EFFECT OF (L-Phe$_7$) AND (D-Phe$_7$) ACTH$_{4-7}$ AND A LONG-ACTING ACTH$_{4-7}$ ANALOG ON RAT BRAIN ACETYLCHOLINESTERASE ACTIVITY

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Fragments of ACTH are known to affect the behavioral activity of animals. In particular the ACTH$_{4-7}$ tetrapeptide (Met-Glu-His-Phe), in microgram doses, acts on the learning process in rats without exhibiting any hormonal properties [1, 7].

Following the results of investigations of correlation between learning and acetylcholinesterase (AChE) activity of specific rat brain structures [2, 3] we studied the effect of (L-Phe$_7$) and (D-Phe$_7$) ACTH$_{4-7}$, and also of a long-acting ACTH$_{4-10}$ analog (Met-Glu-His-Phe-Pro-Gly-Pro) [4] on rat brain AChE activity after intraperitoneal and subcutaneous injection.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 100-150 g. The rats were killed and the brain quickly removed. In the cold the cortex and white matter of the cerebral hemispheres, the hippocampus, brain stem, and cerebellum were separated and homogenized in phosphate buffer (pH 7.4) at the rate of 1 mg tissue to 1 ml, and centrifuged at 3000 rpm for 10 min. AChE activity in the supernatant was determined by Ellman's method [8]. Immediately before testing the oligopeptides were dissolved in sterile physiological saline and injected from a microsyringe into the animals in a dose of 0.5 ml. The (L-Phe$_7$) and (D-Phe$_7$) ACTH$_{4-7}$ were synthesized by the method described previously [5]. Control animals were given injections of physiological saline without the oligopeptide.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of intraperitoneal injection of (L-Phe$_7$) ACTH$_{4-7}$ on AChE activity in different parts of the rats' brain was studied depending on the dose injected. Injection of fragment ACTH$_{4-7}$ was found to activate brain AChE. The stimulant effect of ACTH$_{4-7}$ was exhibited mainly 30 and 60 min after its injection in a dose of 150-300 μg/kg. In this case the white matter displayed greater sensitivity to ACTH$_{4-7}$. Meanwhile there was a general tendency toward an increase in AChE activity in all parts of the brain studied (Fig. 1).

Comparison of the stimulant effect of (L-Phe$_7$) ACTH$_{4-7}$ on brain AChE activity after intraperitoneal and subcutaneous injection showed (Fig. 2) that more marked effects of the oligopeptide can be obtained with subcutaneous injection, in agreement with data in the literature [1]. In the subsequent experiments the oligopeptides were therefore injected subcutaneously.

The results of the experiments with (L-Phe$_7$) and (D-Phe$_7$) ACTH$_{4-7}$ showed that the effect of ACTH$_{4-7}$ on AChE activity depends on the length of exposure of the animals treated with oligopeptides and on the stereoisomerism of the oligopeptide. As Fig. 2 shows, (L-Phe$_7$) ACTH$_{4-7}$ stimulated AChE activity in all parts of the

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TABLE 1. Effect of Subcutaneous Injection of Mixture of Amino Acids Equimolar with $ACTH_{4-7}$ on AChE Activity in Various Parts of Rat Brain (M ± m)

Experimental condition	Cortex	White matter	Hippocampus	Cerebellum	Brain stem
Control	1,79±0,06	2,33±0,12	1,62±0,12	1,50±0,06	3,53±0,30
30 min after injection of amino acid mixture	1,86±0,60	2,90±0,09*	1,60±0,12	1,56±0,60	3,59±0,02

Legend. Here and in Table 2 AChE activity is expressed in moles acetylcholine ($\times 10^{-4}$) per gram wet weight of tissue; *P < 0.05.

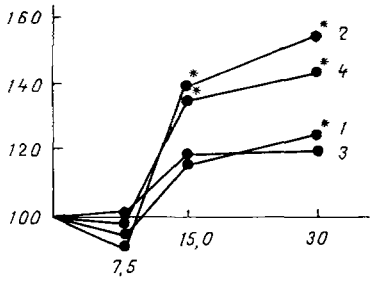

Fig. 1

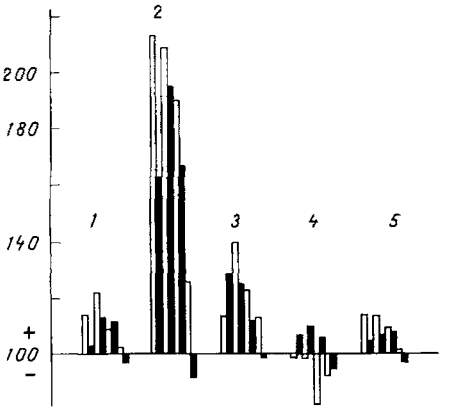

Fig. 2

Fig. 1. Effect of intraperitoneal injection of (L-Phe_7) $ACTH_{4-7}$ on AChE activity of the cerebral cortex (1) and white matter (2), cerebellum (3), and hippocampus (4) of rat brain 30 min after injection of oligopeptide. Abscissa, doses of oligopeptide injected (in μg/100 g body weight); ordinate, AChE activity (in % of control, taken as 100). *P < 0.05.

