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Effects of Adaptation to Intermittent High Altitude Hypoxia on Ischemic Ventricular Arrhythmias in Rats

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Summary

We compared the effects of adaptation to intermittent high altitude (IHA) hypoxia of various degree and duration on ischemia-induced ventricular arrhythmias in rats. The animals were exposed to either relatively moderate hypoxia of 5000 m (4 or 8 h/day, 2-3 or 5-6 weeks) or severe hypoxia of 7000 m (8 h/day, 5-6 weeks). Ventricular arrhythmias induced by coronary artery occlusion were assessed in isolated buffer-perfused hearts or open-chest animals. In the isolated hearts, both antiarrhythmic and proarrhythmic effects were demonstrated depending on the degree and duration of hypoxic exposure. Whereas the adaptation to 5000 m for 4 h/day decreased the total number of premature ventricular complexes (PVCs), extending the daily exposure to 8 h and/or increasing the altitude to 7000 m led to opposite effects. On the contrary, the open-chest rats adapted to IHA hypoxia exhibited an increased tolerance to arrhythmias that was even more pronounced at the higher altitude. The distribution of PVCs over the ischemic period was not altered by any protocol of adaptation. It may be concluded that adaptation to IHA hypoxia is associated with enhanced tolerance of the rat heart to ischemic arrhythmias unless its severity exceeds a certain upper limit. The opposite effects of moderate and severe hypoxia on the isolated hearts cannot be explained by differences in the occluded zone size, heart rate or degree of myocardial fibrosis. The proarrhythmic effect of severe hypoxia may be related to a moderate left ventricular hypertrophy (27 %), which was present in rats adapted to 7000 m but not in those adapted to 5000 m. This adverse effect can be overcome by an unknown protective mechanism(s) that is absent in the isolated hearts.

Key words
High altitude • Chronic hypoxia • Rat heart • Ischemia • Arrhythmias

Introduction

Chronic myocardial hypoxia is the major pathophysiological feature of various cardiopulmonary diseases, such as chronic obstructive pulmonary disease and cyanotic congenital heart defects. It also naturally occurs in fetuses and in populations living at high altitude. High altitude hypoxia simulated in a hypobaric or normobaric chamber serves as a relevant experimental model of chronic hypoxia. It has been known for many years that both humans and animals indigenous or adapted to high altitude hypoxia are more tolerant to an acute ischemic injury of the heart (Poupa *et al.* 1996, for reviews see Moret 1980, Heath and Williams 1981, Ošťádal *et al.* 1998). The majority of experimental studies have demonstrated that the hearts of chronically

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hypoxic animals develop smaller myocardial infarction and exhibit better functional recovery following an ischemia/reperfusion insult than normoxic controls. However, it is not known to what extent this protection depends on the degree, duration and character (permanent, intermittent) of the hypoxic exposure, and which is the optimum condition providing maximum and long-lasting protective effects (for review see Kolář 1996).

From the three above-mentioned major endpoints of myocardial ischemia/reperfusion injury, the incidence and severity of arrhythmias have been the least studied. Moreover, the available information on the effects of chronic high altitude hypoxia is inconclusive. Thus, Meerson *et al.* (1987, 1989) observed a pronounced protection against ischemic arrhythmias in rats adapted to intermittent high altitude (IHA) hypoxia (5000 m, 5-6 h/day, 40-45 exposures) using regional no-flow ischemia in open-chest animals, but not in isolated perfused hearts. In another study (Vovc 1998), a similar protocol (4000 m, 5 h/day, 40 exposures) led to an antiarrhythmic effect that persisted in hearts even after their isolation. Recently, we have also shown a marked protection against ischemia-induced arrhythmias in animals adapted to 5000 m (4 h/day, 10–30 exposures) using an isolated heart preparation (Asemu *et al.* 1999). However, according to our preliminary study (Asemu *et al.* 1997), this protection may be lost and even a promotion of ischemic arrhythmias may be observed in rats exposed to a more severe altitude (7000 m, 8 h/day).

The aim of this study was, therefore, to compare the effects of adaptation to IHA hypoxia of various degree and duration on ischemia-induced ventricular arrhythmias in rats. As different factors may influence the susceptibility to arrhythmias when assessed on the isolated heart preparations or in open-chest animals, these two experimental settings were used for comparison.

