EFFECT OF ENKEPHALINS ON REACTIVITY IN TWO HIPPOCAMPAL SYNAPTIC SYSTEMS

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Studies of the location of enkephalinergic structures and the distribution of opiate receptors in the hippocampus have shown that enkephalin-containing perikarya and nerve endings are present in different parts of the hippocampus [7] and that the density of opiate receptors is high in the layer of pyramidal cells [5, 11]. Enkephalinergic systems are evidently internal systems of the hippocampus [10] and they participate in the processing of incoming information.

In the study of the functional role of enkephalins in the hippocampus most attention has been paid to their action on reactivity of pyramids in area CA-1 [6, 8, 13]. In a few investigations the effect of enkephalins on reactivity of dentate fascia neurons has been studied [8, 12], but the role of enkephalins in regulating transmission in one of the main synaptic systems of the hippocampus, namely connections between dentate fascia neurons and pyramidal cells in area CA-3, has virtually not been studied at all.

Accordingly, the object of the present investigation was to study the effect of enkephalins on reactivity of pyramidal neurons in area CA-3 to stimulation of the dentate fascia (mossy fibers) and to compare it with the effect of enkephalins on reactivity of pyramidal cells in area CA-1 to stimulation of Schaffer's collaterals.

EXPERIMENTAL METHOD

Experiments were carried out on transverse sections through the hippocampus of C57BL mice aged 3-4 weeks by the technique adopted in the laboratory [1]. Focal potentials evoked by single periodic stimulation of the radial layer of the hippocampus or the hilus of the dentate fascia were recorded in the pyramidal layer of areas CA-1 and CA-3, respectively. The strength of stimulation was chosen so that the evoked response included a spike potential (population spike, PS), whose amplitude reflects the number of pyramidal cells involved simultaneously in the response [3].

Evoked potentials were recorded and analyzed by the PDP-8a computer. The action of the substances was evaluated from curves of current amplitude of the PS, measured by a correlation method [2].

The substances, namely leu- and met-enkephalin (synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR), naloxone ("Narcan" from Endo Laboratories, USA), and morphine, were added to the perfusion medium (TK Simms X7 Solution, from Difco Laboratories, USA). The perfusion system provided complete change of medium in the experimental chamber in about 2 min.

EXPERIMENTAL RESULTS

In the present investigation, unlike many others, unmodified enkephalins were used; to estimate active concentrations of the substances, a preliminary investigation accordingly was carried out on area CA-1, for which the facilitatory action of enkephalin analogs, protected from peptidases, has already been described [6, 8].

Addition of enkephalins to the medium in micromolar concentrations rapidly increased the amplitude of the PS recorded in the pyramidal layer of area CA-1 during stimulation of

