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Ischemia and reperfusion-induced arrhythmias: role of hyperoxic preconditioning

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Background **Hyperoxic preconditioning is known to protect the heart against necrosis and contractile dysfunction, but protection against arrhythmias has not been well characterized.**

Objective **The authors hypothesized that pre-exposure to normobaric hyperoxia (H) reduces ischemia and reperfusion-induced arrhythmias in isolated rat hearts.**

Methods **Following 60 and 180 min of hyperoxia treatment, rat hearts were isolated immediately (H60 and H180) or 24 h afterward (H60/24 and H180/24), and subjected to 30 min of regional ischemia followed by 120 min of reperfusion. Occurrence, number, and duration of arrhythmias were analyzed during ischemia and reperfusion. In addition, cardiac infarct size was also assessed.**

Results **Sixty and 180 min of breathing hyperoxic gas induced significant protection against severe ischemia and reperfusion-induced arrhythmias. Total number of premature ventricular beats was markedly attenuated by hyperoxia pre-exposure, especially in H60 and H180 groups. Duration of ventricular tachycardia and ventricular fibrillation was also affected by hyperoxia. Hyperoxia reduced the number of ventricular tachycardia episodes in ischemia and reperfusion phase. Accordingly, severity of arrhythmias (arrhythmia score) and infarct size were lower in hyperoxia-treated groups. The effects were more pronounced using hyperoxia immediately before harvesting the heart.**

Conclusion **These results indicate that hyperoxic preconditioning attenuates ventricular ischemia and reperfusion-induced arrhythmias in isolated rat hearts, decreases cardiac infarct size, and improves postischemic heart function. The effects seem to depend on the time course after hyperoxia treatment.** ***J Cardiovasc Med*** **10:635–642 © 2009 Italian Federation of Cardiology.**

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Introduction

Arrhythmias are one of the important problems in coronary ischemia/reperfusion therapy [1] and constitute a major risk for sudden cardiac death after coronary artery occlusion [2]. Therapeutic strategies currently used for primary prevention of ventricular fibrillation, ventricular tachycardia, or cardiac arrest remain controversial as few trials have shown a survival benefit [3]. Recent therapeutic methods, such as thrombolysis, coronary bypass graft surgery, and angioplasty have reduced mortality of ischemic heart diseases (IHDs), but many patients may not be suitable candidates for these methods [4]. In addition, sudden cardiac death caused by ischemia or reperfusion-induced arrhythmias is a warning to the development of new antiarrhythmic agents [3]. Finding new strategies for reducing the degree of primary injury and/or the rate of dysrhythmias resulting from an ischemic heart insult could have important clinical consequences and could reduce the overall mortality and morbidity of IHDs.

Brief periods of ischemia and reperfusion before a lethal ischemic insult, known as ischemic preconditioning (IPC), have been shown to protect the heart against ischemia/reperfusion (I/R) injury [5]. However, the application of IPC requires a physical cut of the blood supply which can be difficult or impractical in many clinical situations. To circumvent these potential problems, investigations have been directed at finding new therapeutic approaches that mimic intracellular signaling of IPC and induce heart protection.

Hyperoxia is one of the new practical methods for induction of ischemic tolerance in tissues. In-vivo pre-exposure to hyperoxia protects the heart against subsequent I/R injury, as evidenced by attenuated contractile dysfunction

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and myocardial necrosis. These aspects of hyperoxia-induced preconditioning have been confirmed by recent studies [6,7]. The mechanism of cardioprotection by hyperoxia seems to be related to the induction of a low-graded systemic oxidative stress by generation of reactive oxygen species [7,8]. Protection against arrhythmias has been proposed as an additional protective aspect of hyperoxia. Nevertheless, the role of hyperoxia in protection against ischemia and/or reperfusion-induced arrhythmias has not been very well clarified yet. Tahepold *et al.* [9] reported that pretreatment with hyperoxia reduces irreversible reperfusion arrhythmias in a global model of ischemia in rat hearts. In another study, combined pretreatment of hyperoxia and dexamethasone and hyperoxia alone decreased the incidence of reperfusion arrhythmias [10]. In both studies the beneficial effects of hyperoxia have been examined in the reperfusion; however, neither of them assessed the effect of hyperoxia during the ischemic period. By using an isolated rat heart model, we examined the incidence and severity of ventricular arrhythmias during ischemia and reperfusion.

