Hemodynamic Disturbances and Activation of Lipid Peroxidation in the Pathogenesis of Acute Myocardial Injury Induced by Propranolol in Toxic Doses

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We studied the dose-dependent cardiotoxic effect of propranolol. Intraperitoneal injection of propranolol in doses of 1 and 2 mg/100 g body weight produced a potent effect on central hemodynamics and myocardial contractility, impaired diastolic relaxation, and caused damage to cardiomyocyte membranes due to activation of free radical oxidation.

Key Words: *poisoning; propranolol; hemodynamics; isolated heart*

Energy deficiency during chemical trauma and hypoxia is followed by metabolic and structural changes in various organs and tissues, including the heart [6]. Irreversible changes and cell death are related to dysfunction of various metabolic pathways in the cytoplasm and mitochondria [12], development of acidosis [3], activation of free radical oxidation [6,14], and damage to biological membranes [8] (lipid bilayer and membrane proteins, including the enzymes [11]).

Acute poisoning with propranolol is manifested in cardiovascular dysfunction (β-адреноблокирующий синдром – bradycardia and hypotension) [7,14,15]. A selective cardiotoxic effect of this agent is followed by abnormalities in the heart rate, myocardial conductivity, and contractility [9]. Much attention was paid to the action of propranolol in toxic concentrations. Little is known about the dose-dependent effect of propranolol on the myocardium, which is accompanied by an imbalance in LPO and antioxidant system. It should be emphasized that these disorders determine the severity of chemical trauma.

Here we studied functional and metabolic changes in the heart during acute poisoning with propranolol. The role of cardiac dysfunction in the development of circulatory insufficiency was evaluated.

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MATERIALS AND METHODS

Experiments were performed on 50 male outbred albino rats weighing 214±12 g. The animals were divided into 3 groups of 10 specimens each. Group 1 rats did not receive propranolol (control). Propranolol in single doses of 1 and 2 mg/100 g body weight was injected intraperitoneally to group 2 and 3 animals, respectively. After administration of propranolol, the carotid artery was catheterized to measure blood pressure (BP, mm Hg). ECG (lead II) and HR (min^{-1}) were recorded. The state of systemic hemodynamics was estimated from an integral rheogram and its first derivative [4]. The measurements were conducted on a RPG2-02 rheoplethysmograph and N-338-6P recording device. Stroke volume (SV, μl), cardiac output (CO, ml/min), and total peripheral vascular resistance (TPVR, 10^3 dynes×sec×cm^{-5}) were evaluated.

Myocardial contractility was studied on 20 isolated hearts (isovolumetric contractions) from group 1 and 3 rats [16]. The heart was removed after thoracotomy and placed in cold Krebs–Henseleit solution (2-4°C). After partial resection of the left atrium, the heart was fixed (by the aorta) on a cannula of a perfusion device. A latex balloon of fixed volume was inserted into the left ventricle. This balloon was connected to a PM-8000 portable monitor. Retrograde perfusion of the heart was performed through the aorta using the same solution

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saturated with carbogen (95% O_2 and 5% CO_2) at 70 mm Hg, 37°C (maintained on a VT-8 ultrathermostat), and pH 7.33-7.36. The heart was exposed to electrical stimulation with rectangular pulses (duration 3 msec) using an ES-50-1 electrical stimulator. The voltage of electrostimulation was 10% above the baseline level. The frequency of stimulation was 240 min^{-1}. The left ventricular pressure curve was recorded after 30-min normoxic perfusion (necessary for stabilization of heart activity). Systolic pressure, diastolic pressure, developed pressure, and rates of left ventricle contraction (dp/dt max) and relaxation (-dp/dt max) were estimated from graphic data. In parallel, perfusate samples passing the coronary bed were taken and the concentrations of glucose (glucose oxidase method; Ella), lactate (enzymatic method; Vital diagnostics SPb), and pyruvate [2] and activities of AST and LDH (catalytic activity method; Lachema Diagnostika), and creatine kinase myocardial fraction (CK-MB; enzymatic method; Ol'veks Diagnostikum) were measured. The consumption of glucose and pyruvate was calculated per 1 g dry weight of the myocardium and 1 mm Hg developed pressure over 1 min.

The intensity of free radical oxidation was estimated by the method of blood plasma chemiluminescence after addition of iron sulfate. The measurements were performed on a KhL-003 chemiluminometer. Luminescence was induced by addition of 1 ml 50 mM $FeSO_4 \times 7H_2O$, which accelerates LPO. Luminescence was recorded for 10 min. We evaluated the spontaneous luminescence (arb. units), flash (arb. units), and total luminescence (arb. units×min) [13].

The results were analyzed by methods of variation statistics. The arithmetic mean and error of the arithmetic mean were calculated. The significance of differences between the mean values was estimated by Student's *t* test (Microsoft Excel software). The data were tested for conformity to a normal distribution.

