate type [1, 6]. It has also been found that myelopeptides displace labeled opiates competitively from specific binding sites on lymphocytes and brain nerve cells [7].

The aim of this investigation was a further study of the analgesic action of myelopeptides on models of physiological and pathological pain.

EXPERIMENTAL METHOD

Myelopeptides were isolated from the supernatant of cultures of hog bone marrow cells by gel-chromatography on Sephadex G-25 (fine), equilibrated with physiological saline, pH 7.2. The dose of the myelopeptides was estimated on the basis of their protein content, which was determined by Lowry's method.

Noninbred and Wistar rats (males) weighing 200-220 g were used.

An experimental model of pathological pain was produced by creating a generator of pathologically enhanced excitation (GPEE) in the dorsal horn of the lumbosacral segments of the spinal cord by means of penicillin. Penicillin was applied to the dorsal surface of the lumbosacral segments on the left side in an agar wafer (10 × 4 × 1.5 mm). To 1 ml of 1% agar, 15,000 U of penicillin in a volume of 1 ml was added. The method of production of a pain syndrome of spinal origin, and also the method of assessing the separate components of development of the pain syndrome were described previously [2, 3].

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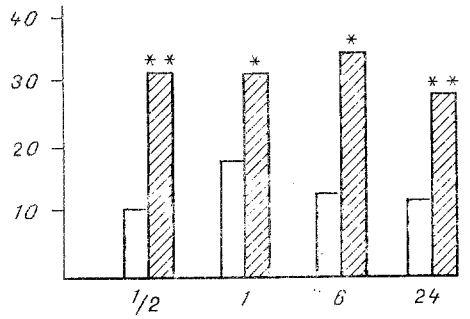

Fig. 1

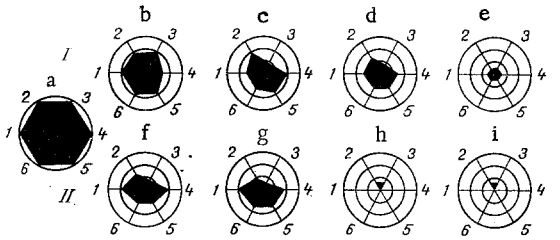

Fig. 2

Fig. 1. Effect of myelopeptides of LP of response of rats to nociceptive thermal stimulation. Abscissa, time after injection of myelopeptides (in h); ordinate, LP of response to nociceptive stimulation (in sec). Unshaded columns, control; shaded, experiment. Each group includes 8 determinations. *P = 0.01, **P = 0.05.

Fig. 2. Reduction of pain syndrome of spinal origin under the influence of myelopeptides. a) Initial background (maximum of manifestation of pain syndrome); I) strongest effect of myelopeptides, recorded 40 min after intraperitoneal injection; b) 20 mg/kg; c) 30 mg/kg; d) 40 mg/kg; e) 50 mg/kg; II) effect of naloxone (0.5 mg/kg, intraperitoneally, against background of maximal action of myelopeptides in a dose of 50 mg/kg (f) ; g) effect of aminopyrine in a dose of 25 mg/kg (intramuscularly); h) effect of morphine, 2 mg/kg (intraperitoneally); i) effect of trimeperidine, 2 mg/kg (intraperitoneally). Main features of pain syndrome: 1) frequency of spontaneous episodes; 2) duration of 1 episode; 3) intervals between episodes; 4) response to provocation; 5) vocalization; 6) total motor response (represented graphically in vector form). Magnitude of individual characteristics of pain syndrome assessed on a 3-point scale; each circle represents 1 point, counting from the center: 0) feature absent; 1) mild; 2) average; 3) strong manifestation.

To determine the effect of the myelopeptides on physiological pain, a hot plate test was used. The latent period (LP) of the nociceptive response of a rat placed on a hot (55 ± 5°C) plate was measured. OP of nociceptive response after injection of the myelopeptide was compared with LP of nociceptive responses in the control. The numerical results were subjected to statistical analysis by nonparametric tests.