Therefore, the aim of the present study was to test the hypothesis that pre-exposure to normobaric hyperoxia attenuates ischemia and/or reperfusion-induced arrhythmias in a regional model of heart ischemia both in the first and second window of protection. We assessed the incidence and severity of ventricular arrhythmias, including total premature ventricular beats (PVBs), number of ventricular tachycardia and ventricular fibrillation episodes, as well as their duration during ischemia and reperfusion.

Methods

Animals

Male Wistar rats fed a standard diet and tap water *ad libitum* and housed at 12 : 12-h light–dark cycle in a stress-free environment. The experimental protocol used in this study was approved by the Tarbiat Modares University Ethics Committee for Animal Research.

Surgical procedure of ischemia/reperfusion injury

Rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and heparinized (300–400 IU, i.p.). After a bilateral thoracotomy, the heart was dissected from surrounding structures and placed in ice-cold Krebs–Henseleit buffer. Then, hearts were immediately cannulated via the aorta and mounted in a Langendorff perfusion system and perfused retrogradely with gassed (5% CO_2, 95% O_2) Krebs–Henseleit solution [11] (NaCl 118.5 mmol/l, $NaHCO_3$ 25.0 mmol/l, KCl 4.7 mmol/l, KH_2PO_4 1.2 mmol/l, $MgSO_4{\cdot}7H_2O$ 1.2 mmol/l, glucose·H_2O 11.1 mmol/l, $CaCl_2{\cdot}2H_2O$ 1.8 mmol/l) at 37°C and a constant pressure of 80 cmH_2O. A 5-0 silk suture was loosely placed under the left anterior descending coronary artery (LAD) 2–3 mm from its origin by inserting the needle into the left ventricular wall. The two ends of the suture were threaded through a 10 mm segment of tubing, forming a snare. Tightening and loosening this snare allowed coronary occlusion and reperfusion, respectively.

An electrocardiogram (ECG) was continuously registered by means of two stainless-steel electrodes – one attached to the apex of the heart and the other placed on the right atrium – and a metal clip was attached to the aortic cannula as the reference electrode. Heart rate was calculated from the ECG. ECG and ventricular pressure were recorded continuously with a computer using a PowerLab analog to digital converter (AD Instruments, Australia). Left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures were obtained by a latex water-filled balloon inserted into the left ventricle via the left atrium and connected to a pressure transducer (MLT 844). The volume of the balloon was adjusted to obtain end-diastolic pressure of 5–7 mmHg and was unchanged for the remainder of the experiment. Left ventricular developed pressure (LVDP) was calculated as 'LVSP–LVEDP'. Rate pressure product (RPP) as an index of cardiac function was calculated by multiplying LVDP by heart rate (HR). Coronary flow was measured by timed collections of the coronary effluent.

Experimental protocol

Animals in hyperoxia groups were kept in a hyperoxic chamber (with oxygen content of $\geq$95%) for 60 or 180 min (hyperoxia groups), whereas control animals were kept in the same chamber breathing normal atmospheric air (normoxic control group). Percentage of oxygen in inspired air was continuously monitored with an Oxygen meter (Lutron–DO 5510, Taiwan). Oxygen was continuously delivered into the chamber through a tube. For assessment of the first window of protection, the hearts were excised immediately after hyperoxia, and for evaluation of the second window of protection the hearts were isolated 24 h later for Langendorff perfusion. After a stabilization time of 20 min, the hearts were exposed to 30 min of regional ischemia, followed by 120 min of reperfusion in all experiments. Respective to the treatment prior to heart isolation, the following groups were investigated: I, normoxic control, breathing normal air (NC); II, 60 min hyperoxia immediately before heart isolation (H60); III, 180 min hyperoxia immediately before heart isolation (H180); IV, 60 min hyperoxia 24 h before heart isolation (H60/24); V, 180 min hyperoxia 24 h before heart isolation (H180/24).

Determination of infarct size

At the end of 120 min reperfusion, the coronary artery was re-occluded, and the risk zone was delineated by perfusing 1 ml of 2% Evans blue solution into the aortic cannula. After freezing at −20°C, hearts were cut into five or six transverse slices of 2 mm thickness from apex to the base and the slices were incubated in 1% triphenyl tetrazolium

chloride (TTC, Sigma) at 37°C for 20 min. Viable myocardium is stained in red by TTC, whereas infarcted tissue remains unstained. The slices were then photographed by a digital camera (Olympus, FE–160). Area at risk (AAR) and infarct size were determined by computerized planimetry using an image analysis software (Image Tool; available at http://ddsdx.uthscsa.edu/dig/itdesc.htm/). The infarcted area was calculated as percentage of AAR.

