August Rats are More Resistant to Arrhythmogenic Effect of Myocardial Ischemia and Reperfusion than Wistar Rats

L. M. Belkina, T. N. Kirillina, M. G. Pshennikova, and Yu. V. Arkhipenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 6, pp. 625-628, June, 2002
Original article submitted March 11, 2002

As differentiated from Wistar rats, myocardial ischemia and reperfusion produce no ventricular fibrillation in August rats. Pretreatment with nitric oxide synthase inhibitor Nω-nitro-L-arginine increased mortality rate in August rats with acute myocardial infarction from 20 to 40%. Under these conditions mortality rate in Wistar rats increased from 50 to 71%. Interstrain differences in the resistance of these animals to the arrhythmogenic effect of ischemia are probably associated with higher activity of the nitric oxide system in August rats compared to Wistar rats.

Key Words: *August rats; Wistar rats; myocardial ischemia and reperfusion; arrhythmias; nitric oxide*

Previous studies showed that mortality from acute myocardial infarction (MI) and incidence of contractile disturbances in the heart in August rats are much lower than in Wistar rats [1]. Severe arrhythmias are the main cause of death in humans and animals with acute myocardial ischemia. The development of these arrhythmias is related to excessive release of norepinephrine in the myocardium, which results from the stress reaction and activation of the sympathetic cardiac regulation accompanying ischemia [15]. High resistance of August rats to myocardial ischemia is probably associated with low reactivity of the stress-realizing system. In August rats activation of the hypothalamic-pituitary-adrenal system, release of corticosterone into the blood, and activation of creatine phosphokinase (marker of damages) in response to stress are less pronounced than in Wistar rats [5]. Moreover, stress reactions in August rats with MI is less pronounced than in Wistar arts. In August rats activation of adrenergic structures in the hypothalamus and release of norepinephrine from sympathetic endings into the heart are less pronounced than in Wistar rats [4]. These interstrain differences in the stress reaction are probably associated with higher activity of the nitric oxide (NO) system in August rats compared to Wistar rats [3,6]. NO acts as a potent vasodilator and stress-limiting agent that inhibits norepinephrine release and, therefore, prevents hyperactivation of the sympathetic system and protects tissues from damages [14]. These data suggest that high survival rate in August rats with MI is related to lower incidence of lethal arrhythmias during acute myocardial ischemia (compared to Wistar rats). High activity of the NO system in August rats probably prevents the development of severe arrhythmias. However, no direct evidence for this suggestion was provided. Here we compared the type and severity of arrhythmias in August and Wistar rats developed after transitory ischemia and reperfusion of the myocardium. The role of NO in the resistance to MI was evaluated by blockade of NO synthesis.

MATERIALS AND METHODS

Experiments were performed on male Wistar and August rats. In series with transitory ischemia followed by reperfusion the weight of animals was 408±18 and 274±4 g, respectively. In series with 2-day MI the weight of animals was 290±6 and 200±16 g, respec-

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/02/1336-0540$27.00

tively. The resistance of rats to the arrhythmogenic effect of myocardial ischemia and reperfusion was studied under nembutal anesthesia (50 mg/kg intraperitoneally). Acute experiments were performed after thoracotomy under conditions of artificial ventilation with atmospheric air on a VITA-1 device.

Transitory local myocardial ischemia was produced by ligation of the left descending coronary artery. The ligature was removed during reperfusion. The severity of arrhythmias was estimated by ECG recorded over 10-min ischemia and 5-min reperfusion on a Mingograf-34 device (Siemens, lead I). We evaluated the incidence and duration of extrasystoles, ventricular tachycardia, and ventricular fibrillation.

MI was modeled by the method of H. Selye. The left descending coronary artery was ligated. The mortality rate was determined over the first 24 h after MI. Activity of the NO system in rats with MI and intact and sham-operated animals (SO, no coronary artery ligation) was estimated by the total content of stable NO metabolites (nitrates and nitrites) in the plasma, which reflected NO content in the body. The animals were decapitated 2 days after ligation of the coronary artery or sham operation. The blood was collected into tubes with heparin and centrifuged at 3000 rpm for 15 min. For removal of proteins from the supernatant (plasma) 30% $ZnSO_4$ was added (1:20 v/v) and the tubes were centrifuged under similar conditions. The supernatant was placed in Nitralyser reactors (World Precision Instruments) to reduce nitrates into nitrites in the presence of 0.5 M NH_4OH (pH 9.0, 9:1 plasma/buffer ratio). Plasma aliquots were mixed 1:1 with Griess reagent and incubated at room emperature for 10 min until staining. Absorption was measured at 540 nm. Nitrite concentration was estimated by the calibration curve (5-50 μM $NaNO_3$). NO synthesis was blocked with NO synthase inhibitor Nω-nitro-L-arginine (L-NNA, Sigma). L-NNA was injected intraperitoneally in a daily dose of 20 mg/kg for 2 days before MI and 1.5 h before surgery. Control rats received an equivalent volume of physiological saline.

