in the frequency of contractions [2, 7]. Incidentally, the mechanisms responsible for this most economical method of urgent adaptation of the heart to an increased load [2] are formed, at least partly, in the heart muscle itself due to changes in its α- and β-adrenoreceptors.

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CHANGES IN ACTIVITY AND REGULATORY PROPERTIES OF Na,K-ATP-ASE FROM THE MYOCARDIAL SARCOLEMMA DURING TOTAL GRADED ISCHEMIA

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UDC 616.127-005.4-092.9-07:616.127-008.931:577.152.361

KEY WORDS: Na,K-ATPase; myocardium; ischemia; digoxin; carbachol

In myocardial ischemia Na^+ accumulates in the cardiomyocytes, which lose K^+ [8]. A probable cause of this phenomenon is disturbance of the function of the Na-pump of the sarcolemma, which actively secretes Na^+ from the cell in exchange for K^+. For instance, it has been shown [4, 12, 15] that Na,K-ATPase activity of membranes isolated from ischemic zones of the myocardium is lower than in the control. However, there are as yet insufficient data to explain the mechanism of ischemic damage to the enzyme. In particular, hardly anything is known of the dynamics of changes in Na,K-ATPase activity and its ability to induce regulatory responses during the development of pathology. Accordingly the aim of this investigation was to study changes in myocardial ATPase activity in rats and guinea pigs and its response to the cardiac glycoside digoxin (DG) and the colinergic agent carbachol (CC) during total graded ischemia.

EXPERIMENTAL METHOD

Albino rats and guinea pigs weighing 150-200 g were decapitated. Graded total ischemia of the myocardium was induced by incubating the hearts in an environment of air at 37°C [9]. The structural and functional changes taking place in the myocardium under these conditions have been shown to be similar to the disturbances observed in models of ischemia created by

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 26-28, July, 1987. Original article submitted December 2, 1986.

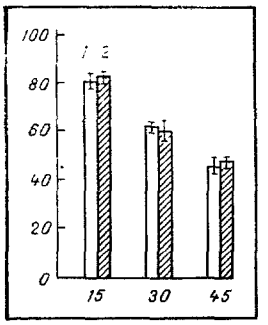

Fig. 1. Changes in Na,K-ATPase activity of myocardial sarcolemma of rats (1) and guinea pigs (2) during graded ischemia. Abscissa, duration of ischemia (in min); ordinate, Na, K-ATPase activity (in %).

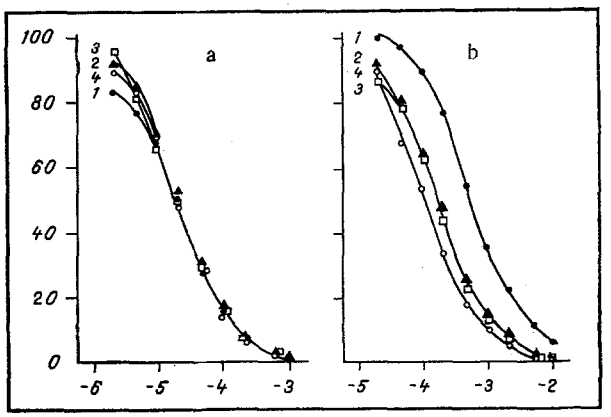

Fig. 2. Dependence of Na,K-ATPase activity of myocardial sacrolemma of guinea pigs (a) and rats (b) on DG concentration during development of ischemia. 1) Control, 2) 15 min, 3) 30 min, 4) 45 min later. K_i values were found from Dixon's graphs (1/V versus [DG], μM); for curves 1-4 its value was 20 μM (a); for curve 1 — 600, curves 2 and 3 — 150, and curve 4 —100 μM (b). Mean results for 11-12 preparations for each species of animal are shown. Abscissa, DG concentration (in log M); ordinate, Na,K-ATPase activity (in %).

other methods [9]. Preparations of sarcolemma were isolated at 2-4°C as follows: Tissue of the left ventricle (2 g) was homogenized in 5 volumes of isolation medium (IM), containing 0.25 M sucrose, 1 mM EDTA, 1 mM ATP, and 20 mM Tris-HCl, pH 7.4, in a homogenizer of Polytron type (three times, for 20 sec each time, with intervals of 30 sec). The homogenate was centrifuged at 2800g for 15 min. The residue was washed three times in IM during centrifugation under the same conditions, suspended in water, and treated with sodium iodide for 1 h by the method [11]. The NaI extract was centrifuged at 10,000g for 10 min, and then at 45,000g for 30 min. The residue was washed three times in 1 mM EDTA (pH 7.4), suspended in medium containing 0.25 M sucrose and 30 mM histidine (pH 7.2), and kept at —15°C.

Total ATPase activity was determined by measuring accumulation of inorganic phosphate (P_i) [13] in medium of the following composition: 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 3 mM Na_2-ATP, 50 mM Tris-HCl, pH 7.4, at 37°C. The Na,K-ATPase activity was calculated as the difference between activities of total and Mg-ATPase, recorded in the same medium, but without KCl. The protein concentration was determined by the biuret method in the presence of 1% sodium deoxycholate.