Methods

Animal model

Adult male Wistar rats weighing 320-360 g were employed. Different protocols of adaptation to IHA hypoxia were used to find optimum conditions that protect the heart against ischemia-induced ventricular arrhythmias. The rats were adapted to an altitude of either 5000 m or 7000 m in a hypobaric chamber. Barometric pressure (P_B) was lowered stepwise, so that the desired terminal levels were achieved after five (P_B 404 mm Hg, 53.8 kPa; PO_2 84 mm Hg, 11.2 kPa) or 13 (P_B 306.8 mm Hg, 40.9 kPa; PO_2 63.8 mm Hg, 8.5 kPa) exposures, respectively. The animals were exposed to hypoxia 5 days a week for either 4 or 8 hours a day. The total number of exposures was either 10-14 (2-3 weeks) or 25-30 (5-6 weeks). Adaptation protocols and designations of the experimental groups are summarized in Table 1. The animals were employed on the next day after the last hypoxic exposure. The tolerance of their hearts to acute ischemia-induced arrhythmias was evaluated using either an *in vivo* open-chest model (groups A2, A4) or an isolated perfused heart preparation (groups A1, A2, A3, A4). The normoxic (control, C) animals were kept for a corresponding period at P_B and PO_2 equivalent to an altitude of 200 m. As no differences were detected in the evaluated parameters among the control groups in the individual series of experiments, the results were pooled.

Table 1. Protocols of adaptation of rats to intermittent high altitude hypoxia

Group	Altitude (m)	Daily exposure (h)	Total number of exposures
A1	5000	4	10-14
A2	5000	4	25-30
A3	5000	8	25-30
A4	7000	8	25-30

Perfusion of the heart

The rats were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg), their hearts were rapidly removed and perfused at constant flow and temperature (37 °C) according to Langendorff under non-recirculating conditions by a modified Krebs-Henseleit solution (mmol/l: NaCl 118.0, KCl 3.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 7.0 and sodium pyruvate 2.0) as described earlier (Asemu *et al.* 1999). Coronary flow was set at ~10 ml/min/g. The expected heart weights were calculated from the regression equations established on the basis of previous data from our laboratory for heart weight to body weight ratio (Asemu *et al.* 1999).

Diastolic perfusion pressure was measured in the aortic cannula by a Hewlett-Packard (HP 1280, USA) transducer. Epicardial electrograms were recorded with platinum electrodes attached to the right atrium and the apex of the heart. Both perfusion pressure and

electrograms were continually registered on a Hewlett-Packard (HP 7702B) recorder; signals were stored in a computer and subsequently analyzed by our computer program. The heart rate was calculated from the electrograms. A silk suture was placed around the left anterior descending (LAD) coronary artery about 1 mm distally from its origin (Ravingerová *et al.* 1995, Kolář and Parratt 1997).

Open-chest animals

Rats were anesthetized as above and their rectal temperature was maintained at 37 °C by a heated table throughout the experiment. After the onset of controlled ventilation with room air by a pump (Ugo Basile, Italy) using a stroke volume of 1.2 ml/100 g BW and a rate of 65-70 strokes/min, a left thoracotomy was performed and a ligature was placed around the LAD coronary artery. A single-lead electrocardiogram (ECG) was recorded (Hellige Servomed, Switzerland) and analyzed as above. The heart rate was assessed from the ECG.

Experimental protocol

In both experimental settings, regional no-flow myocardial ischemia was induced by the tightening of the suture placed around the coronary artery to elicit ischemic ventricular arrhythmias. Coronary occlusion was considered effective if there was a corresponding increase in perfusion pressure in the isolated heart. Characteristic changes in the configuration of the ECG were indicative of ischemia in the open-chest model. Both hypoxic and normoxic control rats were subjected to a single 30-min coronary artery occlusion, following a 30-min stabilization period. In open-chest animals, the ligature was then released to allow reperfusion of the ischemic zone. At the end of experiment, the ischemic zone was delineated by staining with 5 % potassium permanganate and measured by planimetry on 1 mm-thick slices, cut perpendicularly to the long axis of the ventricle. The size of the ischemic zone was normalized to the size of the left ventricle, including the septum.