RESULTS

In animals receiving propranolol, HR and BP tended to decrease over 60 min of propranolol poisoning. The decrease in SV, CO, and TPVR (Table 1) reflected impairment of systemic circulation. The increase in SV in group 3 rats was associated with progressive bradycardia.

Measurement of chemiluminescence parameters in propranolol-treated animals (Table 2) revealed an increase in activity of the prooxidant system (flash amplitude) and decrease in activity of the antioxidant system (decrease in the total luminescence). Activation of free radical oxidation serves as a factor, which causes damage to cardiomyocyte membranes and prooxidant/antioxidant imbalance.

The severity of hemodynamic disturbances was maximum in group 3 animals. Further studies were performed on isolated and isovolumetrically contracting hearts. The cardiotoxic effect of propranolol (Table 3) manifested in a decrease in the force (systolic pressure and developed pressure) and velocity parameters (contraction rate and relaxation rate) for contractile activity of the left ventricular myocardium. These changes resulted in abnormalities of myocardial relaxation.

The membrane-destructing effect of propranolol was accompanied by an increase in the release of cytosolic enzymes AST, LDH, and CK-MB into coronary circulation. This agent in the toxic dose impaired bioenergetics of the heart. We revealed an increase in the consumption of glucose per functional unit (by more than 13%) and contents of pyruvate and lactate in the coronary perfusate. These changes attested to uncoupling of oxidation and phosphorylation processes (*i.e.*, mitochondrial dysfunction; Table 3).

Our results suggest that administration of propranolol in toxic doses is followed by circulatory failure in animals. This state is related to cardiac injury, which depends on the dose of propranolol. Central hemodynamic disturbances and primary cardiotoxic effect (reduction of myocardial contractility; diastolic dysfunction of the myocardium; increase in activities of AST, LDH, and CK-MB in the coronary circulation; and high consumption of glucose per 1 mm Hg developed pressure) attested to destruction of membranes and mitochondrial dysfunction of cardiomyocytes [5].

TABLE 1. Central Hemodynamics in Experimental Animals on Minute 60 of Acute Propranolol Poisoning (*M*±*m*)

Parameter	Group 1 (control)	Group 2	Group 3
HR, min^{-1}	348.0±12.8	294.0±11.6*	261.0±13.4*
BP, mm Hg	122.0±1.8	65.0±2.3*	59.0±1.5*
SV, μl	145.0±2.2	128.5±1.7*	133.1±1.8*
CO, ml/min	54.1±0.9	37.7±1.5*	34.7±1.5*
TPVR, 10^3 dynes×sec×cm^{-5}	181.1±2.1	146.8±6.6*	139.0±7.8*

Note. Here and in Table 3: *$p<0.05$ compared to the control.

TABLE 2. Chemiluminescence of Blood Plasma on Minute 60 of Acute Propranolol Poisoning (*M*±*m*)

Parameter	Group 1 (control)	Group 2	Group 3
Spontaneous luminescence, arb. units	0.29±0.01	0.090±0.004*	0.030±0.005*
Flash, arb. units	1.28±0.06	1.95±0.06*	2.25±0.04*
Total luminescence of the plasma, arb. units×min	0.71±0.02	0.36±0.01*	0.28±0.01*

Note. *$p<0.01$ compared to the control.

TABLE 3. Contractile Function and Metabolism of the Left Ventricle on Minute 60 of Acute Propranolol Poisoning (*M*±*m*)

Parameter	Group 1 (control)	Group 3
SP, mm Hg	56.3±1.4	27.8±0.7*
DP, mm Hg	4.8±0.2	5.7±0.3*
DvP, mm Hg	51.2±1.5	31.4±0.7*
dp/dt max, mm Hg/sec	1112±21	662±16*
-dp/dt max, mm Hg/sec	841±31	380±10*
Glucose, mmol/kg/min	140.5±0.7	161.9±0.9*
Lactate, mmol/kg/min	43.6±0.7	89.8±1.2*
Pyruvate, mmol/kg/min	2.40±0.04	17.9±0.2*
AST, μcat/g/min	1.9±0.1	3.3±0.1*
LDH, μcat/g/min	0.50±0.06	1.30±0.09*
CK-MB, U/liter	1.40±0.09	1.80±0.03*

Note. SP, systolic pressure; DP, diastolic pressure; DvP, developed pressure.

Propranolol in toxic concentrations significantly activated free radical oxidation affecting the state of the cardiovascular system and myocardium [10]. Administration of propranolol in toxic concentrations was followed by the development of functional and metabolic changes in the cardiovascular system. These data should be taken into account in the toxicogenic period of acute poisoning with propranolol. The primary cardiotoxic effect of the β-adrenoceptor antagonist should be prevented at the preadmission stage.

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