

# Myocardial Infarct Size-Limiting Effect of Chronic Hypoxia Persists for Five Weeks of Normoxic Recovery

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## Summary

We examined cardioprotective effect of chronic hypoxia and the time course of its recovery under normoxic conditions. Adult male Wistar rats were exposed to intermittent hypobaric hypoxia (7000 m, 8 h/day, 35 exposures) and susceptibility of their hearts to ischemia-induced ventricular arrhythmias and myocardial infarction was evaluated in anesthetized open-chest animals subjected to 30-min coronary artery occlusion and 4-h reperfusion on the day after the last hypoxic exposure and at 7, 35 and 90 days of normoxic recovery. The infarct size was reduced from  $69.2 \pm 1.7$  % of the area at risk in normoxic controls to  $48.0 \pm 2.2$  % in the chronically hypoxic group and to  $61.6 \pm 2.3$  % in the group recovered for 7 days. This residual protection persisted for at least 35 days of normoxic recovery but it was absent after 90 days. In contrast to the infarct size-limitation, the antiarrhythmic protection disappeared already during the first week; the incidence of ventricular fibrillation was even significantly increased 7 and 90 days after the last hypoxic exposure. In conclusion, the duration of cardioprotection induced by chronic hypoxia differs markedly, depending on the end point of ischemia/reperfusion injury examined. Whereas the increased tolerance to lethal myocardial injury persists for at least 5 weeks after the termination of hypoxia, the antiarrhythmic protection rapidly vanishes, being replaced with transient proarrhythmic effect.

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## Key words

Chronic hypoxia • Normoxic recovery • Myocardial infarction • Arrhythmias • Cardioprotection

## Introduction

Since the first reports in the late 1950s (Kopecký and Daum 1958, Hurtado 1960), adaptation to chronic hypoxia has been recognized as an efficient protective phenomenon, which increases cardiac ischemic tolerance. Similarly as the later discovered ischemic preconditioning, chronic hypoxia reduces all major manifestations of acute ischemia/reperfusion injury such as infarct size, post-ischemic contractile dysfunction or ischemic and reperfusion ventricular arrhythmias (for

review see Kolář and Ošťádal 2004). This form of protection is less powerful than the early phase of ischemic preconditioning (Asemu *et al.* 1999, Neckář *et al.* 2002) and comparable with its second window. However, limited evidence exists suggesting that cardioprotective effects of chronic hypoxia may persist much longer than any form of preconditioning (Ošťádal *et al.* 1985, Faltová *et al.* 1987, Zhong *et al.* 2000, Rafiee *et al.* 2003). This feature makes the molecular mechanism of this phenomenon interesting for potential therapeutic exploitation.

It is well known that the majority of chronic hypoxia-induced cardiopulmonary structural, functional and biochemical alterations return to normal when the adapted animals are allowed to recover under normoxic conditions for a sufficient period of time. Complete reversibility was demonstrated for increased hematocrit and concentration of hemoglobin, pulmonary hypertension, right ventricular hypertrophy, cardiac electrophysiological abnormalities, energy metabolism and other manifestations of chronic hypoxia usually within 3–6 weeks of normoxic recovery, depending on the severity of previous hypoxic exposures (Abraham *et al.* 1971, Ressler *et al.* 1974, Pelouch *et al.* 1985, Bass *et al.* 1989, Kolář and Ošťádal 1991, Chouabe *et al.* 2002, Novák *et al.* 2004). However, some changes appear to persist much longer after the last hypoxic exposure. It was shown, for example, that deranged adenylyl cyclase signaling (Hrbasová *et al.* 2003) and myocardial fibrosis (Pelouch *et al.* 1997) lasted for at least 5 weeks and 2 months, respectively, and that the muscularization of small pulmonary arteries was still increased after 10 weeks (Urbanová *et al.* 1973, Leach *et al.* 1977) or even 20 weeks (Herget *et al.* 1978) of normoxic recovery.

Concerning the duration of cardioprotection induced by chronic hypoxia, the available information is scarce but it seems that increased tolerance to acute injury may also persist longer than other changes. It was shown that myocardial necrotic lesions induced by a toxic dose of isoproterenol were still smaller in previously hypoxic rats recovered in normoxia for 6 weeks as compared with normoxic controls (Faltová *et al.* 1987). Moreover, Ošťádal *et al.* (1985) demonstrated that the increased tolerance of the rat isolated right ventricle to post-anoxic contractile dysfunction persisted even 4 months after the termination of hypoxic exposures. To our knowledge, no study has examined the time course of reversibility of infarct size-limiting effect of chronic hypoxia.