Ventricular arrhythmias were assessed during the ischemic insult according to the Lambeth Conventions (Walker *et al.* 1988). Premature ventricular complexes (PVCs) occurring as single ectopic beats, salvos or tachycardia (a run of 4 or more consecutive PVCs) were counted separately. The incidence and number of episodes of ventricular tachycardia (VT) and fibrillation (VF) were also evaluated. VF lasting more than two minutes was considered as sustained (VFs). The hearts exhibiting VFs were excluded from further evaluation. The severity of arrhythmias in each group was evaluated by an arrhythmia score according to the incidence of the most severe form of arrhythmia that occurred in each individual heart (hearts with single PVCs were given a score of 1, salvos 2, VT 3, reversible VF 4 and VFs 5).

Determination of hydroxyproline

The concentration of 4-hydroxyproline, as a quantitative marker of fibrillar collagen, was determined in the left and right ventricles of A2, A4 and control groups. After the perfusion experiment, the heart was dissected to the free left and right ventricular walls and both parts were weighed separately. Transmural samples were taken from the ventricles, weighed, dried and digested in 6 M HCl for 16 h at 105 °C. The resulting solution was decolorized and the supernatant was analyzed for 4-hydroxyproline using a colorimetric method as previously described (Pelouch *et al.* 1993).

Statistics

The results are expressed as means ± S.E.M. To compare the number of PVCs and the number of episodes of VT and VF between two groups, the Mann-Whitney U test was used. Where necessary, more than two groups were compared by Kruskal-Wallis non-parametric test. The incidence of tachycardia and fibrillation was examined by Fisher's exact test. ANOVA and a subsequent Student-Newman-Keuls test were used for comparison of differences in parametric variables among the groups. The effects of ischemia within the groups were evaluated by the paired *t*-test. Differences were assumed as statistically significant when $P<0.05$.

Results

Weight parameters and collagen concentration

Adaptation of rats to an altitude of 5000 m, 4 h/day for a period of 2-3 weeks (A1) did not affect the body weight and heart weight; therefore, the relative heart weight (HW/BW) remained unchanged (0.266±0.005 and 0.267±0.003 % in controls and A1 groups, respectively). Prolongation of the adaptation period to 5-6 weeks for either 4 h/day (A2) or 8 h/day (A3) slightly decreased the body weight, but the HW/BW remained unchanged (0.261±0.002 and 0.280±0.006 % in A2 and A3 groups, respectively). Adaptation of the rats to an altitude of 7000 m, 8 h/day for a period of 5-6 weeks (A4) markedly slowed down body growth that was accompanied by an increase of the HW/BW ratio to 0.322±0.006.

The left and right ventricular weight and the myocardial concentration of hydroxyproline were only determined in the A2 and A4 groups that exhibited opposite changes in susceptibility to ischemic arrhythmias (see bellow). Thus, the relative left ventricular weight (LV/BW) was unchanged in A2 and moderately increased in A4 groups. The relative weight of the right ventricle (RV/BW) increased in A2 and this effect was more pronounced in A4 group (Table 2). Myocardial concentration of hydroxyproline was higher in the right ventricles than in the left ventricles in all groups ($P<0.05$). Adaptation to IHA hypoxia caused an increase in hydroxyproline concentration to about the same extent in both A2 and A4 groups (Table 2).

Table 2. Relative ventricular weight and concentration of total hydroxyproline in the left and right ventricles of rats adapted to IHA hypoxia and of normoxic controls.

Group	C	A2	A4
Relative ventricular weight (%)			
Left ventricle	0.133 ± 0.003	0.135 ± 0.005	0.169 ± 0.008*†
Right ventricle	0.038 ± 0.001	0.055 ± 0.002*	0.080 ± 0.004*†
Hydroxyproline concentration (mg/g dry weight)			
Left ventricle	2.20 ± 0.18	2.62 ± 0.11*	2.65 ± 0.11*
Right ventricle	3.26 ± 0.20	4.30 ± 0.27*	4.75 ± 0.10*

C, normoxic controls; A2, adapted to hypoxia (5000 m, 4 h/day, 2-3 weeks); A4, adapted to hypoxia (7000 m, 8 h/day, 5-6 weeks). Values are means ± S.E.M. of 8 hearts in each group. Significant differences ($P<0.05$): vs. normoxic controls; † vs. A2 group.*

Table 3. Coronary flow, heart rate and perfusion pressure before and during 30-min coronary artery occlusion in isolated perfused hearts of rats adapted to IHA hypoxia and of normoxic controls.

