EFFECT OF LEUCINE-ENKEPHALIN ON AFTER-DISCHARGE DEVELOPMENT IN THE RAT SENSOMOTOR CORTEX

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Bursts of repetitive electrical stimulation (RES) of the rat sensomotor cortex lead to the appearance of a generator of pathologically enhanced excitation (GPEE) in it [1, 2]. By the time of appearance of stable after-discharges (AD) of the spike-wave type in the electocorticogram (ECoG) in the primary (electrically stimulated) and secondary (in the homotopic zone of the contralateral hemisphere) foci of hyperactivity the concentration of Leu-enkephalin (LEU) falls sharply, whereas the methionine-enkephalin level is essentially unchanged [3]. The question arises, what is the action (pro- or antiepileptic) of the released LEU on neurons of GPEE? It has been shown that endogenous opioids, released into the CSF during acute convulsions, can raise the threshold of evocation of epileptic activity in healthy recipient animals [15]. On the other hand we know that during their action on certain brain structures exogenous LEU and other opioid peptides can evoke epileptiiorm activity [13, 17]. To discover the role of LEU in cortical epileptogenesis, the effect of LEU was studied on sensomotor cortical excitability, the latency and characteristics of AD, and also the lowering of the thresholds of direct (DR) and transcallosal (TCR) responses evoked by bursts of RES [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on 22 noninbred male rats weighing 280-350 g. Rats of the same batch were used and experiments with LEU alternated with control experiments (application of physiological saline). Under ether anesthesia the animal's head was fixed in a frame, and the skull was trephined above the left and right sensomotor cortex [1]. The right hemisphere was stimulated and the ECoG recorded on the left and right sides by means of electrodes located on the dura mater [1, 2]. To apply solutions to the brain surface an automatic micropipet, with its tip introduced into a small hole made in the dura mater, was used.

The experiment began with determination of thresholds of DR and TCR, evoked by single pulses [1]. Next, 2 μl of LEU solution (1 μg/μl, from Serva, Germany) or of physiological saline was applied to the cortical surface of the stimulated hemisphere and measurement of the thresholds was repeated 5 min later. In the course of the experiment 20 bursts of RES (square pulses, intensity of stimulation two thresholds, pulse duration 0.1 msec, frequency 10 Hz, duration of burst 10 sec, interval between bursts of RES 10 min) were applied. After each odd electrical stimulation (after cessation of evoked responses or AD) the LEU solution or physiological saline was applied to the cortical surface. ER, TCR, and AV, evoked by even bursts of RES, were used to determine the effect of the solutions on evoked and self-maintained cortical activity. After cessation of AD evoked by the 20th burst of RFS, thresholds of

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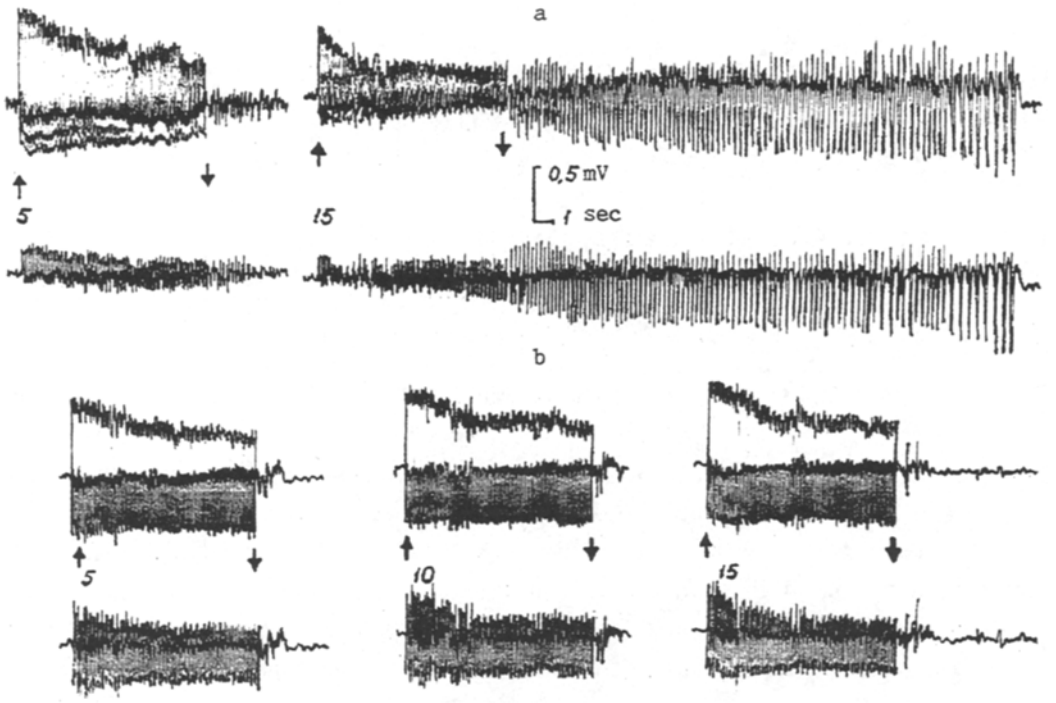