In the present study, therefore, we decided to assess the size of myocardial infarction and the incidence and severity of ventricular arrhythmias induced by acute coronary artery occlusion in open-chest rats recovering from chronic intermittent hypoxia for 1, 7, 35 and 90 days under normoxic conditions.

## Methods

### *Animal model*

Adult male Wistar rats (weighing 250–280 g) were exposed to intermittent high altitude (IHA) hypoxia

in a hypobaric chamber for 8 hours/day, 5 days a week. Barometric pressure ( $P_B$ ) was lowered stepwise so that the level equivalent to an altitude of 7000 m ( $P_B = 307$  mm Hg, 41 kPa;  $PO_2 = 64$  mm Hg, 8.5 kPa) was reached after 13 exposures; the total number of the exposures was 35. Thereafter, the rats were kept at normoxic conditions at  $P_B$  and  $PO_2$  equivalent to an altitude of 200 m ( $P_B = 742$  mm Hg, 99 kPa;  $PO_2 = 155$  mm Hg, 20.7 kPa) for an additional 1 day (hypoxic group) and 7, 35 and 90 days (recovery groups); age-matched normoxic animals served as control groups. Each group contained 11–15 rats. All animals had free access to water and a standard laboratory diet. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

### *Surgical preparation*

Animals were anesthetized by sodium pentobarbitone (Sanofi, France; 60 mg/kg body weight, intraperitoneally). Heparinized cannula was placed in the left carotid artery for blood pressure monitoring with a pressure transducer (Gould P23Gb, USA). Tracheotomy was performed, the rats were intubated with a cannula connected to a rodent ventilator (Ugo Basile, Italy) and ventilated with room air at 65–70 breaths/min (tidal volume of 1.2 ml/100 g body weight). A single-lead electrocardiogram (ECG) was continually recorded (Hellige Servomed, Switzerland). Both blood pressure and ECG signals were stored in a computer and subsequently analyzed by our custom-designed software. The heart rate was assessed from the ECG. The rectal temperature was maintained within 36.5 and 37.5 °C by a heated table throughout the experiment. Hematocrit in the tail blood was estimated by using the capillary micromethod.

Left thoracotomy was performed and the pericardium was removed to reveal the location of the left anterior descending (LAD) coronary artery. Then a polyester ligature 6/0 (Ethibond - Ethicon, Scotland) was placed around the LAD coronary artery about 1–2 mm distal to the origin and an occlusive snare was placed around it. The ends of the suture were threaded through a polyethylene tube. After the surgical preparation, the rats were allowed to stabilize for 10 minutes before the ischemic interventions. Regional myocardial ischemia was induced by tightening of the ligature placed around the coronary artery for 30 min. Characteristic changes in the configuration of the ECG and a transient decrease in

blood pressure verified the coronary artery occlusion. After the occlusion, the snare was released; reperfusion of previously ischemic tissue was indicated by changes of the ECG, transient decrease of blood pressure and appearance of reperfusion arrhythmias.

#### *Analysis of arrhythmias*

The incidence and severity of ischemic arrhythmias during prolonged ischemic insult were assessed according to the Lambeth Conventions (Walker *et al.* 1988). Premature ventricular complexes (PVCs) occurring as singles, salvos or tachycardia (a run of 4 or more consecutive PVCs) were counted separately. The incidence of ventricular tachycardia (VT) and fibrillation (VF) was also evaluated. VF lasting more than two minutes was considered as sustained (VFs); hearts exhibiting VFs were excluded from further evaluation. The severity of arrhythmias in each individual heart was evaluated by means of a 5-point arrhythmia score: single PVCs were given a score of 1, salvos a score of 2, VT a score of 3, reversible VF a score of 4, and VFs a score of 5. An assigned number corresponded to the most severe type of arrhythmias observed in that heart. Scores were used for group analysis of severity of arrhythmias.

#### *Measurement of infarct size*

After 4 h of reperfusion, the hearts were arrested with 0.25 mg verapamil (Isoptin, Krka, Croatia) administered into the jugular vein. The hearts were excised and washed with 20 ml saline through the cannulated aorta. The area at risk and the infarct size were determined as described earlier (Neckář *et al.* 2002) by staining with potassium permanganate (Lachema, Czech Republic) and 2,3,5-triphenyltetrazolium chloride (Sigma, USA) dissolved in 0.1 mol/l phosphate buffer (pH 7.4). The hearts were cut perpendicularly to the long axis of the ventricle into slices 1 mm thick and stored overnight in 10 % neutral formaldehyde solution. The day after the infarct size staining, the right ventricular free wall was separated and both sides of slices were photographed by a digital camera (Olympus, Japan). The infarct size (IS), the size of the area at risk (AR) and the size of the left ventricle (LV) were determined by a computerized planimetric method. The IS was normalized to the AR (IS/AR) and the size of AR was normalized to the LV (AR/LV).

