Original Research

Normobaric, intermittent hypoxia conditioning is cardio- and vasoprotective in rats

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Abstract

Favorable versus detrimental cardiovascular responses to intermittent hypoxia conditioning (IHC) are heavily dependent on experimental or pathological conditions, including the duration, frequency and intensity of the hypoxia exposures. Recently, we demonstrated that a program of moderate, normobaric IHC (FIO_2 9.5–10% for 5–10 min/cycle, with intervening 4 min normoxia, 5–8 cycles/day for 20 days) in dogs afforded robust cardioprotection against infarction and arrhythmias induced by coronary artery occlusion–reperfusion, but this protection has not been verified in other species. Accordingly, in this investigation cardio- as well as vasoprotection were examined in male Wistar rats completing the normobaric IHC program or a sham program in which the rats continuously breathed atmospheric air. Myocardial ischemia and reperfusion (IR) was imposed by occlusion and reperfusion of the left anterior descending coronary artery in *in situ* experiments and by subjecting isolated, perfused hearts to global ischemia–reperfusion. Cardiac arrhythmias and myocardial infarct size were quantified in *in situ* experiments. Endothelial function was evaluated from the relaxation to acetylcholine of norepinephrine-precontracted aortic rings taken from *in situ* IR experiments, and from the increase in coronary flow produced by acetylcholine in isolated hearts. IHC sharply reduced cardiac arrhythmias during ischemia and decreased infarct size by 43% following IR. Endothelial dysfunction in aorta was marked after IR in sham rats, but not significant in IHC rats. Similar findings were found for the coronary circulations of isolated hearts. These findings support the hypothesis that moderate, normobaric IHC is cardio- and vasoprotective in a rat model of IR.

Keywords: Acetylcholine, aorta, coronary circulation, myocardial infarction, myocardial ischemia and reperfusion, nitric oxide

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Introduction

Cardiovascular responses to intermittent hypoxia (IH) are controversial. Severe, repetitive IH during sleep apnea is associated with systemic hypertension and increased risk of coronary heart disease.[1] In rats, brief, repetitive bouts of hypoxia and reoxygenation of sleep apnea-like duration, frequency and intensity impaired endothelium-dependent vasodilation[2–5] and intensified endothelin-induced vasoconstriction[2,5] of conduit arteries, and increased myocardial susceptibility to infarction inflicted by coronary artery occlusion–reperfusion.[6] However, there is mounting evidence that some programs of intermittent hypoxia conditioning (IHC) are cardioprotective,[7–21] anti-hypertensive,[22–28] neuroprotective,[29–34] and vasoprotective.[23,28,29] It is evident that cardiovascular responses to IH depend on the hypoxic regimen, specifically the duration, intensity, and frequency of the hypoxia exposures and also on the total duration of the hypoxia program.[16,26,35,36]

Hypobaric IHC produced in an altitude chamber has proven efficacious for treating patients with idiopathic and ischemic arrhythmias, hypertension, and as a part of rehabilitation therapy after coronary bypass.[37,38] Normobaric IHC, which is simpler and less costly than hypobaric IHC, decreased the frequency of anginal attacks, lessened cardiac arrhythmias, and increased aerobic capacity and exercise tolerance in patients with ischemic heart disease, prior myocardial infarction, or chronic obstructive pulmonary disease.[23,37–44] In addition, normobaric IHC has successfully reduced blood pressure of hypertensive patients.[23,24,27,45]

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Interestingly, experimental studies to support clinical applications of IHC are scarce, and their interpretation is often clouded by widely varying protocols. In 1973, well before the phenomenon of ischemic preconditioning was discovered,[46] Meerson *et al*. reported that ischemia–reperfusion (IR) arrhythmias and infarct size were markedly reduced in rats exposed to simulated high altitude for 5 h/day, five days per week.[7] These observations were confirmed by Meerson's laboratory[8] and by others.[15,18,19,21] In addition, hypobaric IHC has been shown to blunt development of hypertension in young, stroke-prone spontaneously hypertensive rats.[22,47]

Due to the complexity and expense of hypobaric chambers, interest in potential beneficial effects of normobaric IHC has increased for both clinical and experimental applications. We found that 20 days of normobaric IHC profoundly reduced myocardial infarction and cardiac arrhythmias during IR in dogs.[10,12] To date, this finding has not been confirmed in other species.

