EFFECT OF ENKEPHALINS ON ASSOCIATIVE PROCESSES IN PARIETAL CORTICAL NEURONS

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It has been suggested that enkephalins play an important role in learning and memory processes in animals [2, 5, 7]. However, their importance in the neuronal mechanisms of formation of associative connections in vertebrates is not clear. Only solitary investigations, conducted mainly on mollusks, and devoted to this problem have been published. For instance, the action of one of the opioid peptides on electrical and plastic properties of identified neurons of *Helix pomatia* has been described [3].

The aim of this investigation was to study the microiontophoretic effect of enkephalins on the formation of associative connections in parietal cortical neurons of cats.

EXPERIMENTAL METHOD

Experiments were carried out on 19 curarized cats weighing 3-4 kg, under 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, drilling five burr-holes above the region of the middle suprasylvian gyrus, corresponding to the projection of area 5) were done under pentobarbital sodium anesthesia (35-40 mg/kg, intraperitoneally). Extracellular recording of single unit activity (neurons were recorded 8-10 h after injection of pentobarbital sodium) and microiontophoretic application of enkephalins to them were carried out with the aid of multibarreled glass microelectrodes [6]. An "Élektronika D3-28" laboratory computer, coupled with a system for recording unit activity, enabled information obtained on the parameters of spontaneous and evoked neuronal activity to be analyzed actually during the experiment and displayed on a graph plotter. In the course of the experiments responses of the neurons to stimulation of axons of the pyramidal tract (PT; 0.05-0.1 msec, 4-8 V, 200 Hz, 500-600 msec) and to electrodermal stimulation (EDS), induced by stimulation of the foot pads using metal disks (0.3 msec, 30-50 V, 100 Hz, 50 msec) were assessed. The following freshly prepared solutions were used for microiontophoresis: Met- and Leu-enkephalin (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR) 0.02 M, pH 4.0; naloxone hydrochloride (Endo Laboratories, USA) 0.1 M, pH 5.0. The solvent was 0.03 M NaCl, and the recording and compensating channels of the microelectrode were filled with 3 M NaCl solution. The substances were applied by positive currents, with an intensity of 20-50 nA.

EXPERIMENTAL RESULTS

Activity of 185 neurons was studied. Of this number, 88 responded to repetitive stimulation of PT axons. In 64 cases (73%) an increase in discharge activity was observed, in 10 cases (11%) the effect was inhibitory, and 14 neurons (16%) gave a mixed type of response. Of the group of neurons responding to stimulation of PT, 76 (86%) changed their activity in response to EDS. Effects of microiontophoretic application of enkephalins were analyzed on populations of these cells.

It was found that the main type of response to microiontophoretic application of enkephalins was inhibition of spontaneous activity (35 neurons). The degree of inhibition of activity varied widely from almost complete suppression of spontaneous activity to a very small decrease in the frequency of spontaneous discharge generation.

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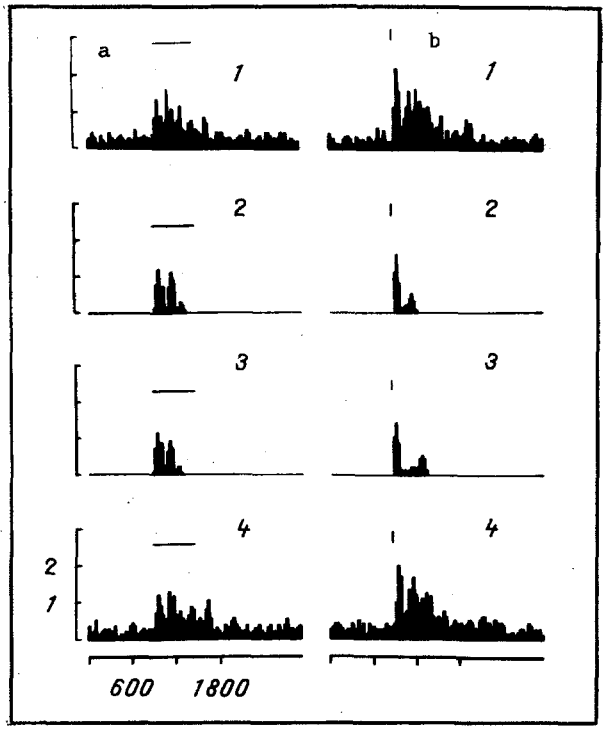