The results were analyzed by Student's *t* test and Mann—Whitney *U* test.

RESULTS

Before thoracotomy heart rate in August and Wistar rats was 423±8 and 408±8 bpm, respectively. After thoracotomy this parameter decreased to 368± 18 and 396±22 bpm, respectively. During ischemia the incidence and duration of extrasystoles and, especially, ventricular tachycardia in August rats were much lower than in Wistar rats. As differentiated from Wistar rats, we did not observe ventricular fibrillation in August rats (Table 1). During reperfusion the incidence and duration of tachycardia in August rats increased and did not differ from those in Wistar rats. However, fibrillation August rats did not develop. The incidence and duration of tachycardia in Wistar rats remained practically unchanged during reperfusion. However, the incidence of ventricular fibrillation in these animals increased by 2.5 times compared to the ischemic period. Thus, the severity of reperfusion arrhythmias in August and Wistar rats surpassed that during ischemia. It should be emphasized that ischemia and reperfusion produce no fibrillation in August rats. These results indicate that August rats are more resistant to the arrhythmogenic effect of acute ischemia and reperfusion than Wistar rats.

We revealed for the first time that higher resistance of August rats to MI is related to lower incidence of lethal arrhythmias during acute myocardial ischemia (compared to Wistar rats). In August rats with 2-day MI plasma nitrate/nitrite content 2-fold surpassed that in Wistar rats (23.8±2.7 and 12.9±2.8 mM, respectively, $p<0.001$). Thus, August rats resistant to ischemic and reperfusion arrhythmias are characterized by intensive NO production. By con-

TABLE 1. Arrhythmias in Wistar and August Rats during Transitory Local Myocardial Ischemia Followed by Reperfusion (*M*±*m*, *n*=10)

Arrhythmias		Ischemia		Reperfusion	
		Wistar	August	Wistar	August
Extrasystoles	incidence, %	100	70	100	90
	duration, sec	8.0±2.2	2±1*	2.0±1.1	0.6±0.1
Tachycardia	incidence, %	70	20	90	70
	duration, sec	22±13	5.4±4.6*	20.0±4.2	20.0±6.8+
Fibrillation	incidence, %	30	0	70	0
	duration, sec	67.0±9.2	0	61±33	0

Note. $p<0.05$: *compared to Wistar rats, +compared to ischemia (*U* test).

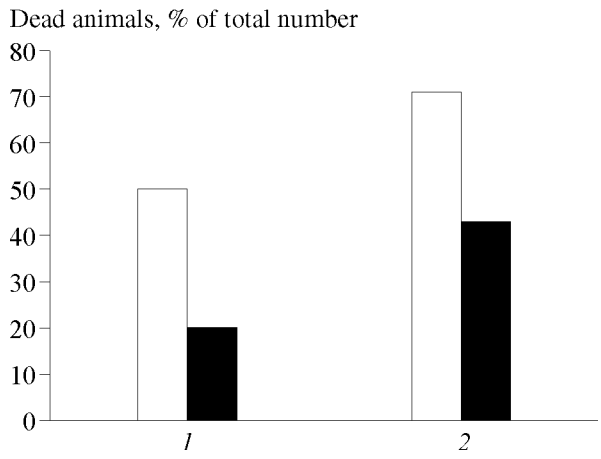

Fig. 1. Effect of pretreatment with L-NNA on mortality from acute myocardial infarction in August (light bars) and Wistar rats (dark bars). Control (*1*) and L-NNA (*2*).

trast, low activity of the NO system is typical of Wistar rats predisposed to arrhythmias.

We observed death of 20% August rats and 50% Wistar rats over the first 24 h after the incidence of acute MI. Pretreatment with L-NNA increased mortality from acute MI in both animal strains. In August rats receiving L-NNA (n=14) the mortality rate increased by 2 times compared to the control (n=15). The mortality rate in Wistar rats injected with L-NNA (n=14) was 40% higher than in control animals (n=14). These results indicate that the mortality rate in Wistar rats receiving L-NNA increased to a lesser extent than in August rats (Fig. 1).