In addition to injuring cardiomyocytes, myocardial IR also damages the coronary vasculature. This injury manifests itself as coronary endothelial dysfunction caused by morphological and functional alterations in endothelial cells,[48] impaired activity of endothelial nitric oxide (NO) synthase (eNOS),[49] and reduced NO bioavailability due to intensive NO oxidation by reactive oxygen species.[50] In our experiments, adaptation to normobaric[28] and hypobaric[47] hypoxia prevented endothelial dysfunction of the aorta in spontaneously hypertensive rats. Adaptation to hypobaric hypoxia also prevented endothelial dysfunction of cerebral vessels in experimental models of Alzheimer's disease.[29] However, the possibility that IHC would lessen coronary endothelial dysfunction during myocardial IR has not been studied.

Recent reports have demonstrated that myocardial IR injury can impair remote, non-coronary blood vessels.[51] Such remote vascular injury may play an important role in complications that accompany myocardial IR. The potential protective effect of normobaric IHC on the endothelium of non-coronary blood vessels is presently unknown and merits study.

Cardiovascular function in rats, among the most extensively studied laboratory animal species, differs appreciably from dogs: rats have substantially higher heart rates and arterial pressures than dogs, so myocardial ATP demand and O_2 requirements are higher in rats; the force–frequency 'staircase' relationship is positive in dogs but negative in rats;[52] and rat myocardium has a sparse collateral circulation,[53,54] while in dogs the collateral circulation is variable and often robust.[12,55] Each of these factors impacts the severity of ischemic injury, so IHC cardioprotection in dogs cannot be extrapolated directly to rats. Accordingly, this study tested the hypothesis that the cardioprotective effects of normobaric IHC previously demonstrated clinically and experimentally in dogs[10,12,27,28,30,39,40] can also be produced in rats. We employed a clinically and experimentally utilized normobaric IHC program in a rat model of myocardial IR.

Materials and methods

Animals

Male Wistar rats weighing 250–280 g at the beginning of the study were maintained at controlled temperature (22–25°C) and humidity (55%). A 12:12 h light–dark cycle was maintained with lights on between 7:00 and 19:00. All animal procedures were performed in accordance with the U.S. National Research Council *Guide for the Care and Use of Laboratory Animals* (publication 85-23, revised 2011) and experimental protocols were approved by the Animal Care and Use Committee of the Institute of General Pathology and Pathophysiology.

Adaptation to hypoxia

Rats were subjected to the previously described[10,28] normobaric IHC program (5–8 cycles/day for 20 days, FIO_2 9.5–10% for 5–10 min/cycle, with intervening 4 min normoxia) summarized in Table 1. For sham IHC, the same 20-day IHC protocol was followed, except that the sham rats breathed atmospheric air (FIO_2 21%) instead of a hypoxic gas mixture.

Effect of IHC on cardiac ventricular weight

Body weights of 15 sham rats and 18 IHC rats were measured after completing the respective sham and IHC programs. The rats were then decapitated, and the weights of the left ventricle plus septum (LV) and the right ventricular free wall (RV) were measured. Ratios of RV/body weight and RV/(LV + RV) were computed. Tissues from these rats were used in experiments not reported here.

Myocardial arrhythmias and injury studied *in situ*

One day after completing the IHC conditioning program, sham ($n = 15$) and IHC rats ($n = 14$) were anesthetized with urethane (150 mg/kg, ip), intubated, placed on mechanical respiration (60 min^{-1} with room air), and then the chest was

Table 1 Intermittent hypoxia conditioning program

Session	FIO_2 (%)	Hypoxia (min)	Normoxia (min)	Cycles	Total hypoxia (min)
1	10	5	4	5	25
2	10	5	4	6	30
3	10	5	4	7	35
4	10	5	4	8	40
5	10	5	4	8	40
6	9.5	6	4	7	42
7	9.5	6	4	8	48
8	9.5	6	4	8	48
9	9.5	7	4	7	49
10	9.5	8	4	7	56
11–20	9.5	10	4	7	70

Note: This table is reproduced from Zong *et al.*[10] with the kind permission of the Society for Experimental Biology and Medicine.
FIO_2: fractional inspired O_2.

opened. After a 30-min stabilization period, the left anterior descending coronary artery was ligated for 30 min followed by a 60-min reperfusion. Myocardial ischemia during LCA ligation was confirmed by regional cyanosis and progressive, marked ST segment elevation. Reperfusion was confirmed by an epicardial hyperemic response. Electrocardiogram lead I was recorded on a Polygraph RM-6000 (Nihon Kohden, Japan) and examined for arrhythmias and ST segment changes.

