DEPRESSION OF ACETYLCHOLINESTERASE ACTIVITY IN THE REGION OF EPILEPTOGENIC FOCI CREATED BY MET-ENKEPHALIN IN THE RAT HIPPOCAMPUS

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Injection of opiate peptides into the cerebral ventricles of rats causes the appearance of pathological activity, characteristic of epilepsy, recordable on the EEG in derivations from the cerebral cortex and many deep brain structures belonging chiefly to the limbic system [11, 15]. The same epileptiform activity (EA) can be obtained by microinjections of peptides into the cerebral cortex, caudate nucleus, hippocampus, and dorsomedial thalamic nuclei [9, 10]. However, the main structure responsible for the appearance of EA after injection of opiate peptides into the cerebral ventricles of rats is considered to be the hippocampus since, first, the strongest EA appears in that structure and is still found even after removal of the region of the medial thalamus and amygdaloid nuclei [11] and, second, when opiate peptides are applied by iontophoresis to neurons in different brain structures these substances have a mainly excitatory action on the pyramidal cells of the hippocampus, whereas they inhibit neurons of other structures [12, 13].

Incidentally, opiate peptides also excite Renshaw cells of the spinal cord [8], and they, like hippocampal pyramidal neurons, increase their activity under the influence of acetylcholine (ACh). It must be emphasized that the inhibitory hippocampal basket cells, whose mediator is presumed to be GABA, are also sensitive to ACh [5, 6].

Since the mechanism of the epileptizing action of opiate peptides on the hippocampal pyramidal cells has not been explained, it was decided to study acetylcholinesterase (AChE) activity in the region of an epileptogenic focus (EF) created by microinjections of met-enkephalin or its longer-acting analog D-alanine-2-met-enkephalin, into the dorsal hippocampus of rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats of both sexes weighing 180-220 g, divided into five groups with 6-10 animals in each group. Ionization electrodes (IE) were implanted into the left and right dorsal hippocampus of the animals by means of an SEZh-2 universal stereotaxic apparatus. The design of the IE and the method of their implantation were described in detail by Mikhailov [5].

An EF was created in the right hippocampus by injection of 0.002 ml of an aqueous solution of met-enkephalin (20-40 μg) or of D-alanine-2-met-enkephalin (40 μg) through the IE (by means of a micromanipulator). The pentapeptides were synthesized on the polymer carrier Sephadex LH-20 [3]. The carboxyl group of the tertiary butyl-hydroxycarbonylamino acids was activated by the symmetrical anhydrides method and the carbodiimide method.

The homogeneity of the products thus obtained was verified electrophoretically, by column and thin-layer chromatography and by amino-acid analysis.

AChE activity was determined spectrophotometrically by Lobanov's method [4]. The principle of the method is hydrolysis of thiocholine substrates by AChE. The velocity of enzymic

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TABLE 1. Changes in AChE Activity during Development of EF in Rat Hippocampus

Experimental conditions	Number of experiments		AChE activity, μmoles substrate/min/ml homogenate	
	LH	RH	LH	RH
Intact rats	10	10	32,8	33,4
Control rats	8	8	29,8	30,1
Experimental rats				
5 min after creation of EF	10	10	26,1*	26,6*
30 min after creation of EF	8	8	26,1*	26,3*
90 min after creation of EF	6	6	26,9*	26,5*

Legend: RH) right hippocampus (primary focus), LH) left hippocampus (mirror focus). *$P < 0.05$.

hydrolysis of the substrate used (ACh) was estimated from the degree of reduction of 2,6-dichlorophenol-indophenol (DCPIP) by SH-groups of thiocholine formed by the reaction of ACh hydrolysis. The decrease in absorption of DCPIP every minute was recorded on an SF-16 spectrophotometer for 5 min at 600 nm.

AChE activity was determined in the hippocampus of intact rats, rats of the control group with IE implanted in the hippocampus after injection of bidistilled water into that structure, and 5, 30, and 90 min after the injection, and rats of the experimental group at the same time intervals as in the control but after injection of the peptides into the hippocampus.

