ENKEPHALINS AND GASTRIN FRAGMENTS: ARE THERE DIFFERENT MECHANISMS OF ANALGESIA?

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Brain oligopeptides (enkephalins) possess morphine-like activity and interact with central and peripheral opiate receptors. Similar properties also are possessed by certain other peptides of both neuronal and peripheral origin. An intensive study of the opioid properties of synthetic derivatives and analogs of enkephalins, other peptides, and also fragments of protein hormones is in progress at the present time with the aim of discovering new pain-relieving and psychoactive compounds.

In the investigation described below the analgesic action of enkephalins and of C-terminal fragments of gastrin (tri- and pentapeptides) was compared and the mechanisms transmitting the analgesic effect of these oligopeptides were analyzed.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g and guinea pigs weighing 300-400 g were used. The analgesic activity of the peptides was determined by the "tail flick method" [3]. The compounds for testing were injected intracranially in a volume of 10 µl (rate of injection 10 µl/min) through a stainless steel cannula 0.45 mm in diameter, introduced into the lateral ventricle (stereotaxic coordinates: A −1, L 1.5, V −4). The tripeptide of the C-terminal fragment of gastrin (MAF), Met- and Leu-enkephalins were injected in a dose of 200 µg, and pentagastrin in a dose of 30 µg. Control animals received the same volume of distilled water. Pentagastrin and MAF also were injected intraperitoneally (the concentrations are indicated below). The latent period of the nociceptive response was determined before the experiment, every 2 min after injection of the enkephalins, and at 5-min intervals after injection of the gastrin fragments. The intensity of analgesia was determined as a percentage of the initial data. The content of biogenic monoamines and their metabolites — noradrenalin (NA), dopamine (DA), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) — was determined in the forebrain of the rats spectrofluorometrically [3, 7, 8] after intraventricular injection of the test compounds. The animals were decapitated 20 min after injection of MAF and pentagastrin and 5 min after injection of the enkephalins. To analyze interaction between the drugs and opiate receptors the method of inhibition of electrically stimulated contraction of a preparation of the longitudinal muscle together with the mesenteric nerve plexus (LMNP) isolated from the guinea pig colon by the method in [6]. A change

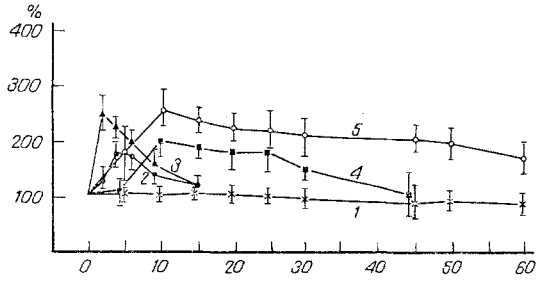

Fig. 1. Analgesic activity of pentagastrin, MAF, Leu- and Met-enkephalins given by intraventricular injection. 1) Distilled water; 2) Met-enkephalin; 3) Leu-enkephalin; 4) MAF; 5) pentagastrin. Abscissa, time after injection of preparation (in min); ordinate, ratio of latent period of nociceptive response to control (in %).

KEY WORDS: enkephalins; pentagastrin; opiate receptors; analgesia; biogenic amines.

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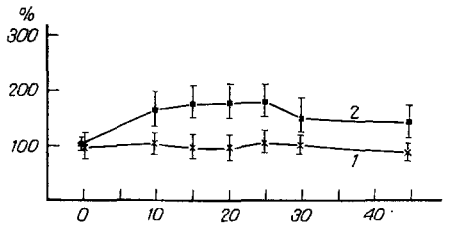

Fig. 2

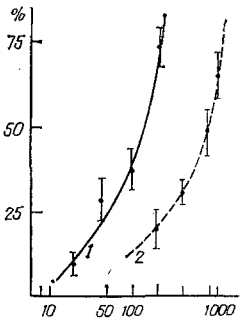

Fig. 3

Fig. 2. Analgesic activity of MAF given by intraperitoneal injection. 1) Distilled water; 2) MAF. Abscissa, time after injection of MAF (in min); ordinate, ratio of latent period of nociceptive response to control (in %).

Fig. 3. Effect of enkephalins on amplitude of contraction of LMNP. 1) Met-enkephalin; 2) Leu-enkephalin. Abscissa, concentration of enkephalins (in mM); ordinate, degree of inhibition of contractions (in %).

in the amplitude of contraction of the LMNP under the influence of different concentrations of the preparations was expressed as a percentage of the initial level of contraction. The compounds used — pentagastrin (BOC-ala-try-met-asp-phe-NH_2), MAF (met-asp-NH_2), Met-enkephalin (try-gly-gly-phe-met-NH_2), and Leu-enkephalin (try-gly-gly-phe-leu-NH_2) — were synthesized in the Research Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR. The naloxone used in the experiments was from Endo Laboratory (USA).

