ACETYLCHOLINE-INDUCED RECIPROCAL CHANGES IN Na,K-ATPase AND ACETYLCHOLINESTERASE ACTIVITY IN NERVE AND IN A MEMBRANE PREPARATION OF Na,K-ATPase

A. M. Mustafin and O. V. Esyrev

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The Na,K-ATPase and acetylcholinesterase (AChE) activity of homogenates of frog nerves and of a membrane preparation of bovine brain Na,K-ATPase was investigated. Preliminary treatment of the nerves and preparation with a solution of acetylcholine in a concentration of 10^{-7}-10^{-6} M increased their Na,K-ATPase activity and reduced their AChE activity. The possible mechanisms of this effect are discussed. KEY WORDS: Na,K-ATPase, cholinesterase, effect of acetylcholine.

According to data in the literature a functional connection exists between the principal membrane proteins – acetylcholinesterase (AChE) and Na,K-ATPase – but its mechanism is not yet clear. According to some workers, this connection is effected through substrates, namely acetylcholine (ACh) and ATP, which can inhibit Na,K-ATPase and AChE respectively [1, 3]. According to other workers, catalytic functions are performed by the same protein molecule [6, 13].

Mustafin [6] has suggested that the change from the acetylcholinesterase function of a protein to its Na,K-ATPase function is induced by ACh. If this suggestion is correct, under the influence of ACh and under appropriate conditions reciprocal relations must be observed between the enzymes, namely: an increase in Na,K-ATPase and a decrease in AChE activity. The investigation described below was devoted to the testing of this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out in February and March on the sciatic nerves of Rana ridibunda and on an Na,K-ATPase preparation isolated from bovine brain by the method of Klodos et al. [12]. The Na,K-ATPase preparation, with an activity of 200 μmoles P_i/mg protein/h, was kept for a long time beforehand at −20°C, then thawed and kept for 15 h at +20°C. The experimental nerves were kept for 30 min in Ringer's solution with ACh (10^{-6} M), and control nerves were kept for the same time in Ringer's solution without ACh. The control and experimental nerves were fixed with liquid nitrogen and homogenized in the frozen state. The homogenate was diluted with Ringer's solution and kept for 5-10 min at the incubation temperature before measurement of activity of the enzymes. In the experiments on the Na,K-ATPase preparation acetylcholine (10^{-7} M) was added 2 min before activity was measured.

ATPase activity was studied in a medium containing 3 mM ATP, salts of sodium, potassium, and magnesium in the optimal proportions of 100:20:3, a Tris-HCl buffer mixture (pH 7.6), protein of the homogenate (0.2 mg) and the Na,K-ATPase preparation (6 μg). The reaction proceeded for 10 min. ATPase activity was determined from the concentration of inorganic phosphate (P_i) in the incubation medium [14]. The temperature coefficient Q_{10} was calculated as the ratio between the activities of the enzymes at 30 and 20°C. AChE activity was estimated by Hestrin's method [11] in the same incubation medium as was used to determine ATPase activity. The concentration of ACh was 1 mM protein of the nerve homogenate 0.2 mg/ml, and protein of the Na,K-ATPase preparation 6 μg. In this case the cholinesterase reaction was studied during inhibition by its own product, and the concentration of undecomposed ACh at the plateau of the reaction was studied as the in-

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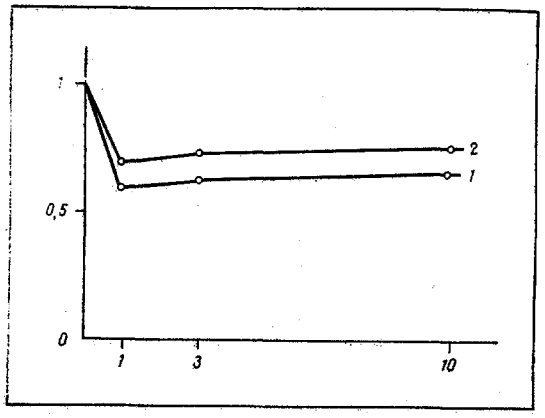

Fig. 1

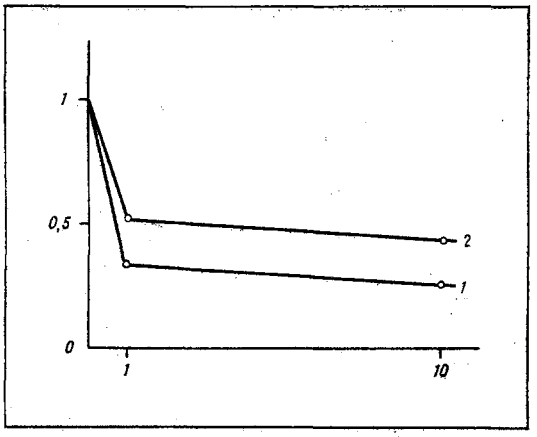

Fig. 2

Fig. 1. Action of ACh on AChE activity of nerve homogenates. Abscissa, incubation time (in min); ordinate, ACh concentration in incubation medium (in mM). 1) Control nerve homogenate; 2) nerve homogenate treated with ACh (10^{-6} M).

