Effect of Digoxin on Acid Hydrolase Activity in the Myocardium

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Dose-dependent effect of digoxin on cardiac lysosomal enzymes is demonstrated. In doses of 2.5 and 5 mg/kg digoxin suppresses and in a dose of 10 mg/kg increases the activity of myocardial acid hydrolases. By sensitivity to digoxin, the enzymes rank as follows: β-glucosidase>β-galactosidase>acid phosphatase.

Key Words: *digoxin; myocardial acid hydrolases*

The pathogenesis of chronic cardiac failure is associated with excessive activation of the neuroendocrine system, increased concentration of catecholamines in the blood [9], enhanced catabolic processed in the myocardium, and injury to intracellular cardiomyocyte structures [6]. The injury to intracellular structures in cardiac failure is explained by increased content of lysosomes, destruction of their membranes, and release of acid hydrolases into the cytosol [5]. Lysosomal enzymes can destroy myofibrils and mitochondria [8]. Lysosomal enzymes suppress myocardial contractility [2,3], and infusion of acid hydrolases in the bloodflow of rabbits appreciably decreases the intensity of cardiac contractions.

Of the drugs possessing positive inotropic effect, only cardiac glycosides, specifically, digoxin, are effective in the treatment of cardiac failure [13]. However, the effect of digoxin on enzyme activity and lysosomal membranes is unknown.

Our purpose was to study the effect of digoxin on the activity of myocardial acid hydrolases at different doses and different times after its administration.

MATERIALS AND METHODS

Experiments were carried out on outbred male rats weighing 120-140 g. Digoxin was injected once intraperitoneally in doses of 2.5, 5, and 10 mg/kg. Distilled water was injected to controls. The animals were decapitated under slight ether narcosis 5 and 30 min and 1, 2, 4, 24, and 48 h after injection. Rat hearts free from blood were pushed through a pump tissue fragmentator and homogenized in a glass Potter-Elweieme homogenizer with a Teflon pestle on the cold. The medium for isolation and homogenization was 0.25 M sucrose solution with 0.001 M EDTA prepared on 0.05 M Tris-HCl buffer, pH 7.4. Activities of β-glucosidase (GL, AP 3.2.1.21), β-galactosidase (GAL, AP 3.2.1.23), and acid phosphatase (AP 3.1.3.2) in the homogenate were measured as described previously [12]. The substrates were 4-nitrophenyl-β-D-glucopyranoside and 4-nitrophenyl-β-D-galactopyranoside. The activity of acid phosphatase (AP) was measured by spectrophotometry with sodium β-glycerophosphate as the substrate [4]. The availability of substrate for enzymes was assessed by the percent ratio of free to total enzyme activities. For measuring total activity, Triton X-100 in final concentration of 0.1% for GAL and AP and 0.05% for GL was used [7].

Enzyme activity was expressed in mmole/min×g protein. Protein content was measured as described elsewhere [10]. The data were processed using variational statistics method [1].

RESULTS

A single intraperitoneal injection of digoxin in a dose of 2.5 mg/kg changed the activities of cardiac lysosomal enzymes. Table 1 shows that 5 min after in-

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jection of the drug free GL activity decreased almost by half and total activity dropped 3-fold. The enzyme activity remained suppressed for 24 h. Free and total GL activities were minimal 1 and 2 h after digoxin injection, respectively. After 48 h, the enzyme activity normalized.

The effect of digoxin (2.5 mg/kg) on GAL activity was weaker. Free and total activities of the enzyme were suppressed 5 min after injection of the drug. Later, both activities continued to decrease and were minimal in 24 h. After 48 h, GAL activity was normal.

AP was still less sensitive to digoxin in this dose than GAL. Only 2 and 4 h after injection of the drug the total AP activity decreased.

Thus, digoxin in a dose of 2.5 mg/kg decreased the activities of all tested acid hydrolases. GL was the most sensitive to it: 1 h after injection its free activity was 3 times lower in comparison with the control. GAL activity was less suppressed than that of GL, and AP activity was less suppressed than that of GAL.

