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ENDOGENOUS ACETYLCHOLINE-INDUCED Na,K-ATPase ACTIVATORS AND INHIBITORS

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It was shown previously that the level of microsomal Na,K-ATPase activity in nerve cells is controlled by acetylcholine (ACh) [3, 4] or its pharmacologic analogs [2, 6]. Since ACh in a cell-free system activates the enzyme and since this effect is abolished by actinomycin D (a transcription inhibitor) and puromycin (a translation inhibitor), it was logical to suggest that ACh induces synthesis either of the enzyme itself or of a protein-activating factor [3, 4]. The investigation described below was undertaken to determine the correct alternative.

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. The animals were quickly decapitated and the whole brain (without the cerebellum and caudal part of the medulla) was homogenized in a cold solution of 0.3 M sucrose, 0.05 M Tris-HCl, pH 8.0 (5 ml per brain). Aliquots of homogenates (3.8 ml each) in the experimental tests were treated with 0.2 ml of an aqueous solution of either ACh (to a final concentration of 10^{-5}-10^{-3} M) or eserine (to a concentration of 10^{-6}-10^{-5} M), and in the control tests with 0.2 ml water, and incubated for 45 min at 37°C with periodic shaking. The reaction was stopped by the addition of 10 ml of cold 0.3 M sucrose containing 5 mM EDTA. The mixture was centrifuged for 15 min at 12,000g. The supernatant was drawn off and centrifuged for 60 min at 30,000g. The residue of microsomes was suspended in 2.4 ml of 0.05 M Tris-HCl, pH 7.55, and their Na,K-ATPase activity was determined by the method described previously [3]. All samples (1 ml) contained 0.5 mM EDTA. The control lev-

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TABLE 1. Effect of Preincubation of Homogenate with ACh and Eserine on Na,K-ATPase Activity of Brain Microsomes (M ± m)

Agent added	Concentration of agents	Conditions of treatment of microsome-cytosol fraction	Enzyme activity, % of control
ACh	10^{-5} M	Without dialysis	115±6 (14)*
"	10^{-4} M	" "	130±7 (11)†
"	10^{-3} M	" "	121±7 (14)*
ACh + actinomycin D	10^{-4} M + +50 μg/ml	" "	99±7 (10)
" "		Dialysis	102±6 (9)
ACh	10^{-4} M	"	91±4 (9)*
Eserine	10^{-5} M	Without dialysis	110±5 (10)*
"	10^{-6} M	" "	115±6 (7)*

Legend. *P < 0.05; †P < 0.01[not cited above — Publisher]; P < 0.001; number of experiments in parentheses.

TABLE 2. Effect of Dialyzable Cytoplasmic Material on Na,K-ATPase Activity of Intact Microsomes (M ± m)

Source of dialyzable material	Enzyme activity, % of control
Microsome-cytosol fraction from brain of animals receiving eserine (0.2 mg/kg)	85±2 (3)*
Microsome-cytosol fraction from brain homogenate preincubated with ACh (10^{-4} M)	116±4 (6)*

Legend. *P < 0.05; number of experiments in parentheses.

el of enzyme activity was 4.0-14.0 μmoles P_i/mg protein/h. In some experiments the supernatant after the first centrifugation (15 min, 12,000g) of the preincubated homogenates was dialyzed against 50 volumes of distilled water with one change in 24 h. The microsomes were sedimented as described above. In cases when the presence of dialyzable factors modifying enzyme activity was studied, the supernatant after the first centrifugation of the homogenate was dialyzed against 15 volumes of water and the dialysate was lyophilized. The dry residue was dissolved in 1.5 ml of distilled water; the presence of factors modifying enzyme activity was tested on intact dialyzed microsomes. The samples were treated with 0.1 ml of the above solution.

In experiments *in vivo*, eserine in aqueous solution (0.1 ml/100g body weight) was injected intraperitoneally into the animals in a dose of 0.02-0.4 mg/kg body weight, whereas the control animals were given an injection of water. The animals were decapitated 45 min later and the brain homogenized in 5 volumes of cold solution of 0.3 M sucrose in 5 mM EDTA. The methods of isolation and dialysis of the microsomes and of obtaining the dialyzed fraction were similar in this case to those described above.

The number of binding sites for [^{3}H]ouabain in the dialyzed microsomes, isolated from the brain of animals receiving eserine (0.2 mg/kg), was determined by Scatchard's method [4]. In this case the samples contained (in a volume of 2 ml) 0.77-15.5 mM ouabain, 4×10^{-6} cpm [^{3}H]ouabain (specific radioactivity 49 Ci/mmole), 1 mg microsomal protein, and 100 μM Tris-HCl, pH 7.55.

EXPERIMENTAL RESULTS

Preincubation of the brain homogenate with ACh caused a significant increase in microsomal Na,K-ATPase activity (Table 1). The effect depended on the ACh concentration and the maximum of activation was observed in a dose of 10^{-4} M. When preincubation with actinomycin D was carried out, ACh had no effect. This fact suggests that the primary effect of ACh is localized at the transcription stage of genes controlling the intracellular Na,K-ATPase level.