Fig. 1. Effect of enkephalins on spontaneous and evoked unit activity in the parietal cortex: a) stimulation of PT (5 V, 0.05 msec, 200 Hz, 500 msec); b) EDS (40 V, 0.3 msec, 100 Hz, 50 msec). 1) Initial responses, 2) application of Met-enkephalin (50 nA, 3 min), 3) application of Leu-enkephalin (50 nA, 3 min), 4) application of Leu-enkephalin (50 nA, 3 min) preceded by naloxone (50 nA, 4 min). Short horizontal line indicates stimulation of PT, vertical line – EDS. Ordinate, number of spikes in 25 msec; abscissa, time (msec). Histograms plotted by averaging 10 presentations of the stimulus.

An attempt was made to study the character of the effect of these preparations on responses formed during combinations of stimulation of PT and EDS. The experiments showed that adaptive restructuring of the responses as a result of the formation and development of associative connections was observed in 20 neurons responding to microiotophoresis of the enkephalins. The quality of the effect of the enkephalins on cell responses newly formed during application of the combinations was found to depend on the duration of the microiotophoretic injections. The study of the effect of the preparations on evoked unit activity also revealed a predominantly inhibitory effect. No differences were found between the effects of Met-enkephalin and Leu-enkephalin. Naloxone blocked the depressant action of the opioid peptides on the neuzonal responses, confirming that opioid receptors are involved in the phenomena described above (Fig. 1).

In response to short injections (under 5 min) inhibition of responses to stimulation of PT as well as to EDS was observed. Cessation of application of the preparations restored the responses; responses to stimulation of PT, moreover, were restored in the form in which they were recorded during the action of combinations of PT stimulation with EDS (Fig. 2). This phenomenon, which was observed in 18 cases, is evidence that short-term injection of enkephalins, which has a phasic inhibitory action on unit activity, nevertheless does not change the character of the associative responses formed to EDS.

Different results were obtained when long injections of enkephalins (over 30-40 min) were used. After cessation of long-term injections, complete recovery of responses to stimulation of PT was not observed in the majority of neurons (11 of 17 cells).

The restored responses corresponded in their parameters to responses of neurons not exposed to the conditioning procedure. This result indicates that the effect of prolonged injection of enkephalins is, on the whole, identical with extinction of the conditioned responses with rupture of associative connections (Fig. 2).

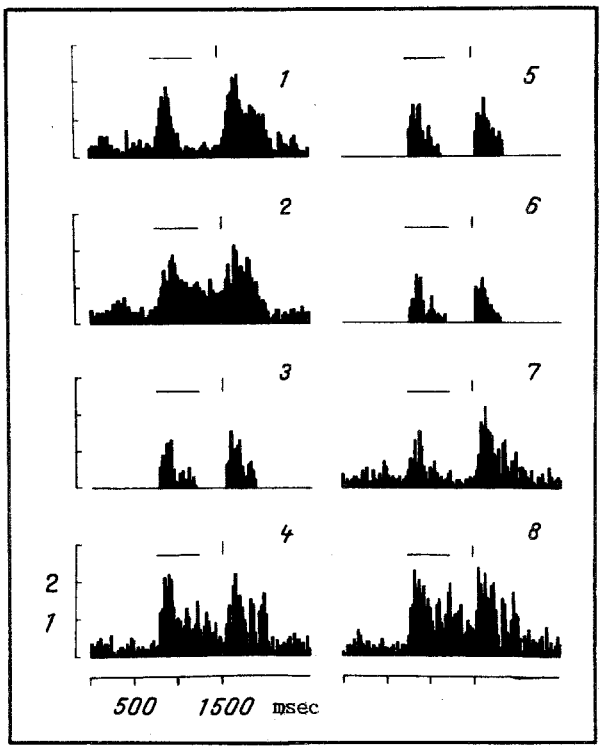

Fig. 2. Effect of Leu-enkephalin on evoked responses of a neuron during combinations of stimulation of PT with EDS. 1) Initial responses to PT + EDS, 1st-10th combinations; 2) PT + EDS, 11th-20th combinations; 3) PT + EDS accompanied by microiotophoresis of Leu-enkephalin (50 nA, 3 min), 21st-30th combinations; 4) PT + EDS after cessation of application of Leu-enkephalin, 31st-40th combinations; 5, 6) accompanied by microiotophoresis of Leu-enkephalin, 41st-60th and 61st-80th combinations respectively; 7) After cessation of application of Leu-enkephalin (50 nA, 37 min), 81st-90th combinations; 8) the same, 91st-100th combinations. Remainder of legend as to Fig. 1.

