Role of ATP-Sensitive K^+-Channels in Antiarrhythmic and Cardioprotective Action of Adaptation to Intermittent Hypobaric Hypoxia

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Mature Wistar rats were exposed to intermittent hypobaric hypoxia (5000 m, 6 h/day, 30 sessions). This mode of adaptation enhanced heart tolerance to the arrhythmogenic action of 45-min coronary occlusion, but does not affect the infarction size/risk area ratio. In some series, the rats were exposed to more severe intermittent hypobaric hypoxia (7000 m, 8 h/day, 6 weeks) followed by 20-min coronary occlusion and 3-h reperfusion one day after the last hypoxia session. In this case, adaptation reduced the infarction size/risk area ratio and enhanced cardiac tolerance to the arrhythmogenic effect of reperfusion, but had no effect on the incidence of ventricular arrhythmia during ischemia. We found that the cardioprotective and antiarrhythmic effects of adaptation to an altitude of 7000 m and the antiarrhythmic effect of 5000-m adaptation were mediated via activation of K_{ATP} channels.

Key Words: *heart; ischemia; reperfusion; adaptation; hypoxia; K_{ATP} channels*

Adaptation to hypoxia is accompanied by enhancement of cardiomyocyte tolerance to the damaging effects of ischemia and subsequent reperfusion [4,6,9]. Specifically, adapted animals demonstrate decelerated development of irreversible alterations in the myocardium and better heart tolerance to the arrhythmogenic effect of ischemia—reperfusion [1,4-6].

The mechanism of cardioprotective and antiarrhythmic effects of adaptation to hypoxia received little attention. Specifically, the role of ATP-regulated K^+-channels (K_{ATP} channels) in adaptation tolerance to ischemia—reperfusion is virtually unknown.

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However, the researchers are aware of the role of K_{ATP} channels in ischemic preconditioning of the heart (cardiac adaptation to ischemia) by preliminary short-term bloodflow arrest and resumption [15]. The cardiotropic effects of ischemic preconditioning and intermittent hypobaric hypoxia are similar [4-6,9].

Our aim was to assess the involvement of K_{ATP} channels into the development of adaptation tolerance of the heart to ischemia—reperfusion.

MATERIALS AND METHODS

The experiments were carried out on Wistar rats weighing 200-250 g. Adaptation to intermittent hypobaric hypoxia was performed in two ways. Group 1 rats were placed in an altitude chamber 5 times a week for 6 h during the period of 6 weeks (in total 30 hypoxic exposures) [4,5]. During the first week, the pressure was gradually decreased starting from

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the "altitude" of 1000 m above sea level and finishing at an altitude of 5000 m at the fifth exposure. During the remaining 5 weeks, the rats were adapted to a pressure corresponding to an altitude of 5000 m. Group 2 rats were placed into an altitude chamber for 8 h 5 times a week [6]. The pressure was decreased every day from 307 mm Hg (41 kPa) on adaptation day 1 to 64 mm Hg (8.5 kPa) on day 13, which corresponded to an altitude of 7000 m.

Russian participants of the study simulated coronary occlusion (CO) and reperfusion on rats intraperitoneally narcotized with α-chloralose (50 mg/kg) and artificially ventilated via modified RO-2 apparatus (Krasnogvardeetz). After 45-min CO, the ligature was loosened and the heart was reperfused for 2 h. Then the ligature was tightened again. To determine the risk area (ischemic region), the rats were intravenously injected patent blue violet dye. After isolation of the heart from the thorax, the transversal sections were made (2-3 mm); the ischemic (non-stained) regions were isolated and placed into NBT solution. Ischemic but viable cardiac tissue turned blue, while necrotized myocardium retained pale. The sizes of the necrotic region (infarction size, IS) and risk area (RA) were determined gravimetrically. The cardioprotective effect of adaptation was assessed by the infarction size/risk area (IS/RA) ratio in percents.

During 45-min CO and 10-min reperfusion, ECG was recorded in thoracic lead I using an UBF4-03 biopotential amplifier and PC with original software. The incidence of the following ECG abnormalities was analyzed: single and multiple ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation. Two phases of ventricular arrhythmias were observed during the first 30-min period after CO: phase 1a appeared within 10 min postocclusion, while phase 1b developed thereafter [10]. Arrhythmias manifested during these phases originated from different electrophysiological mechanisms [10]; therefore, we analyzed them individually in each phase.