Fig. 2. Action of ACh on AChE activity in membrane preparation of Na,K-ATPase. 2) Nerve homogenate treated with ACh (10^{-7} M). Remainder of legend as in Fig. 1.

TABLE 1. Effect of Acetylcholine on Na,K-ATPase Activity of Nerve Homogenates and of Na,K-ATPase

Experimental conditions	Na,K-ATPase activity, μmoles P_i/mg protein/h		
	20° C	30° C	Q_{10}
Control:			
nerve homogenate	0,17	0,33	1.92
membrane preparation	12,17	19,79	1.62
Experiment:			
nerve homogenate	0,36	0,63	1.70
membrane preparation	28,17	45,68	1.62

dicator. It follows from the kinetics of the reaction with inhibition by its own product [10] that the ACh concentration on the plateau must fall with an increase in the content of original enzyme or in the number of catalytic centers.

Wilcoxon's nonparametric criterion was used for the statistical analysis of the results.

EXPERIMENTAL RESULTS

The results of measurement of ATPase activity of the nerve homogenates and Na,K-ATPase preparation are given in Table 1.

In the control the nerve homogenates had comparatively low Na,K-ATPase activity. The values obtained differed only a little from those obtained on the same object by other workers [5]. The Na,K-ATPase activity of the membrane preparation was significantly lower than the initial activity (200 μmoles P_i/mg protein/h at 37°C), and was about 10%. The decrease in activity was evidently the result of keeping the preparation at 20°C, for according to the authors who first described this method of isolation, the activity of the preparation if kept at 37°C remains unchanged only for 1 h [12].

ACh caused a significant ($P < 0.01$) increase in Na,K-ATPase activity in both objects studied. The activation of brain Na,K-ATPase by ACh found in these experiments contradicts the observations of many other workers [2, 3, 7, 9] who found that ACh inhibits Na,K-ATPase activity. However, most of these data were obtained by the use of high concentrations of ACh and freshly isolated preparations. Meanwhile there is evidence that under certain conditions ACh can increase Na,K-ATPase activity [4, 8, 9] The activation by ACh of an Na,K-ATPase preparation kept for a certain time after isolation [9] was of special interest. Changes in Na,K-ATPase preparations during "aging" take place spontaneously at temperatures of 20-37°C.

The increase in Na,K-ATPase activity under the influence of ACh took place on account of a change in the number of catalytic centers or in the number of enzyme molecules. This is shown by the fact that ACh does not change the temperature coefficient of the membrane Na,K-ATPase preparation ($P < 0.05$). Possibly in the experiments with homogenates, ACh caused an increase in Na,K-ATPase activity, and the reduction in the temperature coefficient indicated an increase in the relative content of this enzyme compared with other types of ATPases. This hypothesis was tested in a series of experiments in which the ATP-ase activity of nerve homogenates was determined in medium without Na^+ and K^+. In this case, ACh caused no increase in ATPase activity.

The increase in Na,K-ATPase activity of the test objects under the influence of ACh was accompanied by a reciprocal decrease in AChE activity (Figs. 1 and 2).

The ACh concentrations 1, 3, and 10 min after the beginning of the reaction were always higher in the experiments than in the control. For instance, in the experiments on the homogenate these values were 0.70, 0.73, and 0.76 mM respectively for nerves treated with ACh, and 0.60, 0.63, and 0.67 mM for the controls. It will be clear from Fig. 1 and Table 1 that the most intensive hydrolysis of ACh was observed in the homogenate during the first minute of the reaction, and that later there was a small but significant ($P < 0.05$) increase in the ACh concentration in the medium. Evidently the activity of the ACh-synthesizing system of the nerve homogenate was manifested against the background of inhibition of AChE by hydrolysis products of ACh.

The conditions for ACh synthesis are absent in the Na,K-ATPase preparation, and for that reason the ACh concentration fell during the incubation, although the rate of this decrease was considerably reduced as early as in the first minute (Fig. 2). Whereas after the first minute of the reaction 51 and 34% respectively of the original quantity of ACh remained in the experiment and control, after 10 min of the reaction these values showed little change, namely 43 and 27% respectively.

The results of these experiments do not contradict the earlier hypothesis that ACh causes the formation of new Na,K-ATPase molecules at the expense of acetylcholinesterase molecules. Possibly during aging of the Na,K-ATPase preparation the reaction proceeds in the reverse direction. It is certain that this interpretation of the results offered above is not the only one.

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