ACTION OF CARDIAC GLYCOSIDES ON THE CONCENTRATIONS OF ADENYL NUCLEOTIDES AND NICOTINAMIDE-ADENINE DINUCLEOTIDES IN THE RAT MYOCARDIUM

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Experiments on rats showed that strophanthin K (0.1 mg/100 g body weight) reduces the ATP and ADP content in the rat myocardium by 23 and 26.6% respectively but does not change the AMP level. Convallatoxin (0.07 mg/100 g) increases the ATP concentration by 33%, does not change the ADP level, and lowers the AMP concentration by 51%, and this effect is accompanied by the appearance of inosine monophosphate, inosine, and adenosine. Both glycosides increase the content of reduced forms of nicotinamide coenzymes; convallatoxin also increases the combined concentration of oxidized and reduced forms of NAD, with a tendency for the combined content of NAD and NADP to increase.

In the analysis of the therapeutic effect of the cardiac glycosides special attention is paid to their effect on myocardial metabolism [10, 12]. Previous investigations [8, 9] showed that the glycosides liberate catecholamines from sympathetic nerve endings where they are stored in the form of complexes with adenyl nucleotides, chiefly ATP [1].

This accounts for the considerable interest in the study of the effect of cardiac glycosides on the content of adenyl nucleotides in the heart muscle and the comparison of the state of their phosphorylation with the content of nicotinamide-adenine dinucleotides, substances with a very important role in the metabolic control of energy production in the cell. This was the object of the investigation described below.

EXPERIMENTAL METHOD

Albino rats weighing 180-230 g were used. The cardiac glycosides strophanthin K and convallatoxin were injected intraperitoneally in doses of 0.1 mg/100 g and 0.07 mg/100 g body weight respectively. The animals were decapitated 1 h after injection of the drugs, as when the catecholamine concentrations were investigated [9].

Adenyl nucleotides were identified by electrophoresis on paper [2, 13] and inorganic phosphorus was determined by Delory's method in Grigor'eva's modification [3]. The concentration of adenyl nucleotides was determined on the SF-16 spectrophotometer at wavelengths of 260 and 290 nm and expressed in μmoles/g wet weight of tissues. The combined content of oxidized (NAD + NADP) and reduced forms (NAD$\cdot H_2$ + NADP$\cdot H_2$) of nicotinamide-adenine dinucleotides was determined in a heart muscle homogenate by the method of Huff and Perlzweig [11] and expressed in μg/g wet weight of tissue.

EXPERIMENTAL RESULTS AND DISCUSSION

Three fractions (differing in their position relative to the cathode) corresponding to AMP, ADP, and ATP were found on electrophoretic fractionation of the adenyl nucleotides from the control group of animals. The ATP content was 40% of the total content of adenyl nucleotides (Table 1), in agreement with data in the literature [6]. Injection of strophanthin was followed by a decrease of 23 and 26.6% in the ATP and ADP

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TABLE 1. Effect of Cardiac Glycosides on Content of Adenyl Nucleotides (in μmoles/g tissue) and Inorganic Phosphorus (in mg %) in Heart Muscle of Rats 1 h after Intraperitoneal Injection (M ± m)

Parameter studied	Control	Strophanthin	Convallatoxin
	1	2	3
ATP	2,4±0,16	1,85±0,12	3,2±0,238
		$P_{1-2}<0,01$	$P_{1-3}<0,02$
ADP	2,18±0,115	1,6±0,08	2,23±0,177
		$P_{1-2}<0,001$	$P_{1-3}>0,05$
AMP	1,47±0,08	1,26±0,16	0,72±0,12
		$P_{1-2}>0,05$	$P_{1-3}<0,001$
Combined content of adenyl nucleotides	6,06±0,27	4,6±0,2	6,16±0,26
		$P_{1-2}<0,001$	$P_{1-3}>0,05$
IMP	absent	absent	0,85±0,06
Inosine	absent	absent	0,18±0,02
Adenosine	absent	absent	0,26±0,027
Inorganic phosphorus	26,8±2,0	30,83±1,47	36,14±2,7
		$P_{1-2}>0,05$	$P_{1-3}<0,01$

TABLE 2. Effect of Strophanthin and Convallatoxin on Content of Nicotinamide Coenzymes (in μg/g wet weight of tissue) in Myocardium of Rats 1 h after Intraperitoneal Injection (M ± m)

Preparation and dose	NAD + NADP	NAD·H_2 + NADP·H_2	Combined content of nicotinamide coenzymes	Ratio NAD+NADP / NAD·H_2 + NADP·H_2
1. Control (n = 10)	340,1±25,2	189,9±13	530±37,2	1,75±0,07
2. Strophanthin 0.1 mg/100 g (n = 10)	333,1±20,25	234±15,5	565,1±25,1	1,47±0,12
	$P_{1-2}>0,05$	$P_{1-2}<0,02$	$P_{1-2}>0,05$	$P_{1-2}>0,05$
3. Convallatoxin 0.07 mg/100 g (n = 10)	391,5±25,1	237,1±12,65	628,6±36,6	1,64±0,05
	$P_{1-3}>0,05$	$P_{1-3}<0,01$	$P_{1-3}<0,001$	$P_{1-3}>0,05$

levels respectively compared with their original values. There was no significant change in the AMP concentration. The combined content of adenyl nucleotides fell by 24% mainly on account of the decrease in the ATP and ADP levels. The inorganic phosphorus concentration showed a tendency to increase.

The changes after injection of convallatoxin were more marked and varied in character. Electrophoresis showed six fractions (relative to the cathode) corresponding to adenosine, inosine, AMP, inosine monophosphate (IMP), ADP, and ATP. The ATP level was 33% higher than initially, but ADP was unchanged. Unlike strophanthin, convallatoxin led to a marked decrease (by 51%) in the AMP level and this was accompanied by the appearance of its dephosphorylation products (adenosine). The adenosine formed in all probability was deaminated by deaminase to inosine. The appearance of IMP and inosine could be evidence of activation of the deamination of the adenyl nucleotides. The content of inorganic phosphorus was significantly increased by 34% compared with the control. These results are difficult to interpret because the two glycosides have divergent actions. In Kondrashova's opinion [5], the action of strophanthin on the myocardium is based on mild uncoupling of oxidative and energy-yielding processes. Under these conditions the intensity of phosphorylation lags behind the sharply increased intensity of oxidation, the P/O ratio falls, and as the experiments described above show, this may lead to a decrease in the ATP level. Meanwhile convallatoxin, in the dose used, produced a small decrease in the intensity of both respiration and phosphorylation [4], with no consequent change in the P/O ratio. A sharp increase in the intensity of myocardial contractions may also be accompanied by an increase in ATP formation, even if the P/O ratio is lowered [7].

Strophanthin had no significant effect on the concentration of the oxidized forms of nicotinamide coenzymes (Table 2) but it increased the content of their reduced forms by 23% over the control; the ratio NAD + NADP/NAD·H_2 + NADP·H_2 showed a tendency to fall.

The action of convallatoxin was more marked and it led to an increase in the combined content of nicotinamide coenzymes by 18% and of their reduced form by 24%, a tendency toward an increase in the oxidized form of NAD, but with no significant change in the NAD + NADP/NAD·H_2 + NADP·H_2 ratio. The increase in the content of nicotinamide coenzymes is evidence of active function of the respiratory chain.

These results show that the increase in the contractile power of the myocardium produced by the glycosides is accompanied by changes in the level of adenyl nucleotides and of nicotinamide coenzymes, i.e., factors controlling metabolism at the molecular level.

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