Recent studies showed that NO plays an important role in the pathogenesis of ischemic heart damages [12]. Myocardial ischemia is accompanied by activation of the NO system, which results in the increase in NO concentration in various organs (*e. g.*, heart and blood) [8]. NO produces the cardioprotective effect during ischemic and reperfusion damages to the heart. Blockade of NO synthesis potentiates the formation of ischemic and reperfusion damages to the myocardium [10] and development of arrhythmias [10,12]. Experiments on outbred rats [2] and dogs [7] showed that NO donors possess antiarrhythmic activity during reperfusion after transitory ischemia. Our results are consistent with published data that NO produces a cardioprotective effect during ischemic myocardial damages. These data indicate that the greater resistance of August rats to heart damages is related to higher activity of the NO system (compared to Wistar rats). The antiarrhythmic effect of NO is probably associated with its ability to abolish stress reaction [14]. The resistance of these animals to arrhythmias should be compared taking into account other factors preventing the development of severe arrhythmias. August rats are characterized by low adrenoreactivity of the myocardium [1], which can prevent the development of catecholamine-induced arrhythmias. The main mechanism underlying the development of arrhythmias and formation of cardiomyocyte injuries during reperfusion of ischemic myocardium includes intensive generation of oxygen radicals, whose arrhythmogenic effects are associated with damages to cell membranes and impairment of ion transport [9]. These changes are accompanied by accumulation of reactive oxygen radicals, NO [8], and toxic products formed after interaction of NO with superoxide anions (peroxynitrite [8]) and possessing high oxidative activity. Under these conditions NO present in high concentrations produces not only protective, but also damaging effects [8,12]. We analyzed possible mechanisms preventing the development of fibrillation in August rats during reperfusion. Probably, NO in high concentration interacts with oxygen radicals and inhibits free radical processes in these animals [12]. This assumption is confirmed by published data that NO donors produce an antiarrhythmic effect during reperfusion [2,7]. It cannot be excluded that excessive accumulation of free NO in August rats during reperfusion is compensated by high ability of animals to deposit this compound [6], which prevents its toxic effect. During reperfusion fibrillation did not develop in August rats with high NO content. These results suggest that in August rats activity of antioxidant systems protecting the myocardium from reperfusion damages is higher than in Wistar rats [10]. Previous studies showed that NO activates expression of genes for antioxidant enzymes [12]. These genetically determined characteristics probably contribute to a higher resistance of August rats to the development of lethal arrhythmias compared to Wistar rats.

REFERENCES

1. L. M. Belkina, V. A. Saltykova, and M. G. Pshennikova, *Byull. Eksp. Biol. Med.*, **131**, No. 6, 624-628 (2001).
2. V. A. Kostyuk and G. S. Polyukhovich, *Ibid.*, **127**, No. 2, 137-140 (1999).
3. V. D. Mikoyan, L. N. Kubrina, E. B. Manukhina, *et al.*, *Ibid.*, **121**, No. 6, 634-637 (1996).
4. M. G. Pshennikova, L. M. Belkina, L. Yu. Bakhtina, *et al.*, *Ibid.*, **132**, No. 8, 153-155 (2001).
5. M. G. Pshennikova, L. Yu. Golubeva, B. A. Kuznetsova, *et al.*, *Ibid.*, **122**, No. 8, 156-159 (1996).
6. M. G. Pshennikova, B. V. Smirin, O. N. Bondarenko, *et al.*, *Ros. Fiziol. Zh.*, **86**, No. 2, 174-181 (2000).
7. K. Gyorgy, A. Vegh, M. A. Rastegar, *et al.*, *Cardiovasc. Drugs Ther.*, **14**, No. 5, 481-488 (2000).
8. P. Liu, C. E. Hock, R. Nagele, and P. Y. Wong, *Am. J. Physiol.*, **272**, No. 5, Pt. 2, 327-336 (1997).
9. A. Manning and D. Hearse, *J. Mol. Cell. Cardiol.*, **16**, 497-518 (1984).

10. E. Masini, D. Salvemini, J. F. Ndisavg, P. Gai, *et al*., *Inflamm. Res*., **48**, No. 11, 561-568 (1999).
11. F. Z. Meerson, V. V. Didenko, L. M. Belkina, and E. B. Manukhina, *Cellular Antioxidant Defence Mechanisms*, Boca Raton (1988), p. 215-245.
12. Y. Nonami, *Jpn. Circ*., **61**, No. 2, 119-132 (1997).
13. N. Nunoshiba, T. DeRojas-Walker, J. S. Wishnok, *et al*., *Proc. Natl. Acad. Sci. USA*, **90**, 9993-9997 (1993).
14. P. Schwarz, R. Diem, N. J. Dun, and U. Fostermann, *Circ. Res*., **77**, 841-848 (1995).
15. J. F. Skinner, *Stress and Heart Disease*, Eds. R. E. Beamish *et al*., Boston (1985), pp. 44-49.