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Antiarrhythmic Effect of Heat Adaptation in Ischemic and Reperfusion Injury to the Heart

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Study on a model of 6-day dosed adaptation to heat in rats showed that this adaptation decreased the severity of cardiac arrhythmias during ischemic and reperfusion injury. The duration of arrhythmias decreased not only in the ischemic period, but also under conditions of reperfusion. Adaptation delayed the development of arrhythmias during ischemia, decreased the number of animals with late reperfusion arrhythmias, and improved recovery of the heart after ischemia and reperfusion.

Key Words: *heat adaptation; heart; ischemia; reperfusion; arrhythmias*

The development of models for heart protection from ischemic and reperfusion injury is an urgent problem of modern medicine. Arrhythmias (*i.e.* changes in the frequency, rhythm, or force of cardiac contractions resulting from impairment of myocardial excitability due to progressive energy deficiency) are the major cardiac dysfunctions caused by ischemic and reperfusion injury. Over a long period, necrosis was believed to be the main type of cell death in the myocardium. Recent studies showed that apoptotic death of cardiomyocytes is initiated during various pathological processes in the heart, including ischemic and especially reperfusion injury [2,9]. Necrotic and apoptotic cell death is observed in various zones of the myocardium during various stages of ischemia and reperfusion [3,4].

Adaptation to environmental factors plays the major role in increasing the resistance of the organism. Adaptation to heat is one of the least studied approaches to improving nonspecific organism's resistance to adverse factors. Previous studies demonstrated a protective effect of heat preconditioning in pathological processes accompanied by activation of cell apoptosis [14]. Heat adaptation increases the resistance to heat shock [6]. However, the possibility of adaptive protection of the heart by using adaptation to heat was not studied. In this context, it is important to evaluate the possibility of adaptive protection of the heart from ischemic and reperfusion injury, because this protection can involve inhibition of apoptotic death of cardiomyocytes. The cardioprotective effect of heat adaptation can be also related to activation of nitric oxide (NO) production, which occurs during thermal exposure [6]. NO not only acts as a potent vasodilator, but also prevents activation of the sympathetic nervous system playing a role in the pathogenesis of ischemic injury [5].

Here we studied the effect of dosed heat adaptation on the development of ischemic and reperfusion arrhythmias and activity of the NO system in rats.

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MATERIALS AND METHODS

Experiments were performed on 40 male Wistar rats weighing 200-250 g. The maintenance of animals and performance of the study were in accordance with international requirements.

During heat adaptation, the animals were daily for 6 days placed in a thermostat at 48°C for 20 min. Rectal temperature in rats increased from 37.8±0.6 to 40.0±0.5°C. The study was conducted 24 h after the last adaptation session.

We studied cardiac arrhythmias and evaluated the antiarrhythmic effect of heat adaptation. The animals were narcotized with urethane (1.5 g/kg intraperitoneally). After tracheotomy and thoracotomy the rats were switched to jet ventilation with air using a VITA-1 apparatus. The sternum was pulled upward and fixed with a ligature to gain access to the heart. ECG in lead I was recorded before thoracotomy, after 20-min stabilization, and during ischemia and reperfusion using a special module of a RM-6000 polygraph and VC-9 oscilloscope (Nihon Kohden). Ischemia was induced by 10-min ligation of the left descending coronary artery. The ligature was removed during reperfusion. The duration of reperfusion was 1 h. Myocardial ischemia was verified by *ST* segment elevation and visually by cyanosis of the anterior wall and apex of the left ventricle. The durations of extrasystole, ventricular tachycardia, and ventricular fibrillation during ischemia and various stages of reperfusion were evaluated from ECG recordings.

Activity of the NO system was evaluated by the total content of stable NO metabolites (nitrates and nitrites) in blood plasma from control and treated (heat adapted) animals. This parameter reflects NO concentration in the organism. The rats were decapitated 24 h after the end of adaptation. The blood was collected in tubes with heparin and centrifuged at 3000 rpm for 15 min. The supernatant (plasma) was deproteinized by treatment with $^1/_{20}$ volume of 30% $ZnSO_4$ and centrifuged under similar conditions. The supernatant was placed in Nitralyser reactors (World Precision Instruments) for reduction of nitrates to nitrites in the presence of 0.5 M NH_4OH (pH 9.0) as a buffer. The plasma/buffer volume ratio was 9:1. After reduction, an aliquot of the plasma was mixed with an equivalent volume of Griess reagent and incubated at room temperature for 10 min. Absorption was measured spectrophotometrically at 540 nm. Nitrite concentration was estimated from the calibration curve (5-50 μM $NaNO_2$).

The differences in the duration of cardiac arrhythmias in rats were estimated by exact Fisher test. The differences in the contents of nitrates and nitrites were evaluated by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

The heart rate (HR) did not differ in control and treated animals before and after thoracotomy (adaptation had no effect on HR in the absence of heart damage).

Table 1 shows the duration of various types of arrhythmias, as well as the total time of arrhythmias during ischemia. Preadaptation to thermal exposure significantly decreased the duration of severe arrhythmias and total time of arrhythmias (by 2-3 times). Apart from the duration of arrhythmias, we estimated the latency of ischemia-induced arrhythmias in control and treated rats. The mean latency of arrhythmias in control and adapted rats was 6.6±1.2 and 11.2±1.3 min, respectively. Therefore, adaptation significantly increased the latency of ischemia-induced arrhythmias.

