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EFFECT OF MET- AND LEU-ENKEPHALINS AND THEIR SYNTHETIC ANALOG ON ANALGESIA INDUCED BY STIMULATION AND ACUPUNCTURE

Yu. D. Ignatov, Yu. N. Vasil'ev, V. S. Kovalenko, and M. I. Titov

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KEY WORDS: enkephalins; analgesia; acupuncture; rat brain.

Naloxone, an antagonist of opiate receptors, is known to reduce or even completely suppress analgesia arising in response to stimulation of deep brain structures and activation of acupuncture points [2, 6, 11]. These and other indirect data [12, 14] suggested that endogenous polypeptides (enkephalins and endorphins) play an essential role in the development of acupuncture- and stimulation-induced analgesia. However, the concrete mechanism whereby this effect of the enkephalins in the formation of the pain-relieving action in these types of analgesia is realized has not yet been finally elucidated. Moreover, so far the direct effect of enkephalins on the intensity of stimulation- and acupuncture-induced analgesia has virtually not been studied.

Accordingly the aim of this investigation was to compare the action of enkephalins and of their synthetic analog on analgesia induced by stimulation and acupuncture.

EXPERIMENTAL METHOD

Male albino rats weighing 250-300 g were used. In a preliminary operation monopolar electrodes were implanted into the animals in the region of the central gray matter (CGM) and cannulas for microinjections were inserted into the lateral ventricles. Nociceptive stimulation of the base of the tail was carried out by means of removable bipolar electrodes (100 Hz, 1 msec, 1 sec, 30-150 V) and the animals were able to move freely. The response to nociceptive stimulation was assessed by a scale developed by the writers [2]. The CGM was stimulated for 30 sec by square pulses (100 Hz, 1 msec, 30-350 μA). Electroacupuncture was carried out by the method described in [2]. Polypeptides were injected, in a volume of 5 μl, over a wide range of doses (from 5 to 200 μg). The position of the electrodes was verified in serial brain sections. ED_{50} for the various substances was determined by Prozorovskii's method [5]. Nonparametric methods were used for the statistical analysis of the data.

EXPERIMENTAL RESULTS

The analgesic effect of Met-enkephalin appeared after microinjection of the peptide in a dose of 50 μg. The thresholds of onset of emotional-behavioral components of the pain response such as vocalization and running was raised in this case by 18 and 20% above the initial level, respectively. Initial changes in the structure of the response to nociceptive stimulation were observed 5-10 min after microinjection of the peptide, they reached a maximum after 15 min, and continued for a further 10-15 min. Under the influence of Met-enkephalin in a dose of 100 μg the threshold of development of the vocalization response was increased by 35-40% and of running by 38% (Fig. 1A). The value of ED_{50} for Met-enkephalin was 49.7 μg.

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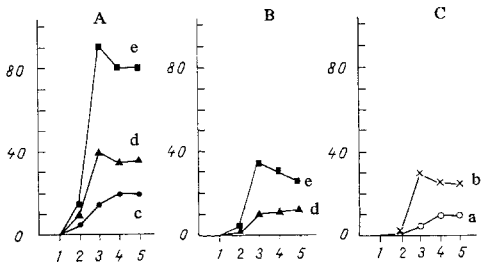

Fig. 1

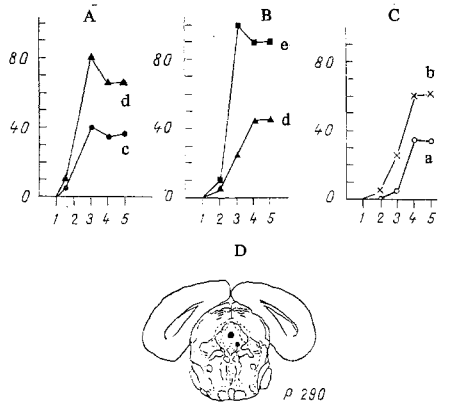

Fig. 2

Fig. 1. Effect of Met- and Leu-enkephalins (A and B, respectively) and of their synthetic analog (C) on nociceptive response in rats. Abscissa, components of nociceptive response: 1) shivering, contraction of the tail; 2) turning the head and trunk, tapping with the paws; 3) squeaking, single rotation; 4) crying; 5) crying accompanied by running; ordinate, changes in thresholds of onset of individual components of nociceptive response (in % of control). Graphs show changes in structure of nociceptive response under the influence of peptides in the following doses: a) 5 μg, b) 10 μg, c) 50 μg, d) 100 μg, e) 200 μg.

Fig. 2. Effect of Met- and Leu-enkephalins (A and B, respectively) and of their synthetic analog (C) on structure of nociceptive response to stimulation of CGM of threshold intensity. D) Diagram of frontal section through mesencephalon showing position of stimulating electrode. Remainder of legend as to Fig. 1.