In a subset of these experiments, myocardial infarct size (IS) was evaluated with the 2,3,5-triphenyl-tetrazolium chloride (TTC)–Evans blue technique in 9 sham and 10 IHC rats. After 60 min of coronary reperfusion, the hearts were excised and perfused from the aorta with 2% Evans blue dye to differentiate the left ventricular area at risk for infarction (AAR, unstained tissue distal to the coronary artery occlusion) from surrounding, blue stained, normally perfused myocardium. The heart was then frozen at -20°C for 20 min, and later cut into five 2-mm thick transverse slices. The slices were incubated for 20 min at 37°C in a 1% solution of TTC in phosphate buffer (pH = 7.4) to differentiate the pale, infarcted myocardium from the non-infarcted red AAR. Slices were fixed in a 10% formaldehyde solution and photographed. The corresponding areas were measured by computerized planimetry, and weights of IS and AAR were estimated assuming $1\,mm^3 = 1\,mg$. IS was calculated as a percentage of the AAR.

Endothelium-dependent relaxation of isolated aorta of rats with *in situ* myocardial IR injury

Four groups of rats were studied: (1) sham with no IR ($n = 8$), (2) sham with IR ($n = 10$), (3) IHC with no IR ($n = 8$), and (4) IHC with IR ($n = 10$). The rats were killed by decapitation under urethane anesthesia. Rats subjected to IR were decapitated immediately after completion of *in situ* myocardial IR as described above. The aorta was isolated, and aortic rings (3.5–4.0 mm length) were mounted in a temperature-controlled, isolated organ bath (Model 4000, Ugo Basile, Comerio, Italy), containing Krebs bicarbonate buffer (130 mM NaCl, 11 mM glucose, 14.9 mM $NaHCO_3$, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.8 mM KH_2PO_4, pH 7.4) at $37 \pm 0.5^{\circ}$C and aerated with 95% O_2–5% CO_2. After a 60-min incubation at a resting tension of 1.2 g, the aortic rings were pre-contracted with 0.5 μmol/L norepinephrine (Abbott Laboratories). Endothelium-dependent relaxation was induced by adding 10 μmol/L acetylcholine (Sigma, St. Louis, MO) to the organ bath and computed as the percentage decline in developed tension of the norepinephrine-mediated pre-contraction.

Endothelium-dependent coronary dilation in isolated hearts before and after IR

After rat decapitation, the hearts were rapidly excised from 6 sham and 7 IHC rats and the aorta cannulated to a Langendorff apparatus. These isolated rat hearts were perfused with aerated (95% $O_2 + 5\%$ CO_2) Krebs–Henseleit solution: NaCl 118.0 mM, KCl 4.7 mM, $NaHCO_3$ 25.0 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, glucose 5.5 mM, pH 7.3–7.4, 37.5°C. The hearts were electrically stimulated at 300 beats/min (impulse duration 2 ms, impulse amplitude 5–7 V). The coronary circulation was perfused retrogradely from the aorta, where pressure was maintained at 100 mmHg, except when myocardial ischemia was produced. IR was imposed by complete cessation of the flow for 15 min with subsequent reperfusion for 10 min. Coronary flow rate was measured as the perfusate outflow. To assess endothelium-dependent coronary dilation before ischemia and at 10 min reperfusion, acetylcholine (10^{-7} M) was infused (LSP04-1 A, LongerPump, Baoding, China) until coronary flow stabilized. The steady-state increase in coronary flow was taken as a measure of endothelium-dependent coronary dilation.