Quantification of arrhythmias

Arrhythmias were defined according to the Lambeth Conventions [12]. Accordingly, arrhythmias were categorized as PVBs (defined as discrete premature ventricular depolarization), ventricular tachycardia (a run of four or more consecutive ventricular premature beats), and ventricular fibrillation (a signal in which individual QRS wave deflections could not easily be distinguished from each other). More complex forms (e.g. bigeminy and salvos) were included in the count of ventricular premature beats and were not analyzed separately.

Electrocardiograms were analyzed for the total number of PVBs and the incidence and duration of ventricular tachycardia and ventricular fibrillation during 30 min of ischemia and 120 min of reperfusion. In addition, severity of arrhythmias was quantified by a scoring system. Each individual heart was evaluated by means of a five-point arrhythmia score, in which single PVBs were given a score of 1, bigeminy/salvos a score of 2, ventricular tachycardia a score of 3, ventricular fibrillation a score of 4, sVF (ventricular fibrillation lasting more than 2 min) a score of 5, and an assigned number corresponded to the most severe type of arrhythmia observed in that heart. Scores were used for group analysis of severity of arrhythmias.

Statistics

Data were expressed as means ± SEM. The incidence of arrhythmias was presented as percentage of occurrence. Comparison of differences in the recovery of post-ischemic functional parameters, infarct size, and number of arrhythmias were tested by one-way ANOVA with Duncan's post-hoc analysis. Fisher's Exact test was used for comparison of the incidences of ventricular tachycardia and ventricular fibrillation. Differences in the arrhythmias score, ventricular tachycardia and ventricular fibrillation duration between the groups were compared by the Mann–Whitney *U*-test. *P*-value of less than 0.05 was considered statistically significant.

Results

Cardiac function parameters

Table 1 summarizes HR, LVDP, RPP, and coronary flow in all groups determined at 20 min of stabilization period; at 30 min after coronary artery occlusion; and at 30, 60, 90, and 120 min of reperfusion. As shown, the baseline functional parameters were not significantly different between the normoxic control and hyperoxia groups.

Table 1 Cardiac function data in isolated rat hearts subjected to 30 min of ischemia and 120 min reperfusion

			Reperfusion			
Parameters/groups	Stabilization (20 min)	Ischemia (30 min)	30 min	60 min	90 min	120 min
HR (beats/min)						
NC	301 ± 8	261 ± 10	263 ± 15	262 ± 17	263 ± 22	241 ± 19
H60	299 ± 11	279 ± 20	322 ± 15*	310 ± 15*	288 ± 14	274 ± 14
H180	333 ± 18	302 ± 23	299 ± 16	300 ± 16	288 ± 17	278 ± 18
H60/24	330 ± 9.0	327 ± 18*	307 ± 4.0	300 ± 6.0	291 ± 5.0	286 ± 14
H180/24	322 ± 22	288 ± 19	314 ± 28*	280 ± 24	256 ± 29	250 ± 28
LVDP (mmHg)						
NC	76.9 ± 3.3	58.5 ± 3.0	74.0 ± 2.8	70.4 ± 2.0	65.9 ± 1.8	61.4 ± 1.4
H60	80.7 ± 3.9	60.2 ± 2.0	72.7 ± 1.0	69.5 ± 1.2	67.1 ± 1.3	66.6 ± 0.8
H180	75.8 ± 3.4	58.6 ± 0.9	77.8 ± 1.2	72.5 ± 1.7	71.0 ± 1.7	68.1 ± 1.0*
H60/24	72.3 ± 1.8	56.6 ± 1.9	73.8 ± 1.9	70.6 ± 2.2	67.5 ± 2.8	66.9 ± 3.4
H180/24	77.3 ± 3.7	59.2 ± 2.3	73.3 ± 2.7	71.8 ± 3.3	69.4 ± 3.4	65.4 ± 2.8
RPP (mmHg beats/min)						
NC	23126 ± 988	14409 ± 713	19149 ± 1283	18383 ± 1527	17132 ± 1289	14752 ± 1134
H60	24032 ± 1259	16685 ± 1122	23382 ± 1051*	21518 ± 979	19350 ± 962	18270 ± 918*
H180	25101 ± 1422	17764 ± 1486	23267 ± 1470*	21864 ± 1463*	20653 ± 619	19000 ± 1314*
H60/24	23245 ± 979	18546 ± 1244	22691 ± 568	21216 ± 338	19621 ± 833	18988 ± 689*
H180/24	24862 ± 1954	16909 ± 948	22678 ± 1351	19892 ± 1266	17498 ± 1505	16130 ± 1447
Coronary flow (ml/min)						
NC	10.4 ± 0.9	4.7 ± 0.7	7.8 ± 1.2	6.6 ± 0.8	5.1 ± 0.5	4.6 ± 0.5
H60	10.1 ± 0.5	5.5 ± 0.9	9.4 ± 0.9*	7.6 ± 0.4	6.3 ± 0.8*	5.5 ± 0.8*
H180	10.3 ± 1.1	5.0 ± 1.0	10.9 ± 0.6*	8.9 ± 0.5*	7.7 ± 0.4*	6.6 ± 0.7*
H60/24	10.9 ± 0.5	4.5 ± 0.8	9.1 ± 0.7	7.5 ± 0.4	6.2 ± 1.0*	5.2 ± 0.7
H180/24	9.8 ± 0.8	4.6 ± 0.4	8.7 ± 0.5	7.6 ± 0.7	6.4 ± 0.7*	5.6 ± 0.6*

