Effect of Acute Alloxan Diabetes on Ischemic and Reperfusion Arrhythmias in Rats with Different Activity of Nitric Oxide System

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Similar degree of glycemia (28-31 mmol/liter) and similar mortality (37-42%) were revealed in August rats exhibiting enhanced activity of NO system and in Wistar rats 3 weeks after alloxan treatment. Under conditions of myocardial ischemia caused by 10-min coronary artery ligation, the intensity of arrhythmias did not differ from the control in Wistar rats with diabetes mellitus and increased in August rats. Under conditions of reperfusion, diabetes produced an antiarrhythmic effect in Wistar rats and did not affect arrhythmia in August rats. Plasma concentrations of nitrates and nitrites in Wistar and August rats increased by 82 and 143%, respectively, compared to the control. The level of hemoxygenase-1 (hsp32) in the myocardium remained unchanged in Wistar rats and decreased by 26% in August rats. Thus, the absence of antiarrhythmic effect of acute diabetes in August rats is probably related to elevated NO content and reduced antioxidant activity.

Key Words: *August and Wistar rats; alloxan diabetes; cardiac arrhythmias; nitric oxide; hemoxygenase-1*

In patients with type 1 diabetes mellitus (DM), the incidence of myocardial infarction and its complications, such as cardiac insufficiency and rhythm disturbances, is higher than in individuals without DM. During the acute stage of DM, paradoxically enhanced resistance of the heart to ischemic and reperfusion damages is observed [2,13]. This effect is related to activation of ATP-dependent K^+-channels [12], inhibition of Na^+/H^+- and Na^+/Ca^{2+}-exchangers of the sarcolemma [8], and activation of the antioxidant system [13]. At the same time, the role of NO in cardioprotective mechanisms of acute DM was little studied. In experimental DM, activity of NO system increases, which is confirmed by activation of eNOS and iNOS in the myocardium [7], accumulation of nitrates and nitrites in the blood and urine in rats [11], and enhanced expression of eNOS in arterioles in mice [14]. In parallel, suppression of nNOS and degeneration of nitroergic terminals in cerebral arteries [5] and reduced bradykinin- and acetylcholine-induced production of NO [15] are observed, which are considered to be a cause of disturbances in endothelium-dependent vascular relaxation. Additional experimental activation of this system limits metabolic disorders in the organism and disturbances in the vascular relaxation caused by DM [10,11], which attest to certain NO deficit under conditions of this pathology. On the other hand, it is known that NO directly participates in β-cell apoptosis, while peroxinitrites formed during interaction between NO and superoxide ions possess pronounced toxic effect. Thus, the role of NO in the pathogenesis of DM is rather ambiguous. Animals with initially different activity of NO system are very useful for evaluation of the role of NO in the development of DM.

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Here we studied the development of acute DM and resistance to ischemic and reperfusion arrhythmias in August rats characterized by innate hyperactivity of the NO system and in Wistar rats.

MATERIALS AND METHODS

Experiments were performed on male Wistar and August rats initially weighing 333.0±5.8 and 236±4 g, respectively. DM was modeled by single subcutaneous injection of alloxan (Sigma) in a dose of 150 mg/kg; the experiments were performed on days 21-22 postinjection. The level of glycemia was measured using a glycometer (Roche) in blood drop taken from rat tail; the dynamics of body weight and heart weight were also evaluated. The sensitivity to the arrhythmogenic effect of myocardial ischemia and reperfusion were studied under urethane narcosis (150 mg/kg intraperitoneally) under conditions of acute experiment (open chest and jet air ventilation with VITA-1 apparatus). Short-term local myocardial ischemia and reperfusion were modeled by 10-min ligation of the descending branch of the left coronary artery followed by 5-min resumption of circulation (loosening of the ligature). The intensity of arrhythmia was evaluated by ECG in standard lead I using a Polygraph RM-6000 (Nihon Kohden). The frequency and duration of extrasystoles, ventricular tachycardia, and ventricular fibrillation and the incidence of cardiac arrest were determined.

Hypertrophy of heart chambers was evaluated by left and right ventricle weight to body weight ratio. Activity of the NO system was evaluated by the total plasma content of stable NO metabolites (nitrates and nitrites) reflecting the intensity of NO production in the organism. Antioxidant activity was evaluated by Western-blotting of inducible hemoxigenase-1 (stress protein hsp32) in the septum using monoclonal antibodies to hsp32 (Stressgen) and peroxidase-labeled second antibodies (Jackson Immuno Research). The antigen was visualized by chemiluminescence using ECL reagents (Amersham) and Kodac X-ray films.

The data were processed using Student's *t* test and Mann–Whitney *U* test.

RESULTS

Wistar and August rats did not differ by the initial blood level of glucose (Table 1). Severe DM developed on day 21 after alloxan injection: glucose concentration in the blood increased by 4.8-5.0 times, *i.e.* to the same extent compared to the control. Maximum mortality was observed on days 3-6 after alloxan injection and was similar in both groups. Body weight loss in Wistar rats was less pronounced than in August rats (14 and 24%, $p<0.05$), which attested to less severe course of DM in Wistar rats. Under conditions of this experimental model, only minor hypertrophy of the left ventricle (by 11%) was observed in Wistar and August rats, while hypertrophy of the right ventricle appeared only in Wistar rats (13%). In rats of both strains, DM led to HR decrease (bradycardia was more pronounced in August rats).

