MUSCARINIC CHOLINOCEPTOR-MEDIATED INHIBITION OF SARCOLEMMAL Na,K-ATPase ACTIVITY OF MYOCARDIUM AND INTESTINAL SMOOTH MUSCLES BY ACETYLCHOLINE

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The sodium pump, the molecular basis of which is the enzyme Na,K-ATPase [11], plays an important role in muscle function [1]. This accounts for the great attention which is being paid to the study of regulation of muscle tissue Na,K-ATPase [1, 3].

It was shown previously that acetylcholine (ACh) specifically inhibits the Na,K-ATPase activity of sarcolemmal preparations from different muscles [2, 5], but the mechanism of the effect of the transmitter remains unexplained.

The aim of this investigation was to determine the role of cholinoceptors (ChC) in the inhibitory effect of acetylcholine (ACh) on Na,K-ATPase activity of the myocardium and small intestinal smooth muscles.

EXPERIMENTAL METHOD

Preparations of the sarcolemma of the frog heart and of the atria and ventricles and small intestinal smooth muscles of a dog were obtained by a modified method in [9]. Total ATPase activity was determined by accumulation of inorganic phosphorus (P_i) [12] in incubation medium containing (in mM): NaCl 100, KCl 20, $MgCl_2$ 3, ATP-Na_2 3, EGTA 0.5, EDTA 0.1, NaN_3 5, Tris-HCl 50 (pH 7.4, at 37°C). Na,K-ATPase activity was calculated as the difference between total activity and Mg-ATPase activity, recorded in the absence of NaCl and KCl. The membranes were treated with sodium dodecylsulfate (SDS, 0.3 mg/mg protein) as in [6]. The number of specific binding sites of the muscarinic ChC [^{3}H]quinuclidinylbenzylates ([^{3}H]-QNB) in the preparations was determined in incubation medium without ATP, containing a saturating (1.5 nM) concentration of [^{3}H]-QNB, as described in [10]. Radioactivity was measured in Bray's solution on an LS-100 C scintillation counter (Beckman, USA). The counting efficiency was 60%. M-ChC of the dog atria were solubilized with a mixture of 0.4% digitonin and 0.8% sodium cholate by the method in [7]. Protein was determined by the microbiuret method [4] in the presence of 1% sodium deoxycholate.

Reagents: NaN_3, SDS, digitonin, and cholic acid were from Serva, West Germany; ouabain, Tris, and EGTA were from Sigma, USA; [^{3}H]-QNB, activity 5 Ci/mmole, was from Amersham Corporation, England; ATP and EDTA were from Reanal, Hungary; the remaining reagents were of Soviet origin and of the highly pure and analytically pure grades.

EXPERIMENTAL RESULTS

The Na,K-ATPase activity of preparations of myocardial sarcolemma was completely inhibited by ouabain ($2 \cdot 10^{-4}$) and was unchanged after treatment with SDS, whereas Mg-ATPase was inactivated by the detergent (Table 1). These results are evidence that the preparations consisted of open membrane fragments in which Na,K-ATPase sites were accessible for substrate, activator ions, and inhibitor, for we know that the active site of Na,K-ATPase is located on the cytoplasmic surface of the cell membrane, and the ouabain-binding site is located on its outer side [11], and that neither ATP nor ouabain can pass through the sarcolemma. Na,K-

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TABLE 1. ATPase Activity of Sarcolemmal Preparations from Cardiac and Smooth Muscles (M ± m)

Object	Na, K-ATPase	Na, K-ATPase	Mg-ATPase	
	−SDS	+SDS	−SDS	+SDS
Frog's heart	31,5±4,6	30,5±3,2	31,2±2,6	0
Dog's heart				
atria	14,5±2,8	14,0±4,1	9,8±1,1	1,2±0,5
ventricles	25,0±3,8	25,1±2,6	19,1±1,8	2,3±1,0
Dog's intestinal smooth muscles	11,6±2,0	60,0±5,8	25,6±3,0	0

Legend. Specific activity in micromoles P_i/mg protein/h. Data given for 7-9 preparations from each object.

TABLE 2. Binding of [^{3}H]-QNB by Sarcolemmal Preparations and Inhibition Constant ($K_{0.5}$) of Na,K-ATPase by ACh (M ± m)

Object	[^{3}H]-QNB, fmoles/mg protein	$K_{0,5}$, μm
Dog's intestinal smooth muscles	1020±48	0,5
Frog's heart	802±26	1,0
Dog's atria	590±51	5,0
Dog's atria treated with mixture of digitonin and cholate	39±10	—
Dog's ventricles	166±22	56

Legend. $K_{0.5}$) ACh concentration giving half of maximal effect.