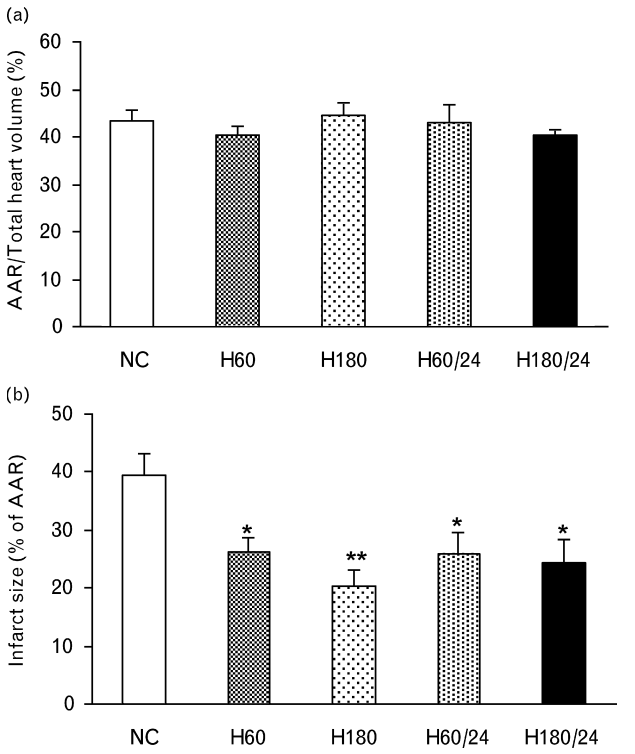
Functional heart parameter recovery was analyzed during reperfusion. Data presented as mean ± SEM. Groups: NC, normoxic control; H60, 60 min hyperoxia immediately before heart isolation; H180, 180 min hyperoxia immediately before heart isolation; H60/24, 60 min hyperoxia 24 h before heart isolation; H180/24, 180 min hyperoxia 24 h before heart isolation. HR, heart rate; LVDP, left ventricular developed pressure; RPP, rate pressure product. * $P < 0.05$ compared to normoxic control group at each time point.

RPP as an index of cardiac function was increased in H60 and H180 groups compared to normoxic control group at different time points during reperfusion ($P < 0.05$). Coronary flow rate was also significantly greater in H60 and H180 groups vs. normoxic control group ($P < 0.05$). Normalizing the data as mean percentage of decrease in cardiac function parameters during reperfusion in comparison to baseline shows that coronary flow was reduced 36% in the normoxic control group with respect to its baseline, which was significantly different from H60 (19%, $P < 0.05$) and H180 (9%, $P < 0.001$) groups. There was also a significant difference between the H180 group and other hyperoxia-treated groups ($P < 0.05$). This change for LVDP and RPP in reperfusion compared to baseline was not significant.

Area at risk and infarct size

Area at risk did not differ significantly among the groups (Fig. 1a). In the normoxic control group $39.5 \pm 3.6\%$ of AAR was necrotic at the end of 2 h reperfusion. Pretreatment with hyperoxia for 60 (H60) and 180 (H180) minutes immediately before heart isolation reduced infarct size to 26.3 ± 2.3 ($P < 0.05$) and $20.4 \pm 2.6\%$ ($P < 0.01$), respectively. Hyperoxia for 60 (H60/24) and 180 (H180/24) minutes, 24 h before heart isolation also significantly reduced infarct size to 25.8 ± 3.8 and $24.3 \pm 3.9\%$, respectively ($P < 0.05$) (Fig. 1b).