Group	n	Coronary flow (ml/min/g)	Heart rate (beats/min)			Perfusion pressure (mm Hg)		
			Preischemic	10 min ischemia	30 min ischemia	Preischemic	10 min ischemia	30 min ischemia
C	43	10.0 ± 0.1	256 ± 4	257 ± 7	260 ± 4	48 ± 2	71 ± 4†	70 ± 3†
A1	31	10.2 ± 0.2	264 ± 4	272 ± 4	270 ± 5	53 ± 7	69 ± 4†	67 ± 5†
A2	12	10.4 ± 0.2	267 ± 9	278 ± 9	280 ± 10	40 ± 3	51 ± 3*†	51 ± 6*
A3	15	10.1 ± 0.2	245 ± 8	259 ± 7	253 ± 5	46 ± 1	50 ± 2*	49 ± 2*
A4	11	10.2 ± 0.2	280 ± 11*	293 ± 14*	287 ± 11*	43 ± 5	68 ± 9†	59 ± 7†

C, normoxic controls; A1, adapted to hypoxia (5000 m, 4 h/day, 2-3 weeks); A2, adapted to hypoxia (5000 m, 4 h/day, 5-6 weeks); A3, adapted to hypoxia (5000 m, 8 h/day, 5-6 weeks); A4, adapted to hypoxia (7000 m, 8 h/day, 5-6 weeks); n, number of rats. Values are means ± S.E.M. Significant differences ($P<0.05$): vs. normoxic controls; † vs. preischemic.*

Table 4. Premature ventricular complexes (PVCs) occurring as singles, salvos and ventricular tachycardia (VT), incidence of VT and ventricular fibrillation (VF), total duration and number of episodes of VT and VF during 30-min coronary artery occlusion in open-chest rats and in isolated perfused hearts of rats adapted to IHA hypoxia and of normoxic controls.

Group	n	Number of PVCs			Incidence (%)		Duration (s)		Number of episodes		n VFs
		Singles	Salvos	VT	VT	VFr	VT	VFr	VT	VFr	
Isolated perfused hearts											
C	43	147 ± 17	70 ± 11	522 ± 69	95.4	20.9	70.8 ± 8.9	8.8 ± 4.1	21.5 ± 2.5	1.0 ± 0.4	7
A1	31	92 ± 15	47 ± 8	292 ± 43*	90.3	6.5	33.7 ± 4.9*	0.5 ± 0.4	14.8 ± 2.2	0.1 ± 0.1	1
A2	12	64 ± 19*	21 ± 7*	195 ± 52*	75.0	0	26.9 ± 7.5*	0	7.8 ± 2.2*	0	0
A3	15	158 ± 34	126 ± 31†	883 ± 93*†	86.7	46.7	102.5 ± 20.5†	40.1 ± 17.9†	35.4 ± 6.5†	5.5 ± 1.8*	1
A4	11	175 ± 31†	123 ± 33†	1230 ± 365*†	90.9	45.5	153.4 ± 41.1†	48.1 ± 23.9†	57.6 ± 14.4*†	2.3 ± 0.8	3
Open-chest rats											
C	14	83 ± 22	19 ± 5	350 ± 94	85.7	7.1	32.8 ± 15.9	7.7 ± 4.7	10.3 ± 3.3	0.4 ± 0.1	0
A2	16	94 ± 4	21 ± 9	117 ± 45	62.5	6.3	9.5 ± 4.5	1.1 ± 1.1	7.4 ± 2.7	0.1 ± 0.1	1
A4	16	52 ± 20	18 ± 11	43 ± 21*	37.5*	0	4.4 ± 2.2	0	2.7 ± 1.4*	0	0

