EFFECT OF STROPHANTHIN AND DIGOXIN ON SUCCINATE AND LACTATE DEHYDROGENASE AND MEMBRANE Na^+, K^+-ATPase ACTIVITY IN THE HEART OF RATS WITH EXPERIMENTAL MYOCARDITIS

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The effect of cardiac glycosides (CG) on transport of bioinorganic ions across cardiomyocyte cell membranes and on structural components and function of the myocardial cell has been studied in fair detail. The ability of CG to affect biochemical processes in the myocardium, however, is reflected in contradictory data [1-3]. Likewise, the problem of the effect of CG on activity of membrane-bound Na^+,K^+-ATPase, which is a receptor for them [14], has not been finally solved. Some workers [8, 13] consider that in therapeutic doses CG reduce activity of membrane-bound Na^+,K^+-ATPase, whereas others [7, 10], on the other hand, have found that glycosides have a stimulating action on the activity of this enzyme.

The aim of this investigation was to determine activity of the key enzymes of the tricarboxylic acid cycle, namely lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH), and also of membrane-bound Na^+,K^+-ATPase, under the influence of therapeutic doses of glycosides.

EXPERIMENTAL METHOD

Two series of experiments were carried out, each of them on four groups of mature noninbred male rats: one control group and three groups with experimental myocarditis. Myocarditis was created in the rats by subcutaneous injections of adrenalin (daily for 4 days in a dose of 0.1 ml of a 0.1% solution/150 g body weight). The presence of myocarditis was confirmed histologically, by processing the material by the usual methods and staining with hematoxylin and eosin (20 experiments altogether) and biochemically – by determining the serum aspartate aminotransferase activity by the method developed by the All-Union Scientific- and Technical Center for Laboratory Investigations, Ministry of health of the USSR (Instruction No. 290 dated April 11, 1972), with the aid of a KFO-U4 single-beam photoelectric colorimeter, OS-6M centrifuge, and thermostat (20 experiments altogether). In the experiments of series I the therapeutic effect of strophanthin and digoxin was evaluated by determining the endurance of rats during physical exertion, produced by swimming in water at 28-33°C, carrying a load equal to 7% of body weight, until complete exhaustion. The rats of group 1 (control 1) received subcutaneous injections of 0.1 ml/150 g body weight of distilled water daily for 4 days, and also on the 5th day, 20-30 min before swimming, in the same volume as the test drugs.

In group 2 rats with myocarditis (control 2) were given a subcutaneous injection of distilled water in the dorsal region on the 5th day of the experiment, 20-30 min before swimming, in the same volume as the drugs. In groups 3 and 4, rats with myocarditis were given a subcutaneous injection of strophanthin and digoxin respectively, 20-30 min before swimming. In the experiments of series II, which also were performed on four groups of rats, a quantitative histochemical study was made of myocardial SDH, LDH, and Na^+,K^+-ATPase activity. The animals were decapitated 20-30 min after subcutaneous injection of CG. Pieces of tissue from the left ventricle were frozen in liquid nitrogen. Myocardial sections 10 μ thick were cut on a cryostat. To detect the enzymes, tetrazolium (for determination of SDH and LDH) and cobalt (for determination of total ATPase activity) methods were used [5].

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TABLE 1. Effect of Strophanthin and Digoxin on Length of Time Rats Swam until Completely Fatigued

	Group of animals	Dose, μg/g	Number of experiments	Duration of swimming until complete exhaustion, min
1	(control 1)	—	10	198
2	(control 2)	—	20	57
3		2,7	20	140*
4		0,89	20	122*

Legend. *$p < 0.05$ Compared with control 2 (myocarditis).

TABLE 2. Effect of Strophanthin and Digoxin on SDH, LDH, and Membrane-Bound Na^+, K^+-ATPase Activity in Sections from Rat Heart

	Group of animals	Dose, μg/g	Number of experiments	LDH	SDH	Na^+, K^+-ATPase
				optical density units		
1	(control 1)	—	12	0,284	0,543	0,061
2	(control 2)	—	12	0,329	0,472	0,045
3		2,7	12	0,253*	0,563*	0,009*
4		0,89	12	0,266*	0,543*	0,023*

Legend. *$p < 0.01$ Compared with control 2 (myocarditis).

Activity of Na^+,K^+-ATPase was determined as the difference between total ATPase activity and activity in the presence of 0.1 mM strophanthin in parallel series of experiments. For cytophotometry of the sections, a single-beam MTsFU-2MP cytophotometer with ×40 objective and with an optial probe 1 μ in diameter was used, by scanning at a wavelength of 545 nm to determine SDH and LDH, and of 520 nm to determine ATPase. The experimental results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney U test [4].

EXPERIMENTAL RESULTS

The results obtained in the experiments of series I are given in Table 1.

The experiments established the doses of strophanthin and digoxin which gave rise to a therapeutic effect in rats with myocarditis. These same conventionally therapeutic doses of CG were used in the experiments of series II to study their biochemical effects (Table 2).

It follows from Table 2 that in adrenalin myocarditis changes take place in LDH and SDH activity.

Activation of LDH observed in sections through the heart of rats with myocarditis indicate a decrease in the ability of the myocardial cells to utilize the pyruvate formed during glycolysis, in the tricarboxylic acid cycle, and of accumulation of lactic acid. Tissue lactacidosis is one of the parameters characterizing the degree of oxygen insufficiency and it is a unique indicator of the depth of tissue hypoxia [6]. Meanwhile SDH activity was reduced, further evidence of inhibition of aerobic processes in the myocardium. The results are in agreement with data in the literature on a decrease in the activity of the respiratory chain in myocarditis [2]. Inhibition of tissue respiration – the main source of energy – is accompanied by a decrease in membrane-bound Na^+,K^+-ATPase activity, which is directly linked with the ATP level in the cardiomyocytes.

CG, injected in conventionally therapeutic doses, depressed LDH activity and enhanced SDH activity. The action of the glycosides was expressed as predominance of oxidative processes and diminution of signs of hypoxia in the heart muscle, which is usually accompanied by restoration of the ATP concentration. However, although the ATP level in the

cardiomyocytes was evidently raised, activity of membrane-bound Na^+,K^+-ATPase was sharply reduced by the action of CG. The mechanism of the inhibitory action of the glycosides on Na^+,K^+-ATPase is dependent on the ability of the drugs to prevent binding of ATP with the enzyme molecule [11] and to insert themselves into the membrane in the immediate proximity of the active centers of the enzyme, and thereby to change its conformation and mobility [9], and to compete with ATP molecules to form bonds with ATPase [15].

The increased resistance of rats with myocarditis to physical exertion, under the influence of cardiac glycosides, is probaly a result of compensation of disturbances of myocardial energy metabolism.

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