#### *Statistics*

The results are expressed as means  $\pm$  S.E.M.

Differences in the number of PVCs between the groups were compared by the Mann-Whitney U test. The incidence of tachycardia and fibrillation was examined by Fischer's exact test. One-way ANOVA and subsequent Student-Newman-Keuls test were used for comparison of differences in normally distributed variables between groups. Differences were considered as statistically significant when  $P < 0.05$ .

## **Results**

#### *Body weight, hematocrit and hemodynamics*

Adaptation of rats to IHA hypoxia significantly decreased body weight (BW) and increased hematocrit as compared with normoxic controls. 35 days after the hypoxic period, BW and hematocrit recovered to the values of age-matched normoxic animals (Table 1). Table 2 summarizes the values of heart rate (HR) and mean arterial blood pressure (MAP) in all groups, determined at baseline (before ischemia), at the end of ischemia and at the end of reperfusion. No significant differences were found in the baseline values of these variables between groups. The 30-min LAD coronary artery occlusion and 4-h reperfusion significantly decreased MAP and HR at the end of the reperfusion period in all groups as compared with baseline values. On the other hand, at the end of reperfusion MAP values were significantly higher in hypoxic and 7-day recovery groups as compared with age-matched normoxic controls.

**Table 1.** Body weight, hematocrit and the relative size of the area at risk (AR/LV) in rats adapted to IHA hypoxia, previously hypoxic rats recovered at normoxia and corresponding age-matched normoxic controls.

Group	n	BW (g)	Hematocrit (%)	AR/LV (%)
Normoxic	15	400 $\pm$ 10.2	48.9 $\pm$ 0.57	43.5 $\pm$ 2.6
Hypoxic	11	354 $\pm$ 4.6 <sup>a</sup>	78.1 $\pm$ 0.74 <sup>a</sup>	42.8 $\pm$ 2.3
Recovery 7 days	15	376 $\pm$ 8.8	71.8 $\pm$ 0.68 <sup>a</sup>	45.5 $\pm$ 2.4
Normoxic	12	466 $\pm$ 6.7	49.7 $\pm$ 0.93	41.5 $\pm$ 1.6
Recovery 35 days	15	450 $\pm$ 6.6	49.9 $\pm$ 0.48	42.2 $\pm$ 2.8
Normoxic	11	482 $\pm$ 11.6	49.8 $\pm$ 0.77	42.3 $\pm$ 2.6
Recovery 90 days	14	500 $\pm$ 10.4	49.5 $\pm$ 0.76	46.7 $\pm$ 2.9

Values are means  $\pm$  S.E.M.; <sup>a</sup>  $P < 0.05$  vs. corresponding normoxic group.

**Table 2.** Heart rate and mean arterial blood pressure after stabilization (baseline), at the end of 30-min coronary artery occlusion and at the end of 4-h reperfusion in rats adapted to IHA hypoxia, previously hypoxic rats recovered at normoxia and corresponding age-matched normoxic controls.

	Baseline	Ischemia 30 min	Reperfusion 4 h
<i>Heart rate (beats/min)</i>			
Normoxic	419±11	407±13	393±9
Hypoxic	432±12	424±7	392±10 <sup>b</sup>
Recovery 7 days	424±10	413±8	374±8 <sup>b</sup>
Normoxic	436±11	430±9	391±12 <sup>b</sup>
Recovery 35 days	418±6	416±5	387±14 <sup>b</sup>
Normoxic	435±8	435±9	387±9 <sup>b</sup>
Recovery 90 days	425±13	431±11	381±10 <sup>b</sup>
<i>Blood pressure (mmHg)</i>			
Normoxic	114±7	119±7	86±4 <sup>b</sup>
Hypoxic	131±4	135±6	110±8 <sup>a b</sup>
Recovery 7 days	128±4	128±6	107±6 <sup>a b</sup>
Normoxic	123±6	105±6 <sup>b</sup>	81±4 <sup>b</sup>
Recovery 35 days	113±4	112±6	91±5 <sup>b</sup>
Normoxic	128±6	115±5	91±6 <sup>b</sup>
Recovery 90 days	125±5	116±7	78±7 <sup>b</sup>