The location of IE was verified histologically. The experimental results were subjected to statistical analysis using the Wilcoxon—Mann—Whitney tests.

EXPERIMENTAL RESULTS

After injection of solutions of both peptides into the hippocampus, increased motor and investigative activity of the rats was observed for 10-20 min, followed by a state of inhibition accompanied by some muscular rigidity, which lasted 40-60 min. When spikes were recorded on the EEG, the animals' muscles twitched all over the body.

Single high-amplitude epileptiform discharges (200-250 μV) appeared on the EEG immediately after injection of the opiate peptides. From 8 to 12 min after injection of the two peptides, the first electrographic correlate of the epileptiform seizure (ES) could be observed on the EEG, where it was recorded in both parts of the hippocampus with equal intensity, and consisted of continuous, prolonged (5-16 sec) hypersynchronous discharges with a frequency of 5-7 Hz and an amplitude of 170-250 μV.

The number of seizures during the first 80 min of the experiment averaged two in the course of 10 min of observation. Starting from the 80th-90th minute of the experiment the seizures ceased.

The number of interictal epileptiform discharges (IED) per minute of continuous EEG recording in each 10-min interval of the experiment was 30-60 from 0 to the 40th minute and 30-40 from the 40th to the 120th minute. The parameters of IED and ES given above were characteristic of EF created both by met-enkephalin and by D-ala-2-met-enkephalin.

EA of the foci created by D-ala-2-met-enkephalin ceased completely 4 h after their creation. The EF created by injection of met-enkephalin functioned for 100-120 min, in agreement with data of other workers [9], who injected leu-enkephalin into the hippocampus of cats and rats.

The results of the study of behavioral electroencephalographic manifestations of pathological activity of "enkephalin-induced" EF made it possible to choose, in order to determine AChE activity, those time intervals of their functioning which reflect the most characteristic stages of epileptogenesis: 1) the 5th minute from the time of injection of the peptides into the hippocampus — this is the initial stage of development of EF, when only IED

are recorded on the EEG; 2) the 30th minute reflects the period of maximal intensity and number of IED and ES; 3) 90 min after creation of the EF, ES were no longer recorded on the EEG and the IED were reduced in amplitude and number.

The data given in Table 1 show that AChE activity in the hippocampus of the control animals was a little lower ($P > 0.05$) after repeated injection of bidistilled water into this brain structure than in intact rats. AChE activity in different periods of functioning of the EF was about equally significantly reduced compared with the control experiments.

Lowering of AChE activity in the mirror foci of the experimental animals, incidentally, was the same as in the primary foci.

These experiments revealed a significant decrease in AChE activity in the region of EF created in the rat hippocampus by microinjections of met-enkephalin or of D-alanine-2-met-enkephalin.

The authors previously showed that ACh activity is lowered in EF created by injection of penicillin into the rat hippocampus [2], from which it can be concluded that a fall in AChE activity in hippocampal EF is a regular feature.

It has been suggested [7, 11, 14] that the cause of the epileptizing action of opiate peptides on the hippocampus is the weakening by these substances of the inhibitory effect of basket cell interneurons on pyramidal neurons of the hippocampus. It can be tentatively suggested that the fall in AChE activity in the region of EF takes place, not because of a decrease in ACh synthesis and release (this is difficult to imagine in EF) but, on the contrary, because it is an adaptive mechanism, aimed at strengthening the effects of the neurotransmitter in the region of EF. The result is not so much excitation of pyramidal neurons as stimulation of the acetylcholine-sensitive hippocampal basket cells, and it is aimed at terminating the pathological hypersynchronous activity of the epileptized pyramidal cells. Stimulation of basket cells, leading to increased liberation of GABA, their hypothetical inhibitory mediator, may perhaps counteract the action of enkephalins in "blocking" the endings of these cells.

This hypothesis is based, in particular, on the fact that microinjections of small doses of muscarinic and nicotinic cholinomimetics (0.05 μg) into the hippocampus prevent epileptization of neurons of that structure by penicillin [1].

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