C, normoxic controls; A1, adapted to hypoxia (5000 m, 4 h/day, 2-3 weeks); A2, adapted to hypoxia (5000 m, 4 h/day, 5-6 weeks); A3, adapted to hypoxia (5000 m, 8 h/day, 5-6 weeks); A4, adapted to hypoxia (7000 m, 8 h/day, 5-6 weeks); n, number of rats; VFr, reversible VF; n VFs, number of hearts exhibiting sustained VF (excluded from the evaluation). Values are means ± S.E.M. Significant differences (P<0.05): vs. normoxic controls; † vs. A2 group.*

Effects of IHA hypoxia on arrhythmias

Isolated perfused heart

The heart rate did not differ among the control and hypoxic groups and was not altered by coronary artery occlusion, except for the group A4, which exhibited slightly but significantly higher heart rate as compared with normoxic controls. Coronary flow and perfusion pressure were similar in all groups. Perfusion pressure increased immediately after coronary artery occlusion and did not change significantly over the 30-min ischemic period (Table 3).

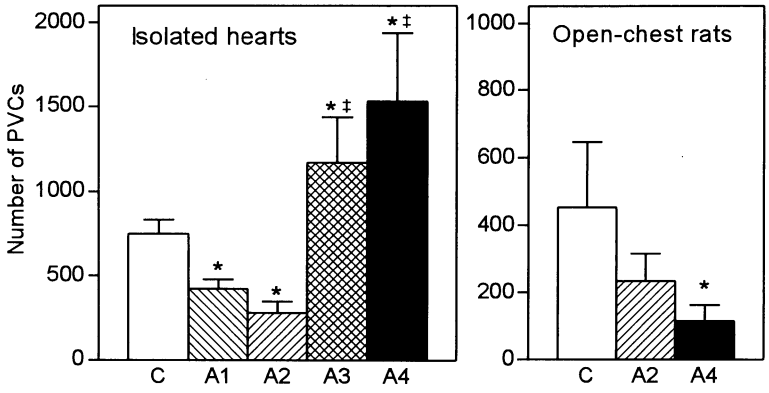

Fig. 1. *Total number of premature ventricular complexes (PVCs) over 30-min coronary artery occlusion in the isolated perfused hearts (left panel) and in open-chest rats (right panel) adapted to IHA hypoxia (A), compared to normoxic controls (C). See Table 1 for designation of the A groups. Values are means ± S.E.M. of 11-43 experiments (see Table 4). Significant differences (P<0.05): * vs. normoxic controls; † vs. A2 group.*

Adaptation of rats to IHA hypoxia of 5000 m (4 h/day) for either 2-3 weeks (A1) or 5-6 weeks (A2) significantly decreased the total number of PVCs during 30-min coronary artery occlusion in the isolated perfused hearts. Maximum ectopic activity occurred between 10 and 20 min of ischemia and this temporal profile was not affected by adaptation to chronic hypoxia (Fig. 2, upper panel). Ventricular tachycardia was the predominant type of arrhythmias in all groups. Whereas the incidence of VT did not differ among the groups, the hearts of A1 and A2 animals exhibited a significantly lower number of ectopic beats occurring as VT and a reduced total duration of VT. The number of episodes of VT was significantly decreased in the A2 group only, the same being true for the number of single PVCs and salvos. The incidence of VF was not influenced by chronic hypoxia as it was already rather low in the control group (Table 4).