Fig. 1



Graphs represent percentages of area at risk by the total heart area (a), and percentages of infarct size (IF) by the area at risk (b). Area at risk (AAR) in all groups is identical. NC: normoxic control group ($n = 10$); H60: 60 min hyperoxia (>95% O_2) pretreatment immediately before heart isolation ($n = 7$); H180: 180 min hyperoxia pretreatment immediately before heart isolation ($n = 8$); H60/24: 60 min hyperoxia pretreatment 24 h before heart isolation ($n = 6$); H180/24: 180 min hyperoxia pretreatment 24 h before heart isolation ($n = 6$). Data are presented as mean ± SEM. (**) $P < 0.01$ compared to normoxic control and (*) $P < 0.05$ compared to NC.

Arrhythmia studies during ischemia

Table 2 summarizes the effects of hyperoxic preconditioning on the severity of arrhythmias during 30 min of ischemia. Episodes of PVBs occurred in all groups. The total number of PVBs in the normoxic control group was 344 ± 54. Hyperoxia reduced the total number of PVBs in H60 and H180 groups (192 ± 30 and 219 ± 21, respectively, $P < 0.05$) compared to the normoxic control group. The reduction was also significant in the H180/24 group ($P < 0.01$). Duration of ventricular tachycardia and ventricular fibrillation was assessed during 30 min of ischemia. The mean duration of ventricular tachycardia episodes in the normoxic control group was 45.18 ± 7.5 s. Sixty minutes' hyperoxia immediately before heart isolation significantly decreased ventricular tachycardia duration (17.9 ± 4 s, $P < 0.05$). The reduction of ventricular fibrillation duration by hyperoxia was not statistically significant during ischemia. In addition, number of ventricular tachycardia episodes was also affected by hyperoxia pretreatment, so that there was a significant decrease in number of ventricular tachycardia episodes in H60, H180, and H180/24 groups compared to the normoxic control group. Arrhythmia score was assigned on the basis of the most severe arrhythmia detected in each experiment.

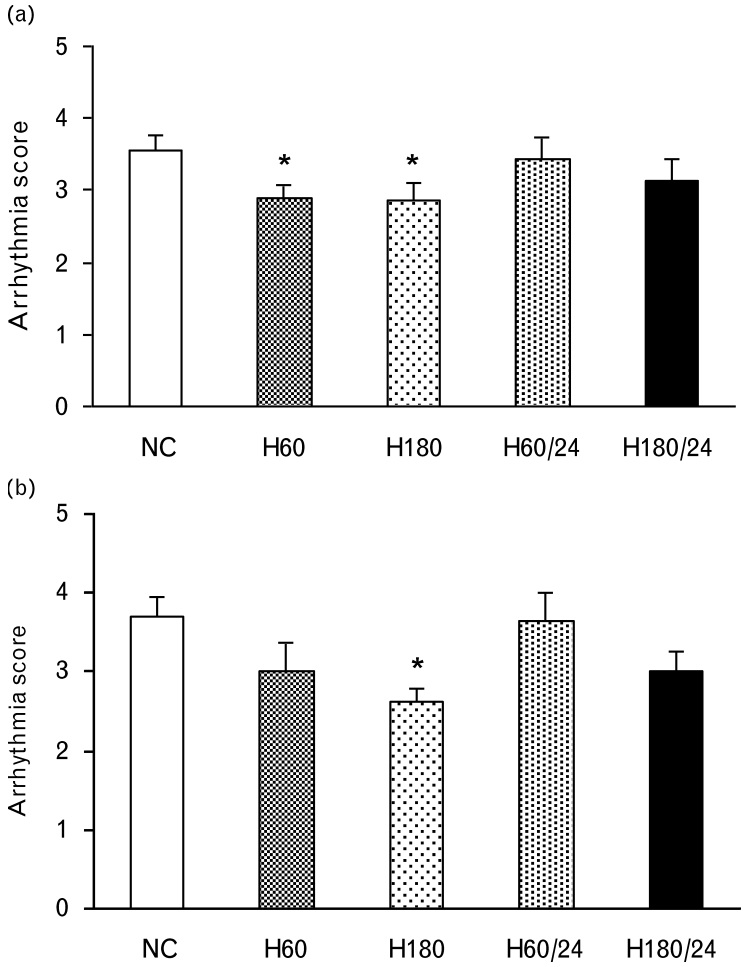
Arrhythmia score in the normoxic control group was 3.54 ± 0.2 which was significantly attenuated to 2.9 ± 0.18 by 60 and 2.87 ± 0.22 by 180 min of hyperoxia