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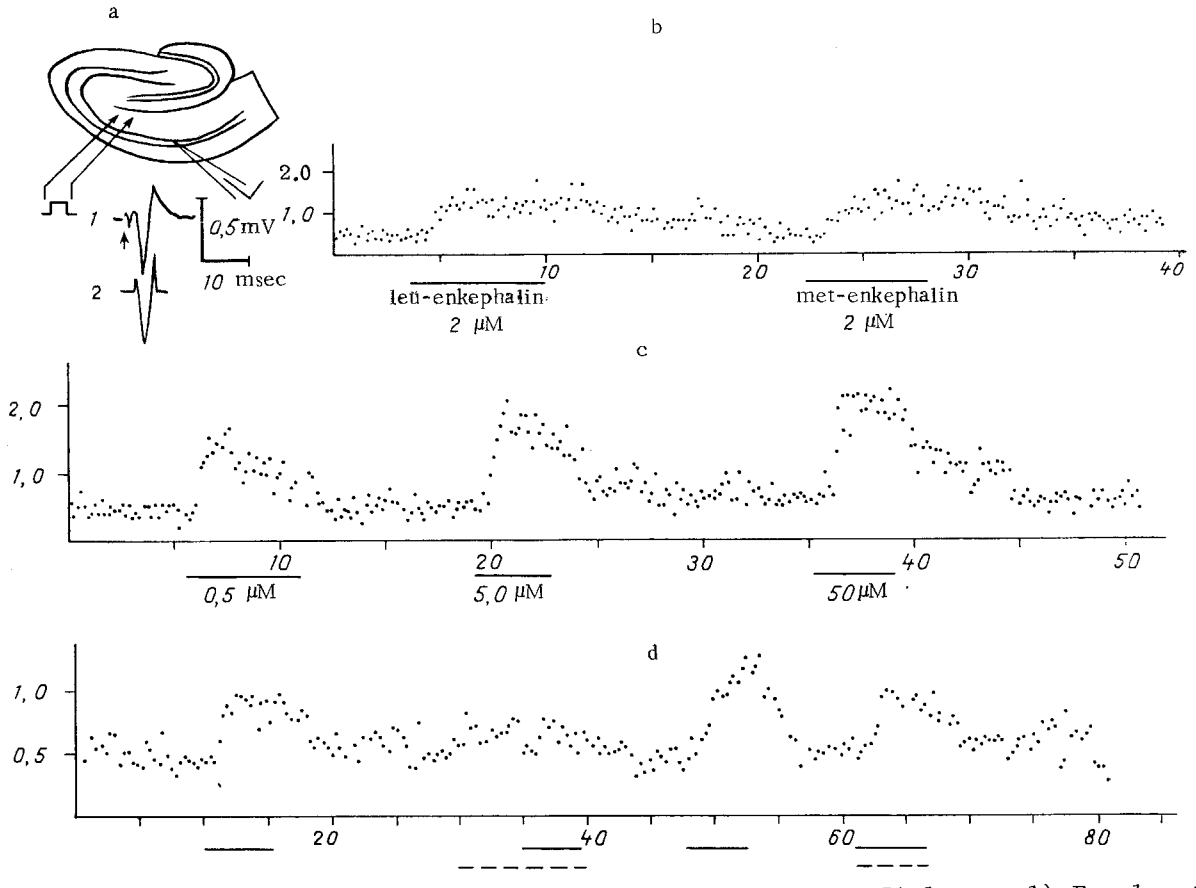

Fig. 1. Effect of **enkephalins** on reactivity of pyramidal cells in area CA-1. a: 1) Focal potential recorded in pyramidal layer CA-1 during stimulation of radial layer (averaged results of 50 experiments); 2) measured part of focal potential, PS. Above – scheme of arrangement of recording and stimulating electrodes in section through hippocampus. b-d) Curves of current amplitude of PS in three different experiments: b) similarity of facilitatory action of leu- and met-enkephalin, c) action of different concentrations of leu-enkephalin (0.5, 5, and 50 μM), d) inhibition of responses to leu-enkephalin (5 μM) by preliminary addition of 10 μM naloxone (broken line). Abscissa, time (in min); ordinate, amplitude of PS (in mV). Short horizontal lines below abscissa indicate addition of substances.