Statistical analyses

Data are presented as mean values ± SEM. Data were analyzed by analyses of variance (ANOVA) followed by *post hoc* Newman–Kuels tests if more than two groups were present. A repeated measures ANOVA was used to analyze arrhythmia duration and coronary flow data collected from the same hearts. Homogeneity of variances for arrhythmia data was achieved by log transformation ($Y = \log_{10}(X + 1)$). Between-group comparisons of body and ventricular weights (Table 2) were accomplished by unpaired Student's *t*-tests. Chi-square tests, with Yates corrections for continuity, were used to compare instances of arrhythmias between sham and IHC rats (Table 3), except for ventricular fibrillation data during ischemia and ventricular tachycardia and fibrillation data during reperfusion, where the Fisher exact probability test was used. Differences were considered statistically significant at $P < 0.05$.

Results

Effect of IHC on cardiac ventricular weight

The 20-day IHC regimen did not induce any significant changes in right ventricular weight, whether expressed in absolute terms or as a function of the combined weight of the ventricles (Table 2). These data mitigate concerns about hypoxia-induced right ventricular hypertrophy.

Table 2 Body and ventricular weights of unconditioned sham and IHC rats

	n	BW (g)	RV (g)	LV (g)	RV/(RV + LV)	RV/BW (g/kg)
Sham	15	316 ± 8	0.21 ± 0.01	0.71 ± 0.03	0.23 ± 0.005	0.67 ± 0.02
IHC	18	319 ± 8	0.23 ± 0.01	0.76 ± 0.02	0.23 ± 0.007	0.72 ± 0.03

Note: Values are means ± SEM. There were no statistically significant differences between respective values for sham and IHC rats.
n: number of rats; BW: body weight; LV: left ventricular weight; RV: right ventricular weight.

Table 3 Incidence of specific arrhythmias during ischemia and reperfusion in sham and IHC rats

	Ischemia				Reperfusion		
	n	ES	VT	VF	ES	VT	VF
Sham	15	14	14	5	9	2	0
IHC	14	3	4	1	4	7	0
P value		<0.001	0.001	0.17	<0.18	0.05	-

Note: Values show number of rats in which at least one arrhythmia was observed. Group comparisons were made by Chi-square tests, with Yates corrections for continuity, except for ischemia VF data and reperfusion VT data, where the Fisher exact probability test was used. *P* values indicate probability of no difference between sham and IHC data.
n: number of rats; ES: extrasystole; VT: ventricular tachycardia; VF: ventricular tachycardia.

Myocardial arrhythmias and injury studied *in situ*

During myocardial ischemia, sustained ventricular fibrillation occurred in two of the sham rats (12%) and in one of the IHC rats (7%). Data from these hearts were excluded. Heart rates (min^{-1}) were similar ($P = 0.28$) in non-IHC sham (358 ± 10) and IHC (352 ± 14) rats. Table 3 shows that the incidence of extrasystole and ventricular tachycardia was significantly less during ischemia for IHC rats. Figure 1 shows the total durations of arrhythmias recorded during myocardial ischemia and reperfusion. The total durations of ischemic extrasystole, ventricular tachycardia, and ventricular fibrillation were all sharply decreased in IHC versus sham rats. The durations of extrasystole and ventricular tachycardia were shorter during reperfusion versus ischemia, and were not affected by IHC. In neither group was transient ventricular fibrillation observed during reperfusion.

Ischemic areas at risk (AAR) were 360 ± 20 mg in hearts of the sham rats and 320 ± 20 mg in the IHC rat hearts, and were not significantly different ($P = 0.179$). However, the masses of the infarcts were significantly lower ($P = 0.01$) in hearts of the IHC rats (22 ± 4 mg) than those of the sham rats (54 ± 6 mg). In sham rats, IS was $30.6 \pm 5.1\%$ of AAR, whereas in IHC rats, infarct size was significantly reduced ($P = 0.04$) to $17.5 \pm 2.6\%$ of AAR (Figure 2).