TABLE 3. Effect of Eserine *in vivo* on Na,K-ATPase Activity of Brain Microsomes (M ± m)

Dose of eserine, mg/kg	Conditions of treatment of microsome-cytosol fraction	Enzyme activity, % of control
0,02	Without dialysis	100±6 (9)
0,2	"	90±3 (14)*
0,4	"	84±4 (11)*
0,2	Dialysis	139±6 (9)†

Legend. *P < 0.05, †P < 0.001; number of experiments in parentheses.

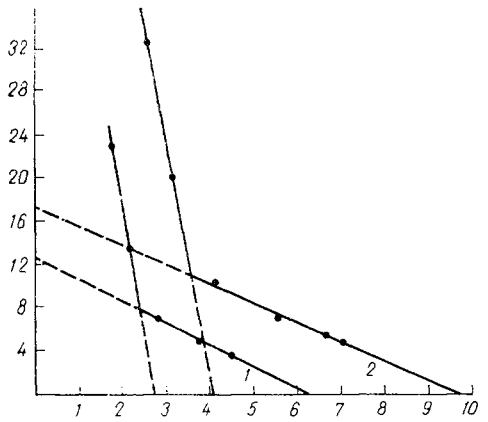

Fig. 1. Binding of [^{3}H]ouabain (Scatchard plot) with dialyzed brain microsomes from control animals (1) and from animals receiving eserine (2). Abscissa, bound ouabain (in nM × 10^{-2}); ordinate, ratio of bound to free ouabain (× 10^{-3} M).

The same effect in principle as that of ACh was given by eserine, used in concentrations completely inhibiting cholinesterase activity (Table 1). If ACh and eserine were added to the homogenate immediately before isolation of the microsomes, i.e., without preincubation, their NaK-ATPase activity remained unchanged.

After dialysis of the microsome-cytosol fraction obtained from homogenate preincubated with ACh the isolated microsomes had lower Na,K-ATPase activity than in the control (Table 1); this effect likewise was absent if incubation was carried out in the presence of actinomycin D. Lyophilized material which passed through the membrane during dialysis of the microsome-cytosol fractions (from homogenate preincubated with ACh) had the ability to activate Na,K-ATPase of the intact microsomes (Table 2). These facts must evidently be interpreted as meaning that the regulatory influence of ACh on the level of Na,K-ATPase activity, in the model used consists of inducing the synthesis of an activating factor while, at the same time, depressing synthesis of the enzyme itself.

Eserine in nontoxic doses (0.2 and 0.4 mg/kg), but increasing the ACh concentration in the brain by several times [7], had the opposite action: depression of Na,K-ATPase activity of undialyzed microsomes and a substantial increase in activity of the dialyzed microsomes (Table 3). In this case addition of lyophilized material which had passed through the membrane during dialysis of the microsome-cytosol fraction isolated from the brain of animals receiving eserine to intact microsomes was found to reduce their Na,K-ATPase activity (Table 2). It can be concluded from these facts that as a result of the action of ACh *in vivo*, unlike its action *in vitro*, an inhibiting factor is formed and synthesis of the enzyme itself is activated.

Evidence that an increase in the number of enzyme molecules in the membranes takes place under the influence of eserine is given by the increase in the number of binding sites for [^{3}H]ouabain (Fig. 1). It was shown that [^{3}H]ouabain has two types of binding sites in the membrane; the number of both types increased after treatment with eserine.

Differences in the action of eserine *in vivo* and *in vitro* suggest that when the integrity of the brain is disturbed, certain stages in the processes of regulation of Na,K-ATPase activity are blocked. One of them may be a disturbance of intercellular interaction, such as disappearance of the influence of interneurons, releasing inhibitory mediators (noradrenalin, dopamine) which have an action on synthetic processes opposite to that of ACh, on acetylcholine-sensitive cells [1, 5].

The results of these experiments thus indicated the existence of a complex system of mediator regulation of the level of Na,K-ATPase activity in nerve cells, the principal features of which can be represented as follows: When synthesis of the enzyme is activated, this is accompanied by synthesis of an inhibiting factor and, conversely, when enzyme synthesis is inhibited, an activating factor may be formed. These opposite processes are evidently stages in a single system maintaining activity of the enzyme at the optimal functionally determined level.

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PHOSPHOLIPID LEVEL AND LIPID PEROXIDATION ACTIVITY IN THE MYOCARDIUM OF RABBITS WITH CHRONIC BRONCHOPULMONARY INFLAMMATION

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The results of the study both of chronic nonspecific lung diseases (CNLD) themselves and of complications caused by them have demonstrated a causative link between the degree of disability and mortality among such patients and the level of accompanying cardiopulmonary failure [1, 2, 4, 8, 11, 12].

The object of this investigation was to study the role of changes in activity of free-radical reactions in disturbance of metabolism of individual phospholipid (PL) fractions — highly important components of membranes — in the myocardium in chronic bronchopulmonary inflammation.

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