Ischemic and reperfusion arrhythmias in Wistar rats were more pronounced than in August rats (Table 2). For instance, severe arrhythmias (ventricular fibrillations and ventricular tachycardia) were observed in Wistar, but not in August rats. During reperfusion, long-term ventricular fibrillation always led to cardiac arrest (62%), while in August rats, short-term fibrillation developed in only 20% cases and none cases of cardiac arrest were recorded.

In Wistar rats, DM had no effect on arrhythmia during myocardial ischemia, but had a protective effect during reperfusion: the incidence and duration of ventricular fibrillations and the incidence of cardiac

TABLE 1. Status of Wistar and August Rats on Day 21 after Alloxan Injection (*M*±*m*)

Parameter	Wistar		August	
	control (*n*=12)	DM (*n*=7)	control (*n*=15)	DM (*n*=7)
HR, bpm	363±20	313±12^{+}	300±19	266±18^{+}
Body weight, g	398±12	273±7***	265±11	180±10***
Blood glucose level, mmol/liter	5.7±0.4	31.6±0.9***	5.8±0.2	27.2±3.7***
Relative weight of the left ventricle	1.71±0.06	1.91±0.09*	2.10±0.07	2.34±0.09*
Relative weight of the right ventricle	0.79±0.03	0.89±0.02**	1.10±0.09	1.02±0.04
Relative heart weight	2.50±0.09	2.80±0.08*	3.20±0.04	3.36±0.07*

Note. $^{+}p<0.05$ and $^{*}p<0.05$, $^{**}p<0.02$ and $^{***}p<0.001$ compared to the control by Mann–Whitney *U* test and Student *t* test, respectively.

TABLE 2. Effect of DM on Intensity of Ischemic and Reperfusion Arrhythmias in Wistar and August Rats (*M*±*m*)

Group	*n*	Extrasystole		Ventricular tachycardia		Ventricular fibrillation		Incidence of cardiac arrest, %
		incidence, %	duration, sec	incidence, %	duration, sec	incidence, %	duration, sec	
Ischemia								
Wistar								
control	8	50	8.9±5.5	62	6.3±2.2	25	4.5±3.2	0
DM	11	72	4.4±2.0	64	7.0±4.0	18	6.4±3.9	0
August								
control	8	37	0.2±0.2	0	0	0	0	0
DM	11	82	30.5±19.0	64	25.2±5.5	9	0.1	0
Reperfusion								
Wistar								
control	8	0	0	50	14.5±8.5	62	112±22	62
DM	11	27	0.4±0.3	80	20.2±2.1	27	15.4±12.0*	9
August								
control	8	25	0.8±0.2	62	13.8±5.4	12	4.3±4.8	0
DM	11	9	0.1±0.1	72	22.7±10.2	27	4.6±1.8	9

Note. *$p<0.001$ compared to the control by Student *t* test.

arrests decreased. On the contrary, in August rats DM did not increase the severity of ischemic arrhythmias, which manifested in increased incidence and duration of tachycardia episodes, but had little effect on reperfusion arrhythmias.

In the control (n=8), the content of nitrates and nitrites in rats of both strains was similar (Fig. 1). In DM, the content of these metabolites in August rats (n=11) increased more markedly than in Wistar rats (n=8): by 143 and 82%, respectively; their absolute content in August rats was higher by 39% ($p<0.02$ by Mann–Whitney *U* test) than in Wistar rats. This finding suggests that DM more pronouncedly activates the NO system in August rats.

The initial level of hsp32 in the heart in August rats (n=5) was increased by more than 2 times (Fig. 2) compared to that in Wistar rats (n=6). In DM, the content of hsp32, the parameter highly sensitive to hypoxia and the level of reactive oxygen forms, considerably decreased in August rats (by 26%, n=5) and remained unchanged in Wistar (n=5). Since hsp32 is a marker of activity of free-radical processes, we can say that this stage of acute DM in August rats is characterized by suppression of antioxidant systems, while unchanged level of hsp32 in Wistar rats with DM can be determined by preserved balance between the pro- and antioxidant systems.