Leu-enkephalin had weaker analgesic activity than Met-enkephalin (ED_{50} = 168.0 μg). A distinct analgesic effect of the peptide appeared only after microinjection in a dose of 200 μg, and this effect was comparable in strength with that of Met-enkephalin in a dose of 100 μg (Fig. 1B).

The synthetic analog Tyr-dAla-Gly-Phe-NH_2 had a stronger (ED_{50} = 8.7 μg) and longer-lasting (up to 50-60 min) analgesic action than Met-enkephalin. In a dose of 25 μg the synthetic preparation raised the thresholds of appearance of vocalization and running by 40-50%. An increase in the dose of the preparation to 50-100 μg was accompanied by considerable intensification of analgesia, as reflected in complete suppression of crying in the structure of the nociceptive response (Fig. 1C).

The effect of the peptides on the degree of stimulation-induced analgesia was studied during subthreshold and threshold intensities of stimulation of CGM. Met- and Leu-enkephalins in subanalgesic doses (25 and 50 μg, respectively) led to the appearance of an analgesic effect during stimulation of CGM at subthreshold intensity, which itself caused no changes in the structure of the nociceptive response. This was shown by elevation of the thresholds of vocalization and running by 16-20%. Against the background of the action of enkephalins in the above doses, threshold stimulation of CGM was accompanied by greater changes in the thresholds of appearance of the main components of the nociceptive response compared with the changes observed during stimulation of CGM alone (Fig. 2). Stimulation of CGM against the background of the action of enkephalins in initial analgesic doses (50 and 100 μg, respectively) was accompanied by greater elevation of the thresholds of the generalized response than the changes observed during the action of enkephalins in the control (Fig. 3A, B).

Tyr-dAla-Gly-Phe-NH_2, like the enkephalins, in a subanalgesic dose (5 μg) led to the appearance of stimulation-induced analgesia during subthreshold stimulation of CGM and increased the intensity of analgesia stimulation of threshold strength. The thresholds of the crying and running responses were increased by 80-100% compared with changes observed in response to stimulation of CGM threshold intensity only (Fig. 3C).

Met- and Leu-enkephalins and the synthetic preparation Tyr-dAla-Gly-Phe-NH_2 potentiated acupuncture analgesia. Whereas in the control the thresholds of appearance of the emotional-behavioral components of the nociceptive response during activation of acupuncture points were raised by 32-46%, against the background of the action of enkephalins and the synthetic preparation in subanalgesic doses, electroacupuncture raised the thresholds by 52-65%.

The results of these experiments thus show that enkephalins and their synthetic analog differ in their analgesic activity, in agreement with data in the literature [9, 10], and this difference may be due to differences in the affinity of these polypeptides for opiate receptors. The stronger and more prolonged action of the synthetic analog of the enkephalins may be

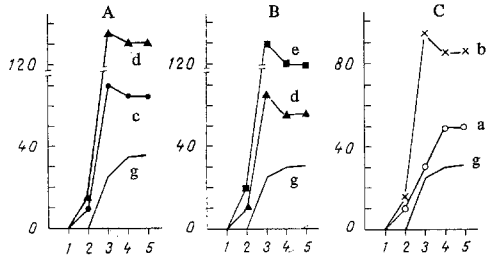

Fig. 3. Effect of Met- and Leu-enkephalins (A and B, respectively) and of their synthetic analog (C) on structure of nociceptive response to stimulation of CGM of threshold intensity. g) Structure of nociceptive response to stimulation of CGM of threshold intensity. Remainder of legend as to Fig. 1.

associated with the slower enzymic degradation of the peptide because of replacement of the glycine in the second position of its structure by the d-stereoisomer of alanine [7].

All the peptides studied facilitated the appearance of stimulation-induced analgesia during subthreshold stimulation of CGM, potentiated analgesia during stimulation of threshold strength, and increased the intensity of acupuncture analgesia. These findings, together with the results of the writers' previous investigations [3], indicating that analgesia develops in the same manner during activation of acupuncture points, stimulation of CGM, and injection of enkephalins, can be regarded as proof of the participation of enkephalins in the development of stimulation- and acupuncture-induced analgesia. Previous investigations [15] showed that microinjections of opiates into the cerebral ventricles are followed by accumulation of these substances chiefly in the periaqueductal structures, which are distinguished by a high concentration of opiate receptors and enkephalins [13]. Microinjections of opiates and electrical activation of these brain zones are known to be accompanied most frequently by the development of analgesia [1, 8]. Accordingly it may be submitted that stimulation- and acupuncture-induced analgesia arises as a result of activation of enkephalinergic systems of the periaqueductal structures which, in turn, trigger the inhibitory mechanisms of the raphe nuclei and reticular formation [8, 12]. As a result inhibitory influences on activity of the segmental system controlling the efferent input are potentiated, with the result that the intensity of the ascending nociceptive flow is limited and the severity of the emotional-behavioral manifestations of pain reduced.

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