The duration of various types of arrhythmias and total time of arrhythmias were evaluated during reperfusion. The duration of ventricular fibrillation decreased more than by 2 times after heat adaptation (Table 1). The incidence of arrhythmias in the early stage of reperfusion (5 min) did not differ in animals of both groups. By the 20th minute of reperfusion, the heart rhythm in adapted rats returned to normal more rapidly than in control animals

TABLE 1. Duration of Cardiac Arrhythmias during Ischemia and Reperfusion (sec, *M*±*m*)

Parameter		Ischemia		Reperfusion	
		control	adaptation	control	adaptation
Extrasystole		12.6±4.7	9.0±5.0	5.4±2.5	8.7±3.6
Ventricular	tachycardia	20.1±10.2	8.7±6.5	2.4±1.8	1.7±1.7
	fibrillation	24.7±19.2	8.0±5.7*	24.3±13.2	9.6±5.3*
Total time		57.4±32.7	25.6±14.8*	32.2±14.5	19.9±6.8*

Note. *$p<0.05$ compared to the control (Fisher test).

TABLE 2. Percent of Rats with Cardiac Arrhythmias at Various Stages of Reperfusion

Time of reperfusion, min	Group	
	control	treatment (adapted animals)
5	45	45
20	55	20
50	80	65

(Table 2). By the end of reperfusion, the percent of adapted rats with arrhythmias was lower compared to the control.

Activity of the NO system was studied to evaluate the mechanisms of the protective effect of heat adaptation. Adaptation increased the content of nitrates and nitrites by 35.7% compared to the control (73.7±6.5 and 54.30±3.25 μM, respectively). Hence, activation of NO-producing systems is one of the mechanisms of the antiarrhythmic effect of heat adaptation. Recent studies showed that NO plays an important role in the pathogenesis of ischemic injury to the heart [9]. For example, NO has a cardioprotective effect during ischemic and reperfusion injury to the myocardium and prevents the development of arrhythmias [8,1]. It can be hypothesized that the antiarrhythmic effect of NO during ischemia can be associated with its ability limit the stress response [13].

The major mechanism of injury to cardiomyocytes and development of arrhythmias during reperfusion of the ischemic myocardium is a sharp increase in production of oxygen radicals and peroxynitrites. The arrhythmogenic effect of these compounds is associated with damage to cell membranes and impairment of ion transport [7]. The study of mechanisms of prevention of reperfusion fibrillation suggests that NO in high concentration contributes to quenching of free radical processes due to the interaction with oxygen radicals [9]. Adapted rats were characterized by a lower severity of fibrillation and high concentration of NO during reperfusion. These specific features were probably related to high activity of antioxidant systems in the organism that play a role in myocardial protection from reperfusion injury. Our hypothesis is confirmed by published data that NO activates expression of genes for antioxidant enzymes [11]. It cannot be excluded that the development of late reperfusion arrhythmias is associated with apoptosis in myocardial cells, which results from free radical generation under conditions of oxygen supply to damaged heart tissue [10,12]. Previous studies showed that heat adaptation specifically protects cardiomyoblasts from induced apoptosis, but has no effect on necrotic death [2]. We showed that adaptive protection is most effective at the late stage of reperfusion. Our results and published data suggest that preadaptation protects the heart due to inhibition of cardiomyocyte apoptosis.

We conclude that dosed heat adaptation in rats protects the heart from ischemic and reperfusion arrhythmias. One of the mechanisms for a protective effect is activation of NO production.

REFERENCES

1. V. A. Kostyuk and G. S. Polyukhovich, *Byull. Eksp. Biol. Med.*, **127**, No. 2, 137-140 (1999).
2. E. A. Monastyrskaya, M. R. Duchen, L. V. Andreeva, *et al.*, *Ibid.*, **135**, No. 2, 143-146 (2003).
3. H. Fliss and D. Gattinger, *Circ. Res.*, **79**, No. 5, 949-956 (1996).
4. G. Majno and I. Joris, *Am. J. Pathol.*, **146**, No. 1, 3-15 (1995).
5. R. E. Malmstrom, H. Bjorne, K. Alving, *et al.*, *Nitric Oxide*, **5**, No. 2, 98-104 (2001).
6. I. Yu. Malyshev, L. A. Bayda, A. I. Trifonov, *et al.*, *Physiol. Res.*, **49**, No. 1, 99-105 (2000).
7. A. S. Manning and D. J. Hearse, *J. Mol. Cell. Cardiol.*, **16**, No. 6, 497-518 (1984).
8. E. Masini, D. Salvemini, J. F. Ndisang, *et al.*, *Inflamm. Res.*, **48**, No. 11, 561-568 (1999).
9. F. Z. Meerson and E. B. Manukhina, *Stress and Heart Disease*, Eds. R. E. Beamish *et al.*, Canada (1984), pp. 422-435.
10. P. Nerheim, S. C. Krishnan, B. Olshansky, and K. Shivkumar, *Cardiol. Clin.*, **19**, No. 1, 155-163 (2001).
11. Y. Nonami, *Jpn. Circ. J.*, **61**, No. 2, 119-132 (1997).
12. C. Ozcan, H. Bienengraeber, P. P. Dzeja, and A. Terzica, *Am. J. Physiol. Heart Circ. Physiol.*, **282**, H531-H539 (2002).
13. R. Pabla and M. J. Curtis, *Circ. Res.*, **77**, No. 5, 984-992 (1995).
14. Y. Z. Qian, J. B. Shipley, J. E. Levasseur, and R. C. Kukreja, *J. Mol. Cell. Cardiol.*, **30**, No. 6, 1163-1172 (1998).