Table 2 Effects of hyperoxic preconditioning on the number of premature ventricular beats, ventricular tachycardia and ventricular fibrillation duration, and their episode number during 30 min of ischemia

Groups	*n*	PVBs	Ventricular tachycardia duration (s)	Ventricular fibrillation duration (s)	Ventricular tachycardia episodes	Ventricular fibrillation episodes
NC	11	344 ± 54	45.18 ± 7.5	18.8 ± 12.5	37.3 ± 4.7	0.81 ± 0.32
H60	10	$192 \pm 30^{*}$	$17.9 \pm 4^{*}$	4.5 ± 4.5	$13.2 \pm 3^{**}$	0.20 ± 0.20
H180	8	$219 \pm 21^{*}$	23.8 ± 6.3	0.9 ± 0.9	$20 \pm 5.8^{*}$	0.12 ± 0.12
H60/24	7	329 ± 28	39.7 ± 5.7	19.5 ± 20.2	34.3 ± 6	0.71 ± 0.47
H180/24	8	$180 \pm 23^{**}$	29.4 ± 7.7	16 ± 16	$18.9 \pm 4.5^{*}$	0.12 ± 0.12

PVBs, premature ventricular beats. Data are mean ± SEM. $^{*}P < 0.05$ compared with normoxic control. $^{**}P < 0.01$ compared with normoxic control.

Fig. 2



Arrhythmias severity evaluated by means of arrhythmia score. (a) arrhythmia score in ischemia. (b) Arrhythmia score in reperfusion. Data are mean ± SEM. (*) $P < 0.05$ compared to normoxic control group (NC).

immediately before heart isolation ($P < 0.05$) (Fig. 2a). The decrease in ventricular tachycardia and ventricular fibrillation incidence was not significant during ischemia (see Table 4).

Arrhythmia studies during reperfusion

Incidence and severity of arrhythmias were also analyzed during 120 min of reperfusion. The total number of PVBs in normoxic control group was 701 ± 82. Pretreatment with hyperoxia reduced the total number of PVBs in H60 and H180 groups to 266 ± 60 and 225 ± 36, respectively. The ventricular tachycardia duration for normoxic control group was 17.7 ± 3.3 s. This time markedly decreased in H60 and H180 groups: 1.5 ± 0.7 s ($P < 0.01$) and 7.5 ± 2.5 s ($P < 0.05$), respectively. Duration of ventricular fibrillation was also decreased in the H180 group compared to normoxic control (0 vs. 50 ± 31 s, $P < 0.05$). Duration of ventricular tachycardia and ventricular fibrillation was not significantly reduced by hyperoxia 24 h before heart isolation (Table 3).

The incidence of ventricular tachycardia did not differ significantly among the normoxic control and the hyperoxia pretreatment groups. There were no significant differences in the incidence of ventricular fibrillation between H60, H60/24, and H180/24 with normoxic control group. On the contrary, pretreatment with 180 min of hyperoxia immediately before heart isolation (H180) completely abolished the incidence of ventricular fibrillation (0 vs. 50% of normoxic control, $P < 0.05$) (Table 4). Severity of arrhythmias that was evaluated by means of arrhythmia score was also affected by hyperoxia. Arrhythmia score in the normoxic control group was 3.7 ± 0.26, which was significantly attenuated to 2.6 ± 0.18 by 180 min hyperoxia immediately before heart isolation ($P < 0.05$) (Fig. 2b).

Discussion

The main findings of the present study show that preexposure to normobaric hyperoxia (≥95% O_2) has an antiarrhythmic effect against ischemia and reperfusion-

Table 3 Effects of hyperoxic preconditioning on the number of PVBs, ventricular tachycardia and ventricular fibrillation duration, and their episode number during 120 min of reperfusion

Groups	*n*	PVBs	Ventricular tachycardia duration (s)	Ventricular fibrillation duration (s)	Ventricular tachycardia episodes	Ventricular fibrillation episodes
NC	10	701 ± 82	17.7 ± 3.3	50 ± 30.6	8.9 ± 1.9	0.9 ± 0.4
H60	7	266 ± 60***	1.5 ± 0.7**	26.5 ± 26.5	2.3 ± 1*	0.14 ± 0.14
H180	8	225 ± 36***	7.5 ± 2.5*	0*	5.1 ± 1.9	0
H60/24	6	490 ± 97	9.9 ± 2.5	58.4 ± 35.3	5 ± 1.1	1 ± 0.51
H180/24	6	365 ± 94**	15.2 ± 3.6	2.8 ± 2.8	5.8 ± 1.7	1 ± 1

PVBs, premature ventricular beats. Data are mean ± SEM. * $P < 0.05$ compared with normoxic control. ** $P < 0.01$ compared with normoxic control. *** $P < 0.001$ compared with normoxic control.

induced arrhythmias and protects hearts from I/R injury. The effects were more obvious for hyperoxia exposure immediately before heart isolation. Our work may have clinical relevance because 50% of sudden death in humans is due to ventricular tachycardia or ventricular fibrillation and the remainder to bradyarrhythmias or electromechanical dissociation [13].