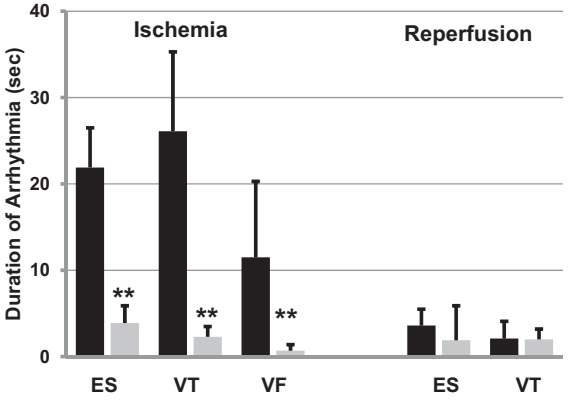

Figure 1 IHC reduced cardiac arrhythmias during IR. Data show duration of cardiac arrhythmias recorded during *in situ* 30-min myocardial ischemia and 60-min reperfusion. Dark columns show data from control, sham IHC rats ($n = 15$). Light columns show data from IHC rats ($n = 14$).
ES: extrasystole; VT: ventricular tachycardia; VF: ventricular fibrillation with spontaneous conversion. Ventricular fibrillation was not observed during reperfusion. Values are means ± SEM. $^{**}P < 0.01$ versus respective control value

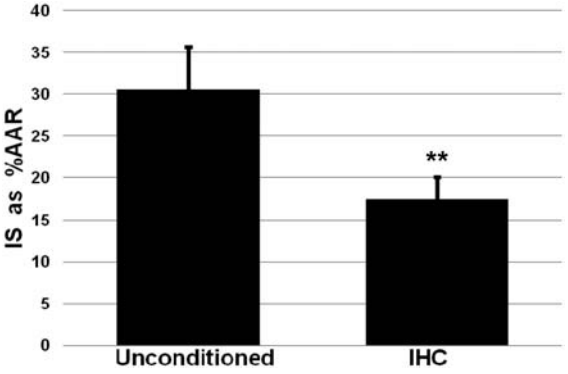

Figure 2 IHC decreased myocardial infarction following coronary artery occlusion–reperfusion. The main left coronary artery was occluded *in situ* for 30 min, then reperfused for 60 min. Left ventricular ischemic area at risk was demarcated with Evans blue dye; infarcted tissue was measured after staining with 2,3,5-triphenyl-tetrazolium chloride to detect non-infarcted tissue.
IS: infarct size; AAR: ischemic area at risk. Values are means ± SEM from 9 sham and 10 IHC rats. $^{**}P < 0.01$ versus sham value

Endothelium-dependent relaxation of isolated aorta of rats with *in situ* myocardial IR injury

Figure 3 shows endothelium-dependent relaxation of isolated aorta of rats with *in situ* myocardial IR injury. For non-IHC sham rats with no IR, 10 μmol/L acetylcholine caused baseline aortic tension to decrease by $45 \pm 4\%$ of its norepinephrine-precontracted value. IR induced pronounced endothelial dysfunction in the aorta, as evident from the much smaller, $9.7 \pm 1\%$, decrease in tension produced by acetylcholine ($P < 0.01$ *vs.* sham, no IR). This detrimental effect of IR on endothelium dependent relaxation of the aorta was largely mitigated by IHC, since the acetylcholine-induced fall in aortic tension was $30 \pm 3\%$ compared to $33 \pm 3\%$ in IHC rats with no IR, and this endothelium-dependent relaxation was much greater than that observed in aortas of sham rats ($P < 0.01$).

Endothelium-dependent coronary dilation in hearts with *in vitro* IR injury

In hearts from sham rats before IR, acetylcholine increased coronary flow from 12.1 ± 0.8 to 14.0 ± 0.9 mL/min ($P < 0.01$). After IR, acetylcholine had no significant effect on coronary flow (10.9 ± 0.9 *vs.* 11.2 ± 0.9 mL/min). In hearts from IHC rats before IR, acetylcholine increased coronary flow from 11.0 ± 1.0 to 12.6 ± 1.2 mL/min ($P < 0.01$). After IR, acetylcholine again increased coronary flow from 11.1 ± 0.5 to 12.2 ± 0.5 mL/min ($P < 0.01$). Figure 4 shows the difference in incremental increases in coronary flow produced by acetylcholine before and after IR in hearts isolated from sham versus IHC rats. Following IR in sham rats, the acetylcholine-induced flow increment was 19% of that observed before IR

