ACETYLCHOLINE AND THE LIPOLYTIC ACTIVITY OF BRAIN TISSUE

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The main chemical component of the humoral mechanism of transmission of the activity of cholinergic nerve elements, acetylcholine (AC), besides possessing its mediator function, also plays the part of a regulator of some aspects of metabolism [2-5]. Because of the great importance of lipids in nerve tissue, it is important to remember that AC, in particular, stimulates the incorporation of the phosphate group into some phospholipids of nerve tissue [7]. As yet, however, no information has been obtained regarding its relationship to the lipolytic processes in the nervous system.

For this reason, a series of experiments was undertaken to study the effect of AC on the lipolytic activity of brain tissue homogenate.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing 180-200 g. After decapitation of two or three animals, their brain (without the cerebellum) was quickly extracted, washed with physiological saline (0.9% NaCl), freed from its meninges and large blood vessels on ice, dried with filter paper, and quickly suspended. Samples of the total suspension weighing 2-3 g were homogenized for 2 min in McIlwain's buffer, in a ratio of 10:1 to the tissue; the pH of the solution was between 6.8 and 7.8 depending on the purpose of the experiment. To inhibit cholinesterase activity, a solution of eserine sulfate in a final concentration of $1 \cdot 10^{-5}$ g/ml was added to the suspensions of tissue homogenate. The lipolytic activity was determined by the method of Fiore and Nord [6], based on titration of the free fatty acids formed by the action of the lipolytic enzyme. The substrate for the lipolytic reaction was an emulsion of sunflower oil, prepared with the use of polyvinyl alcohol as emulsifying agent. The suspension of brain tissue homogenate thus obtained was used for preparing several samples. The reaction mixture in each sample was as follows: 2.5 ml brain tissue homogenate suspension, 5 ml of the freshly prepared emulsion of sunflower oil, 2.4 ml of buffer solution, and 0.1 ml of AC solution in the concentration to be investigated; the total volume of the mixture was 10 ml. Parallel with the samples containing AC, control samples were used, including the same ingredients except for the AC solution, instead of which an equal volume of bidistilled water was added. The samples with AC and the controls were incubated at 37° for 2 h in a water thermostat, with constant mixing. At the end of the experiment, the enzyme reaction was stopped by adding a mixture of alcohol and acetone (1:1). At the beginning and the end of incubation, the pH of the medium was verified with a glass electrode. The samples were titrated with a 0.05 N solution of NaOH in the first minute after addition of AC, and 1 h and 2 h after the beginning of incubation. The numerical results (the mean results of two parallel determinations) were expressed as the number of milliliters of 0.05 N NaOH solution used up in titrating the samples. The statistical analysis of the results was carried out by means of the formulas

$$\sqrt{\frac{\sum(\Delta x)^2}{n(n-1)}} = \sigma_{\Delta x}, \quad t = \frac{\Delta x}{\sigma_{\Delta x}}.$$

The differences between the mean results of titration of the control samples and of the samples with AC were taken as significant when $P < 0.01$.

EXPERIMENTAL RESULTS

The control determination of the pH showed that, in these experimental conditions during incubation of the samples, no change took place in the pH of the medium. In special experiments with samples containing a boiled suspension of brain tissue homogenate, no increase in acidity was observed during incubation. Consequently, the changes in acidity in the experiments with incubation of suspension of untreated homogenates were in fact due to

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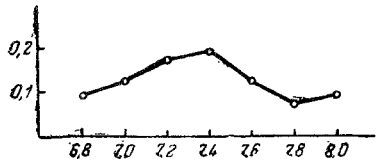

Fig. 1. Relationships between lipolytic activity of homogenate of rats' brain tissue and pH. Along the axis of ordinates – increase in titration results 1 h after beginning of incubation (in ml of 0.05 N NaOH solution); along the axis of abscissas – pH.