There are only limited numbers of studies showing antiarrhythmia effect of hyperoxia [9,10]. In these studies the beneficial effects of hyperoxia were reported in the reperfusion period; however, none of them reported the effect of hyperoxia during the ischemic period. In our previous study we showed that pre-exposure to 60, 120, and 180 min of normobaric hyperoxia (>95%) 24 h before ischemia significantly decreases incidence and severity of ischemic arrhythmias in anesthetized rat hearts [14].

Here we observed that hyperoxia lowered ventricular fibrillation incidence during the reperfusion phase in a regional model of rat heart ischemia. This result is in line with the results presented by Tahepold *et al.* [9]. Moreover our results show that hyperoxia dramatically lowered the number of PVBs, ventricular tachycardia and ventricular fibrillation duration, and the number of ventricular tachycardia and ventricular fibrillation episodes during reperfusion. We also provided the first evidence that, in addition to reducing the incidence of reperfusion arrhythmias, hyperoxia also decreases the severity of arrhythmias during ischemia. To our knowledge, this is the first experimental evidence showing that hyperoxia reduces the severity of arrhythmias during the ischemic phase. Hyperoxia for 60 and 180 min immediately before heart isolation decreased infarct size, improved coronary flow, and increased recovery of heart function. In addition, some of the protective effects of hyperoxia persisted 24 h after treatment, showing a second window of protection. The reduction of infarct size in our study was in concordance with the reduction of LDH and creatine kinase release into the coronary effluent (data are not shown). The most powerful cardioprotective effects of hyperoxia were seen in 180 min pretreatment with hyperoxia immediately before heart isolation.

One of the major determinants of arrhythmogenesis is the size of ischemic zone [15]; however, this factor was disregarded since there were no differences in the size of AAR between the groups. On the contrary, changes in infarct size correlate well with antiarrhythmic effects of hyperoxia. Therefore, it appears that the extent of myocardial infarct size may directly affect arrhythmogenesis.

Despite its cardioprotective properties, the actual mechanisms of hyperoxia antiarrhythmia effects have not been elucidated. Several studies indicate that reactive oxygen species (ROS) participate in the signaling pathway involved in preconditioning triggering [16–18]. On the contrary, antioxidant administration abolished the cardioprotective effects of IPC [19]. The mechanisms by which ROS are responsible for this cardioprotective effect are not well recognized; however, it seems that ROS both indirectly, through the activation of protein kinases, and directly lead to the activation of some transcription factors such as nuclear factor kappa B (NF-κB), which in turn trigger genes related to tissue protection, including Bcl-2 [7,20]. ROS also could activate protein kinase C (PKC) which in turn prevents the opening of mitochondrial permeability transition pores (MPTP). Breathing a hyperoxic gas mixture leads to an elevated level of ROS generation due to higher O_2

Table 4 Incidence of ventricular tachycardia and ventricular fibrillation

	Ischemia		Reperfusion	
Groups	Ventricular tachycardia (%)	Ventricular fibrillation (%)	Ventricular tachycardia (%)	Ventricular fibrillation (%)
NC	100	45.4	100	50
H60	80	10	71.4	14.28
H180	75	12.5	62.5	0*
H60/24	100	28.5	83.3	50
H180/24	87.5	12.5	83.3	16.7

* $P < 0.05$ compared with normoxic control.

tension in inspired air than normal values in the body [21,22]. Therefore, observed cardioprotective effects of hyperoxia may be mediated via ROS generation.

In addition to ROS, hyperoxia also induces its cardioprotective effects through the generation of nitric oxide. In a study of the response to hyperoxia, 1 h of exposure to hyperbaric hyperoxia (2 atm, 100%) increased nitric oxide synthase 3 (NOS_3) expression that resulted in elevated nitric oxide levels in the rat heart [23]. Valen *et al.* [24] found that mice deficient for the inducible nitric oxide synthase gene could not be protected by classic ischemic or hyperoxic preconditioning. In another study Nω-nitro-1-arginine methyl ester (L-NAME) administration prior to hyperoxia pretreatment blocked the cardioprotective effects of hyperoxia [25]. Taken together, these results suggest that nitric oxide is an important mediator of hyperoxic preconditioning. Moreover, studies show that nitric oxide itself is responsible for induction of both the first and second window of protection by IPC [26]. Thus it could be speculated that observed immediate and 24 h later cardioprotective effects of hyperoxia are mediated by nitric oxide.