Fig. 2. Effect of subcutaneous injection of L-isomer (unshaded columns) and D-isomer (black columns) in a dose of 15 μg/100 g body weight on AChE activity of cerebral cortex (1) and white matter (2), hippocampus (3), cerebellum (4), and brain stem (5) of rats 30 min (first pairs of columns), 60 min (second pairs of columns), 120 min (third pairs of columns), and 24 h (fourth pairs of columns) after injection of oligopeptide. Remainder of legend as to Fig. 1.

brain studied except the cerebellum. This was seen particularly clearly 30 and 60 min after injection of the oligopeptides. After exposure of 2 h the stimulating effect on AChE activity decreased and the initial level was reached after 24 h. The exception was the white matter of the cerebral hemispheres: AChE activity after an exposure of 24 h was once again significantly raised by 20-25%.

(D-Phe_7) $ACTH_{4-7}$, like its L-isomer, also stimulated AChE but a lesser degree (Fig. 2); for example, whereas 1 h after injection of (L-Phe_7) $ACTH_{4-7}$ AChE activity in the white matter was increased by more than 100%, its D-isomer activated the action of the enzyme by only 60%. The stimulating effect 2 h after injection of the D-isomer was 90% compared with the control, taken as 100.

The effect of the long-acting $ACTH_{4-10}$ analog on AChE activity is particularly interesting. There is reason to suppose that this analog, because of its increased resistance to protease hydrolysis, possesses greater physiological efficacy. It has been shown that this analog is distinguished also by greater biochemical activity. AChE activity in the white matter of the cerebral hemispheres 30 min after its subcutaneous injection in a dose of 150 μg/kg was increased by 120%. The positive effect of the oligopeptide on AChE activity also persisted significantly after an exposure of 24 h, but to a lesser degree. Unlike in the white matter, AChE activity in the hippocampus was increased after exposure of the animals for 30 and 60 min by 30 and 38% respectively, and reached the initial level after 24 h (Fig. 3).

TABLE 2. Effect of Subcutaneous Injection of Actinomycin D and (L-Phe$_7$) ACTH$_{4-7}$ on AChE Activity in White Matter of Cerebral Hemispheres of Rats (M ± m)

Control	Actinomycin D	Actinomycin D + ACTH$_{4-7}$	ACTH$_{4-7}$
2,32±0,06	2,31±0,05	2,39±0,07	5,22±0,27*

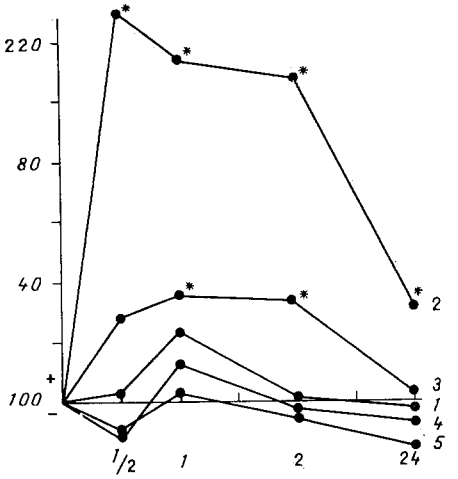

Fig. 3. Effect of subcutaneous injection of long-acting ACTH$_{4-10}$ analog in a dose of 15 μg/g body weight on rat brain AChE activity. Abscissa, length of exposure (in h; ordinate, AChE activity (in %). Remainder of legend as to Fig. 2.

Investigation of the effect of a mixture of amino acids equimolar with ACTH$_{4-7}$, injected subcutaneously, showed that this mixture had no effect on AChE activity of the cerebral cortex, hippocampus, or brain stem but stimulated activity of the enzyme in the white matter by 20-24% (Table 1). Activation of AChE by the oligopeptide exceeded the corresponding effect of the amino-acid mixture about fivefold. Consequently, it can be concluded that the increase in AChE activity under the influence of ACTH$_{4-7}$ is the result of the action of the oligopeptide itself, and not of its hydrolytic degradation products.

