It can be tentatively suggested that the marked ability of cesium and rubidium to weaken analgesia induced by morphine and enkephalin analogs depends on elevation of the basal cAMP level by these ions [3].

It may be that the antagonism of these three ions toward the effect of enkephalin analogs is connected with their direct action on individual components of opiate receptors. For example, there is evidence that lithium and rubidium can modify binding of ligands of opiate receptors with cerebroside sulfate, a component of opiate receptors [7].

The data obtained in this investigation on the ability of PG, cAMP, dibutyrl cAMP, and lithium, rubidium, and cesium ions to weaken analgesia induced by enkephalin analogs obtained in the present investigation suggests the existence of a complex type of regulation of the functional state of opiate receptors.

The tetrapeptide-amide and its nitro analog were obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center.

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EFFECT OF OPIOID PEPTIDES, MORPHINE, AND ELECTROACUPUNCTURE ON UNIT ACTIVITY IN THE SENSOMOTOR CORTEX AND BRAIN-STEM RETICULAR FORMATION

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KEY WORDS: opioid peptide; electroacupuncture; naloxone; microiontophoresis.

Most workers nowadays associate the phenomenon of electroacupuncture analgesia mainly with the endogenous opioid system, for it is naloxone-dependent in character [8, 9, 12]. This view has also been confirmed by our own experiments [1, 4]. However, naloxone abolishes the analgesic effect of all opioid peptides without exception, and it is therefore not completely clear which substances — enkephalins, endorphins, or both together, are responsible for the analgesia with electroacupuncture (EA). The results of radioimmunologic investigations are contradictory: some workers observed an increase in the concentration of met-enkephalin but not of β-endorphin in the CSF and blood after electroacupuncture [5], whereas others observed the contrary [7, 11].

With these facts in mind it was decided to study, by the method of microiontophoresis, responses of single neurons in the sensomotor cortex and brain-stem reticular formation to application of enkephalins and endorphins and to compare it with the neuronal effects of EA. Morphine, as the classical representative of the opiates, was used.

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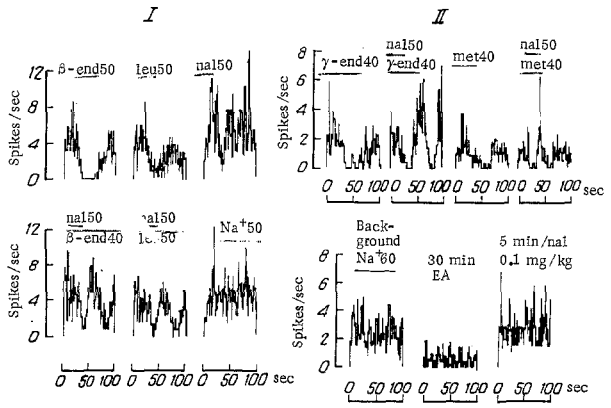

Fig. 1. Effect of opioid peptides and EA on spontaneous unit activity in rabbit sensomotor cortex. met) Met-enkephalin; leu) leu-enkephalin; γ-end) γ-endorphin; β-end) β-endorphin; nal) naloxone. Horizontal line above histograms indicates time of application of substances. Numbers near abbreviations denote strength of iontophoretic currents (in nA). Histograms obtained by averaging unit responses to 3-4 consecutive microapplications of the drugs. I and II) Two different neurons.

TABLE 1. The Effect of Enkephalins, Endorphins, Morphine, and EA on Spontaneous Unit Activity