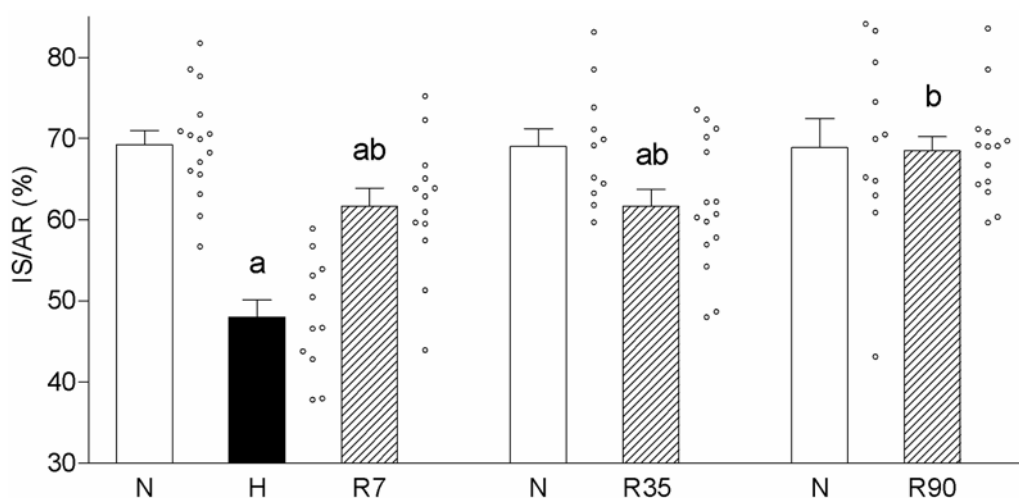
Values are means ± S.E.M.; <sup>a</sup>  $P < 0.05$  vs. corresponding normoxic group; <sup>b</sup>  $P < 0.05$  vs. baseline.

### Infarct size

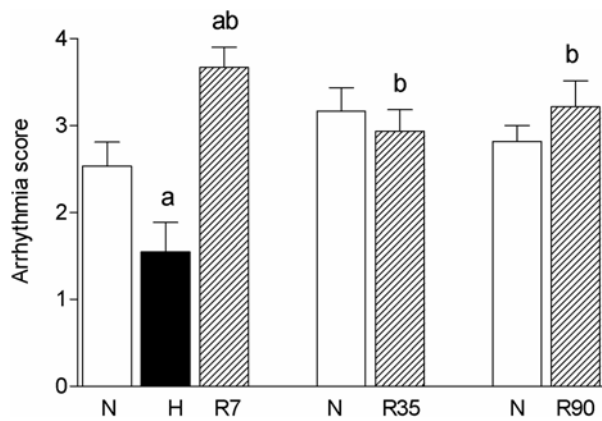
The normalized area at risk (AR/LV) did not differ between the groups (Table 1). The infarct size reached about 70 % of the AR in all normoxic groups; IHA hypoxia significantly decreased IS/AR to  $48.0 \pm 2.2$  %. 7, 35 and 90 days of normoxic recovery increased the infarct size as compared with the hypoxic group. Nevertheless, it was still significantly lower (by about 11 %) in 7- and 35-day recovery groups as compared with age-matched normoxic controls. 90 days after the last hypoxic exposure, the infarct size did not differ from that in the controls (Fig. 1).

### Arrhythmias

All normoxic groups of animals exhibited similar incidence and severity of various types of ischemic ventricular arrhythmias. Chronic hypoxia decreased the arrhythmia severity, expressed as arrhythmia score (Fig. 2), and the incidence of VT but the other indices of antiarrhythmic protection did not reach statistical significance due to rather high variability (Table 3). The antiarrhythmic effect of chronic hypoxia disappeared already after 7 days of normoxic recovery when arrhythmia score reached a higher level than in age-matched normoxic controls (Fig. 2). Moreover, after 7 and 90 days of recovery (but not after 35 days), the incidence of VF was elevated as compared with normoxic animals (Table 3). All groups recovering from hypoxia for 7–90 days exhibited significantly higher arrhythmia score than hypoxic animals (Fig. 2).



**Fig. 1.** Myocardial infarct size expressed as percentage of the area at risk (IS/AR) in rats adapted to IHA hypoxia (H), previously hypoxic rats recovered at normoxia for 7 (R7), 35 (R35) and 90 days (R90) and corresponding age-matched normoxic controls (N). Circles indicate individual experiments. Values are means ± S.E.M.; <sup>a</sup>  $P < 0.05$  vs. corresponding normoxic group; <sup>b</sup>  $P < 0.05$  vs. hypoxic group.



**Fig. 2.** Arrhythmia score over 30-min coronary artery occlusion in hearts of rats adapted to IHA hypoxia (H), previously hypoxic rats recovered at normoxia for 7 (R7), 35 (R35) and 90 days (R90) and corresponding age-matched normoxic controls (N). Values are means  $\pm$  S.E.M.; <sup>a</sup>  $P < 0.05$  vs. corresponding normoxic group; <sup>b</sup>  $P < 0.05$  vs. hypoxic group.