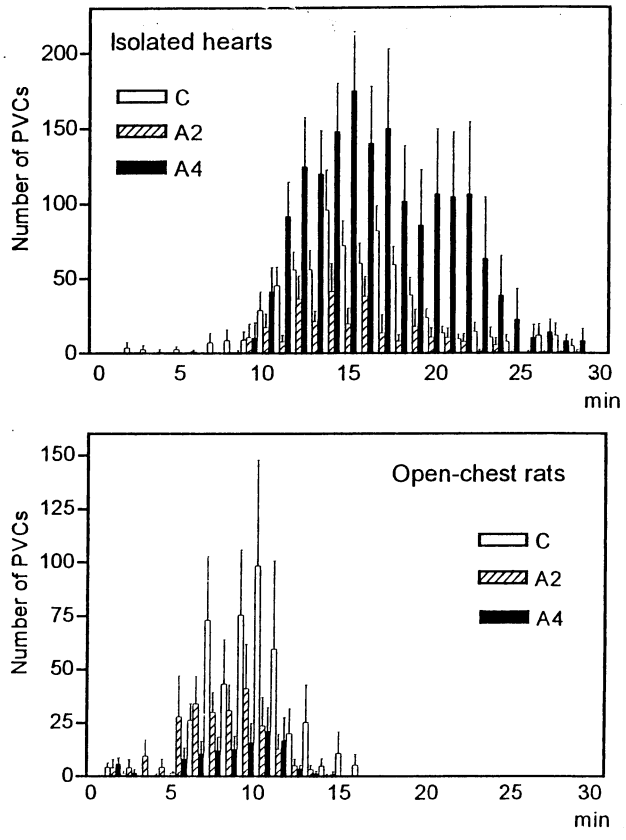

Fig. 2. *Distribution of premature ventricular complexes (PVCs) over 30-min coronary artery occlusion in the isolated perused hearts (upper panel) and in open-chest rats (lower panel) adapted to IHA hypoxia (A), compared to normoxic controls (C). See Table 1 for designation of the A groups. Values are means ± S.E.M. of 11-43 experiments (see Table 4).*

Extending the daily exposure of rats to the same altitude (5000 m) from 4 h to 8 h (A3) eliminated the antiarrhythmic effect of chronic hypoxia, as observed in the A1 and A2 groups. In fact, both the total number of PVCs (Fig. 1) and the number of PVCs occurring as VT were significantly increased as compared with the controls. Most of the other indices exhibited a marked deterioration of arrhythmias in this group as compared with the A2 group (Table 4).Increasing the severity of chronic hypoxia in the A4 group (7000 m) caused an even more pronounced proarrhythmic effect, increasing the total number of PVCs twice (Fig. 1). Although the incidence of VT remained similar to that in the controls, the total duration of VT and the number of episodes were increased as compared with both control and A2 groups. Despite a tendency to higher incidence of VF in the A3 and A4 groups, the increase did not reach statistical significance (Table 4). The proarrhythmic effect of severe chronic hypoxia was not associated with an altered temporal profile of arrhythmias (Fig. 2, upper panel).

The arrhythmia score, indicating the severity of arrhythmias, was significantly lower in the A2 group than in the controls. This parameter in A1, A3 and A4 groups did not differ from the controls, but it was significantly higher as compared with A2 group (Fig. 3).

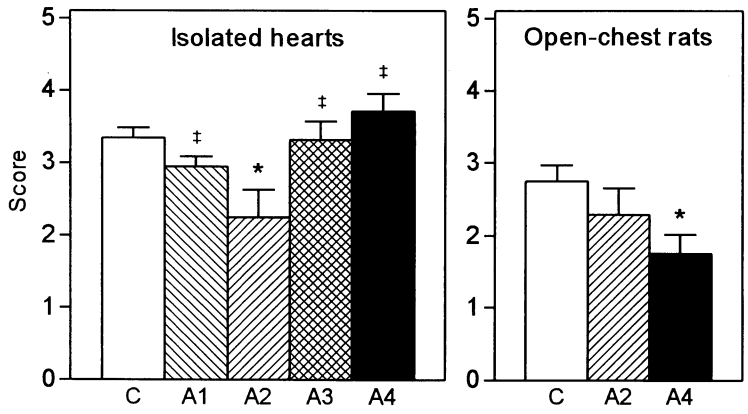

Fig. 3. *Arrhythmia score in the isolated perfused hearts (left panel) and in open-chest rats (right panel) adapted to IHA hypoxia (A), compared to normoxic controls (C). See Table 1 for designation of the A groups. Values are means ± S.E.M. of 11-43 experiments (see Table 4). Significant differences (P<0.05): * vs. normoxic controls; † vs. A2 group.*

Open-chest animals

In the control open-chest animals, the total number of PVCs and the severity of arrhythmias were lower as compared with the *in vitro* study (P<0.05). We compared only two chronically hypoxic groups (A2 and A4) that exhibited opposite changes in susceptibility to ischemic arrhythmias in the isolated heart model. In the A2 group, there was a tendency to antiarrhythmic protection, similar to that observed in the isolated heart, but this effect did not reach statistical significance in any indices of arrhythmias (Fig. 1, Table 4).