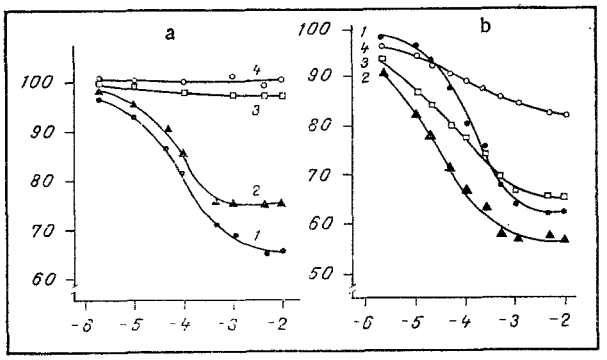

Fig. 3. Effect of carbachol (CC) on Na,K-ATPase activity of myocardial sarcolemma of guinea pigs (a) and rats (b) during graded ischemia. 1) Control, 2) 15, 3) 30, and 4) 45 min later. K_i values obtained from graphs (V_0/V_1 versus [CC], μM [3]); for curves 1-2 its value was 80 μM (a); for curve 1 — 130, 2, 3, and 4 — 50 μM (b). Data for 7-10 preparations for each species of animal are given. Abscissa, CC concentration (in log M); ordinate, Na,K-ATPase activity (in %) .

EXPERIMENTAL RESULTS

The Na,K-ATPase activity of the myocardial sarcolemma of rats and guinea pigs in the control was 9.30 ± 0.24 (n = 9) and 13.35 ± 1.57 (n = 11) μmoles P_i/mg protein/h respectively. Exposure to ischemia for 15, 30, and 45 min led to gradual decline, which was similar in character in animals of both species (Fig. 1).

We know that the Na,K-ATPase molecule contains a pharmacological receptor for cardiac glycosides, which are specific inhibitors of this enzyme [5]. Analysis of concentration dependences of the action of DG on Na,K-ATPase activity of the guinea pig myocardium revealed no differences between the sarcolemma of the control and ischemic hearts (Fig. 2a), whereas the affinity of the rat enzyme for the glycoside increased with an increase in the duration of ischemia (Fig. 2b). CC (10^{-7}—10^{-3} M) caused inhibition of Na,K-ATPase activity, amounting under normal conditions to 40% in animals of both species. Its action was completely blocked by atropine (10^{-6} M), an antagonist of muscarinic acetylcholine receptors (MACR). During ischemia the level of inhibition of Na,K-ATPase by CC fell. The sensitivity of the enzyme for the MACR agonist, determined in the guinea pig myocardium, was almost unchanged, whereas in the rat myocardium it was actually increased (Fig. 3). Under these circumstances it was found that, unlike Na,K-ATPase from the rat heart, which largely preserved the property of reacting to CC even after 45 min, the enzyme from the guinea pig heart lost its ability to respond to addition of the agonist as early as 30 min after the development of ischemia.

This investigation thus showed that during myocardial ischemia the Na,K-ATPase activity of the cardiomyocyte sarcolemma falls. This is in good agreement with results obtained by other workers [4, 12, 15]. Meanwhile we obtained new data to show that for at least the first 45 min of development of ischemia the degree of inhibition of this enzyme depends essentially on the duration of exposure to ischemia. Although the trend of changes in Na,K-ATPase activity in the myocardium of rats and guinea pigs is similar, a significant difference was observed in the character of the changes in its regulatory reactions in animals of these species. We know that the rat myocardium has low sensitivity to cardiac glycosides, which is attributed to the presence of a form of Na,K-ATPase with low affinity for these substances [5]. Under certain conditions, however, and during perfusion of the heart with calcium-free solution, for example, increased affinity of the rat enzyme was found for ouabain, which is attributed to the appearance of a form of Na,K-ATPase with high affinity, which is latent under normal conditions [10]. The phenomenon we observed, namely increased affinity of the rat enzyme to digoxin, may perhaps be based on the same mechanism. The results of experiments with cholinergic ligands agree with data published previously on the inactivating action of acetylcholine on Na,K-ATPase activity of the myocardial sarcolemma of different species of animals [1, 3], due conjecturally to functional interaction between this enzyme and MACR in the sarcolemma. The relatively high values (about 10^{-4} M) of the inhibition constants of Na,K-ATPase by CC, determined by the method described in [3], indicate that receptors in a

state of very low affinity for the agonists, similar to those which mediate cholinergic stimulation of inositol-containing phospholipids in the myocardium [7], are implicated in this process. Disturbance of the response to CC in ischemia may be connected with many factors and, in particular, with injury to molecules of Na,K-ATPase and MACR by fatty acids and by free radicals [6, 14], accumulating in ischemia [2], or with disturbance of coupling between the enzyme and receptor.

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