TIME COURSE OF Na,K-ATPase ACTIVITY DURING DEVELOPMENT OF ISOPROTERENOL-INDUCED MYOCARDIAL NECROSIS IN RATS

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UDC 616.127.002.4-02:615.217.22]-092.9-97:616.127-008.931:577.152.14

KEY WORDS: rat myocardium, necrosis, isoproterenol; Na,K-ATPase.

Massive doses of catecholamines and, in particular, of the synthetic β-adenomimetic isoproterenol, produce infarct-like necrotic lesions in the myocardium of experimental animals [12]. A distinguishing feature of isoproterenol-induced necrosis of the myocardium is early damage to the sarcolemma of the cardiomyocytes, manifested as a sharp increase in its permeability for macromolecular tracers (horseradish peroxidase, ferritin, colloidal lanthanum, etc.) [2, 5, 11]. Unlike other models of myocardial pathology, such as ischemia, in isoproterenol necrosis the permeability of the intracellular membranes for macromolecules is distubed much later than the permeability of the sarcolemma [2, 5].

It was this feature of isoproterenol necrosis (IN) which led us to study the activity of Na,K-ATPase, a biochemical marker of the sarcolemma, at different stages of development of the pathological conditions. The following time intervals were chosen, counting starting from the last injection of isoproterenol: 1 h, when the period of the β-adrenergic response of the vessels ended and passage of the tracers through the plasma membrane of the cardiomyocytes was beginning to be found [5]; 6 h, the time of the greatest increase in permeability of the sarcolemma for macromolecular tracers and Ca^{++} [11]; 24 h, when necrosis of the heart muscle has completely developed [12].

To shorten the time between sacrifice of the animals and determination of enzyme activity as much as possible, the work was done on heart tissue homogenate [14], using an anatomically well-demarcated area—the apex of the left ventricle, where necrotic lesions are most marked [12], and the greatest increase in permeability for tracers is observed [11]. Considering the vector character of Na,K-ATPase, i.e., that its activity depends on accessibility of ligands on different sides of the membrane and, consequently, on the degree of closure of the sarcolemmal particles formed during homogenization, parallel determinations of enzyme activity were made in the presence and absence of low concentrations of detergent, making the membrane completely permeable for all ligands and ensuring complete manifestation of Na,K-ATPase activity [3].

EXPERIMENTAL METHOD

IN was induced in Wistar rats weighing 200-250 g by injection (100 mg/kg) of isoproterenol sulfate (in the form of "Novodrin," East Germany) during two successive days with an interval of 24 h. The animals were decapitated 1, 6, and 24 h after the last injection of isoproterenol, the heart was isolated, the atria and right ventricles removed, the apex of the left ventricle taken and washed in homogenization medium (see below), weighed, and homogenized in 40 volumes of medium consisting of 10 mM Tris-HCl, pH 6.5, in a Teflon homogenizer powered by a small motor. The homogenate (110 μg protein) was transferred to tubes containing incubation medium: 100 mM NaCl, 20 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM EGTA, 5 mM NaN_3, 50 mM Tris-HCl, pH 7.4, at 37°C, with or without 0.5 mM ouabain. Some samples contained Lubrol WX (from Sigma, USA) in the required concentration. After 15 min of preincubation the reaction was started by the addition of 2.5 mM ATP-Na (total volume 1 ml), and after 20 min the reaction was stopped by the addition of 1 ml of 2.5 M Tris-acetate buffer, pH 4.3, containing 0.2% of ammonium molybdate for determination of inorganic phosphate, after which 0.2 ml of a freshly prepared solution of 1% ascorbate in 5 mM $CuSO_4$ was immediately added. Extinc-

Laboratory of Molecular Pathology and Biochemistry, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 8, pp. 184-186, August, 1984. Original article submitted October 14, 1983.

 0007-4888/84/9808-1068$08.50

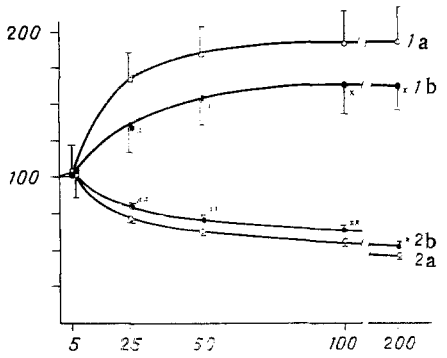

Fig. 1

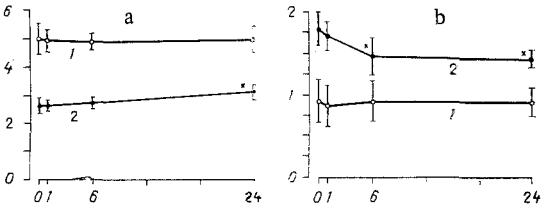

Fig. 2

Fig. 1. Na,K-ATPase (1) and Mg-ATPase (2) activities of rat heart homogenate as functions of concentration of Lubrol WX (M ± m, 4-5 observations). Abscissa, concentration of Lubrol WX (in % × 10^4); ordinate, ATPase activity (in % of initial level in absence of detergent). a) Homogenate of control rat heart; b) homogenate of rat heart 24 h after administration of isoproterenol. *P < 0.05, **P < 0.01 compared with level in absence of detergent.

