EFFECT OF (L-Phe) AND (D-Phe) ACTH₁₋₇ AND A LONG-ACTING ACTH₁₋₁₀ 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 ACTH₁₋₇ 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) and (D-Phe) ACTH₁₋₇, and also of a long-acting ACTH₁₋₁₀ 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) and (D-Phe) ACTH₁₋₇ 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) ACTH₁₋₇ on AChE activity in different parts of the rats' brain was studied depending on the dose injected. Injection of fragment ACTH₁₋₇ was found to activate brain AChE. The stimulant effect of ACTH₁₋₇ 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 ACTH₁₋₇. 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) ACTH₁₋₇ 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) and (D-Phe) ACTH₁₋₇ showed that the effect of ACTH₁₋₇ 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) ACTH₁₋₇ stimulated AChE activity in all parts of the
TABLE 1. Effect of Subcutaneous Injection of Mixture of Amino Acids Equimolar with ACTH₄₋₇ on AChE Activity in Various Parts of Rat Brain (M ± m)

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Cortex</th>
<th>White matter</th>
<th>Hippocampus</th>
<th>Cerebellum</th>
<th>Brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.79±0.06</td>
<td>2.33±0.12</td>
<td>1.62±0.12</td>
<td>1.50±0.06</td>
<td>3.53±0.30</td>
</tr>
<tr>
<td>30 min after injection of amino acid mixture</td>
<td>1.86±0.60</td>
<td>2.90±0.09*</td>
<td>1.60±0.12</td>
<td>1.56±0.60</td>
<td>3.59±0.02</td>
</tr>
</tbody>
</table>

Legend. Here and in Table 2 AChE activity is expressed in moles acetylcholine (x10⁻⁴) per gram wet weight of tissue; *P < 0.05.

Fig. 1. Effect of intraperitoneal injection of (L-Phe₇) ACTH₄₋₇ 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-Phe₇) ACTH₄₋₇, like its L-isomer, also stimulated AChE but a lesser degree (Fig. 2); for example, whereas 1 h after injection of (L-Phe₇) ACTH₄₋₇ 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 ACTH₄₋₁₇ 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).