Effect of Hypoxia on Metabolism and Contractile Function of the Heart in Rats with Type 2 Diabetes Mellitus and Abdominal Obesity

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Type 2 diabetes mellitus was modeled in newborn albino rat pups. Metabolism and contractile activity of isolated heart under conditions of hypoxia were studied on adult rats. Contractile activity of the myocardium in animals with type 2 diabetes mellitus and abdominal obesity decreased during hypoxia. It was manifested in a decrease in systolic and developed pressure and disturbances in diastolic relaxation of the myocardium. Damage to cell membranes and increased secretion of aspartate transaminase into the coronary circulation were observed under conditions of energy deficit and activation of the anaerobic pathway of energy production. These changes became more pronounced with increasing the period of hypoxic exposure.

Key Words: *experimental diabetes mellitus; abdominal obesity; heart*

In addition to cardiovascular diseases and tumors, type 2 diabetes mellitus (DM2) is of considerable medical and social significance. This disorder is a frequent cause of disability and mortality [1-4]. DM2 is accompanied by hyperglycemia, low effectiveness of insulin, neuropathy, and ischemia. These disturbances contribute to the development of tissue hypoxia. Under these conditions cardiomyocytes cannot provide metabolic and functional adaptive changes, which would allow the myocardium to react adequately to overload. Here we studied the effect of hypoxia on functional activity and metabolism of the isolated heart from rats with DM2 and obesity.

MATERIALS AND METHODS

DM2 was modeled in newborn albino rat pups by the method of B. Portha *et al.* [8]. On day 1 after birth the animals intravenously received 100 μg/g streptozotocin in 25 μl citrate buffer (0.05 mmol/liter, pH 4.5). Glucosuria was monitored from the 2nd day after birth using Bioscan test strips. Further study was performed on animals with severe glucosuria. The rats were housed under standard conditions (12:12-h light/dark cycle, 22±3°C) and isolated from mothers on day 21. To produce abdominal obesity, the rat pups with DM2 fed a special high-fat diet (30% of total daily requirement). The experimental group included 8-12-month-old rats with DM2 and obesity (n=30). The control group consisted of 30 outbred rats. Control animals received 25 μl citrate buffer (0.05 mmol/liter, pH 4.5) on day 1 after birth, fed a standard diet, and were housed under similar conditions. The study was conducted according to the Policy on the Humane Care and Use of Experimental Animals.

The rats were intraperitoneally narcotized with 100 mg/kg calipsol (Gedeon Richter). The hearts were removed and placed in cold Krebs—Henseleit solution (2-4°C). The atria were partially excised. The aorta was fixed with a cannula using a device for isolated heart perfusion. A constant-pressure latex balloon connected to an original tensiometric transducer was inserted into the left ventricle. Mechanical oscillations

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of intraventricular pressure were transformed into electrical oscillations using an analog-digital converter and then recorded on an IBM personal computer and H338 automatic recorder.

Myocardial hypoxia was modeled as follows: the isolated hearts were perfused with oxygenated Krebs—Henseleit solution at 37°C and pH 7.33-7.36 for 30 min (Po_2=600 mm Hg) followed by perfusion with hypoxic solution (Po_2=150 mm Hg). The heart was paced at a rate of 240 bpm using rectangular pulses generated by an ES-50-1 electrical stimulator. Intraventricular pressure and power indexes (systolic, diastolic, and developed pressure) were recorded on the 2nd, 5th, and 10th minutes of hypoxic perfusion and after 10-min reoxygenation. The perfusate passing through the coronary bed was sampled and the concentrations of glucose (Fotoglyukoza), lactate (Olvex), and pyruvate [2] were measured. Consumption of glucose and release of lactate and pyruvate were calculated per 1 kg dry myocardium weight and 1 mm Hg developed pressure over 1 min. Aspartate transaminase (AST) activity was measured in the coronary perfusate [9]. Enzyme loss was determined per 1g dry myocardium weight over 1 h.

The results were analyzed by methods of variational statistics. The arithmetic mean ($\bar{X}$) and standard error (m) were calculated. The significance of differences between the mean and relative values (p) was estimated by Student's t test (Excel 7.0 software).

RESULTS

The hearts from animals with DM2 were more sensitive to hypoxia. On the 2nd minute of perfusion systolic and developed pressure decreased by 34.4 and 94.8%, respectively, while diastolic pressure increased compared to the baseline level (by 3.6 times, Table 1).

Diastolic pressure increased by 2.2 times and significantly exceeded the normal by the 10th minute of hypoxic perfusion. On the 10th minute of hypoxia developed pressure in the hearts of animals with DM2 and control rats decreased by 13.2 and 6.7 times, respectively, compared to the baseline level (Table 1).