Myelopeptides were injected intraperitoneally in doses of 20 to 50 mg/kg body weight in a volume of 0.3-0.5 ml. The same volume of culture medium 199 or physiological saline was injected into animals of the control group. The intensity of the analgesic effect of the myelopeptides was compared with the effect of aminopyrine (analgin) (25 mg/kg), morphine (2-5 mg/kg), and trimeperidine (Promedol) (2-5 mg/kg). In separate experiments, naloxone was d injected intraperitoneally in a dose of 0.5 mg/kg.

EXPERIMENTAL RESULTS

The action of myelopeptides on LP of the pain response to nociceptive thermal stimulation (physiological pain) was studied in the experiments of series I. An increase of 40% was shown by LP 30 min after injection of the myelopeptides in a dose of 20 mg/kg. A more prolonged analgesic effect was observed when the preparation was given in a dose of 40 mg/kg. When nociceptive responses were tested after 30 and 60 min and 6 and 24 h, significant differences were found between the control and experiment (Fig. 1). Lengthening of LP in animals of the experimental group after the above-mentioned time intervals amounted to 42, 33, 46, and 36% respectively.

In the experiments of series II the analgesic action of the myelopeptides was studied against a pain syndrome of spinal origin (pathological pain). The myelopeptides were injected at the height of development of the pain syndrome, which was characterized by paroxysms of great intensity, accompanied by motor excitation, flexion of the hind limb, vocalization, and biting and chewing the pin projection area -- the trigger zone (that part of the lumbosacral region in which the GPEE had been created; Fig. 2a). The pain syndrome lasted about

3.5 h. Myelopeptides had an analgesic action starting from a dose of 20 mg/kg (Fig. 2b); all signs of the pain syndrome were equally reduced to 2 points (the strongest manifestation of the pain syndrome was rated at 3 points). An increase in the dose of the preparation to 30-40 mg/kg potentiated its analgesic effect, but not significantly (Fig. 2c, d). Injection of the myelopeptide in a dose of 50 mg/kg almost completely abolished the pain syndrome: After 40-60 min it was reduced to 0.5 point (Fig. 2e). The action of the compound lasted throughout the period of observation (3-3.5 h). The total duration of the effect of the myelopeptides in this series of experiments could not be determined because the pain syndrome evoked by creation of a GPEE with the aid of penicillin ceased after 3-3.5 h.

Even in a large dose (50 mg/kg) the myelopeptides had no muscle-relaxing or narcotic effect.

A special series of investigations was carried out to compare the analgesic action of myelopeptides with the effect of commonly used standard analgesics: aminopyrine, morphine, and trimeperidine. In the doses chosen these drugs had a maximal analgesic action without any side effects. As will be clear from Fig. 2g-i, aminopyrine in a dose of 25 mg/kg had an effect equal to that of the myelopeptides in a dose of 30 mg/kg. Morphine and trimeperidine, in a dose of 2 mg/kg, were as effective as myelopeptides in a dose of 50 mg/kg. Incidentally the dose (20-50 mg/kg) indicated for myelopeptides is conventional, for, besides active peptides, the preparation also includes amino acids of the culture medium, which are detected by Lowry's reaction.

The effect of medium 199, in which the bone marrow cells were cultured, was tested in a control series of experiments. The culture medium (8 experiments) was ineffective.

Injection of naloxone, an antagonist of opiate receptors, abolished the action of the myelopeptides. Restoration of the pain syndrome from 0.5 to 2 points was observed 30-40 min after injection of naloxone (0.5 mg/kg, intraperitoneally) against the background of the maximal analgesic effect of the myelopeptides (Fig. 2f). When the action of naloxone, superposed on that of the myelopeptides, reached its maximum (2 h after injection of the myelopeptides) the pain syndrome was reduced to 2 points, i.e., naloxone completely restored the pain syndrome. These results indicate that the myelopeptides contain substances which interact with opiate receptors and they confirm previous observations, showing that myelopeptides have opiate-like properties [6, 7].

Myelopeptides, which have an analgesic action, thus actively depress pathological pain and a severe pain syndrome induced by a GPEE in the dorsal horns of the spinal cord; however, a larger dose of the preparation is necessary for this latter effect. Since myelopeptides have no potential narcotic action, unlike morphine and trimeperidine, they may be of practical interest for clinical use.

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