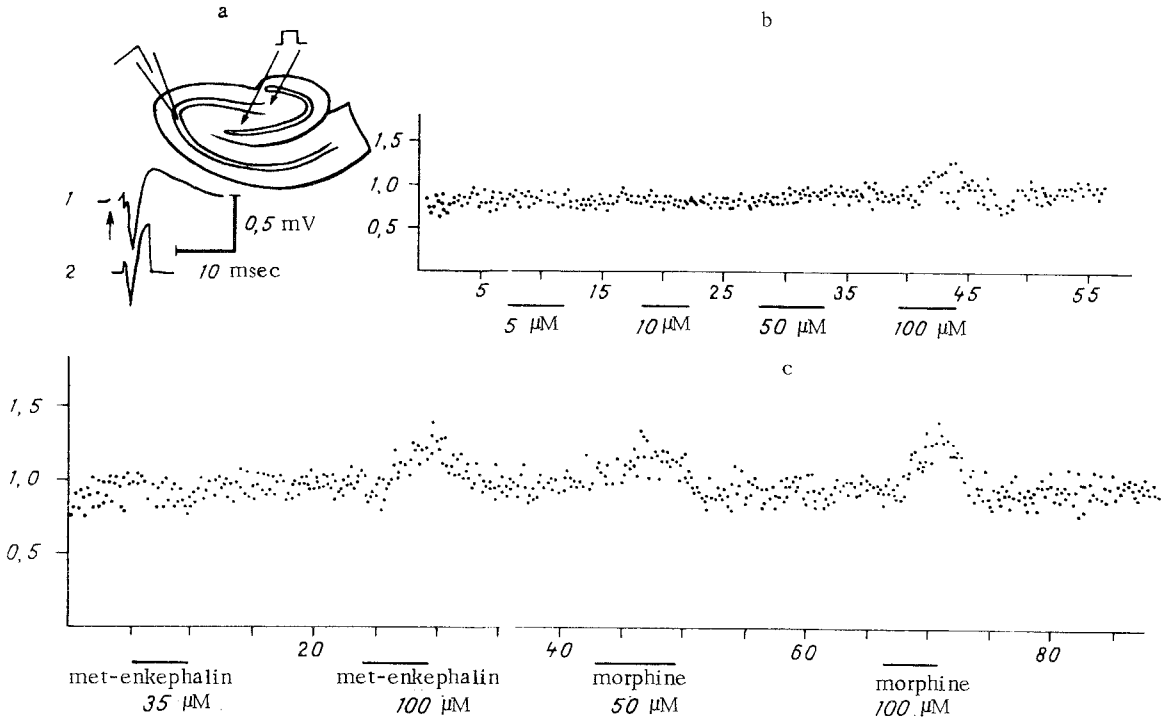

Fig. 2. Effect of enkephalins on reactivity of pyramidal cells in area CA-3. a: 1) Averaged focal potential recorded in area CA-3 during stimulation of hilus of dentate fascia; 2) measured part of potential, PS. Above — scheme showing arrangement of electrodes in section. b, c) Curves of current amplitude of PS in two different experiments: b) addition of various concentrations of leu-enkephalin, c) addition of various concentrations of met-enkephalin and morphine. Remainder of legend as in Fig. 1.

the radial layer (Schaffer's collaterals). Leu- and met-enkephalins were equally effective (Fig. 1b). An increase in reactivity was observed in all 15 experiments. Estimation of dependence of the magnitude of the effect on concentration of the substance, undertaken on three sections, showed that a two-threefold increase in response amplitude occurred after addition of 0.5-1 μM of enkephalins, and that a further increase in their concentration caused no significant potentiation of the effect (Fig. 1c), but changed only the time course of its development and disappearance.

To test the specificity of action of the enkephalins, in most experiments (12) their influence on different effects of addition of naloxone, an antagonist of opiate receptors, was studied. Addition of naloxone itself in a concentration of 10 μM usually did not affect the magnitude of the responses, but in three experiments it caused a small increase in amplitude of PS, due perhaps to the inhibitory action of naloxone on GABA-ergic inhibitory systems [4]. After preliminary addition of 10 μM naloxone, partial or total inhibition of responses to the subsequent addition of 1-5 μM enkephalins was observed in five of eight experiments (Fig. 1d). In testing by a different system, when naloxone and enkephalins were added simultaneously (four experiments) as a rule naloxone did not abolish the responses to enkephalin (three experiments). Hence, although under certain conditions complete blockade of responses to enkephalins by naloxone could be observed, the effectiveness of naloxone was not very great, possible evidence that the leading role in the mechanism of responses to enkephalins is played by opiate receptors of the δ type, whose affinity for naloxone is much lower than that of μ-receptors [9].

The results obtained by recording activity of pyramidal cells in area CA-1 of the mouse hippocampus agreed well with those obtained by other workers on sections of the rat hippocampus [6, 8, 12].

In 10 experiments on the same preparations, in which the effect of enkephalins on reactivity in area CA-1 was studied, changes in reactivity of pyramidal cells in area CA-3 were studied during stimulation of the hilus of the dentate fascia (mossy fibers). The amplitude of PS was found to be unchanged after addition of micromolar concentrations of enkephalins to the medium. Only when concentrations of about 10^{-4} M were used was a small increase in amplitude of the response (by 30-50%) observed (Fig. 2b, c). Similar changes were produced by morphine when the same concentrations were used (Fig. 2c). Naloxone in a concentration of 10-50 μM did not affect the amplitude of the PS when added alone, and did not inhibit responses to high concentrations (50-100 μM) of enkephalins and morphine.