ATPase activity of the sarcolemma of intestinal smooth muscles was inhibited by ouabain by only 60%, and after incubation with SDS it increased fivefold, indicating that the preparations contained both open and vesicular formations.

ACh in concentrations of $1 \cdot 10^{-4}$ to $5 \cdot 10^{-4}$ M completely inhibited the Na,K-ATPase activity of the sarcolemma of the frog's heart and the dog's atria; enzyme from the dog's ventricles was inhibited by 90% only by very large doses ($5 \cdot 10^{-3}$ to $1 \cdot 10^{-2}$ M doses of ACh). In preparations of sarcolemma from smooth muscles ACh inhibited only ouabain-sensitive Na,K-ATPase activity (Fig. 1), i.e., activity of open membrane fragments. Treatment with detergent did not change the action of ACh on myocardial Na,K-ATPase but abolished its effect on the enzyme on smooth muscles. This is probably the result of structural-functional differences between membranes of cardiac and smooth muscles. Mg-ATPase activity of all the preparations studied did not respond to addition of ACh ($1 \cdot 10^{-7}$ to $1 \cdot 10^{-2}$ M).

Unlike the nicotinic cholinolytic D-tubocurarine (10^{-6}-10^{-4} M), the M-ChC antagonist atropine (10^{-7}-10^{-6} M) blocks the inhibitory effect of the mediator. This suggested that inhibition of Na,K-ATPase by ACh is due to activation of M-ChC. A high density of binding sites of the M-ChC antagonist [^{3}H]-QNB was found in preparations of the sarcolemma. It was noted that the more numerous these sites in the preparation, the higher the affinity of Na,K-ATPase for ACh (Table 2). Treatment of the atrial sarcolemma with a mixture of digitonin and sodium cholate (1:0.2 mg to 1 mg protein) which, according to data in the literature [7], leads to solubilization of up to 90% of M-ChC, in the present experiments also caused considerable loss of membrane-bound M-ChC (Table 2) and some degree (up to 20%) of inactivation of Na,K-ATPase. Extraction of M-ChC was accompanied by disappearance of the reaction of the enzyme to a ACh.

Guanyl nucleotides are known to reduce the affinity of N-ChC for antagonists [8], evidently by uncoupling the receptor molecules and protein molecules binding guanosine triphosphate (GTP) [13]. In the presence of 10 μM GTP the sensitivity of Na,K-ATPase to ACh fell sharply; dependence of the effect of the mediator on con-

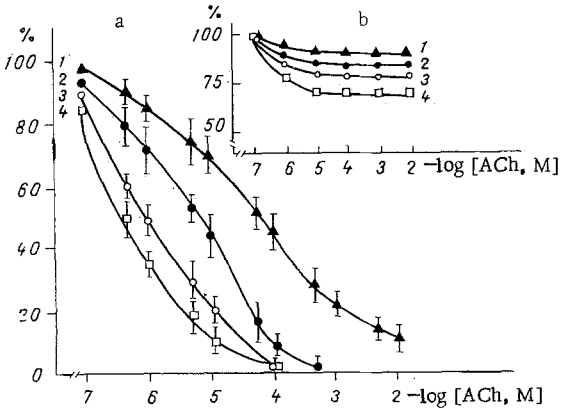

Fig. 1. Effect of ACh on Na,K-ATPase activity (in %) of sarcolemmal preparations: a) without GTP; b) with 10 μM GTP. 1) Smooth muscles of dog's small intestine (ouabain-sensitive Na,K-ATPase activity); 2) frog's heart; 3) dog's atria; 4) dog's ventricles.

centration disappeared (Fig. 1). GTP itself (10-100 μM) did not affect ATPase activity. Other nucleotide triphosphates (ITP, CTP) did not modify the action of ACh on Na,K-ATPase.

The results thus indicate that inhibition of Na,K-ATPase by ACh is not the result of its direct action on the enzyme, but is mediated through ChC. It remains to be explained how activation of M-ChC leads to inhibition of Na,K-ATPase. Inhibition of activity of the enzyme was not due to a change in membrane permeability caused by agonist–receptor interaction, for the effect of ACh was observed on open fragments of sarcolemma. The absence of Ca^{++} in the incubation medium and of conditions for cyclic nucleotide synthesis rules out any participation of secondary messengers as the connecting link between receptors and enzyme.

The coupling mechanism between Na,K-ATPase and M-ChC can be represented in terms of the general principle of function of membrane receptor systems [14]. As a result of the agonist–receptor reaction, the functional complex of M-ChC–GTP-binding protein–Na,K-ATPase is able to appear. Under conditions preventing the formation of this triple complex (blockade of M-ChC by atropine or its solubilization by detergents, and also uncoupling of the receptor and GTP-binding protein by guanyl nucleotides), inhibition of Na,K-ATPase by acetylcholine takes place only insignificantly or not at all.

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