Fig. 2. Changes in Mg-ATPase (a) and Na,K-ATPase (b) activity of rat heart homogenate after injection of IP (M ± m, from 12-20 observations). Abscissa, time after injection of IP (in h); ordinate, ATPase activity (in μmoles P_i/mg protein/h). 1) Measurements made in the absence of Lubrol WX; 2) in presence of 0.01% Lubrol WX. *P < 0.002 compared with control.

tion at 735 nm was measured after 20 min. Na,K-ATPase activity was calculated as the difference between hydrolysis of ATP in the absence and presence of ouabain. The "basal" Mg-ATPase activity was determined as the difference in samples with ouabain and in control samples to which protein was added after the reaction had stopped. Activity of the experimental and control preparations was always determined in parallel. Protein was determined by Lowry's method and Student's test was used for statistical analysis.

EXPERIMENTAL RESULTS

Macroscopically visible necrosis of the myocardium developed in the rats 24 h after the last injection of isoproterenol. Despite the severity of the tissue damage, the yield of protein of the homogenate, calculated per gram wet weight of tissue, was unchanged. According to the results of ten experiments, in the control it was 152 ± 16 mg/g tissue, falling after 1 h to 146 ± 15 mg/g and after 6 h to 140 ± 11 mg/g, whereas 24 h after addition of isoproterenol it was 147 ± 16 mg/g tissue.

Activity of Na,K-ATPase as a percentage of total ATPase activity of the heart homogenate was quite low, on average 16%, despite the fact that mitochondrial ATPase was inhibited by 5 mM sodium azide, and that the different Ca-ATPases were inhibited by addition of 0.5 mM EGTA to the determination medium.

Much of the activity of Na,K-ATPase is known not to be revealed without special treatment, as a result of incomplete accessibility of the ligands for the enzyme. Total Na,K-ATPase activity was found by the action of low concentrations of the nonpolar detergent Lubrol WX [3]. Activity of the enzyme rose sharply after the addition of 0.0025% Lubrol WX, subsequently flattening out on a plateau in the presence of 0.01-0.02% of detergent, whereas the "basal" Mg-ATPase activity fell steadily with an increase in the Lubrol WX concentration (Fig. 1).

The activation curve of Na,K-ATPase by Lubrol WX was similar in character both for the control preparations and for preparations obtained after administration of isoproterenol, the only difference being in the degree of activation. Differences in the degree of activation evidently reflect the presence of particles (vesicles) of the sarcolemma that are completely permeable for ligands of the enzyme even without detergent, in preparations obtained from necrotic tissue, as is clear from data in the literature showing increased permeability for tracers [2, 5, 11].

Na,K-ATPase activity, measured in the presence of 0.01% Lubrol WX, was appreciably depressed (by 18%, P < 0.001) during the first 6 h after treatment with IP, and then remained

unchanged until 24 h (Fig. 2). In the absence of the detergent no changes in Na,K-ATPase activity were found (Fig. 2). Other workers [7] also observed a decrease in Na,K-ATPase activity 24 h after injection of IP, accompanied by an increased outflow of protein. However, the time course of these changes in the earlier stages of myocardial damage was not known.

By contrast with the sarcolemmal enzyme, activity of "basal" Mg-ATPase was unchanged in the early stage of development of isoproterenol necrosis; not until 24 h after injection of IP was this activity, measured in the presence of detergent, 14% higher ($P < 0.002$) than in the control (Fig. 2). It will be clear from Fig. 1 that inhibition of Mg-ATPase activity by Lubrol WX was reduced in preparations obtained from necrotic tissue. It is not known what caused this shift in the concentration-effect curve for detergent at the peak of necrosis, for "basal" Mg-ATPase activity is a component of several Mg-ATPase activities, differing in their location in membranous and nonmembranous formations of the cell.

Changes in activity of Na,K-ATPase, as enzyme located in the sarcolemma, were in good agreement with data in the literature showing a sharp and selective increase in permeability of the cardiomyocyte plasma membrane after exposure to high doses of IP [2, 5, 11] and accumulation of Ca^{++} in damaged heart muscle cells [4, 8] at the same stage of injury, and they suggest that these lesions of the sarcolemma may be based on a single mechanism.

Adrenochrome, a product of oxidative metabolism of catecholamines, is known to depress Na,K-ATPase activity of sarcolemmal preparations of the rat heart sharply *in vitro*, and to reduce its activity moderately (by 25%) *in vivo* during perfusion of the isolated heart under conditions leading to the development of necrosis [13]. The mechanisms of action of adrenochrome *in vitro* and *in vivo* probably differ. Some workers ascribe a leading role in the pathogenesis of isoproterenol necrosis to oxidation products of catecholamines of the adrenochrome type [6].

Overloading of heart muscle cells with Ca^{++}, which takes place in response to injection of cardiotoxic doses of IP [4, 8], can trigger a chain of reactions leading to inactivation of many enzymes, disturbance of membrane permeability, and subsequent cell death. In addition, an increase in the Ca^{++} concentration inhibits activity of Na,K-ATPase. Data on possible modification of Na,K-ATPase molecules under the influence of Ca-dependent phosphorylation could not be found in the literature.

A direct effect of IP, by a β-adrenergic mechanism, on Na,K-ATPase through cAMP-dependent phosphorylation can evidently be disregarded, for the period of β-adrenergic stimulation ends before any significant fall in Na,K-ATPase activity is found. Data in the literature on the action of cAMP on Na,K-ATPase activity in the heart are contradictory. cAMP has been found to have no effect on the sarcolemmal Na,K-ATPase of the heart [10, 15]. Some workers found [1, 9] that cAMP (10^{-6}-10^{-4} M), like adrenalin [9], in experiments *in vitro* inhibits (evidently through a stage of cAMP-dependent phosphorylation) Na,K-ATPase of the heart.

Further studies not only of Na,K-ATPase, but also of other sarcolemmal enzymes during myocardial necrosis will provide a better understanding of the mechanisms of the cardiotoxic action of isoproterenol.

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