On the other hand, and in sharp contrast to the proarrhythmic effect demonstrated in the isolated hearts, adaptation to severe hypoxia of 7000 m (A4) provided a marked antiarrhythmic protection in the open-chest animals. The total number of PVCs during the ischemic period decreased to 25 % of the control value (Fig. 1) and this effect was mainly due to a reduction in the number of PVCs occurring as VT. Both the incidence of VT and the number of episodes were significantly reduced, whereas the incidence of VF remained unchanged, as it was already low in the control group (Table 4).

The maximum arrhythmogenesis occurred between 5 and 12 min of coronary artery occlusion, i.e. earlier than in the isolated hearts, and this distribution of arrhythmias over the ischemic period was not altered in chronically hypoxic animals (Fig. 2, lower panel). The severity of arrhythmias was significantly reduced in the A4 group only, as is evident from the lower value of the arrhythmia score compared to the controls (Fig. 3).

Although this study was not designed to assess reperfusion arrhythmias, we observed ventricular arrhythmias during the first few minutes of reperfusion in 69 % of control normoxic animals. The incidence of arrhythmias was decreased to 31 % and 19 % in the A2 and A4 groups, respectively, the latter effect being statistically significant.

The ischemic zone produced by coronary artery occlusion occupied 42.9±3.1, 41.6±3.1 and 42.8±2.2 % of the ventricle size in control, A2 and A4 groups, respectively. There was no difference among the groups. The preischemic heart rate was slightly higher in the A2 group (445±8 beats/min) than in the controls (408±10 beats/min) and this difference essentially persisted

throughout the experiments. The heart rate in the A4 group (429±9 beats/min) did not differ from that in the controls.

Discussion

The major result of this study concerns the finding that the effect of adaptation of rats to IHA hypoxia on ischemic arrhythmias induced by coronary artery occlusion differs markedly when assessed on isolated perfused hearts or on open-chest animals. In the isolated hearts, the effect was strongly dependent on the degree (altitude) and duration of hypoxic exposure. Whereas the adaptation to a relatively "moderate" altitude of 5000 m for 4 h/day decreased ectopic activity and severity of ischemic arrhythmias, prolongation of the daily exposure to 8 h and/or increasing the degree of hypoxia to 7000 m led to an opposite, proarrhythmic effect. On the other hand, the open-chest rats adapted to IHA hypoxia exhibited antiarrhythmic protection, which was even more pronounced at the higher altitude. The protective effect of moderate chronic hypoxia is in agreement with previous reports on open-chest rats (Meerson *et al.* 1987, 1989) and isolated hearts (Vovc 1998, Asemu *et al.* 1999). The loss of protection in the isolated hearts following a prolongation of hypoxic exposure from 4 to 8 h/day is in line with the observation of Meerson *et al.* (1989) using the same altitude for 6 h/day. However, to our knowledge, no studies have been published about the influence of "severe" chronic hypoxia on cardiac susceptibility to ischemic arrhythmias. We have observed distinct effects in the two experimental settings, despite the fact that both involved the same model of zero-flow regional ischemia. The reason for this difference is not known at present. Obviously, the presence of blood components and/or neurohumoral control mechanisms in open-chest animals appears to play a crucial role in maintaining the antiarrhythmic protection in rats adapted to more severe hypoxia.

The results obtained on isolated denervated hearts, perfused by a crystalloid solution, suggest that chronic hypoxia is associated with an enhanced tolerance of the heart to ischemic arrhythmias, unless its severity exceeds a certain upper limit. The mechanism of this protection is not known, although several factors have been proposed to play a role (for review see Kolář 1996). Recently, we have demonstrated that ATP-sensitive potassium channels (K_{ATP}), possibly those localized on the mitochondrial membrane, appear to be involved in the antiarrhythmic effect of IHA hypoxia. A selective mitochondrial K_{ATP} blocker, 5-hydroxydecanoate, abolished this protection without affecting arrhythmias in normoxic controls (Neckář *et al.* 1999). In agreement with this, a selective mitochondrial K_{ATP} opener, diazoxide, was antiarrhythmic in the controls, but not in chronically hypoxic hearts (Asemu *et al.* 1999). Thus, it appears that enhanced activation of these channels in chronic hypoxia is of importance in cardioprotection.