Digoxin in a dose of 5 mg/kg had a weaker effect on cardiac lysosomal enzymes (Table 2). The total GL activity was suppressed only 5 min and 1 and 4 h postinjection, GAL activity 5 min, and AP activity 5 min and 4 h after injection. Free AP activity was decreased 5 min after injection of digoxin.

Digoxin in a dose of 10 mg/kg caused two-phase changes in the activities of myocardial acid hydrolases. Table 3 shows that 5 min after injection, total and free GL activities decreased more than by half in comparison with the control and normalized after 30 min. Later, the enzyme activity increased and

TABLE 1. Activities of Myocardial Acid Hydrolases after a Single intraperitoneal Injection of 2.5 mg/kg Digoxin ($M\pm m$, n=6-10)

Time postinjection	Free activity	Total activity	Free/total, %
	μmole/min×g protein		
β-Glucosidase			
Control	0.1750±0.0250	0.3280±0.0210	50.8±7.9
5 min	0.0901±0.0291	0.1150±0.0302*	85.5±20.7
30 min	0810±0.0171*	0.1140±0.0151*	71.1±10.5
h	0.0580±0.0130*	0.0750±0.021*	77.3±38.8
2 h	0.0610±0.008*	0.0680±0.019*	90.0±11.0
4 h	0.0601±0.0113*	0.0951±0.0240	63.2±17.1
24 h	0.0570±0.008*	0.0846±0.017*	67.9±20.9
48 h	0.2001±0.035	0.2215±0.037	90.3±15.0
β-Galactosidase			
Contro	0.2662±0.05	0.3392±0.04	79.0±15.9
5 min	0.1770±0.038*	0.2310±0.022*	77.8±14.9
30 min	0.2120±0.04*	0.2750±0.008	79.8±17.5
1 h	0.1645±0.029*	0.2029±0.016*	84.7±13.9
2 h	0.2096±0.037*	0.2220±0.0405*	94.5±28.5
4 h	0.1218±0.021*	0.1525±0.037*	80.1±13.0
24 h	0.1062±0.01*	0.1428±0.023*	75.0±25.9
48 h	0.4001±0.06	0.3960±0.05	101±15.2
Acid phosphatase			
Control	8.45±0.06	11.70±1.80	63.20±2.96
5 min	6.94±1.05	10.51±1.20	66.01±5.2
30 min	8.05±2.10	12.50±1.91	64.4±3.8
1 h	7.05±1.12	9.92±1.35	71.1±13.5
2 h	5.10±1.90	6.6±0.89*	77.2±6.0
4 h	4.52±0.92	5.9±0.98*	76.6±2.7
24 h	9.75±1.11	13.46±1.88	74.00±5.28

Note. Here and in Tables 2 and 3: *$p<0.05$ *vs.* the control.

after 4 h reached the maximum. After 24 h it did not differ from the control.

The effect of digoxin on GAL activity was weaker in comparison with its effect on GL activity. The enzyme activity was similarly suppressed at the beginning, normalized after 30 min, and increased after 1 h. After 48 h GAL activity was normal.

The activity of AP was suppressed by digoxin in a dose of 10 mg/kg 5 min and increased 1 and 4 h after injection. After 24 h, the total AP activity was again suppressed and after 48 h normalized.

Thus, digoxin in a dose of 10 mg/kg at first (5 min after injection) suppressed the activities of acid hydrolases, then increased the activities of glycosidases, increased and again suppressed (after 24 h) the activity of AP.

Hence, the pattern and intensity of digoxin effect on cardiac lysosomes depends on its dose, time elapsed after administration, and individual sensitivity of hydrolases to cardiac glycoside. In doses of 2.5 and 5 mg/kg digoxin suppressed and in a dose of 10 mg/kg first suppressed and then increased the activities of acid hydrolases. The most expressed changes were observed 1, 2, and 4 h after injection of the drug. GL was most sensitive to digoxin, whereas AP most resistant.

There are no published reports about the effect of digoxin on the activity of cardiac lysosomal enzymes. The pathogenic role of acid hydrolases in irreversible injury to cardiomyocytes [14] and in the development of cardiac failure [2,3,5] prompts us to regard our data on the suppressive effect of digoxin in therapeutic doses on the activities of acid hydrolases as a favorable effect and a component of the positive inotropic action of this drug. By contrast, an increase in the activity of lysosomal enzymes with catabolic direction of the effect by toxic doses of the drug can lead to development of dystrophic and necrotic foci in the myocardium of patients dying from glycoside intoxication [11].

