Effect of Stress Adaptation on Cyclic Nucleotide Content in Myocardial Tissue during Acute Ischemia/Reperfusion

N. V. Naryzhnaya, L. N. Maslov, Yu. B. Lishmanov, and F. Kolář*

Translated from *Byulleten' Eksperimenta''noi Biologii i Meditsiny*, Vol. 145, No. 5, pp. 525-528, May, 2008
Original article submitted March 20, 2007

We studied the effect of adaptation to chronic immobilization stress on the contents of cyclic adenosine monophosphate and cyclic guanosine monophosphate in myocardial tissue during coronary occlusion and reperfusion. The contents of cyclic adenosine monophosphate and cyclic guanosine monophosphate in the ischemic area and non-ischemic myocardium of unadapted rats increased during coronary artery ligation for 10 min. Reperfusion for 10 min was followed by an increase in the content of cyclic adenosine monophosphate. During coronary occlusion, the content of cyclic adenosine monophosphate in the myocardium of stress-adapted rats increased less significantly than in control animals. No significant differences were found in the content of cyclic guanosine monophosphate in control and adapted rats. Our results suggest that poor response of the myocardial cyclic nucleotide system to ischemia/reperfusion in adapted animals is associated with the antiarrhythmic and cardioprotective effect of adaptation.

Key Words: *adaptation and immobilization stress; ischemia; reperfusion; cyclic adenosine monophosphate; cyclic guanosine monophosphate*

The myocardium of animals subjected to stress training is resistant to adverse arrhythmogenic effect of ischemia/reperfusion [3-5]. However, the mechanism of the cardioprotective effect of adaptation is poorly understood. The understanding of this mechanism will allow us to develop new approaches to the therapy and prevention of ischemic/reperfusion injuries to the heart. It was hypothesized that adaptation-mediated increase in myocardial resistance to the pathogenic effect of ischemia/reperfusion is related to variations in the intracellular response of cardiomyocytes to catecholamines [2]. However, this hypothesis was not confirmed. Cyclic adenosine monophosphate (cAMP) is one of the major signal messengers of the adrenergic response in cells. Cyclic guanosine monophosphate (cGMP) is a functional antagonist of cAMP. The increase in cAMP concentration in the myocardium is the cause of ventricular fibrillation during cardiac ischemia/reperfusion. The drugs that decrease synthesis of this intracellular messenger may prevent arrhythmias and delay the development of necrosis during myocardial ischemia [7,8]. Published data show that the increase in guanylate cyclase activity and cGMP synthesis in the myocardium is accompanied by the improvement of cardiac resistance to an adverse effect of ischemia/reperfusion [7,11]. These data suggest that the cyclic nucleotide system is associated with the cardioprotective and antiarrhythmic effect of adaptation.

Laboratory of Experimental Cardiology, Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Russia; *Institute of Physiology, Prague, Czech Republic. ***Address for correspondence:*** maslov@cardio.. L. N. Maslov

0007-4888/08/1455-0588

Here we studied the effect of stress adaptation on the content of cAMP and cGMP in myocardial tissue during coronary occlusion and reperfusion.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. The animals were adapted to stress by a series of short-term immobilizations in the supine position: day 1, 15 min; day 2, 30 min; day 3, 45 min; and days 4-12, 60 min per day [4]. This procedure of adaptation increases heart resistance to ischemia/reperfusion [4].

After adaptation, the animals were anesthetized with ketamine (50 mg/kg intravenously). Myocardial ischemia was induced by left coronary artery ligation (10 min) [11] and reperfusion (10 min). Artificial pulmonary ventilation was performed using a modified RO-6 device. Unadapted rats served as the control.

The heart was rapidly removed from the thorax after ischemia/reperfusion. Tissue fragments (40-80 mg) were dissected from the ischemic area and zone of normal perfusion. The ischemic area was verified by myocardial blanching. The right ventricle served as the nonischemic region. Comparative study with the right ventricle is based on the results of previous experiments. No differences were found in cAMP concentration in the right and left ventricles of intact animals.