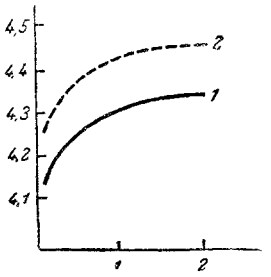

Fig. 2. Effect of acetylcholine in a concentration of $1 \cdot 10^{-4}$ g/ml on lipolytic activity of homogenates of rats' brain tissue: 1) samples with acetylcholine; 2) control samples. Along the axis of ordinates – volume of 0.05 N NaOH solution (in ml); along the axis of abscissas – time (in hours).

enzyme activity. The other control experiments showed that, in the samples incubated with the addition of eserine alone in a final concentration of $1 \cdot 10^{-6}$ g/ml, without addition of AC, eserine had no effect on the lipolytic activity of the homogenate of rats' brain tissue.

Little attention has hitherto been paid to the study of the lipolytic activity of brain tissue. For this reason, as a first step a series of experiments was carried out to determine its pH optimum in the chosen conditions. The mean results obtained are shown in Fig. 1. They demonstrate that the optimal pH of lipolytic activity was 7.2-7.4. For this reason, in the subsequent experiments, the pH of the reaction medium was maintained at 7.4.

The experiments with AC were carried out to show how it acts on lipolytic activity when added in the following final concentrations: $1 \cdot 10^{-8}$, $1 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, and $1 \cdot 10^{-3}$ g/ml. They showed that AC has a marked effect on the liberation of fatty acids only in a concentration of $1 \cdot 10^{-4}$ g/ml. The mean results of the experiments with AC in this concentration are given in Fig. 2. It is clear that AC in these conditions produced a decrease in the already weak lipolytic activity of the brain tissue homogenate. This result was obtained in 14 of the 15 experiments (in one experiment AC had no effect). The fact was noted that this inhibition was slight, yet it developed quickly after the addition of AC to the sample, in the first minutes of its action. The statistically significant difference which developed in these experiments between the control samples and the samples with AC on the average remained practically unchanged during further incubation, and determinations made 1 h and 2 h after the beginning of incubation gave the same results as before. The lipolytic activity was also slightly inhibited by AC in a concentration of $1 \cdot 10^{-5}$ g/ml. In this case, however, the difference between the results of titration of the samples with AC and the control samples was not statistically significant, although it was reduced in the samples with AC in 9 of the 11 experiments.

An attempt was also made to discover whether an increase in the concentration of endogenous AC in the brain tissue has any effect on its lipolytic activity in experiments in vivo. For this purpose, the experimental rats received a subcutaneous injection of phosphacol, an inhibitor of cholinesterase activity, in the form of a 0.01% acetone solution in a dose of 0.2 mg phosphacol/kg body weight. After this injection, no external signs of changes in their state were observed (injection of a slightly larger dose of phosphacol was followed by convulsions). The animals were sacrificed 1.5 h after the injection of phosphacol. No difference was found between the lipolytic activity of the brain tissue of the rats receiving phosphacol injection and of the control animals. It is possible that in these experimental conditions, the mean AC concentration in the brain tissue did not reach a high enough value for the manifestation of its inhibitory effect.

It may be concluded from these preliminary experiments with homogenates of whole tissue of rats' brain, using vegetable oil as substrate, that AC may inhibit the lipolytic activity slightly in those parts of the brain where its concentration reaches $1 \cdot 10^{-4}$ g/ml or very slightly less. More definite conclusions must await the study of the effect of AC separately on the microsomal and the structureless fraction of the homogenates, in which the lipase is concentrated, and the use of specific substrates.

At present, it is impossible to assess the possible biological significance of this slight inhibitory effect of AC on the lipolytic activity of brain tissue. However, it is worth remembering that adrenalin, which in some respects is antagonistic to acetylcholine, may act in the opposite direction on the lipolytic activity. It has been shown [1-8], for instance, that adrenalin activates the lipase of the epididymal fatty tissue.

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