## Discussion

We confirmed earlier reports showing that adaptation of rats to intermittent high altitude hypoxia increased the tolerance of their hearts to ischemia/reperfusion injury determined as diminution of infarct size and reduction of the incidence and severity of

ischemia-induced ventricular arrhythmias. In addition, the residual protective effect on infarct size was detected 7 and 35 days after termination of the adaptation period; it was absent after 90 days of normoxic recovery. In contrast, the antiarrhythmic protection by chronic hypoxia disappeared already during the first week after restoration of normoxic conditions.

To the best of our knowledge, this is the first study that examined the reversibility of the infarct size-lowering effect of chronic hypoxia. The observation that significantly smaller infarction induced by coronary artery occlusion persists for at least 5 weeks in previously hypoxic animals recovering at normoxia is in line with the scarce earlier reports. Similarly, Faltová *et al.* (1987) demonstrated that chronic hypoxia resulted in a long-lasting protection of rat hearts against acute myocardial necrotic lesions induced by a toxic dose of the  $\beta$ -adrenoceptor agonist isoproterenol. The protective effect decreased 6 weeks after the last hypoxic exposure but the extent of injury was still significantly smaller than in normoxic controls. These results suggest that the increased tolerance of chronically hypoxic hearts to injury may last longer than most of the other cardiopulmonary adaptive responses.

**Table 3.** Premature ventricular complexes (PVCs) occurring as singles, salvos and ventricular tachycardia (VT), incidence of VT and ventricular fibrillation (VF) and total duration of VT and VF during 30-min coronary artery occlusion in hearts of rats adapted to IHA hypoxia, previously hypoxic rats recovered at normoxia and in corresponding age-matched normoxic controls.

Group	Number of PVCs				Incidence (%)		Duration (s)		n VFs
	Singles	Salvos	VT	Total	VT	VFr	VT	VFr	
Normoxic	105 $\pm$ 28	32 $\pm$ 12	205 $\pm$ 78	341 $\pm$ 112	73.3	6.6	23.3 $\pm$ 10.0	5.1 $\pm$ 5.1	0
Hypoxic	66 $\pm$ 29	25 $\pm$ 16	56 $\pm$ 31	147 $\pm$ 70	27.3 <sup>a</sup>	0	5.7 $\pm$ 3.1	0	0
Recovery 7 days	179 $\pm$ 42 <sup>b</sup>	41 $\pm$ 11	422 $\pm$ 122 <sup>b</sup>	642 $\pm$ 153 <sup>b</sup>	85.7 <sup>b</sup>	66.7 <sup>a,b</sup>	65.4 $\pm$ 23.3 <sup>b</sup>	28.1 $\pm$ 15.8	2
Normoxic	124 $\pm$ 21	42 $\pm$ 15	294 $\pm$ 120	461 $\pm$ 147	90.9	25.0	31.1 $\pm$ 12.3	7.0 $\pm$ 6.9	1
Recovery 35 days	119 $\pm$ 37	32 $\pm$ 11	190 $\pm$ 64 <sup>b</sup>	340 $\pm$ 101 <sup>b</sup>	80.0 <sup>b</sup>	26.7	29.8 $\pm$ 14.9 <sup>b</sup>	12.2 $\pm$ 9.3	0
Normoxic	150 $\pm$ 34	59 $\pm$ 35	178 $\pm$ 65	387 $\pm$ 116	90.9	0	16.8 $\pm$ 6.0	0	0
Recovery 90 days	182 $\pm$ 33 <sup>b</sup>	53 $\pm$ 19	636 $\pm$ 198 <sup>b</sup>	870 $\pm$ 224 <sup>b</sup>	85.7 <sup>b</sup>	50.0 <sup>a,b</sup>	66.5 $\pm$ 21.0 <sup>b</sup>	6.9 $\pm$ 3.4	0

Values are means  $\pm$  S.E.M.; VFr, reversible VF; VFs, sustained VF (lasting > 2 min); <sup>a</sup>  $P < 0.05$  vs. corresponding normoxic group; <sup>b</sup>  $P < 0.05$  vs. hypoxic.