The discovery of the negative effect of long-term application of enkephalins on the stability of associative connections was confirmed by experiments in which attempts were made to obtain primary adaptive restructuring of neuronal responses with continuous application of enkephalins. It was found that inhibition of spontaneous unit activity and reduction of responses to EDS involved marked inhibition of conditioning. In 11 of 15 cases reconstruction did not develop even when a very large number of combinations (over 100) was used, and in four cases although some reconstruction did take place, the newly formed responses were unstable.

Finally it will be noted that the results described above correlate with data obtained during animal training. For instance, the inhibitory action of enkephalins on the formation and consolidation of defensive conditioned reflexes in rats was clearly demonstrated in [5, 8].

So far as the elementary mechanisms of these phenomena are concerned, it must be accepted that the principal change is a disturbance of the activity of the neuronal micropool during iontophoretic application of the enkephalins [1, 4, 9]. The possibility cannot be ruled out that a disturbance of information flows in the composition of the micropool and a disturbance of associative processes are the result of long-term inhibition of convergent integrative neurons of the micropool. The neurons recorded in the present experiments, which responded by inhibition to iontophoresis of the enkephalins, were mainly neurons with extensive receptive fields and with marked responses to stimuli of different modalities.

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LITERATURE CITED

1. A. A. Aleksandrov, The Method of Microiotophoresis in Physiology [in Russian], Leningrad (1983).
2. I. P. Ashmarin, Biochemical and Physiological Bases of Learning Processes [in Russian], Leningrad (1982), pp. 105-111.
3. T. L. D'yakonova and K. Sh.-Rozha, Zh. Vyssh. Nerv. Deyat., No. 4, 751 (1986).
4. B. I. Kotlyar, Mechanisms of Formation of the Temporary Connection [in Russian], Moscow (1977).
5. R. I. Kruglikov, L. S. Bikbulatova, and V. M. Getsova, Zh. Vyssh. Nerv. Deyat., No. 6, 1011 (1986),
6. V. A. Pravdivtsev, N. M. Osipov, and V. V. Yasnetsov, Farmakol. Toksikol., No. 2, 224 (1981).
7. I. Izquierdo, Psychopharmacology, **66**, No. 2, 199 (1979).
8. I. Izquierdo and R. D. Dias, Neurosci. Lett., **22**, No. 2, 189 (1981).
9. L. P. Renaud and J. S. Kelly, Brain Res., **79**, No. 1, 29 (1974).

AFFERENT CONNECTIONS OF POSTERIOR COLUMN NUCLEI OF THE SPINAL CORD

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Electrical stimulation of the forelimb or its nerves in cats is known to induce evoked potentials (EP) in nuclei of the posterior columns of the spinal cord (NPC) not only on the side of stimulation [5], but also on the opposite side [2-4]. This observation contradicted the classical view that the posterior columns of the spinal cord have unilateral connections with NPC and are responsible for generation of the EP-complex in response to stimulation of the corresponding forelimb.

The aim of this investigation was to study the spread of posterior-column afferent projections to contralateral NPC, to compare ipsilatefal and contralateral EP in these structures, and to look for the structural sources of conduction of afferent projections of the posterior spinal columns to the contralateral NPC.

EXPERIMENTAL METHOD

Experiments were carried out on adult cats. There were two methods of investigation: electrophysiological and electron-microscopic, both described in detail previously [3]. The first method consisted of recording and analyzing EP at different points of symmetrically opposite NPC during stimulation of the forelimbs in intact cats and after preliminary hemisection of the tegmentum mesencephali under acute experimental conditions. For electron-microscopic investigation of the afferent connections of the posterior columns of the spinal cord with NPC unilateral damage was inflicted on the posterior columns of the spinal cord in the animals of Group 3 at the level of cervical segments 3-5. For this purpose, after removal of the covering cervical spinal muscles the corresponding part of the spinal cord was exposed for a distance of 3-5 cm. The posterior arches of the vertebrae were nibbled away, the dura mater was divided sagittally, and the posterior columns of the spinal cord were identified and then divided.

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