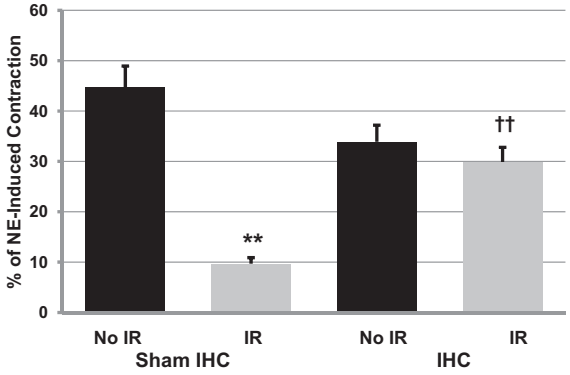

Figure 3 IHC blunted IR-induced endothelial dysfunction in isolated aortic rings. Data show acetylcholine-induced tension decrease, expressed as a percentage of NE-induced contraction of aortic rings, from (1) sham rats with no IR ($n = 8$), (2) sham rats with IR ($n = 10$), (3) IHC rats with no IR ($n = 8$), and (4) IHC rats with IR ($n = 10$). Dark columns show data from rats with no IR, and light columns show data from rats subjected to IR. Values are means ± SEM. $^{**}P < 0.01$ versus sham IHC with no IR. $^{††}P < 0.01$ versus sham IHC with IR

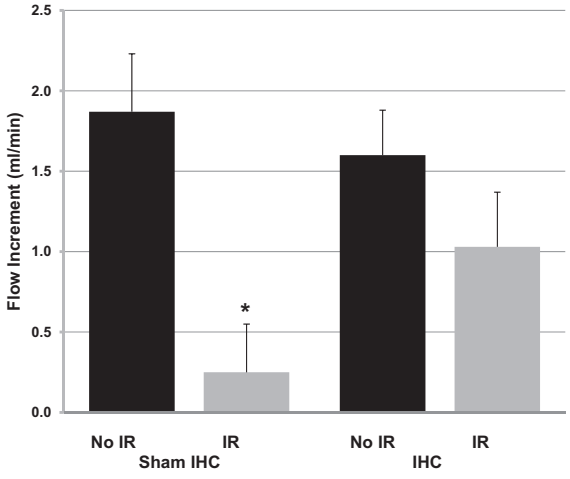

Figure 4 Acetylcholine-induced increment in coronary flow before and after IR. Values are means ± SEM from 6 sham and 7 IHC rats. $^{*}P < 0.05$ versus no IR for the same treatment regimen

($P < 0.01$), whereas in IHC rats this flow increment was 64% of that observed before IR. Thus, antecedent IHC substantially preserved endothelium-dependent coronary dilation in the face of myocardial IR.

Discussion

This study demonstrated that normobaric IHC in rats markedly increased cardiac resistance to IR injury and lessened IR-induced endothelial dysfunction of coronary and non-coronary blood vessels. Specifically, the antecedent IHC program dampened cardiac arrhythmias during myocardial ischemia and reduced cardiac infarction caused by myocardial IR. Moreover, normobaric IHC lessened IR-induced endothelial dysfunction of coronary and extracoronary blood vessels.

Previously, we demonstrated appreciable antiarrhythmic and anti-infarction effects in dogs completing a 20-day program of normobaric IHC.[10,12] By extending these findings to rats, this study demonstrates that the cardioprotective effects of normobaric IHC are not peculiar to dogs. These findings are also supportive of recently reported cardioprotective effects of hypobaric IHC in rats.[15,19–21] Although there are clinical reports from Eastern Europe of beneficial cardiac effects of IHC,[25,26,37–44] there is limited experimental evidence to support clinical use of IHC for cardiac protection or therapy. While the current findings are supportive of clinical applications of IHC, more importantly, these findings may stimulate further research to establish optimal IHC programs and to decipher the mechanism of its cardioprotective action. It is also important to establish the duration of the cardioprotective action of IHC. In this regard, it is interesting to note that antihypertensive actions of IHC have been reported to persist for up to six months.[27,45]

While mechanisms of IHC-induced cardioprotection are not well understood, hypobaric IHC is known to induce gene expression of protective proteins,[56,57] augment antioxidant defense,[18,19] modulate NO synthesis,[45,58,59] activate ATP-sensitive K^+ channels,[14,19] prevent Ca^{2+} overload of cardiomyocytes,[11] and improve O_2 transport in tissues.[60] IHC also induces changes in mitochondrial nicotinamide adenine dinucleotide (NAD-)dependent metabolism that increase the efficiency of oxygen utilization in ATP production.[17]

Alternation of hypoxia and normoxia apparently plays an important role in IHC-induced cardioprotection. Béguin *et al*. showed that 4 h of cyclic IH produced acute IR cardioprotection, whereas 4 h of continuous moderate hypoxia (10% O_2) increased IR injury.[16] Since antioxidants abolished the cardioprotection produced by an IH program similar to that employed in the current study,[61] it appears that intermittent activation of free-radical processes during cyclic periods of hypoxia-normoxia contributes to the cardioprotective action of IHC.[13,61] This hypothesis merits further investigation.