The study of cardioprotective and antiarrhythmic activity of adaptation was conducted by a blind method: one researcher prepared solutions of pharmacological agents, while CO and data processing was performed by another person, who did not know which rats belonged to the control or experimental (adapted) groups.

The Czech side of the study performed CO and reperfusion on rats intraperitoneally narcotized with pentobarbitone (60 mg/kg) and artificially ventilated using an Ugo Basile volume ventilator. Occlusion of the left anterior descending artery was performed 1-2 mm distally from its origin from the basic coronary trunk. The durations of ischemia and reperfusion were 20 min and 3 h, repectively [6]. Identification of RA and necrotic region was made using potassium permanganate and 2,3,5-triphenyltetrazolium chloride solutions [6]. Ventricular arrhythmias were scored as follows: single extrasystole (1 point), multiple extrasystoles (2 points), ventricular tachycardia (3 points), reversible ventricular fibrillation (4 points), and repeated ventricular fibrillation (5 points). For each rat, assessment of arrhythmia was based on the most severe arrhythmic episode.

For blockade of sarcolemmal and mitochondrial K_{ATP} channels, Russian participants of the study used glibenclamide (0.3 mg/kg, 1 ml/kg intravenously, 45 min before CO) dissolved in 0.2 ml ethyl alcohol/DMSO mixture and then in 20% hydroxypropyl-β-cyclodextrin. The Czech group used MCC-134 (1-[4-(1H-imidazole-1-yl)benzoyl]-N-methylcyclobutane-carbothioamide, Mitsubishi Parma Corporation), which blocks mitochondrial K_{ATP} channels and opens sarcolemmal K_{ATP} channels. MCC-134 was dissolved in DMSO and injected intravenously by two boluses 10 min before CO and 5 min before reperfusion, respectively. The total dose of MCC-134 was 0.3 or 3.0 gm/kg.

The preparations were from Sigma (glibenclamide, chloralose, and the stains), Tocris Cookson Ltd (hydroxypropyl-β-cyclodextrin), and Sanofi (pentobarbitone).

The data were analyzed statistically using Student's *t* test, Mann—Whitney U test, and χ^2-test. Normalcy of distribution was assessed by analysis of variances.

RESULTS

Daily 6-h adaptation of rats to hypoxia at an "altitude" of 5000 m had no effect on IS/RA ratio, while adaptation at 8.5 kPa (7000 m) decreased this ratio 1.5-fold (Fig. 1). It was previously demonstrated (F. Z. Meerson and co-workers) that adaptation at an altitude of 6000 m decelerated the development of irreversible damages to cardiomyocytes after CO [4]. We observed similar effects of adaptation at 5000 m, but they developed only if the rats were exposed to daily hypoxia for at least 8 h [7]. Moreover, in rats adapted to 5000 m altitude, the IS/RA ratio was only by 15% below the control ($p<0.05$) [7]. Thus, these data showed that adaptation altitude of 5000 m was insufficient for deceleration of irreversible damage to cardiomyocytes during subsequent ischemia. Nevertheless, this adaptation improved heart tolerance to the arrhythmogenic action of CO and reperfusion.

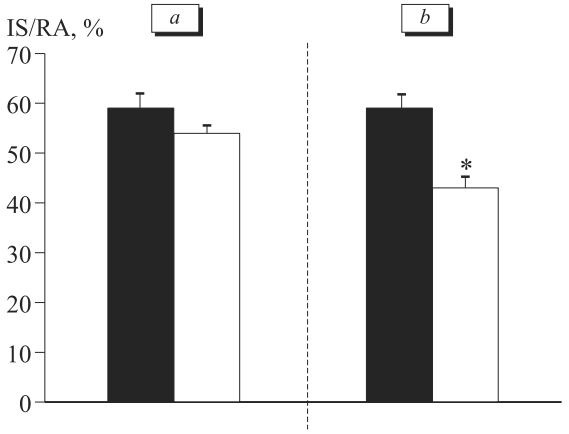

Fig. 1. Effect of adaptation to hypoxia at an "altitude" of 5000 m (*a*) and 7000 m (*b*) on IS/RA ratio. Dark and light bars correspond to control and adapted rats. **p*<0.05 compared to the control.