Region studied	Type of response	Met-enkephalin	Leu-enkephalin	γ-endorphin	β-endorphin	Morphine	EA
Sensomotor cortex	Total number of neurons	43	64	43	59	38	21
	Inhibition	15 (34,9)	26 (40,6)	15 (34,9)	24 (40,6)	16 (42,1)	9 (42,6)
	Excitation	8 (18,6)	10 (15,6)	8 (18,6)	9 (15,3)	5 (13,3)	2 (9,5)
	No response	20 (46,5)	28 (43,8)	20 (46,5)	26 (44,1)	17 (44,7)	10 (47,6)
Mesencephalic reticular formation	Total number of neurons	56	56	27	56	27	17
	Inhibition	30 (53,6)	30 (53,6)	13 (48,1)	30 (53,6)	13 (48,1)	8 (47,1)
	Excitation	4 (7,1)	4 (7,1)	2 (7,4)	4 (7,1)	2 (7,4)	0
	No response	22 (39,3)	22 (39,3)	12 (44,4)	22 (39,3)	12 (44,4)	9 (52,9)
Gigantocellular reticular nucleus	Total number of neurons	30	30	—	30	18	11
	Inhibition	16 (53,3)	16 (53,3)	—	16 (53,3)	9 (50,0)	5 (45,5)
	Excitation	2 (6,7)	2 (6,7)	—	2 (6,7)	1 (5,6)	0
	No response	12 (40,0)	12 (40,0)	—	12 (40,0)	8 (44,4)	6 (54,5)

Legend. 1) Group of cells not reacting also includes cells with the relatively rare polyphasic reactions to these drugs. 2) Percentages shown in parentheses.

EXPERIMENTAL METHOD

Experiments were carried out on 11 rabbits and eight cats. Unit activity (sensomotor cortex — the focus of maximal activity of the sciatic nerve, mesencephalic reticular formation, gigantocellular reticular nucleus of the medulla) was recorded extracellularly and microiontophoretic application of the test substances, by means of multibarreled glass microelectrodes, which the writers described previously [2], was carried out on curarized unanesthetized animals. An "Elektronika DZ-28" minicomputer, coupled with an apparatus for recording unit activity, processed information on spontaneous and evoked unit activity in the course of the experiments and presented it graphically. The following freshly prepared solutions were used for microiontophoresis: met-enkephalin 0.02 M, pH 4.0; leu-enkephalin 0.001 M, pH 4.0; γ-endorphin 0.005 M, pH 7.2; β-endorphin 0.001 M, pH 7.4 (the peptides were synthesized by M. I. Titov at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR); naloxone hydrochloride (from Endo Laboratores) 0.1 M, pH 5.0; morphine hydrochloride 0.05 M, pH 5.0. The solvent was 0.03 M NaCl, with which the recording (3 M) and compensating (2 M) channels on the microelectrode also were filled. The substances were transported by positive currents with a strength of 10-60 nA for 20-120 sec. Some neurons in the sensomotor cortex with a low initial firing rate (under 5 spikes/sec) were activated by continuous microiontophoretic application of L-glutamate (1 M solution, 5-20 nA); the

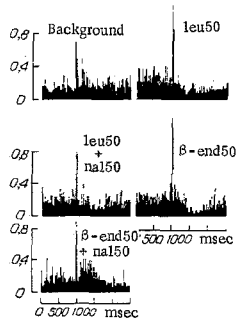

Fig. 2

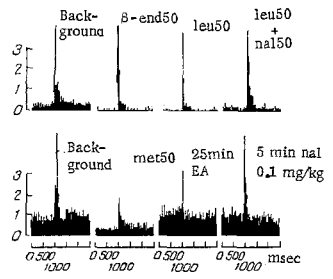

Fig. 3

Fig. 2. Effect of opioid peptides on evoked sensomotor cortical unit activity. Post-stimulus histogram plotted by averaging 20 (n = 20) neuronal responses to nociceptive stimulation. Bin width 25 msec. Arrow indicates sciatic nerve stimulation. Abscissa, time (in msec); ordinate, number of spikes in 25 msec. Remainder of legend as to Fig. 1.

Fig. 3. Effect of opioid peptides and EA on evoked activity of first (a) and second (b) types of neurons in mesencephalic reticular formation. Bin width 25 msec; n = 20 for A and n = 25 for B. Remainder of legend as to Figs. 1 and 2.

appropriateness of this approach to study neuronal responses to microapplication of morphine and enkephalins was demonstrated previously [10, 13]. Nociceptive stimulation was applied in the form of electrical stimulation of the sciatic or radial nerve (10-15 V, 50 mC, 100 Hz). The technique of EA and of evaluation of its effectiveness (analgesic effect) was described previously [1].