After reoxygenation diastolic pressure remained high in rats of both groups: in treated and control animals this parameter exceeded the baseline level by 4 and 3 times, respectively. Published data show that activity of enzymes involved in Ca^{2+} transport sharply decreases under conditions of hypoxia and energy deficit. These changes are followed by accumulation of Ca^{2+} in the myoplasm, disturbances in diastolic relaxation of the myocardium, and suppression of contractile function [5-7].

Metabolic studies showed that the intensity of glucose consumption per 1 mm Hg developed pressure

TABLE 1. Effect of Hypoxia on Function and Metabolism of the Isolated Heart in Rats with DM2 ($\bar{X}\pm m$)

Parameter	Baseline level		Hypoxia				Reoxygenation, 10 min	
			2 min		10 min			
	control	treatment	control	treatment	control	treatment	control	treatment
Systolic pressure, mm Hg	54.1±7.5	43.5±2.2*	38.3±2.1	31.1±5.9*	30.3±2.1	27.0±2.2**	55.3±2.5	35.9±2.4**
Diastolic pressure, mm Hg	5.4±0.3	5.8±0.8	14.3±2.3	20.8±3.9*	18.6±2.2	25.9±0.9***	17.8±1.7	22.9±2.5**
Developed pressure, mm Hg	49.6±2.5	33.2±2.1*	27.4±3.2	7.7±1.8*	17.4±1.5	2.5±0.7***	39.5±2.7	17.2±1.8**
Glucose, mmol/kg/min	146.0±3.1	178.0±6.9*	—	—	1024.0±9.4	1617.0±14.2***	358.0±7.7	586.0±9.3**
Lactate, mmol/kg/min	48.0±1.5	85.0±5.4*	—	—	107.0±3.8	180.0±7.4***	73.0±2.4	146.0±9.0**
Pyruvate, mmol/kg/min	2.9±0.1	7.4±0.1	—	—	7.4±0.1	39.6±3.3**	5.7±0.1	22.6±1.9***
AST, μmol/g/h	17.0±0.8	85.0±5.0*	—	—	26.0±1.1	130.0±5.0*	27.0±1.1	97.0±6.3***

Note. *p<0.001 and **p<0.05 compared to the control; *p<0.001 and **p<0.01 compared to the baseline level.

increased by 9 times in the hearts from DM2 rats exposed to 10-min hypoxia (compared to the baseline level). In these rats the release of lactate and pyruvate into the coronary bed was higher than in control animals by 40.3 and 81%, respectively (Table 1). During reoxygenation the intensity of glucose consumption per 1 mm Hg developed pressure in rats with DM2 3.2-fold surpassed the baseline level and was 38.9% higher than in control animals. Lactate release in hearts of DM2 rats and control animals exceeded the baseline level by 1.7 and 1.5 times, respectively. Similar changes were observed in the release of pyruvate. After 10-min reoxygenation pyruvate content in rats of the DM2 group exceeded the baseline and control level (Table 2). Secretion of AST in the myocardium of rats with DM2 decreased after reoxygenation, but did not reach the baseline level and significantly exceeded the control. Hypoxia is accompanied by activation of the anaerobic pathway of energy production and increased formation of lactate. These changes result in the development of intracellular acidosis, which plays a role in the impairment of electromechanical coupling and decrease in contractile activity of the myocardium during hypoxia [7]. The increased release of pyruvate into the coronary bed can be due to damage to cardiomyocyte mitochondria [5].

Adverse effects of hypoxia were manifested in a considerable decrease in contractile activity of the myocardium in rats with DM2 and abdominal obesity. We observed the decrease in systolic and developed pressure and disturbances in diastolic relaxation of the myocardium. These changes became more pronounced with increasing the period of exposure to hypoxia. Metabolic disturbances in the diabetic myocardium are related to energy deficit, which results from abnormal glycolysis, uncoupling of oxidation and phosphorylation, acidosis, and damage to cardiomyocyte membranes. These changes were manifested in increased release of lactate and pyruvate, inefficient glucose consumption, and high secretion of enzymes into the coronary bed. Severe metabolic disturbances in the diabetic myocardium were not optimized even after reoxygenation.

Our results suggest that rats with DM2 and abdominal obesity hypoxic exposure suppresses heart function. Pathological changes primarily concern relaxation of the myocardium. Hypoxia alleviates symptoms of energy deficit in the diabetic myocardium and is followed by the development of metabolic disorders, decrease in the efficiency of consumption of energy-rich compounds, and damage to cardiomyocytes.

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