In the experimental rats adapted to an altitude of 5000 m, the incidence of ventricular extrasystoles and ventricular tachycardia in phase 1a was 2-fold lower than in controls (Table 1). Among non-adapted rats, only 2 of 16 animals (13%) had no rhythm disturbances during the first 10 min of ischemia, while in adapted group 9 of 14 rats were tolerant to the arrhythmogenic effect of CO in phase 1a. Tolerance to arrhythmogenic effect of long-term ischemia (phase 1b) was observed in 44% control rats and 86% adapted rats. During reperfusion, all rats (control and experimental) demonstrated only solitary ventricular extrasystoles, which explained the absence of significant differences between the groups.

In rats adapted to 7000 m, no antiarrhythmic effect of adaptation towards arrhythmias induced by a 20-min ischemia was observed, but the severity of reperfusion-induced heart rhythm disturbances decreased significantly from 2.4±0.5 points (n=10) in control rats to 0.8±0.5 points (n=10) in experimental rats (p<0.05).

Thus, only adaptation to a pressure of 8.5 kPa (7000 m) can efficiently prevent irreversible damages to cardiomyocytes during ischemia/reperfusion. Cardiac tolerance to the arrhythmogenic action of ischemia results only from long-term stay (no less than 6 h every day) at an altitude of 5000 m. Reperfusion-induced arrhythmias can be prevented by adaptation at an altitude of 7000 m. Comparison of these facts suggests that the mechanisms of the adaptive action of chronic ischemia are different at altitudes of 5000 and 7000 m.

Blockade of sarcolemmal and mitochondrial K_{ATP} channels with glibenclamide completely eliminated the antiarrhythmic effect of adaptation to an "altitude" of 5000 m (Table 1). By contrast, injection of glibenclamide to non-adapted rats produced no effect on the character and incidence of occlusion- and reperfusion-induced arrhythmias. Therefore, K_{ATP} channels play a key role in the realization of the antiarrhythmic effect of adaptation to an "altitude" of 5000 m.

In addition, K_{ATP} channels are the key players in the mechanism of cardioprotective effect of adaptation to a pressure of 8.5 kPa (7000 m). While 0.3 mg/kg MCC-134 produced no effect on IS/RA ratio in adapted rats, the dose of 3 mg/kg of this blocker

TABLE 1. Effect of Adaptation to Hypoxia at 5000 m "Altitude" on Character and Incidence of Ventricular Arrhythmias Caused by 45-min CO and 10-min Reperfusion in Rats

Parameter	Group								
	ischemia, 10 min			ischemia, 35 min			reperfusion, 10 min		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
No ventricular arrhythmias	2 (13)	9** (64)	4 (36)	7 (44)	12* (86)	7 (64)	7 (44)	8 (57)	6 (54)
Solitary ventricular extrasystoles	4 (25)	6 (42)	4 (36)	8 (50)	7 (50)	6 (54)	9 (56)	6 (43)	5 (46)
Multiple ventricular extrasystoles	14 (87)	5** (36)	7 (64)	9 (56)	2* (14)	4 (36)	— —	— —	— —
Ventricular tachycardia	12 (75)	5* (36)	5 (45)	3 (19)	1 (7)	1 (9)	— —	— —	— —
Ventricular fibrillation	2 (13)		1 (9)	3 (19)		1 (9)	— —	— —	— —

Note. Group 1, control (n=16); group 2, adaptation (n=14); group 3, adaptation+glibenclamide, 0.3 mg/kg (n=11). The percentage is shown in parentheses. **p*<0.05, ***p*<0.01 compared to the control.