Fig. 1. Effect of LEU on direct and transcallosal cortical responses to single electric pulse and to seizure after-discharges evoked by bursts of RES of rat sensomotor cortex. a) DR, TCR, and AD to a combination of RES with application of physiological saline; b) the same, but with application of solution of Leu-enkephalin (2 μg/2 μl) to cortex. Top trace shows derivation of ECoG from zone of electrical stimulation (right hemisphere), bottom trace shows ECoG derived from zone of synaptic stimulation (symmetrical region of left hemisphere). Arrows indicate beginning and end of RES. Amplification and paper tape winding speed are the same in a and b.

DR and TCR were again tested. The amplitude of AD was measured as the maximal spike-wave oscillation (from spike to spike), the duration of AD from the beginning of the first until the end of the last spike-wave oscillation. The results were subjected to statistical analysis by Student's t test for tied pairs and independent variables.

EXPERIMENTAL RESULTS

In the experiments in which physiological saline was applied to the cortex (control) bursts of RES led to changes in the ECoG [1]. For an AD to appear 4 or 5 bursts of RES were required (latency of AD), and application of subsequent stimuli led to an increase in the amplitude and duration of AD and to the appearance of AD with relatively stable characteristics (Fig. 1a). In response to a combination of bursts of RES and poststimulus application of LEU, significant inhibition of potentiation of the amplitude and duration of AD was observed in the course of repetitive stimulation. In the control, the amplitude of spike-wave oscillations of the 5th AD was twice the amplitude of the first AD (Fig. 2b), whereas in the experiments with application of LEU this difference was not significant (Fig. 2b). Repetitive cortical electrical stimulation in these experiments did not lead to an increase in the duration of AD (Fig. 2a, c). On the other hand, application of LEU caused no changes in latency of AD (Fig. 2a). Thresholds of DR and TCR, measured 5 min after application of LEU, were unchanged compared with values before application of

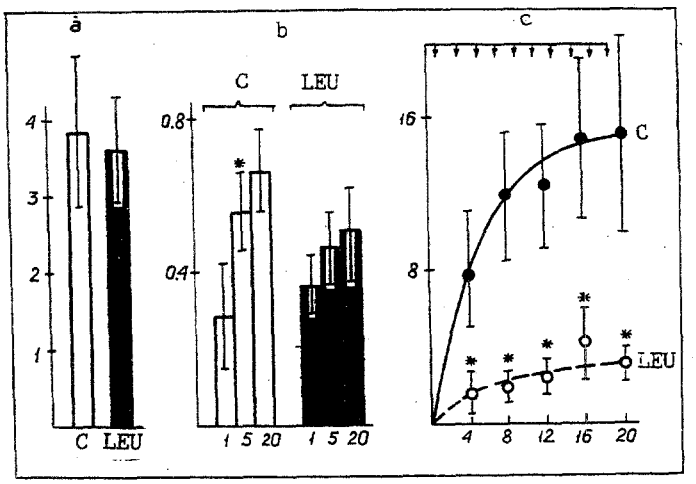

Fig. 2. Effect of Leu-enkephalin on latency, amplitude, and duration of AD. Abcissa, number of bursts of RES. Ordinate: a) latency of AD (number of RES bursts required to evoke the first AD); b) amplitude of AD (in mV); c) duration of AD (in sec). C) Averaged data for control group of animals in which RES was combined with application of physiological saline (n = 10). LEU) The same for animals to whose cortex Leu-enkephalin was applied (n = 12). Amplitude of 5th AD in control significantly (b, *$p < 0.05$) higher than that of 1st. Arrows (c) indicate times of application of solutions. All points on curve for LEU were significantly ($p < 0.05$) different from corresponding points for C, starting with 3rd burst of RES.

LEU or with the thresholds of DR and TCR after application of physiological saline (Fig. 3). The character of changes in evoked responses was similar during odd (after application of LEU) and even (before application of LEU) bursts of RES.

