ROLE OF CYCLIC NUCLEOTIDES IN THE MECHANISM OF ACTION OF ENKEPHALINS

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Recent investigations have demonstrated the marked effect of enkephalins on functions of the CNS and, in particular, on learning and memory [1, 6, 9, 14]. However, the mechanisms of their activating and inhibitory action on conditioned-reflex activity have so far received little study. Considering that many neuropeptides have only a short half-life, the role of intermediate components in the realization of their effect on brain functions may be postulated. It has been suggested [13, 14] that under the influence of ACTH and other polypeptide hormones changes take place in cyclic AMP synthesis in the tissues. However, data so far published on changes in the cyclic nucleotide level in the tissue under the influence of neuropeptides are contradictory and are based mainly on the results of experiments *in vitro* [5, 8, 11] or were obtained by the use of test objects such as neuroblastoma, hybrid glioma cells [7], and the cerebral ganglia of mollusks [10, 12].

It was accordingly decided to study the effect of leu-enkephalin, a peptide with very short half-life [2], on the concentrations of cyclic AMP and cyclic GMP in individual brain structures of rats.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-170 g were given a subcutaneous injection of 5 μg leu-enkephalin in 0.2 ml physiological saline. Control rats received an injection of the same volume of 0.85% NaCl solution. The rats were killed 5 min or 1 h later by immersion in liquid nitrogen. The brain structures isolated (sensomotor cortex and hippocampus) were additionally frozen in nitrogen, weighed, and homogenized in EDTA buffer. After extraction and centrifugation the concentrations of cyclic AMP and cyclic GMP in the supernatant were determined by a radioimmunologic method. Standard kits (from the Radiochemical Center, Amersham, England) were used. The cyclic AMP and cyclic GMP concentrations were expressed in pmoles/mg tissue or protein, determined by Lowry's method. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney method.

TABLE 1. Cyclic AMP Content in Cerebral Cortex and Hippocampus of Rats after Injection of Leu-Enkephalin

Experimental conditions	Cortex		Hippocampus	
	time after injection of leu-enkephalin, min			
	5	60	5	60
	pmoles/mg tissue			
Control (n = 10)	0,90		0,78	
Leu-enkephalin (n = 10)	0,86	0,91	0,77	0,64*
% of control	95,5	101,1	98,7	82,0
	pmoles/mg protein			
Control (n = 10)	7,40		6,10	
Leu-enkephalin (n = 10)	6,38*	7,71	5,88	4,83*
% of control	86,0	104,2	96,4	79,0

Legend. Here and in Table 2, asterisk denotes $P < 0.05$.

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TABLE 2. Cyclic GMP Content in Cerebral Cortex and Hippocampus of Rats after Injection of Leu-Enkephalin

Experimental conditions	Cortex		Hippocampus	
	time after injection of leu-enkephalin, min			
	5	60	5	60
	pmoles/mg tissue			
Control (n = 10)	0,15		0,15	
Leu-enkephalin (n=10)	0,15	0,09*	0,13	0,12*
% of control	100	60	87	80
	pmoles/mg protein			
Control (n=10)	2,41		2,44	
Leu-enkephalin (n=10)	2,65	2,0	2,03*	1,87*
% of control	110	83	83	77

EXPERIMENTAL RESULTS

The cyclic AMP concentration in the cerebral cortex (per milligram tissue) was not significantly changed either 5 min or 1 h after injection of leu-enkephalin (Table 1). The cyclic AMP level in the hippocampus remained stable after 5 min, but was significantly reduced after 1 h. Similar results were obtained when the cyclic AMP content was calculated per milligram protein. In this case the fall in the cyclic AMP level in the cerebral cortex in the early stages after injection of the peptide was more clearly demonstrable.

Changes in the cyclic GMP level in different parts of the brain under the influence of leu-enkephalin are given in Table 2. The cyclic GMP content (per milligram tissue) in the cortex was unchanged 5 min after injection of the peptide, but it was significantly lowered (by 40%; $P < 0.05$) 1 h after the injection. A fall in the cyclic GMP level was also observed in the hippocampus at all times, and these changes were significant after 1 h. The cyclic GMP content per milligram protein also was significantly reduced in the hippocampus ($P < 0.05$) both 5 min and 1 h after injection of leu-enkephalin.

The results are evidence that the cyclic nucleotide level in the brain is lowered by leu-enkephalin. The changes were more marked in the hippocampus in the late stages (1 h) after injection of the peptide.

These changes correspond in timing to the effects observed after injection of neuropeptides into the animals and, in particular, when their effect on learning and memory processes is studied.

In previous investigations a stimulating effect on the consolidation of temporary connections and of protein synthesis in the rat hippocampus also was observed 1 h after subcutaneous injection of 25 μg lysylvasopressin deglycylamide [4].

These effects of enkephalins, observed in the late stages after injection, are difficult to explain by their direct effect on temporary connection fixation and on activation of the protein-synthesizing system of the brain. The half-life of many neuropeptides is known to be measured in minutes and, in particular, for leu-enkephalin it is 1 min [2]. It can accordingly be postulated that the action of the neuropeptides is indirect.

Considering the known views on the role of cyclic AMP in the mechanism of action of hormones and neurotransmitters as a second mediator, and also the results now obtained, it can be tentatively suggested that the effect of neuropeptides and, in particular, of enkephalins on CNS functions is realized indirectly through the cyclic nucleotide system.

The experimental results confirm the hypothesis [13, 14] that cyclic AMP participates in the mechanism of action of peptides, and also data on enkephalins as adenylate cyclase inhibitors [7]. The authors cited observed opposite changes in the cyclic AMP and cyclic GMP content under these circumstances in neuroblastoma cells under the influence of opiates. This fact was not confirmed in the present experiments. After injection of leu-enkephalin the content of both cyclic AMP and cyclic GMP in the hippocampus was reduced. These differences may be attributed both to differences in the action of exogenous opiates and enkephalins on the cyclic GMP system and also differences in the test objects.

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SOME PROPERTIES OF FRUCTOSE-1,6-DIPHOSPHATE ALDOLASE FROM HUMAN MUSCLES IN ATHEROSCLEROSIS

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The study of enzyme activity actually in human tissues in patients with atherosclerosis is very interesting. Characteristics of fructose-1,6-diphosphate aldolase (FDA), obtained in a purified form, could help to shed light on some aspects of the pathogenesis of this disease.

In the present investigation, in order to isolate and study the properties of human muscle aldolase, it was first necessary to work out a method of obtaining FDA in crystalline form, which involved the taking up of a new experimental approach based on the use of human tissue obtained at autopsy.

EXPERIMENTAL METHOD

Muscles obtained at autopsy were used to isolate FDA. The material was taken 12-18 h after death, which occurred as a result of automobile and railroad accidents. The experimental group consisted of subjects with the typical morphological features of atherosclerosis, unaccompanied by any other acute or chronic physical diseases. The control group consisted of persons with no pathological manifestations whatsoever. Using the technical approach suggested by Gulyi [1], the conditions were chosen for isolation and crystallization of FDA from human muscles. FDA was isolated in crystalline form from a common muscle extract for glyceraldehyde phosphate dehydrogenase and FDA. Maximal transfer of FDA into the residue free from ballast proteins and other enzymes took place at pH 6.0-6.25 and with a 0.55% degree of saturation with ammonium sulfate. At all stages of isolation and purification, activity of the enzyme was verified spectrophotometrically [2].

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