It is unknown at present how long a recovery period is needed to achieve complete reversibility of protection. In our study, the infarct size-lowering effect of chronic hypoxia disappeared between days 35 and 90 of normoxic recovery. However, it was suggested earlier

that increased cardiac tolerance to injury might persist much longer. The contractile function of the right ventricle of previously hypoxic rats recovered better during reoxygenation following acute anoxia *in vitro* than normoxic controls even 120 days after the last hypoxic

exposure (Ošťádal *et al.* 1985). It cannot be excluded that the different time course of recovery between these two studies is due to different types of insult used to induce acute injury (ischemia *vs.* anoxia), the end point of injury examined (myocardial infarction *vs.* contractile dysfunction) or regional differences (left ventricle *vs.* right ventricle). Measurement of infarct size *in vivo* appears to be a more reliable method to quantify ischemia/reperfusion injury than the assessment of contractile dysfunction in the isolated superfused right ventricle. It was shown that diffusion is sufficient to ensure the exchange of oxygen and metabolites between the medium and the core of the rat myocardial preparation only when its diameter does not exceed 0.2 mm (Schouten and ter Keurs 1986). This critical diameter is greatly exceeded in the isolated right ventricular preparation used in the above-mentioned study (Ošťádal *et al.* 1985) that most likely suffers from tissue core hypoxia even under full oxygenation of the medium. Obviously, this limitation may affect both the pre-anoxic contractile performance of the preparation as well as the degree of its recovery during reoxygenation.

Chronic hypoxia induces a large variety of adaptive changes in the myocardium that can be considered as potentially protective. It increases the expression and/or subcellular distribution of several cytoprotective proteins including nitric oxide synthases, antioxidant enzymes, protein kinase C, MAP kinases, heat shock proteins, heme oxygenase etc. (Rouet-Benzineb *et al.* 1999, Rafiee *et al.* 2002, 2003, Deindl *et al.* 2003). The precise molecular mechanism of myocardial protection afforded by chronic hypoxia is unknown but limited available information suggests that it utilizes the same pool of signaling elements as various forms of preconditioning. It has been shown, for example, that an important role is played by reactive oxygen species, nitric oxide, protein kinases and ATP-sensitive potassium channels (for review see Kolář and Ošťádal 2004). To date, however, no experimental evidence exists as to whether these elements are involved also in the residual infarct size-limiting effect, which persists for weeks under normoxic conditions. The infrequent earlier reports on myocardial recovery from chronic hypoxia do not offer any unambiguous explanation. We may perhaps speculate that desensitization of the adenylyl cyclase signaling system, that persists for at least 5 weeks after the last hypoxic exposure (Hrbasová *et al.* 2003), contributes to the protective effect by blunting adrenergic stimulation of the heart during ischemia and decreasing

its energy demand. This pathway may be also responsible for protection of previously hypoxic animals against myocardial necrotic lesions induced by a toxic dose of isoproterenol (Faltová *et al.* 1987). Inducible heat shock protein 70 is another potential candidate because the hypoxia-induced increase of its transcript level (Zhong *et al.* 2000) and protein redistribution from the particulate to the cytosolic fraction (Rafiee *et al.* 2003) were maintained upon subsequent recovery from hypoxia for at least 2 or 3 weeks, respectively, together with the cardioprotective effect (assessed as the improved post-ischemic recovery of contractility, Rafiee *et al.* 2003).

In contrast to the reduction of infarct size, the antiarrhythmic protection induced by chronic hypoxia disappeared rapidly during the first week of normoxic recovery. Moreover, the incidence and severity of ischemic ventricular arrhythmias were even increased 7 days after the last hypoxic exposure as compared with normoxic controls. This observation is in contradiction with the single study available, which showed that the antiarrhythmic effect of chronic hypoxia in rats was still present 2 weeks after the last hypoxic exposure (Zhong *et al.* 2000). The reason for this discrepancy is unknown but different protocols of adaptation should be taken into account. We have shown previously that the influence of chronic intermittent hypoxia on ventricular arrhythmias occurring during acute ischemia is critically dependent on the degree and duration of daily hypoxic exposures (Asemu *et al.* 2000). Whereas Zhong *et al.* (2000) adapted the animals to hypoxia corresponding to 5000 m for 6 h/day which was associated with right ventricular hypertrophy only, we employed a more severe model of hypoxia (7000 m, 8 h/day), which is known to cause also moderate hypertrophy of the left ventricle (Kolář and Ošťádal 1991, Pelouch *et al.* 1997, Hrbasová *et al.* 2003). Hypertrophy is a well-recognized risk factor, which increases cardiac susceptibility to arrhythmogenic stimuli. The degree of fibrosis, as an important component of the arrhythmogenic substrate in hypertrophied myocardium, further increased during the recovery from normoxia because the turnover of fibrillar collagen is considerably slower than the regression of the ventricular mass (Pelouch *et al.* 1997). The prolongation of action potential duration of hypertrophied cardiac myocytes from chronically hypoxic rats, that may promote triggered ischemic arrhythmias and suppress reentry arrhythmias, returned to normal within 3 weeks of normoxic recovery, i.e. at a time where hypertrophy was still significant (Chouabe *et al.* 2002). In addition,

chronic intermittent hypoxia exceeding 7000 m decreased the plasma potassium level and increased the plasma calcium and magnesium levels (Vats *et al.* 2001). Thus, severe hypoxia seems to induce both antiarrhythmic and proarrhythmic changes; the balance between them apparently shifts in favor of the latter soon after the termination of the adaptation period. However, experimental data available to date are insufficient to confirm this hypothesis. The mechanism underlying antiarrhythmic protection by chronic hypoxia and transient increase in susceptibility to ischemic arrhythmias during the early phase of normoxic recovery is obviously complex and requires further detailed investigation.