The effect of enkephalins on reactivity of pyramidal cells in areas CA-3 and CA-1 was identical **in direction and** consisted of increased reactivity, although the intensity of these changes and the doses of the compounds required to exhibit effects in CA-3 and CA-1 differed significantly. Reactivity of pyramidal cells in area CA-1 was increased considerably by addition of micromolar concentrations of enkephalins (the amplitude of PS was increased by several times), whereas in CA-3 concentrations 2 orders of magnitude higher increased the amplitude of the responses by not more than 1.5 times.

Whereas the weaker facilitation of responses in CA-3 can be regarded as evidence of the relatively less important role of enkephalinergic systems in the regulation of reactivity of pyramidal cells in area CA-3 than in area CA-1, the reasons why only high concentrations of enkephalins are active in area CA-3 still remain not quite clear. These two regions of the hippocampus do not differ significantly in density or character of distribution of opiate receptors [5, 11]. The neurophysiological mechanisms suggested to explain the action of enkephalins on reactivity of pyramidal cells in area **CA-1**, namely inhibition of inhibitory interneurons [13], or presynaptic regulation of transmitter release [8], apply equally to pyramidal cells in CA-3 also. All that can be suggested, therefore, is that because of certain structural differences the zones of reception in CA-3 are less accessible for substances added to the perfusion fluid, and that is why high concentrations have to be used.

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POLYMODALITY OF DISTRIBUTION OF MINIATURE END-PLATE POTENTIAL AMPLITUDES DUE TO THE ACTION OF SPATIALLY SEPARATE AREAS OF TRANSMITTER RELEASE

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Investigations have shown that during recording of miniature end-plate potentials (MEPP) two populations of signals are found — of low and high amplitude [2, 5]. Low-amplitude signals, accounting for 2-5% of all MEPP, form an independent peak at the beginning of the histogram of distribution, whereas high-amplitude signals constitute the main population of spontaneous potentials and constitute classical MEPP. Peaks, multiples of the mean amplitude of the small MEPP, were found on histograms of high-amplitude signals [6, 8]. These experimental data lay at the basis of the subquantum hypothesis of transmitter secretion in the neuromuscular synapse. According to this hypothesis, small MEPP are the response to liberation of one subquantum of acetylcholine, whereas high-amplitude signals, corresponding to classical MEPP, are formed through synchronous release of a certain number of subquanta [6].

It is also known that a quantum of mediator is released in particular areas of the nerve ending, or release points, which are arranged some distance apart [3, 7]. The membrane of the muscle fiber has a high input resistance, and accordingly the signal arising in response to secretion of a quantum of mediator, as it spreads over the membrane, dies away [4]. In this paper the hypothesis is submitted and proved that peaks on histograms of amplitudes of spontaneous synaptic potentials may be due to the activity of spatially separate areas of transmitter secretion in the nerve ending.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscles of small frogs at room temperature. During the experiment the preparation was fixed in a bath containing continuously flowing Ringer's solution of the following composition (in mM): NaCl 115.0, KCl 2.0, $CaCl_2$ 0.3, $MgCl_2$ 2.0, $NaHCO_3$ 2.4.

To record MEPP two glass microelectrodes filled with 3 M KCl solution, with a resistance of 7-10 MΩ, were used. Under a binocular microscope a myelinated nerve twig running along the surface of the muscle was dissected. Microelectrodes were inserted into a muscle fiber near this nerve twig, 50-100 μ apart. The distance between the electrodes was measured by means of an ocular micrometer. The criterion that the microelectrodes were in the region of a synapse was recording of MEPP with a leading edge about 1 msec in duration.

Spontaneous potentials recorded by two microelectrodes were amplified by means of two amplifiers and recorded from the screen of a two-channel oscilloscope on photographic film by means of an FOR-2 camera. For convenience of recording and measuring, signals from one elec-

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