One of the proposed mechanisms of nitric oxide action is through a nitric oxide–cGMP–protein kinase G (PKG) signaling pathway, and mitochondrial K^+-ATP channels and PKC as the downstream targets [27,28]. The activation of these channels may improve the recovery of regional contractility of myocardium by shortening the duration of action potentials and by attenuating membrane depolarization, both of which would decrease myocardial contractility and reduce energy expenditure during ischemia [26]. Also, opening of mitochondrial K^+-ATP channels may lead to ROS generation, which in turn would be responsible for the activation of PKC [29]. The role of mitochondrial K^+-ATP channels as one the end effectors of hyperoxic preconditioning has been also established [22]. Thus according to these results, and evidence for the proved antiarrhythmic role of mitochondrial K^+-ATP channels in IPC [30], it can be suggested that the antiarrhythmia effect of hyperoxia mediates through the pathway of nitric oxide–cGMP–PKG–mitochondrial K^+-ATP channels.

Another possible mechanism of nitric oxide action is through the combination of nitric oxide with superoxide to from peroxynitrite which is responsible for the induction of the second window of protection by up-regulation of PKC, which in turn up-regulates inducible nitric oxide synthase (iNOS) by activation of the transcription factor NF-κB. Experimental results also support a cardioprotective and antiarrhythmic role for peroxynitrite [31]. Peroxynitrite has been shown to contribute to the cardioprotective effects of IPC in rats [31]. Thus it is conceivable that hyperoxia may induce its cardioprotective effects through release of nitric oxide, with subsequent accumulation of peroxynitrite produced through interaction with superoxide. This suggestion is supported by a recent review in which part of the effects of hyperoxia were attributed to the formation of peroxynitrite from nitric oxide and superoxide anion following hyperoxic gas exposure [32].

Although the results of the present study and others [9,10,14] clearly show a beneficial effect of hyperoxia pre-exposure against ischemia and/or reperfusion-induced arrhythmias in rat hearts, there are also contradictory results, indicating an arrhythmogenic effect of severe hyperoxia. Wittnich *et al.* [33] showed that exposing neonatal pig hearts to severe hyperoxia (500 mmHg, 5 h) increases the incidence of ventricular fibrillation during global ischemia. In another study Pifarre *et al.* [34] reported a ventricular fibrillation incidence increase following regional ischemia in dog hearts. At present we have no explanation for this discrepancy, but it may be due to a species difference.

Usage of hyperoxia in a clinical human setting would be very attractive. It has been shown that hyperoxygenation therapy is well tolerated by humans if administrated according to the standard protocols. In this regard, administration of 80% oxygen for less than 24 h is considered well tolerated [35]. Because in rats the first histological findings of oxygen toxicity will appear in the lung tissue only after about 40 h of pure oxygen administration [36], it seems that there is a wide safety window between protective effects of oxygen pretreatment and its toxic effects. However, this is the case in healthy lungs, and in lungs that are already diseased, or in lungs that are exposed to multiple risk factors and/or for acute lung injury (as seen in coronary artery bypass grafting using extracorporeal circulation); a much shorter exposure to hyperoxia has the potential to cause lung injury.

The most important advantage of short-term normobaric hyperoxia pretreatment as a preconditioning model compared to other methods is its simplicity and easy clinical applicability. In addition, increasing O_2 tension in blood affects other organs in the body and may even induce a whole-body preconditioning-like effect which could be very useful in critical surgery. Although perioperative oxygen administration has known advantages, such as decreasing wound infection or nausea and vomiting [35], there may still be a long way to go before approving the use of oxygen with this new potential indication (PC induction) before surgery.

On the basis of the results obtained in present study it could be concluded that pretreatment with normobaric hyperoxia alleviates ischemia and reperfusion arrhythmias in isolated rat heart and that this protective effect is more obvious immediately after hyperoxia pretreatment. Additionally hyperoxia improves cardiac function

parameters and reduces infarct size. Irrespective of the precise mechanisms of these beneficial effects, hyperoxia could be a potentially useful preconditioning method in clinical situations.

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