Myocardial IR may cause acute NO overproduction and oxidative stress,[58,62] which would damage both myocardium and the coronary vasculature.[63] Recently, we reported that myocardial infarction caused endothelial dysfunction in rat aorta,[64] and Zhao *et al*. showed that myocardial IR produced endothelial dysfunction in mesentery vessels.[51] Accordingly, endothelial dysfunction was studied in the coronary circulation and the aorta of rat hearts with IR. At both sites, there was marked endothelial dysfunction, as reflected by the attenuated vasodilatory response to acetylcholine after IR. This dysfunction was significantly blunted by IHC. In previous canine studies, we found that the same 20-day IHC program reduced myocardial NO production during IR.[62] Whether the IHC-induced reduction in the endothelial dysfunction observed in the current study resulted from reduced NO overproduction and oxidative stress remains to be determined.

IHC programs are not universally vasoprotective. Indeed, the duration and frequency of the hypoxia stimulus have a marked impact on the outcome of the conditioning

regimen. Programs utilizing hypobaric hypoxia, in which rats were exposed to uninterrupted hypoxia of several hours each day, enhanced endothelium-dependent aortic vasodilation to acetylcholine.[47,57] In contrast, normobaric hypoxia models of sleep apnea, in which rats were subjected to numerous brief (40–60 s), intense (5–10% FIO_2) cycles of hypoxia, repeated at 1–4-min intervals for 8–12 h per day, impaired acetylcholine-induced dilation of aorta,[5] gracilis muscle artery,[3,4,65] middle cerebral artery,[3] and pulmonary artery.[2] Such intensive IH was found by Wang *et al*. to down-regulate eNOS expression.[66] In contrast, a normobaric IHC regimen utilizing somewhat longer cycles of 6-min hypoxia (8% FIO_2) and 6-min reoxygenation-protected post-ischemic acetylcholine induced vasodilation in cheek pouch of hamsters.[67] Collectively, these studies suggest that the 5–8 min hypoxia bouts and 4-min reoxygenations constituting the present IHC program were of appropriate durations to protect, not impair, endothelium-dependent dilation of coronary and systemic arteries.

The current 20-d IHC program of brief, normobaric, moderately intense (FIO_2 9.5–10%) hypoxia bouts did not produce right ventricular hypertrophy in Wistar rats. In contrast, normobaric or hypobaric hypoxia programs in which hypoxia exposures of a similar intensity are continuous[68–70] or protracted (e.g. 4–8 h bouts)[71,72] provoke right ventricular hypertrophy and medial thickening of the pulmonary vasculature in this strain. Thus, the brief, moderately intense hypoxia exposures of the current IHC program elicit favorable cardiac and vascular adaptations without causing the potentially maladaptive remodeling typically produced by more protracted hypoxia.

In summary, this investigation confirmed in rat hearts that a program of moderate, normobaric intermittent hypoxic conditioning is cardioprotective, both in regard to ischemia-induced arrhythmias and to IR-induced infarction. Furthermore, intermittent hypoxic conditioning markedly reduced IR-induced endothelial dysfunction of coronary vasculature and aorta. Results support clinical applications of intermittent hypoxic conditioning for cardiac and vascular protection from ischemic insults.

Author contributions: EBM designed the study, performed experiments on isolated rat aorta, and drafted the manuscript. LMB and OLT performed *in situ* experiments and analyzed cardiac arrhythmias. EAS measured area at risk and infarct size in these experiments. DVA, OLT, and OPB evaluated coronary endothelial function in isolated hearts. OPB performed hypoxic and sham adaptation of rats and assisted with experiments on isolated rat aorta. RTM expanded, edited, and revised the manuscript. HFD performed statistical analyses, assisted with data interpretation, edited, and revised the manuscript.

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