EXPERIMENTAL RESULTS

Opioid peptides and morphine, on microiontophoretic application to neurons of the sensomotor cortex and brain-stem reticular formation had a predominantly inhibitory effect on spontaneous activity (Table 1). EA gave a similar effect. Inhibitory responses of the neurons to application of opioid peptides and morphine were evidently specific, i.e., they were connected with opiate receptors, for they were abolished by naloxone (in the cortex in 24 of 25 cells; in the subcortex in 18 of 19 cells; Fig. 1). Activating responses of neurons evoked by opioid peptides and morphine were observed much less frequently than inhibitory and were virtually not abolished by naloxone (in the cortex naloxone abolished excitation in two of seven cells, in the subcortex in none of four cells). This fact suggests that their nature in these brain structures is predominantly "nonspecific." No opposite reactions were observed to application of morphine and the various peptides, i.e., if the response of a neuron to microapplication of morphine (enkephalin) was inhibitory (excitatory), it responded in the same way to application of endorphin. In the overwhelming majority of cases EA gave rise to effects similar to those evoked by the drugs. All changes in unit activity produced by EA were abolished by systemic (0.1 mg/kg, intravenously) or microiontophoretic administration of naloxone (Fig. 1).

The opioid peptides, morphine, and EA thus evoked similar changes, in the same direction in spontaneous unit activity in the sensomotor cortex and brain-stem reticular formation. The main reaction in this case was inhibitory and naloxone-dependent in character.

Investigation of evoked unit activity in the sensomotor cortex (focus of maximal activity of the sciatic nerve) to nociceptive stimulation (sciatic nerve stimulation) showed that the opioid peptides, morphine, and EA modified the pattern of the evoked response of the nerve cell equally. Characteristically the late (long-latency) components of the neuronal response

were reduced by microiontophoretic application and EA whereas the early (short-latency) components were virtually unchanged (sometimes they even showed a tendency to increase). These effects were "specific" for they were abolished by naloxone (Fig. 2). Similar changes in the pattern of the evoked response to nociceptive stimulation of the sciatic nerve during systemic injection of morphine were observed previously by Emmers [6] in thalamic neurons.

To analyze this phenomenon the response of a cortical neuron in the focus of maximal activity of the radial nerve to nociceptive stimulation of both the radial and the sciatic nerve was recorded in a series of experiments in cats. The opioid peptide, morphine, and EA were found to inhibit the evoked response of the neuron to sciatic nerve stimulation in a similar and naloxone-dependent manner, and to modify it in the way described above (by reducing the late components) in response to radial nerve stimulation. These data can evidently be compared with the well-known fact that morphine does not change (or increases) the primary response of the cortical-evoked potential to stimulation of somatic nerves in the projection zone, while inhibiting it in nonprojection areas of the cerebral cortex [3].

Investigation of evoked responses of neurons of the brain-stem reticular formation to nociceptive stimulation showed that there are at least two types of cells in which the pattern of evoked responses is changed differently during microiontophoretic application of opioid peptides, morphine, and EA. Cells of the first type reacted to afferent stimulation against the background of microapplications of the drugs or EA in the same way as sensomotor cortical neurons, i.e., the early components of the response were unchanged and the late components were reduced (Fig. 3A). In neurons of the second type, the whole evoked response was reduced (Fig. 3B). Naloxone abolished these effects.

In both the cortex and the subcortex changes in evoked unit responses to nociceptive stimulation during microiontophoresis of the pharmacologic agents and EA were similar.

Microiontophoretic investigation of spontaneous and evoked unit activity in the sensomotor cortex, mesencephalic reticular formation, and gigantocellular reticular nucleus of the medulla to application of enkephalins, endorphins, morphine, and EA thus revealed similar responses, uniform in direction, and abolished by naloxone. It can accordingly be postulated that the effects of EA in these brain structures are mediated by opiate receptors and they are evidently connected with the liberation of either enkephalins or endorphins.

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