Application of 20 bursts of RES was accompanied by increased sensomotor cortical excitability in animals of the control group. This was manifested as a significant fall of the thresholds of DR and TCR (Fig. 3a, b). Stimulation that was previously below threshold for TCR for electrical stimulation of the cortex, after 20 bursts of RES caused the appearance of an appreciable TCR (Fig. 3c). The amplitude of DR after 20 bursts of RES was almost doubled compared with DR before stimulation (post-tetanic potentiation; Fig. 3c, control). On combination of RES with application of LEU, the poststimulus (after 20 bursts of RES) lowering of the thresholds of DR and TCR observed in the control and also potentiation of the amplitudes of DR and TCR were not observed (Fig. 3).

The data given above on the effect of exogenous LEU on the parameters of AD enable the functional role of endogenous LEU, released from cortical neurons during RES [3] to be assessed. LEU is not involved in the initiation of seizure activity in the rat sensomotor cortex. Neither application of LEU to the sensomotor cortex (Fig. 2a) nor injection of naloxone [3], a blocker of opiate receptors, had any effect on the latency of appearance of AD. The functional role of LEU release from neurons during cortical hyperactivity evidently consists of limitation of the intensity (Fig. 2b) and duration (Figs. 1 and 2) of seizure activity and prevention of the appearance of a state of increased predisposition to seizures. Similar conclusions regarding the role of opioid peptides in epileptogenesis have been drawn from the study of other models of epilepsy [5].

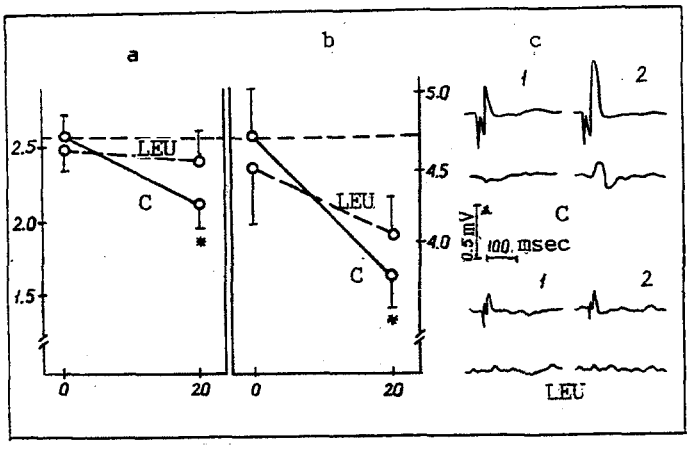

Fig. 3. Effect of Leu-enkephalin on thresholds of DR and TCR in rat sensomotor cortex. Abscissa, times of testing thresholds: 0) before application of RES, 20) after application of 20 bursts of RES. Ordinate, thresholds (in V) for DR (a) and TCR (b). C) Application of physiological saline to cortex, LEU – application of Leu-enkephalin. c) Traces of DR (above) and TCR (below) before (1) and after (2) application of 20 bursts of RES. Intensity of stimulation the same in 1 and 2: above threshold for DR, but below threshold for TCR (before application of RES). Thresholds of DR (a) and TCR (b) in control after 20th burst of RES significantly (*$p < 0.05$) below prestimulus level in the same animals.

The evident difference in sensitivity of evoked (DR and TCR) and self-maintained activity to the action of LEU may be partly connected with the fact that these potentials are generated by different groups of neurons. In response to direct cortical stimulation, neurons of the surface layers ought to be involved in the first instance, whereas neurons of layer 4 are predisposed for generation of seizure activity [6].

The targets of action of LEU under our experimental conditions are evidently trace processes in neurons. We know that postictal trace processes can activate depolarization (calcium) and hyperpolarization after-potentials [11], stable shifts of membrane potential [11], and long-term (4 h) phase changes of seizure activity. Our experiments revealed poststimulus potentiation of evoked responses of the sensomotor cortex (Fig. 3c). Evidence of more prolonged trace processes, exceeding the 10-min interval between bursts of RES, is given by potentiation of the amplitudes and duration of AD in the course of bursts of cortical RES (Fig. 1a, 2b, c). As was pointed out above, application of LEU is accompanied by effective suppression both of potentiation of evoked responses and of potentiation of self-maintained seizure activity.

The mechanism of action of LEU on these processes is evidently linked with suppression of Ca^{2+} inflow into the neurons. It has been shown that LEU and other opioid peptides, interacting with G protein, suppress Ca currents [10, 16]. Under the influence of a structural analog of LEU in the rat brain, including in the cortex, the calcium concentration, but not that of monovalent cations, falls [8]. Opiates and opioid peptides induce a fall in the calcium concentration in synaptosomes [4]. Conversely, long-term potentiation of evoked responses [12] and epileptization of neurons [9] require a greater intake of Ca^{2+} into neurons [7]. A probable mechanism of the anticonvulsant action of LEU in the sensomotor cortex may thus perhaps be its effect on calcium homeostasis of neurons.

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