the formation of cerebral edema. The GABA-ergic system plays an important role in this process. Its functional activity in edema is probably depressed, and this is also confirmed by data in the literature [1]. For the combined treatment of cerebral edema it is therefore important to use preparations with a GABA-positive action.

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EFFECT OF β-NEOENDORPHIN, A κ-OPIATE RECEPTOR AGONIST, ON CEREBRAL CORTICAL UNIT ACTIVITY

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KEY WORDS: β-neoendorphin; various types of opiate receptors; microiontophoresis of opioid peptides.

β-neoendorphin (Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro) is a peptide with opioid activity which was isolated from pig hypothalamus in 1981 [4]. In experiments *in vitro*, using various isolated organs (guinea pig and rabbit ileum, vasa deferentia of mice, rats, and rabbits), containing peripheral opiate receptors of various types, it was shown that β-neoendorphin is an agoinst of κ-opiate receptors [5]. However, the action of the peptide on electrical activity of CNS neurons, on whose membranes opiate receptors also are located, had not been studied.

The aim of this investigation was to study the effect of microiontophoretic applications of the preparation on unit activity in the cerebral cortex. The effects of β-neoendorphin were compared with the action of morphine (an agonist of μ-opiate receptors), Leu-enkephalin (an agonist of δ-opiate receptors), and β-endorphin (an agonist of μ-, δ-, ε-opiate receptors) in experiments on the same cortical neurons.

EXPERIMENTAL METHOD

Experiments were carried out on six curarized cats of both sexes weighing 2.6-4.1 kg, with artificial ventilation of the lungs. The animals' body temperature was maintained at 37-38°C by means of an electric heater. The preliminary surgical manipulations (tracheotomy, scalping, etc.) were performed under general anesthesia (pentobarbital sodium, 50 kg/mg, intraperitoneally). Single unit activity was recorded extracellularly (14-16 h after injection of pentobarbital sodium) and the physiologically active substances were applied microiontophoretically by means of multichannel glass microelectrodes [2]. An Élektronika DZ-28 microcomputer, coupled with the apparatus for recording unit activity, analyzed information on spontaneous and evoked unit activity in the course of the experiment and plotted it graphi-

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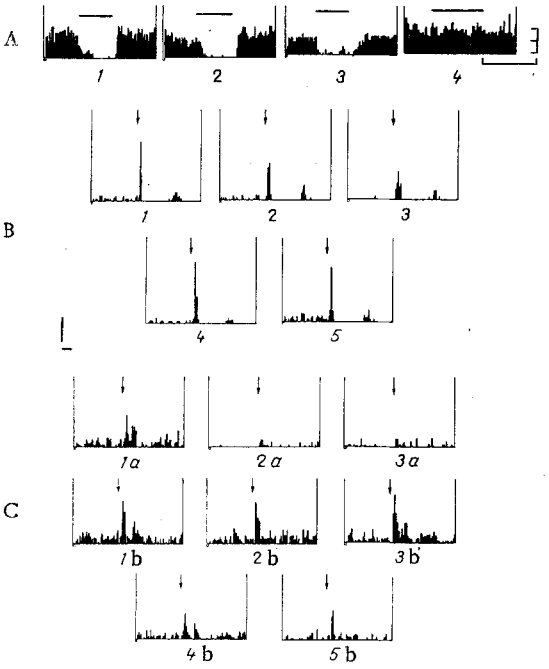

Fig. 1. Effect of β-neoendorphin and other opioid peptides on spontaneous and evoked unit activity in somatosensory area I of the cortex. A, B, C) Different neurons. A) Effect of preparations on spontaneous activity of PT-neuron; 1) β-neoendorphin (20 nA), 2) β-neoendorphin (20 nA) preceded by naloxone (60 nA, 9 min), 3) Leu-enkephalin (20 nA), 4) Leu-enkephalin (40 nA) preceded by naloxone (60 nA, 9 min). Short horizontal line above histograms indicates time of iontophoretic application. Histograms obtained by averaging responses of neurons to two consective microapplications of substances. Calibration: abscissa, 50 sec; ordinate, 20 Hz; B) effect of preparations on activity of PT-neuron evoked by nociceptive stimulation (stimulation of sciatic nerve): 1) initial response, 3) preceded by action of β-neoendorphin (20 nA, 90 sec), 3) preceded by β-neoendorphin (60 nA, 135 sec), 3) morphine (60 nA, 135 sec), 50 morphine (60 nA, 150 sec) preceded by naloxone (60 nA, 7 min). Poststimulus histograms plotted by averaging 12 responses of neuron to nociceptive stimulation. Histogram bin 10 msec. Arrow indicates sciatic nerve stimulation: C) effect of preparations on evoked activity of unidentified neuron: 1a) initial response to stimulation of PT, 2a) β-neoendorphin (60 nA, 2 min), 3a) Leu-enkephalin (60 nA, 150 sec). Poststimulus histograms plotted by averaging 12 responses of a neuron to stimulation of PT. Histogram bin 10 msec. Arrow indicates stimulation of PT; 1b) initial response to radial nerve stimulation, 2b) Leu-enkephalin (60 nA, 195 sec), 3b) Leu-enkephalin (60 nA, 210 sec) preceded by naloxone (60 nA, 9 min), 4b) β-neoendorphin (40 nA, 135 sec), 5b) β-neoendorphin (60 nA, 270 sec). Averging of 10 responses to radial nerve stimulation. Histogram bin 10 msec. Calibration (for B and C): abscissa, 100 msec; ordinate, 1 spike/10 msec.