Myocardial tissue was immediately frozen and maintained in liquid nitrogen until cAMP and cGMP assay. Cyclic nucleotides were extracted as described elsewhere [9]. A sample of myocardial tissue was homogenized in 2 ml acidified ethanol (100 ml 96% ethanol and 1 ml 1 N HCl), and centrifuged at 3000*g* for 15 min. The supernatant was collected in a tube. A mixture of ethyl alcohol and water in the 2:1 ratio was added to the pellet. The sample was mixed and repeatedly centrifuged. Both supernatants were combined and dried in a vacuum apparatus at 55°C. cAMP concentration in these samples was measured using commercial radioimmune kits (RIA AMPc/cAMP and RIA GMPc/cGMP, Immunotech). Dry residual was dissolved in a special buffer of the kit. Radioactivity of samples was measured on a gamma counter (Gamma-12, Russia).

The results were analyzed by methods of variational statistics. Student's *t* test was applied in testing the hypothesis for equality of the means.

RESULTS

cAMP concentration in the ischemic area and nonischemic cardiac region increased by 38.7 and 54.9%, respectively, after 10-min ischemia (compared to intact animals, Table 1). Our results are consistent with published data that cAMP concentration in cardiac tissue increases during short-term coronary occlusion [7,10]. No differences were revealed in the content of this cyclic nucleotide in the ischemia area and nonischemic myocardium (Table 1).

cAMP concentration in cardiac tissue after reperfusion was much lower than during ischemia. cAMP concentration did not differ in treated and intact animals, which is consistent with published data [7].

Ischemia for 10 min had no effect on cAMP concentration in the myocardium of stress-adapted rats. The content of cyclic nucleotide in treated rats did not differ from that in intact animals. These data are consistent with the results of our previous experiments. We showed that stress exposure of adapted animals is followed by an insignificant release of catecholamines from myocardial sympathetic terminals and adrenal medulla [2]. Acute ischemia is usually accompanied by severe strain. The sympathoadrenal system of trained specimens was characterized by poor response to coronary occlusion, which reflects the stress-limiting effect of adaptation. It is manifested in reduced activation of adenylate cyclase-coupled β-adrenoceptors. Hence, cAMP concentration in the ischemic myocardium is lower in adapted rats. The increase in cAMP concentration in cardiomyocytes during ischemia/reperfusion probably contributes to cardiac electrical instability and ventricular fibrillation [10]. However, the arrhythmogenic effect of coronary occlusion and reperfusion is less significant in rats adapted to chronic stress [3]. These changes probably result from a decrease in cAMP concentration in the ischemic myocardium of adapted specimens (compared to control animals).

The decrease in adrenoreactivity of adapted specimens can be mediated by activation of the opioid system. This hypothesis is confirmed by published data and results of our studies [2,3,9,13]. Our previous experiments showed that opioid content increases in blood plasma and myocardial tissue of adapted rats [2]. Moreover, intravenous injection of opioid peptides decreases the degree of catecholamine depletion in the myocardium and adrenal glands [2] and prevents the increase in myocardial cAMP concentration during coronary occlusion [9]. Opioid peptides decrease adenylate cyclase activity [13]. Our hypothesis is also confirmed by the fact that opioid receptor blockade in rats adapted to chronic stress abolishes the antiarrhythmic effect of adaptation during acute ischemia and reperfusion [3].

TABLE 1. Concentrations of cAMP and cGMP in the Myocardium of Rats Adapted to Chronic Stress after Ischemia/Reperfusion (*M*±*m*, nmol/g)

Parameter	Unadapted rats					Adapted rats				
	intact (*n*=11)	ischemia		reperfusion		ischemia		reperfusion		
		ischemic area (*n*=10)	intact region (*n*=9)	ischemic area (*n*=10)	intact region (*n*=10)	ischemic area (*n*=10)	intact region (*n*=10)	ischemic area (*n*=10)	intact region (*n*=10)
cAMP	7.64±0.63	10.60±0.07***	11.84±1.40**	7.23±0.79	7.58±0.61	6.55±0.77+	6.74±0.48+	6.25±0.62	7.51±0.65
cGMP	0.18±0.04	0.86±0.16***	1.04±0.20***	1.34±0.47*	0.840±0.095**	0.79±0.19**	1.08±0.24**	1.29±0.23***	0.85±0.19**

Note. *n*, number of animals per group. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to intact rats; +$p<0.001$ compared to unadapted rats after ischemia/reperfusion.

cGMP concentration in the myocardium increased by 4.5 times during coronary occlusion (compared to intact rats, Table 1). Our results are consistent with published data on the increase in cGMP synthesis in the myocardium during ischemia [7, 11]. The increased synthesis of cGMP during ischemia is probably related to activation of NO synthases and production of NO in the ischemic myocardium [12]. NO activates the synthesis of cGMP. This assumption is confirmed by published data that blockade of NO synthase contributes to the decrease in cGMP concentration in the myocardium during ischemia [7]. By contrast, administration of NO donors is followed by an increase in cGMP concentration under pathological conditions [11]. We revealed that cGMP concentration after reperfusion does not differ from that during ischemia (Table 1). Similar results were obtained by other investigators [12].