In conclusion, our results demonstrate that adaptation of rats to chronic intermittent hypoxia reduced the infarct size and this protective effect, although

attenuated, was still significant 5 weeks after the termination of hypoxia. This long-lasting protection makes chronic hypoxia superior to a transient effect of any form of preconditioning. However, the persistence of the infarct size-lowering effect of chronic hypoxia was not accompanied by antiarrhythmic protection, which waned rapidly during the first week of normoxic recovery. The transient increase in susceptibility to ischemic arrhythmias following severe hypoxia may represent a serious limitation to this cardioprotective phenomenon and points to potential risks associated with the return from extreme hypoxic environment.

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### References

- ABRAHAM AS, KAY JM, COLE RB, PINCOCK AC: Haemodynamic and pathological study of the effect of chronic hypoxia and subsequent recovery on the heart and pulmonary vasculature of the rat. *Cardiovasc Res* **5**: 95-102, 1971.
- ASEMU G, NECKÁŘ J, SZÁRSZOI O, PAPOUŠEK F, OŠŤÁDAL B, KOLÁŘ F: Effects of adaptation to intermittent high altitude hypoxia on ischemic ventricular arrhythmias in rats. *Physiol Res* **49**: 597-606, 2000.
- ASEMU G, PAPOUŠEK F, OŠŤÁDAL B, KOLÁŘ F: Adaptation to high altitude hypoxia protects the rat heart against ischemia-induced arrhythmias. Involvement of mitochondrial K<sub>ATP</sub> channels. *J Mol Cell Cardiol* **31**: 1821-1831, 1999.
- BASS A, OŠŤÁDAL B, PROCHÁZKA J, PELOUCH V, ŠAMÁMEK M, STEJSKALOVÁ M: Intermittent high altitude-induced changes in energy metabolism in the rat myocardium and their reversibility. *Physiol Bohemoslov* **38**: 155-161, 1989.
- CHOUABE C, AMSELLEM J, ESPINOSA L, RIBAUUX P, BLAINEAU S, MEGAS P, BONVALLET R: Reversibility of electrophysiological changes induced by chronic high-altitude hypoxia in adult rat heart. *Am J Physiol* **282**: H1452-H1460, 2002.
- DEINDL E, KOLÁŘ F, NEUBAUER E, VOGEL S, SCHAPER W, OŠŤÁDAL B: Effect of intermittent high altitude hypoxia on gene expression in rat heart and lung. *Physiol Res* **52**: 147-157, 2003.
- FALTOVÁ E, MRÁZ M, PELOUCH V, PROCHÁZKA J, OŠŤÁDAL B: Increase and regression of the protective effect of high altitude acclimatization on the isoprenaline-induced necrotic lesions in the rat myocardium. *Physiol Bohemoslov* **36**: 43-52, 1987.
- HERGET J, SUGGETT AJ, LEACH E, BARER GR: Resolution of pulmonary hypertension and others features induced by chronic hypoxia in rats during complete and intermittent normoxia. *Thorax* **33**: 468-473, 1978.
- HRBASOVÁ M, NOVOTNÝ J, HEJNOVÁ L, KOLÁŘ F, NECKÁŘ J, SVOBODA P: Altered myocardial G<sub>s</sub> protein and adenylyl cyclase signaling in rats exposed to chronic hypoxia and normoxic recovery. *J Appl Physiol* **94**: 2423-2432, 2003.
- HURTADO A: Some clinical aspects of life at high altitudes. *Ann Intern Med* **53**: 247-258, 1960.
- KOLÁŘ F, OŠŤÁDAL B: Right ventricular function in rats with hypoxic pulmonary hypertension. *Pflügers Arch* **419**: 121-126, 1991.
- KOLÁŘ F, OŠŤÁDAL B: Molecular mechanisms of cardiac protection by adaptation to chronic hypoxia. *Physiol Res* **53** (Suppl 1): S3-S13, 2004.