ically. The following freshly prepared solutions were used for microiontophoresis: 0.02 M β-neoendorphin (Peptide Institute, Osaka, Japan),* 0.02 M Leu-enkephalin (Peninsula Laboratory, USA), 0.001 M β-endorphin (Serva, West Germany), 0.05 M morphine hydrochloride, and 0.1 M naloxone hydrochloride (Endo Laboratories, USA). The solvent was 0.03 M NaCl, and the recording and compensating channels of the microelectrodes were filled with 3 M NaCl solution. The substances were expelled by currents of positive polarity of 10-60 nA. The dose of the preparation applied to the neuron could be varied by changing the strength of the electrophoretic current and the duration of injection. Two regions of the cerebral cortex were chosen for investigation: somatosensory area I (the focus of maximal activity of the sciatic or radial nerve) and Brodmann's area 5 in the parietal association cortex. The choice of these cortical areas was dictated by the fact that in cats the largest number of cells with connections with area 5 of the parietal association cortex is located in somatosensory area

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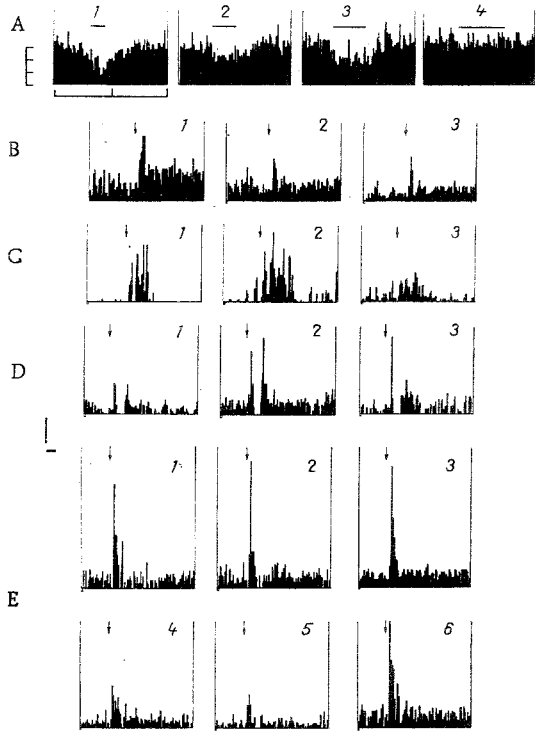

Fig. 2. Effect of β-neoendorphin and other opioid peptides on spontaneous and evoked unit activity in area 5 of the parietal association cortex. A, B, C, D, E) Different neurons. A) Effect of preparations on spontaneous activity: 1) β-neoendorphin (30 nA), 2) β-neoendorphin (30 nA) preceded by naloxone (60 nA, 6 min), 3) morphine (30 nA), 4) morphine (30 nA) preceded by naloxone (60 nA, 6 min). Short horizontal line above histograms indicates time of microiontophoresis. Calibration: abscissa, 100 sec; ordinate, 30 spikes/sec; B) effect of preparations on unit activity evoked by nociceptive stimulation: 1) initial response to sciatic nerve stimulation, 2) β-neoendorphin (60 nA, 320 sec), 3) morphine (60 nA, 270 sec). Averaging of 11 neuronal responses. Histogram bin 25 msec; C) effect of preparations on unit activity evoked by visual stimulation: 1) initial response to flashes, 2) β-neoendorphin (40 nA, 160 sec), 3) Leu-enkephalin (40 nA, 160 sec). Averaging of 15 neuronal responses. Histogram bin 25 msec; D) effect of preparations on unit activity evoked by visual stimulation: 1) initial response to flashes, 2) β-neoendorphin (50 nA, 210 sec), 3) morphine (50 nA, 210 sec). Averaging of 12 neuronal respones. Histogram bin 25 msec; E) effect of preparations on unit activity evoked by visual stimulation: 1) initial response to flashes, 2) β-neoendorphin (60 nA, 2 min), 3) β-neoendorphin (60 nA, 4 min 15 sec), 4) Leu-enkephalin (60 nA, 2 min), 5) Leu-enkephalin (60 nA, 4 min), 6) Leu-enkephalin (60 nA, 4 min) preceded by naloxone (60 nA, 9 min). Averaging of 11 neuronal respones. Histogram bin 25 msec. Calibration (for B, C, D, E): abscissa, 250 msec; ordinate, spike/25 msec.