We previously showed that August rats are highly resistant to ischemic and reperfusion arrhythmias [1]. Under conditions of acute myocardial ischemia, arrhythmias are a result of activation of the adrenergic system, due to which catecholamines damage ischemic and non-ischemic myocardium. Reduced adrenoreactivity of the myocardium in August rats [1] formed due to inherent elevated catecholamine content in the

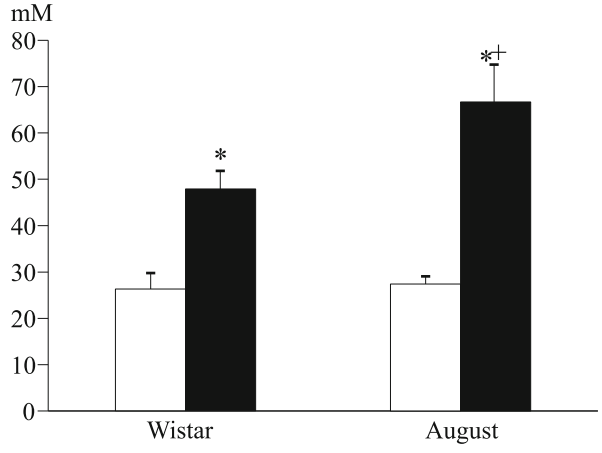

Fig. 1. Plasma concentration of nitrates and nitrites in Wistar and August rats with acute DM. Open bars: control; dark bars: DM. *$p<0.001$ compared to the control by Student *t* test; *$p<0.02$ compared to Wistar rats by Mann–Whitney *U* test.

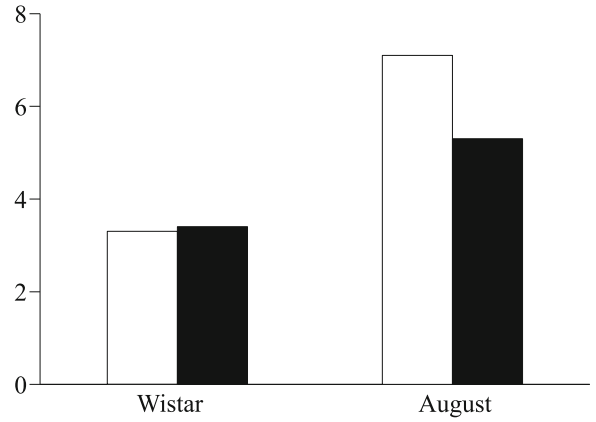

Fig. 2. Content of hsp32 in the heart of Wistar and August rats with acute DM. Open bars: control; dark bars: DM. Ordinate: content of protein in relative densitometric units.

blood [4] is a mechanism underlying high resistance of this rat strain to arrhythmias. This phenomenon and enhanced activity of the NO system limiting activity of the sympathetic nervous system largely determine this peculiarity of August rats. High resistance to reperfusion arrhythmias in August rats can be determined to a certain extent by enhanced resistance of their myocardium to oxygen damage [1] caused by myocardial reperfusion.

The phenomenon of antiarrhythmic effect of experimental DM in myocardial ischemia and reperfusion was discovered long ago [9]. Experiments on Wistar rats with experimental streptozotocin-induced DM demonstrated reduced incidence and duration of ventricular tachycardia and fibrillation during ischemia and reperfusion of the isolated heart [13]. The mechanisms of this phenomenon are not quite clear. This effect is observed only at early stages of experimental DM (weeks 1-9) and is absent at later terms, when the resistance to arrhythmias decreases. It is assumed that this effect is related to the following factors: activation of antioxidant enzymes in the myocardium in response to activation of free-radical processes [7]; activation of ATP-dependent K^+-channels leading to hyperpolarization of the membrane and preventing arrhythmia development. Moreover, reduced activity of Na^+/H^+- and Na^+/Ca^{2+}-exhangers of cardiomyocyte sarcolemma in DM impairs myocardial contractility, on the one hand, and prevents arrhythmia development, on the other [8]. Reduced adrenoreactivity of the myocardium in DM should also limit the development of these arrhythmias.

NO produces a cardioprotective effect in ischemic and reperfusion myocardial injuries [3]. This assumption is confirmed by our findings on innate increased resistance of August rats to ischemic arrhythmias. The blockade of NO synthesis with L-NAME in rats with 3-week streptozotocin-induced DM positively affects the contractile function of the myocardium, but abolishes the antiarrhythmic effect of DM during reperfusion [6], which also attested to the positive role of NO in DM. At the same time, NO excess in DM can produce a negative effect on the studied processes due to massive production of peroxinitrites potentiating myocardial damage. These findings suggest that NO excess in August rats can play an important role in their increased vulnerability to ischemic and reperfusion arrhythmias in DM. Sharp decrease in HR caused by DM in August rats should also be taken into account, because bradycardia aggravates myocardial heterogeneity by conductivity characteristics, which by itself is an arrhythmogenic factor. More pronounced dispersion of myocardial conductivity in August rats compared to Wistar rats can also be determined by more severe injury due to impairment of antioxidant protection. This assumption is also confirmed by the fact that the content of hsp32 in August rats is lower than in Wistar rats. It should be also noted that under conditions of DM increased blood level of epinephrine in August rats aggravates damage, because epinephrine not only stimulates glucose release from the liver, but also inhibits its insulin-dependent utilization.

Thus, acute DM produces an arrhythmic effect in myocardial reperfusion, while in August rats DM has no effect on reperfusion arrhythmias, but potentiates the development of ischemic arrhythmias. It can be hypothesized that the arrhythmogenic effect of acute DM in August rats is related to hyperactivation of the NO system.

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