Meanwhile, the quantitative increase in the brain AChE activity with time after subcutaneous injection of ACTH$_{4-7}$ is evidence of induction of synthesis of the enzyme in the brain. In experiments in vitro ACTH$_{4-7}$, in a concentration of between 7 and 30 μg/3 ml, had no significant effect on AChE activity of a homogenate of whole rat brain. Accordingly, in the next series of experiments the effect of ACTH$_{4-7}$ was studied on AChE activity after preliminary treatment of the animals with the transcription inhibitor actinomycin D. The antibiotic was injected subcutaneously (150 μg/100 g body weight) 30 min before injection of the oligopeptide, and after the subsequent 30 min of exposure the AChE activity was measured in the white matter of the cerebral hemispheres. The results showed that actinomycin D completely inhibits the stimulating effect of ACTH$_{4-7}$ on AChE activity of the white matter of the cerebral hemispheres (Table 2). On the basis of these data it was concluded that the increase in AChE activity under the influence of oligopeptides was due to induction of synthesis of new AChE molecules. Restoration of the original level of enzyme activity 24 h after treatment of the animals with the oligopeptides indicates that it is the AChE isozyme with the shortest half-life that was responsible for increased activity of the enzyme. According to data in the literature, this half-life for one functional AChE isozyme does not exceed 3 h [6].

Unfortunately it is difficult as yet to judge the mechanisms of controlled induction of AChE synthesis at the level of the genetic apparatus.

It can thus be concluded that the positive effects of ACTH$_{4-7}$ and of the long-acting ACTH$_{4-10}$ analog on learning and memory processes must be linked with changes in the acetylcholine system. The fact that 24 h

after injection of the oligopeptide ability of the rats to remember was again maintained at a high level, when AChE activity regained its initial value (Figs. 2 and 3), indicates that changes in the acetylcholine system must play an essential role only in the initial phase of memory fixation. It is more likely that the increase in AChE activity under the influence of oligopeptides after 30 and 60 min creates favorable conditions for effective functioning of the consolidation apparatus [3].

LITERATURE CITED

1. V. M. Vinogradov, N. I. Medvedev, A. T. Grachko, et al., Fiziol. Zh. (Ukr.), 16, 409 (1980).
2. P. A. Kometiani, N. G. Aleksidze, and E. Ê. Klein, Neurochemical Aspects of Memory [in Russian], Tbilisi (1980).
3. R. I. Kruglikov, Neurochemical Mechanisms of Learning and Memory [in Russian], Moscow (1981).
4. M. A. Ponomareva-Stepnaya, V. N. Nezavibat'ko, I. P. Ashmarin, et al., Inventor's Certificate No. 939440 (USSR) (1981).
5. M. A. Ponomareva-Stepnaya, L. Yu. Alferova, L. A. Maksimova, et al., Khim.-Farm. Zh., No. 10, 37 (1981).
6. G. A. Davis and B. W. Agranoff, Nature, 220, 227 (1968).
7. D. de Wied, A. Witter, and H. M. Greven, Biochem. Pharmacol., 24, 1463 (1975).
8. G. L. Ellman et al., Biochem. Pharmacol., 7, 88 (1961).

REDUCTION IN THE TISSUE ASCORBIC ACID LEVEL IN GUINEA PIGS BY THALIDOMIDE

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Many investigations have been devoted to a study of the mechanisms of the teratogenic action of thalidomide (T) [2, 16]. Nevertheless the problem is still unsolved after more than 20 years. It has been postulated that fetal malformations are caused by T itself [9, 14]. Yet at the same time it has been shown that T can undergo both aqueous hydrolysis and biotransformation in vivo in mammals [8, 12]. It has accordingly been suggested that degradation products of T have a teratogenic action, that they are antagonists of glutamine, glutamate, or folic acid [2, 16], that they are toxic arenoxides [10], and that they inhibit protocollagen proline hydroxylase and thereby inhibit collagen formation and disturb embryonic limb development [12]. The species-specificity of the action of T (mice and rats are insensitive to this teratogen, whereas monkeys, man and, to a lesser degree, rabbits are sensitive) has been linked with species differences in its metabolism [15]. The possibility cannot be ruled out that the target of T is the connecrive tissue of the limb anlagen [18], an essential element of whose composition is collagen. The rate of collagen synthesis is known to depend largely on the ascorbic acid concentration in the tissue [4].

It was accordingly decided to study the effect of T on the ascorbic acid concentration in the organs of guinea pigs which, like primates, cannot synthesize this vitamin [4]. Since induction of enzymes of the liver microsomal fraction can sharply increase the rate of ascorbic acid metabolism [7], the action of T on microsomal hydroxylase activity from the liver of several species of mammals also was studied in experiments in vivo and in vitro. On the basis of the results a mechanism of the teratogenic action of T, capable of experimental verification, was put forward.

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