EXPERIMENTAL RESULTS

Intraventricular injection of both enkephalins and gastrin fragments led to the development of analgesia (Fig. 1). Enkephalins in a dose of 200 μg increased the lag period of the nociceptive response to 200%, but this effect was short in duration and lasted from 3 to 9 min. Analgesia induced by pentagastrin (30 μg) reached a maximum (250%) only after 10 min, but remained at a high level for 45 min. The antinociceptive effect of MAF was rather weaker than that of pentagastrin but similar to it in its course. It is an interesting fact that when the drugs were given intraperitoneally, only MAF preserved its analgesic property and all the other compounds were inactive in this respect (Fig. 2). Analysis of the behavioral effects developing in the animals under the influence of enkephalins and gastrin fragments showed that in all cases catalepsy developed. However, the catalepsy was characterized by some particular features: After injection of enkephalins the catalepsy was accompanied by the appearance of "head shaking" in the dog, which was not found after MAF and pentagastrin. These observations agree with the behavioral stereotype induced by oligopeptides, described previously [1, 2].

Consequently, definite similarity was found between the enkephalins and gastrins with respect to behavioral and nociceptive responses on intraventricular injection, whereas significant differences were found in the kinetics of development of the analgesic effect and behavioral responses.

Opiate alkaloids and peptides are known to act by modulating the secretion of neuromediators in specialized regions of the CNS. This process is preceded by interaction with specific opiate receptors; the efficiency of their biological action correlates with the degree of affinity for receptor molecules.

To elucidate the mechanisms determining similarity and differences in the action of the compounds studied, both with one another and compared with the classical opiates, the character of their effects on the concentration and metabolism of biogenic amines in the CNS (Table 1) and interaction with peripheral opiate receptors were investigated. The results indicate that enkephalins do not change the content of NA and DA in the whole brain and they significantly raise the level of the 5-HT metabolite 5-HIAA. The effect of MAF observed in these experiments, incidentally, resembled the action of morphine described in the literature. This tripeptide depressed DA metabolism (reduced the HVA concentration) and stimulated 5-HT metabolism (raised the 5-HIAA level). Pentagastrin, like the other peptides studied, had certain

TABLE 1. Effect of Enkephalins, Pentagastrin, and MAF on Content of Biogenic Amines (in %) in Rat Brain (M ± m)

Experimental conditions	Dose, μg	NA	DA	HVA	5-HT	5-HIAA
Control	—	100±18	100±10	100±16	100±10	100±12
Met-enkephalin	200	100±19	94±13	88±7	102±13	143±10*
Leu-enkephalin	200	84±12	95±16	109±12	95±15	128±5*
Pentagastrin	30	75±23	70±16*	184±11*	189±13*	103±14
MAF	200	105±17	59±16*	69±12*	157±17*	135±15*

*$P \leq 0.05$ relative to control.

unique features in its action on biogenic amines. As a result of its injection the 5-HT content was significantly increased. By contrast with MAF it caused the HVA level to fall somewhat but did not raise the 5-HIAA concentration in brain tissue.

Analysis of the results of the study of the effect of the peptides used in this investigation on biogenic amines revealed a number of similar features, indicating the existence of common stages in the mechanism of their action. All affected 5-HT metabolism to some degree or other but did not affect NA.

Further information on the mechanisms of action of enkephalins and C-terminal fragments of gastrin was obtained by testing their effect on contraction of the LMNP. The synthetic Met- and Leu-enkephalins tested were found to have a morphine-like action on contraction of LMNP. Dependence of the effect on dose for these pentapeptides is demonstrated in Fig. 3. The amplitude of contraction of LMNP decreased when Met-enkephalin was present in the incubation medium in a concentration of 40 nM, and the dose causing a reduction of 50% in the amplitude of contractions (ID_{50}), determined from the graph, was 158 nM. ID_{50} for Leu-enkephalin was 790 nM, somewhat higher than values given in other reports. ID_{50} determined in the present experiments for morphine was 110 nM.

To test the specificity of action of the enkephalins on opiate receptors naloxone was used. This opiate antagonist completely prevented the development of the inhibitory effect of the enkephalins on contraction of LMNP in concentrations as low as 30 nM. Pentagastrin and MAF had no effect on contraction of LMNP even when their concentration in the medium was raised to 1.0 and 20 μM, respectively. It can be concluded from these data that the analgesic action of pentagastrin and of MAF is not mediated through the classical opiate receptors. Meanwhile the analgesic properties of these substances can be manifested as the resultant effect of their action on effector regions of the brain responsible for analgesia.

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