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CHOLINERGIC REGULATION OF Na,K-ATPase ACTIVITY FROM PIG KIDNEY

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Many investigations have shown that acetylcholine (ACh) can regulate Na,K-ATPase activity. High ACh concentrations inhibit, whereas low stimulate Na,K-ATPase activity of various biological objects [1, 4]. It has been suggested that in Na,K-ATPase there are subunits of acetylcholine receptors [7].

A link also is found between two membrane enzymes, namely Na,K-ATPase and acetylcholinesterase (AChE), but its nature is not yet clear. It has been suggested that this link is effected by means of an intermediary, ACh, for example, which is the substrate for one enzyme and regulator for the other.

According to one view, catalytic centers of both enzymes lie on the same protein subunit [9] and function simultaneously; however, according to another view, the catalytic subunit of AChE is the precursor of the catalytic subunit of Na,K-ATPase. It has been shown that ACh in membrane preparations of Na,K-ATPase and homogenates of various tissues can increase the number of catalytic centers of Na,K-ATPase and reduce their number in AChE [5, 6]. In the investigation described below the effect of ACh was studied on the AChE activity of a purified preparation of Na,K-ATPase, generously provided by the staff of the Institute of Bioorganic Chemistry, Academy of Sciences of the USSR [3].

The preparation is a homogeneous protein with mol. wt. of 540 kilodaltons, containing equal amounts of α- and β-subunits.

EXPERIMENTAL METHOD

The original preparation of Na,K-ATPase, containing 3.6 mg protein in 1 ml of histidine-sucrose medium, was diluted in a solution of 250 mM sucrose and 30 mM histidine, pH 7.2, to a concentration of 40 μg protein/ml. The total sample was divided into 1.5-ml portions and frozen at −12°C for keeping until required. Each portion was thawed before the experiment.

AChE activity was determined as the rate of hydrolysis of ACh by Ellmann's method [8], and Na,K-ATPase activity was determined as accumulation of inorganic phosphorus [10].

EXPERIMENTAL RESULTS

The results of the experiments with ACh are given in Table 1.

The control preparation of Na,K-ATPase did not react significantly to ACh irrespective of the concentration of the regulator used. Preincubation of the preparation at 22°C for 30 min did not change the sensitivity of Na,K-ATPase for ACh. Similar treatment of unpurified preparations in some cases revealed a stimulating action of ACh on Na,K-ATPase [2].

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TABLE 1. Action of ACh on Na,K-ATPase Activity of Na,K-ATPase Preparation from Pig Kidney (in μmoles P_i/mg protein/h)

Experimental conditions	Without ACh	With ACh	
		10^{-7} M	10^{-4} M
Control	916 (890—940)	920 (890—950)	908 (882—938)
Incubation at 22 °C	896 (872—921)	898 (871—918)	892 (878—918)
Freezing and thawing	740 (718—760)	748 (722—768)	728 (706—756)

TABLE 2. AChE Activity of Na,K-ATPase Preparation in Control and After Various Treatments

Treatment	AChE activity, μmoles substrate/mg protein/h	Na, K-ATPase activity, μmoles P_i/mg protein/h
Control	15,8 (12,3—22,05)	916 (890—940)
Triton X-100 (0,2%)	17,9 (16,3—19,1)	0
Incubation for 5 min	34,9 (30,8—39,0)	—
ACh, 10^{-7} M	13,3 (11,1—16,8)	—

As a result of freezing and thawing 3 times activity of the enzyme diminished, but sensitivity to ACh was virtually unchanged.

During isolation of Na,K-ATPase, the regulatory action of ACh on the enzyme thus disappears irreversibly and this contradicts the view that Na,K-ATPase has an active ACh receptor [7].

The results of measurement of AChE activity of the Na,K-ATPase preparation are given in Table 2.

The initial Na,K-ATPase activity of the preparation was 916 μmoles P_i/mg protein/h, and its AChE activity was 15.8 μmoles substrate/mg protein/h. ACh in a concentration of 10^{-7} M had no significant effect on either of the enzyme activities of the preparation.

Treatment of the preparation with the detergent Triton X-100 caused disappearance of Na, K-ATPase activity but did not affect AChE activity.

Since a change in structure of the protein by the action of detergent caused a change in only one activity, the view that the detectable AChE activity is the result of functioning of a second catalytic center (Na,K-ATPase) is incorrect. The most probable explanation is that we discovered contaminating activity in the purified Na,K-ATPase preparation.

Reducing the incubation time by two-thirds (to 5 min) increased the calculated AChE activity by a little more than twice. This is evidence that AChE catabolism followed a nonlinear course in the experiment, with delay. A similar effect was described previously during the study of acetylcholinesterase activity of frog nerve homogenate and of a membrane preparation of Na,K-ATPase by Hestrin's method [6].

At our request experiments with an immune serum containing antibodies against the test Na,K-ATPase were carried out in the laboratory of the Institute of Developmental Biology, Academy of Sciences of the USSR [3]. It was found that the serum affected neither the AChE nor the Na,K-ATPase activity of dog brain.

When comparing the results of the present investigation with previous data [2], we are forced to acknowledge once again that in the course of purification of the enzyme information on its regulatory properties is lost. Careful analysis of these properties at every stage of

isolation of Na,K-ATPase is therefore necessary in order to understand the mechanisms of action of different regulators and, in particular, of ACh on enzymes.

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CHANGES IN SOME LIPID PEROXIDATION PARAMETERS OF ALBINO RAT LIVER MITOCHONDRIA DURING ANOXIC INJURY SIMULATED *IN VITRO*

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The results of experiments to study the role of lipid peroxidation (LPO) in anoxia now available can be divided into two groups. On the one hand, a decrease in antioxidant activity (AOA) is observed in lipid extracts from mitochondrial and microsomal fractions of various organs in ischemia [1, 2]. On the other hand, there is evidence of increased AOA in homogenates and mitochondrial fractions of organs surviving *in situ* [3, 4, 12]. Probably these two groups of experimental facts are not contradictory, but mutually complementary, for in one case the test object consisted of lipid extracts, whereas in the other it was organelles or homogenates.

The object of this investigation was to study the state of LPO in mitochondria during anoxia in a simpler system than the cell, namely mitochondria + Ca^{++} + anoxia *in vitro*, in which virtually all features of anoxic injury to organelles can be adequately simulated [11]. Changes in the kinetics of Fe^{++}-induced chemiluminescence (CHL) of mitochondria and liposomes from the total lipid fraction, the content of intermediate and end products of LPO, and also the effect of Mn^{++} and Cu^{++} on parameters of CHL in mitochondria, for they may be potential regulators of LPO in the cell [15], were investigated.

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