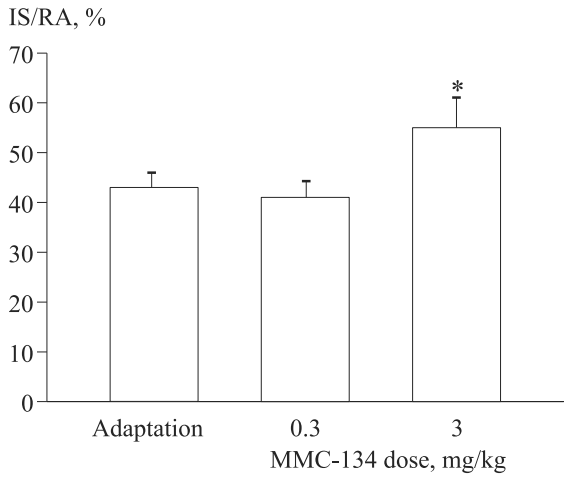

Fig. 2. Effect of K_{ATP} channel blockade on cardioprotective effect of adaptation to hypoxia at an "altitude" of 7000 m. *$p<0.05$ compared to adaptation without blockade.

completely eliminated the cardioprotective effect of hypobaric adaptation (Fig. 2). In contrast, MCC-134 did not affect the size of IS and RA.

MCC-134 eliminated the antiarrhythmic effect of adaptation even in the dose of 0.3 mg/kg. Although the mechanism of this action is unknown, it attests to an important role of K_{ATP} channels in the cardioprotective and antiarrhythmic effects of adaptation to hypobaric hypoxia. It can be hypothesized that activation of these channels during adaptation enhances mitochondrial tolerance to the pathogenic action of ischemia—reperfusion stress. This hypothesis can be substantiated by published data, which showed that pharmacological stimulation of mitochondrial K_{ATP} channels accelerates ATP resynthesis during reoxygenation of the myocardium and prevents irreversible damages to cardiomyocytes during reperfusion [2]. According to other hypothesis, activation of mitochondrial K_{ATP} channels before ischemia/reperfusion exposure intensifies production of ROS that play the role of intracellular messengers and activate effector stages involved in improvement of heart tolerance to hypoxia and reoxygenation [3,4].

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REFERENCES

1. Yu. B. Lishmanov, N. V. Naryzhnaya, L. N. Maslov, and G. J. Gross, *Pat. Fiziol. Eksper. Ter.*, No. 1, 19-21 (2003).
2. R. M. Fryer, A. K. Hsu, Y. Wang, *et al., Basic Res. Cardiol.*, **97**, No. 1, 47-54 (2002).
3. E. R. Gross, J. N. Peart, A. K. Hsu, *et al., J. Mol. Cell. Cardiol.*, **35**, No. 8, 985-992 (2003).
4. F. Z. Meerson, O. A. Gomazkov, and M. V. Shimkovich, *Am. J. Cardiol.*, **31**, No. 1, 30-34 (1973).
5. F. Z. Meerson, E. E. Ustinova, and E. H. Orlova, *Clin. Cardiol.*, **10**, No. 12, 783-789 (1987).
6. J. Neckar, B. Ostadal, and F. Kolar, *Physiol. Res.*, **53**, No. 6, 621-628 (2004).
7. J. Neckar, O. Szarszoi, L. Koten, *et al., Cardiovasc. Res.*, **55**, No. 3, 567-575 (2002).
8. J. N. Peart and G. J. Gross, *Am. J. Physiol. Heart Circ. Physiol.*, **285**, No. 1, H81-H89 (2003).
9. O. Poupa, K. Krofta, J. Prochazka, and Z. Turek, *Fed. Proc.*, **25**, No. 4, 1243-1246 (1966).
10. D. C. Russell, J. S. Lawrie, R. A. Riemersma, M. F. Oliver, *Am. J. Cardiol.*, **53**, No. 2, 307-312 (1984).
11. N. Sasaki, M. Murata, Y. Guo, *et al.*, *Circulation*, **107**, No. 8, 1183-1188 (2003).
12. J. E. Schultz, Y. Z. Qian, G. J. Gross, and R. C. Kukreja, *J. Mol. Cell. Cardiol.*, **29**, No. 3, 1055-1060 (1997).
13. J. E. J. Schultz, Z. Yao, I. Cavero, G. J. Gross, *Am. J. Physiol.*, **272**, No. 6, Pt. 2, H2607-H2615 (1997).
14. M. J. Walker, M. J. Curtis, D. J. Hearse *et al., Cardiovasc. Res.*, **22**, No. 7, 447-455 (1988).
15. D. M. Yellon and J. M. Downey, *Physiol. Rev.*, **83**, No. 4, 1113-1151 (2003).