The cause of a reversal of protection by moderate hypoxia to a proarrhythmic effect of more severe hypoxia is not known. We can exclude the influence of ischemic zone size which is an important determinant of arrhythmias (Ridley *et al.* 1992), because the left anterior descending coronary artery occlusion produced the ischemic zone of the same size in both A2 and A4 groups. Another factor, which should be taken into account, is the heart rate, as the higher rate is known to promote ischemic arrhythmogenesis (Bernier *et al.* 1989). Although the heart rate was slightly higher in the A4 group as compared with the controls, it is unlikely to play a major role, because it did not differ between A4 and A2 groups, despite a five-fold difference in the number of PVCs.

As a consequence of a sustained pulmonary hypertension, the right ventricular hypertrophy developed in both chronically hypoxic groups (A2 and A4) and this effect was more pronounced in the latter one. Unlike the A2 hearts, the A4 hearts also exhibited mild hypertrophy of the left ventricle, in agreement with our former reports on the same model (Kolář *et al.* 1989, Kolář and Ošťádal 1991). This is probably due to stress associated with intermittent exposure to severe hypoxia (Ošťádal *et al.* 1984). Susceptibility to ventricular arrhythmias is increased in the patients with left ventricular hypertrophy (Messerli *et al.* 1984) and the experimental hypertrophy aggravates the severity of arrhythmias caused by ischemia (Kohya *et al.* 1988, Kolář and Parratt 1997). Myocytes and extracellular matrix remodeling in the hypertrophied myocardium can serve as a structural basis for electrophysiological abnormalities in action potential duration and conduction velocity, and their inhomogeneous distribution within the ventricular tissue (Keung and Aronson 1981, Cameron *et al.* 1983). Consequently, the impaired generation and spread of excitation may result in arrhythmias. Myocardial hypertrophy is usually associated with fibrosis, which is well known to predispose the heart to ischemic

ventricular arrhythmias by promoting formation of re-entrant circuits. However, in our experiments, the concentration of collagen was increased to the similar level in both hypertrophied left ventricles of the A4 group and non-hypertrophied ventricles of the A2 group. Moreover, no individual correlation was found between the collagen concentration and severity of arrhythmias (data not shown). Therefore, myocardial fibrosis appears unlikely to play a major role in the proarrhythmic effect of severe hypoxia, as assessed on the isolated heart.

In the hypertrophic myocardium, including that of chronically hypoxic rats (Chouabe *et al.*, 1997), prolonged action potential duration is a common observation, reflecting changes in mechanisms of ionic transport. It cannot be excluded that altered sodium and calcium handling by hypertrophied myocytes (Golden *et al.* 1994, Allard *et al.* 1994) can contribute to greater arrhythmogenesis during ischemia. In our experiments on isolated hearts, low potassium and high calcium concentrations in the perfusate were used to promote triggered activity due to calcium influx. Polymorphic ventricular tachycardia, in particular *torsade de pointes*, which are triggered by early afterdepolarizations induced by calcium influx through L-type calcium channels (Ming *et al.* 1994), were quite frequent in our model. As calcium influx depends on the action potential duration, its prolongation in hypertrophied myocytes of chronically hypoxic rats may promote this type of arrhythmias during the ischemic insult.

In conclusion, adaptation of rats to IHA hypoxia may protect their hearts against ischemia-induced ventricular arrhythmias. Whether this protection occurs or not depends on the severity and duration of the hypoxic exposure and on the experimental model utilized to assess arrhythmias. Whereas the relatively moderate hypoxia has an antiarrhythmic effect in both open-chest rats and isolated hearts, more severe hypoxia is antiarrhythmic only in the open-chest model and proarrhythmic in the isolated hearts. Further studies are necessary to clarify these differences.

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