- KOPECKÝ M, DAUM S: Tissue adaptation to anoxia in rat myocardium (in Czech). *Čs Fysiol* **7**: 518-521, 1958.
- LEACH E, HOWARD P, BARER GR: Resolution of hypoxic changes in the heart and pulmonary arterioles of rats during intermittent correction of hypoxia. *Clin Sci Mol Med* **52**: 153-162, 1977.
- NECKÁŘ J, PAPOUŠEK F, NOVÁKOVÁ O, OŠŤÁDAL B, KOLÁŘ F: Cardioprotective effect of chronic hypoxia and ischaemic preconditioning are not additive. *Basic Res Cardiol* **97**: 161-167, 2002.
- NOVÁK F, TVRZICKÁ E, PELOUCH V, JEŽKOVÁ J, SMIK D, NOVÁKOVÁ O: Phospholipid composition of immature rat myocardium exposed to chronic hypoxia and the effect of normoxic recovery. *Collect Czech Chem Commun* **69**: 1-15, 2004.
- OŠŤÁDAL B, PROCHÁZKA J, PELOUCH V, URBANOVÁ D, WIDIMSKÝ J, STANĚK V: Pharmacological treatment and spontaneous reversibility of cardiopulmonary changes induced by intermittent high altitude hypoxia. *Prog Resp Res* **20**: 17-25, 1985.
- PELOUCH V, KOLÁŘ F, OŠŤÁDAL B, MILEROVÁ M, ČIHÁK R, WIDIMSKÝ J: Regression of chronic hypoxia-induced pulmonary hypertension, right ventricular hypertrophy, and fibrosis: effect of enalapril. *Cardiovasc Drugs Ther* **11**: 77-185, 1997.
- PELOUCH V, OŠŤÁDAL B, PROCHÁZKA J, URBANOVÁ D, WIDIMSKÝ J: Effect of high altitude hypoxia on the protein composition of the right ventricular myocardium. *Prog Resp Res* **20**: 41-48, 1985.
- RAFIEE P, SHI Y, KONG X, PRITCHARD KA, TWEDDELL JS, LITWIN SB, MUSSATTO K, JAQUISS RD, SU J, BAKER JE: Activation of protein kinases in chronically hypoxic infant human and rabbit hearts: role in cardioprotection. *Circulation* **106**: 239-245, 2002.
- RAFIEE P, SHI Y, PRITCHARD KA, OGAWA H, EIS AL, KOMOROWSKI RA, FITZPATRICK CM, TWEDDELL JS, LITWIN SB, MUSSATTO K, JAQUISS RD, BAKER JE: Cellular redistribution of inducible Hsp70 protein in the human and rabbit heart in response to the stress of chronic hypoxia. *J Biol Chem* **278**: 43636-43644, 2003.
- RESSL J, URBANOVÁ D, WIDIMSKÝ J, OŠŤÁDAL B, PELOUCH V, PROCHÁZKA J: Reversibility of pulmonary hypertension and right ventricular hypertrophy induced by intermittent high altitude hypoxia in rats. *Respiration* **31**: 38-46, 1974.
- ROUET-BENZINEB P, EDDAHIBI S, RAFFESTIN B, LAPLACE M, DEPOND S, ADNOT S, CROZATIER B: Induction of cardiac nitric oxide synthase 2 in rats exposed to chronic hypoxia. *J Mol Cell Cardiol* **31**: 1697-1708, 1999.
- SCHOUTEN VJ, TER KEURS HE: The force-frequency relationship in rat myocardium. The influence of muscle dimension. *Pflügers Arch* **407**: 14-17, 1986.
- URBANOVÁ D, RESSL J, WIDIMSKÝ J, OŠŤÁDAL B, PELOUCH V, PROCHÁZKA J: Pulmonary vascular changes induced by intermittent altitude hypoxia and their reversibility in rats. *Beitr Pathol* **150**: 389-399, 1973.
- VATS P, SINGH SN, KUMRIA MM, RANGANATHAN S, ARORA MP, JAIN CL, SRIDHARAN K: Effect of hypoxia on the circulating levels of essential mineral elements in rats. *J Environ Biol* **22**: 277-282, 2001.
- WALKER MJA, CURTIS MJ, HEARSE DJ, CAMPBELL RWF, JANSEN MJ, YELLON DM, COBBE SM, COKER SM, HARNESS JB, HARRON WG, HIGGINS AJ, JULIAN DG, LAB MJ, MANNING AS, NORTHOVER BJ, PARRATT JR, RIEMERSMA RA, RIVA E, RUSSELL CD, SHERIDAN DJ, WISLOW E, WOODWARD B: The Lambeth Convention: guidelines for study of arrhythmias in ischaemia, infarction and reperfusion. *Cardiovasc Res* **22**: 447-455, 1988.
- ZHONG N, ZHANG Y, FANG QZ, ZHOU ZN: Intermittent hypoxia exposure-induced heat-shock protein 70 expression increases resistance of rat heart to ischemic injury. *Acta Pharmacol Sin* **21**: 467-472, 2000.

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#### Reprint requests

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