I [1]. Both spontaneous unit activity and activity evoked by various afferent volleys were investigated. Nociceptive stimulation was induced by electrical stimulation of the sciatic or radial nerve (15-20 V, 0.5 msec). Flashes from a flash tube were used as visual stimulus (the diameter of the pupil was stabilized with 0.1% atropine solution).

To identify neurons in somatosensory area I whose axons run in the pyramidal tract (PT) stimulation of the pyramids in the medulla was used (5-7 V, 0.1 msec, 20-100 msec, frequency over 200 pulses/sec). If the cortical cells were excited antidromically with constant latent period (under 2 msec) and during repetitive stimulation of PT frequency binding at 200 pulses/sec or more took place, they were identified as PT neurons. Cells not satisfying this criterion were subsequently described conventionally as unidentified neurons.

EXPERIMENTAL RESULTS

β-Neoendorphin, when applied by microiontophoresis to neurons of this particular cortical area, either inhibited their spontaneous activity or did not affect it. For instance, of 31

cells studied 14 (45.2%) neurons were inhibited, whereas 17 (54.8%) did not respond (Fig. 1A). The other opioid peptides and morphine acted similarly to β-neoendorphin on spontaneous unit activity. No variation in the direction of the responses to the preparations was observed, i.e., if the response to a cell to microapplication of β-neoendorphin was inhibitory or absent, the cell responded in the same way to morphine and to the morphine-like substance (Fig. 1B). No differences likewise were found in chemical sensitivity of PT-neurons and unidentified cells to the substances tested. Naloxone, a specific blocker of opiate receptors, if applied microiontophoretically in doses which blocked the depressant action of morphine, Leu-enkephalin, and β-endorphin (i.e., agonists of μ-, δ-, and ε-opiate receptors), did not abolish the inhibitor effect of β-neoendorphin, an agonist of κ-opiate receptors (Fig. 1A). Consequently, it can be tentatively suggested that κ-opiate receptors in this area of the cortex are less sensitive to naloxone than other types of opiate receptors. This was established previously for peripheral opiate receptors [5].

Investigations of the effect of the preparations on evoked unit activity showed that they all equally inhibited responses of unidentified cells to PT stimulation (Fig. 1C). Differences in the action of β-neoendorphin and the other opioids were discovered when responses of the neurons to nociceptive stimulation were studied. For instance, in small doses, β-neoendorphin inhibited the early (short-latency) components of the neuronal response to an afferent nociceptive volley but had virtually no effect on the late (long-latency) components; in large doses, however, the peptide also inhibited the late components of the response (Fig. 1B, C). Unlike β-neoendorphin, the other opioid peptides and morphine caused dose-dependent inhibition of the late components of the neuronal response but did not change the early ones (Fig. 1B, C), as we observed previously [3]. No qualitative differences were observed in the effect of the preparations on responses of PT-neurons and unidentified cells to nociceptive stimulation (Fig. 1B, C).

When applied by microiontophoresis β-neoendorphin reduced the spontaneous discharge frequency of 11 (47.8%) of the 23 neurons studied in this area of the cortex (Fig. 2A). The remaining 12 (52.2%) cells were insensitive to the peptide. The other opioid peptides and morphine acted similarly to β-neoendorphin on spontaneous unit activity. In this area of the cortex, just as in somatosensory area I, variation in the direction of the responses to the preparations likewise was not observed (Fig. 2A). In doses which prevented the depressant action of opioids and morphine, due to stimulation of μ-, δ-, and ε-opiate receptors, naloxone did not block the inhibitory effects of β-neoendorphin, and agonist of the κ-opiate receptors (Fig. 2A). This was evidently because κ-opiate receptors in the parietal association cortex are less sensitive to naloxone than other types of opiate receptors.