Published data show that pharmacological inhibition of cGMP synthesis during ischemia/reperfusion impairs contractility and has an arrhythmogenic effect on the isolated rat heart and under *in vivo* conditions [7,14]. It may be suggested that the increase in cGMP concentration during ischemia/reperfusion serves as a compensatory mechanism, which prevents the adverse effects of activation of the adenylate cyclase/cAMP system (arrhythmias and cardiomyocyte injury).

cGMP concentration in the myocardium of stress-adapted rats during ischemia/reperfusion did not differ from that in unadapted animals. It was observed in various periods of ischemia/reperfusion (Table 1). Our results contradict published data. Previous studies showed that NO production increases in the myocardium of rats adapted to chronic stress [6]. NO serves as the major activator of guanylate cyclase [12]. These changes should be followed by a greater increase in cGMP concentration in treated rats compared to unadapted animals. However, we failed to observe it. Myocardial ischemia in rats adapted to chronic stress is accompanied by the inhibition of endothelium-dependent aortic relaxation [1], which reflects a decrease in the synthesis of NO and cGMP.

NO synthesis increases in the myocardium of rats adapted to chronic stress [6]. However, these changes are not accompanied by the increase in cGMP concentration and endothelium-dependent vascular relaxation [1]. Probably, the rate of NO degradation and activity of adenylate cyclase decrease in the myocardium of adapted rats.

We conclude that adaptation to chronic stress contributes to the decrease in cAMP concentration, but has no effect on cGMP content in the myo-

cardium of rats during coronary occlusion. The observed changes improve cardiac resistance to adverse effects of acute ischemia/reperfusion.

REFERENCES

1. A. V. Lapshin, E. B. Manukhina, and F. Z. Meerson, *Fiziol. Zh. SSSR*, **77**, No. 3, 70-78 (1991).
2. Yu. B. Lishmanov, N. V. Naryzhnaya, and L. N. Maslov, *Opioidergic Mechanisms of Morphofunctional Changes in the Myocardium during Stress and Adaptation* [in Russian], Tomsk (2003).
3. L. N. Maslov, N. V. Naryzhnaya, A. V. Krylatov, and G. J. Gross, *Pat. Fiziol. Eksper. Ter.,* No. 4, 11-14 (2004).
4. F. Z. Meerson, D. M. Belkina, S. S. Dyusenov, *et al*., *Kardiologiya*, **26**, No. 8, 19-24 (1986).
5. F. Z. Meerson, A. D. Dmitriev, and V. I. Zayats, *Ibid*., **24**, No. 9, 81-88 (1984).
6. F. Z. Meerson, A. V. Lapshin, P. I. Mordvichev, *et al*., *Byull. Eksp. Biol. Med.*, **117**, No. 3, 242-244 (1994).
7. E. F. Du Toit, J. Meiring, and L. H. Opie, *J. Cardiovasc. Pharmacol*., **38**, No. 4, 529-538 (2001).
8. Y. Jia, L. Yu, and Y. Chen, *Zhongguo Zhong Xi Yi Jie He Za Zhi*, **19**, No. 11, 678-681 (1999).
9. L. N. Maslov and Yu. B. Lishmanov, *Int. J. Cardiol*., **40**, No. 2, 89-94 (1993).
10. L. H. Opie, C. Muller, and D. Nathan, *Adv. Cyclic Nucleotide Res. N. Y.*, **12**, 63-69 (1980).
11. G. Rossoni, B. Manfredi, V. D. Colonna, *et al*., *J. Pharmacol. Exp. Ther*., **297**, No. 1, 380-387 (2001).
12. Y. T. Xuan, X. L. Tang, Y. Qiu, *et al*., *Am. J. Physiol. Heart Circ. Physiol*., **279**, No. 5, H2360-H2371 (2000).
13. X. C. Yu, H. X. Wang, J. M. Pei, *et al*., *J. Mol. Cell. Cardiol*., **31**, 1809-1819 (1999).
14. Y. Zhang, J. W. Bissing, L. Xu, A. J. Ryan, *et al*., *J. Am. Coll. Cardiol*., **38**, No. 2, 546-554 (2001).