The different sensitivities of the studied acid hydrolases to digoxin can be explained by their different localization. Glycosides are localized mainly in cardiomyocytes [14], being more sensitive to digoxin

TABLE 2. Effect of 5 mg/kg Digoxin on Activities of Myocardial Acid Hydrolases of Intact Rats ($M \pm m$, n=6-10)

Time postinjection	Free activity	Total activity	Free/total, %
	μmole/min×g protein		
β-Glucosidase			
Control	0.175±0.024	0.328±0.021	60.8±12.0
5 min	0.161±0.005	0.215±0.04*	74.4±10.6
30 min	0.185±10.029	0.246±0.035	74.5±10.3
1 h	0.169±0.031	0.192±0.038*	88.0±15.0
4 h	0.142±0.03	0.181±0.045*	77.3±6.7
4 h	0.245±0.051	0.320±0.06	76.5±3.8
24 h	0.245±0.051	0.320±0.06	76.5±3.8
β-Galactosidase			
Control	0.265±0.030	0.339±0.04	88.8±15.0
5 min	0.205±0.028	0.200±0.023*	102.5±4.5
30 min	0.255±0.02	0.287±0.024	90.8±6.2
1 h	0.197±0.019	0.298±0.031	64.0±11.0
2 h	0.245±0.0173	0.306±0.0199	80.1±5.7
4 h	0.200±0.036	0.207±0.011	96.6±8.1
24 h	0.310±0.040	0.390±0.037	79.5±5.9
Acid phosphatase			
Control	8.45±0.630	11.7±1.81	73.6±3.41
5 min	5.90±0.912*	7.1±1.00*	83.1±5.9
30 min	9.50±0.52	13.6±1.09	71.9±5.09
1 h	8.00±0.98	10.0±0.75	80.5±6.3
4 h	5.89±0.49	6.04±1.55*	97.02±10.5
24 h	11.0±2.05	12.15±1.46	93.7±8.1

TABLE 3. Effect of 10 mg/kg Digoxin on Activities of Myocardial Acid Hydrolases of Intact Rats (*M±m*, *n*=6-10)

Time postinjection	Free activity	Total activity	Free/total, %
	μmole/min×g protein		
β-Glucosidase			
Control	0.288±0.018	0.296±0.021	125.8±7.5
5 min	0.111±0.035*	0.115±0.040*	96.5±12.7
30 min	0.209±0.041	0.216±0.05	99.8±10.8
1 h	0.507±0.060*	0.515±0.041*	103.5±15.7
4 h	0.520±0.102*	0.540±0.052*	109.7±10.8
24 h	0.396±0.110	0.420±0.062	107.9±7.8
48 h	0.290±0.12	0.300±0.18	103.4±10.5
β-Galactosidase			
Control	0.431±0.028	0.473±0.031	91.8±6.7
5 min	0.296±0.045*	0.300±0.037*	99.6±10.1
30 min	0.400±0.080	0.456±0.061	90.9±3.6
1 h	0.628±0.051*	0.652±0.071*	100.2±25.0
4 h	0.695±0.080*	0.725±0.1001*	105.6±8.1
24 h	0.629±0.05*	0.645±0.048	98.8±5.9
48 h	0.395±0.071	0.405±0.105	98.5±10.9
Acid phosphatase			
Control	5.81±0.85	13.48±1.12	43.2±5.4
5 min	3.2±0.56*	5.85±0.93*	39.8±3.2
30 min	6.0±0.76	12.6±2.8	49.0±2.6
1 h	8.95±0.95*	19.54±1.52*	45.4±5.0
4 h	12.6±2.01*	18.9±1.15*	66.7±7.0*
24 h	5.6±1.25	8.0±0.75*	70.0±1.2*
48 h	6.5±1.5	14.7±2.01	44.2±6.8

effect than AP typical of interstitial cells. GL is localized in lysosomal membranes and GAL in their matrix [7]. This may account for a more potent effect of lipophilic digoxin on the activity of GL.

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