Investigation of the effect of the preparations on evoked unit activity showed that all the morphine-like substances inhibited responses of the cells to nociceptive stimulation similarly (Fig. 2B). However, when the effect of the opioids and morphine was studied on neuronal responses evoked by afferent volleys of a different modality (photic stimulation), definite differences were found in the effects of the preparations. β-Neoendorphin and morphine, for instance, facilitated respones of the neurons to flashes (Fig. 2C, D, E). By contrast, Leu-enkephalin inhibited respones of the cells evoked by visual stimulation (Fig. 2C, E). Consequently, it can be postulated that δ-opiate receptors in this zone of the cortex play a different functional role in visual information processing from μ- and κ-opiate receptors.

This comparative investigation of the effect of β-neoendorphin, morphine, Leu-enkephalin, and β-endorphin on neuronal activity in two areas of the cerebral cortex (somatosensory area I, parietal association area) thus showed that the preparations produces changes in the same direction in spontaneous unit activity, i.e., basically inhibitory responses due to stimulation of different types of opiate receptors. However, the κ-opiate receptors in the cortex were found to be less sensitive to naloxone than μ-, δ-, and ε-opiate receptors. Opioid peptides and morphine also were found to have a different modulating effect on neuronal activity evoked by nociceptive stimulation in somatosensory area I and on neuronal responses to photic stimulation in area 5 of the parietal association cortex. This suggests that different types of opiate receptors and also their endogenous ligands, namely opioid peptides (enkephalins, endorphins, etc.), may perhaps pay a different functional role in nociceptive and visual information processing at the cortical level.

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STIMULATION OF [^{3}H]SPIROPERIDOL BINDING AFTER PROLONGED NEUROLEPTIC THERAPY BY THE CHOLECYSTOKININ OCTAPEPTIDE ANALOG CERULEIN

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612.822.1

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There is evidence that peptides of the cholecystokinin series, in low concentrations, modulate interaction of spiroperidol with dopamine$_2$- and serotonin$_2$-receptors [2]. Studies of behaviorial reactions have shown that cholecystokinin and its analogs, given by intracerebral and peripheral routes, have an action similar to that of both neuroleptics [4, 15] and apomorphine [1]. Parallel with behavioral changes, cholecystokinin and its analogs also cause depression of dopamine and serotonin metabolism in structures of the forebrain [1, 7]. Evidence has recently been obtained that cholecystokinin and its analog cerulein have a marked antipsychotic action on patients with schizophrenia who are resistant to neuroleptics [10, 11].

These facts explain the practical interest of a study of the effect of cerulein, a high-affinity analog of the octapeptide cholecystokinin [15], on binding of [^{3}H]spiroperidol *in vivo*. Considering the apormorphine-like action of cerulein, this biochemical analysis was undertaken in the form of a comparative study with N-propylnorapomorphine (NPA), a high-affinity analogy of apomorphine.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 20-25 g. The animals were given haloperidol (0.25 mg/kg, Gedeon Richter, Hungary), pirenperone (0.25 mg/kg, Janssen Pharmaceutica, Belgium), or physiological saline twice a day for 2 weeks. Binding experiments *in vivo* were carried out 72 h after the last dose of this prolonged course: Six animals from each group (physiological saline, haloperidol, and pirenperone) were given a subcutaneous injection of [^{3}H]spiroperidol alone, in a dose of 5 µg/kg (specific radioactivity 17 Ci/mmole, Amersham Corporation, England), and they were decapitated 20 min later. The remaining mice of the same groups (six animals in each group) were treated with displacing agents before receiving the labeled spiroperidol. Haloperidol was injected intraperitioneally in a dose of 2.5 mg/kg 40 min before injection of the labeled ligand, and cerulein, in a dose of 0.4 mg/kg (subcutaneously, Farmitalia, Italy), and MPA in doses of 5 and 50 µg/kg (Research Biochemicals Inc. USA) were injected 15 min before [^{3}H]spiroperidol. After decapitation of the animals the brain was quickly removed on ice and deep forebrain structures (limbic system and striatum) and the frontal cortex were dissected. The isolated structures were homogenized in 25 volumes of Tris-HCl buffer (50 mM, pH 7.4, 20°C). The samples were then centrifuged at 9000 rpm for 10 min. The supernatant was decanted and the residue carefully washed several times with cold Tris-HCl buffer. The radioactivity of the samples (five parallel tests) was determined in Bray's scintillator on an Ultro-Beta 1210 β-counter (LKB, Sweden). The experiments were repeated three times.

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