*J Appl Physiol* 99: 1064–1069, 2005.
First published May 5, 2005; doi:10.1152/japplphysiol.00056.2005.

# Acute intermittent hypoxia improves rat myocardium tolerance to ischemia

**P. C. Béguin, M. Joyeux-Faure, D. Godin-Ribuot, P. Lévy, and C. Ribuot**

*Laboratoire HP2, Hypoxie Physio-Pathologie Respiratoire et Cardiovasculaire, Equipe d'Accueil Ministère de la Recherche, Equipe Soutenue par la Région et l'Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine-Pharmacie, Université Grenoble I, La Tronche, France*

Submitted 18 January 2005; accepted in final form 3 May 2005

**Béguin, P. C., M. Joyeux-Faure, D. Godin-Ribuot, P. Lévy, and C. Ribuot.** Acute intermittent hypoxia improves rat myocardium tolerance to ischemia. *J Appl Physiol* 99: 1064–1069, 2005. First published May 5, 2005; doi:10.1152/japplphysiol.00056.2005.—In this study, we investigated the influence of depth and duration of intermittent hypoxia (IH) on the infarct size development in isolated rat heart. The role of nitric oxide synthase (NOS) and ATP-sensitive $K^+$ ($K_{ATP}$) channel was also studied. Wistar male rats were exposed to IH [repetitive cycles of 1 min, 40 s with inspired oxygen fraction ($FI_{O_2}$), 5 or 10%, followed by 20-s normoxia], during 30 min or 4 h. Another group was exposed to 4 h of continuous hypoxia with 10% $FI_{O_2}$. Twenty-four hours later, their hearts were isolated and subjected to a 30-min no-flow global ischemia-120-min reperfusion sequence. For some hearts, $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) (a nonselective inhibitor of NOS) or 5-hydroxydecanoic acid (5-HD) (a selective mitochondrial $K_{ATP}$ blocker) was infused before ischemia. Infarct size (in percentage of ventricles) was significantly reduced by prior IH for 4 h (10% $FI_{O_2}$) (21.8 ± 3.1 vs. 33.5 ± 2.5% in sham group). This effect was abolished by L-NAME or 5-HD. Infarct size was not different in groups subjected to either 30 min of IH or to continuous hypoxia compared with sham group. In contrast, IH for 4 h (5% $FI_{O_2}$) significantly increased infarct size (45.1 ± 3.6 vs. 33.5 ± 2.5% in sham group). Acute IH for 4 h with a minimal $FI_{O_2}$ of 10% induced a delayed preconditioning against myocardial infarction in the rat, which was abolished by NOS inhibition and mitochondrial $K_{ATP}$ channel blockade. Depth, duration, and intermittence of hypoxia appeared to be critical for cardioprotection to occur.

ischemia-reperfusion; infarction; $K_{ATP}$ channel; nitric oxide

CONTINUOUS HYPOXIA (CH) IS known to be an early preconditioning (PC) stimulus. Thus, in neonatal mouse cardiomyocytes, prior hypoxia (0% $O_2$ for 30 min and 1 h reoxygenation) improves cellular viability following lethal ischemia (15). Moreover, isolated rat hearts submitted to an anoxia (10 min)-reoxygenation (10 min) sequence show a preserved mechanical function and a reduced myocardial necrosis following ischemia-reperfusion (9, 10, 16). CH (10% $O_2$ for 4 h) is also able to induce delayed cardioprotection. Indeed, isolated hearts from mice submitted to this stimulus are more resistant to infarction, 24 h later (26). Moreover, in a rat model of permanent occlusion, it decreases infarct size as a result of myocardial angiogenesis (22). In contrast, little is known about the effects of intermittent hypoxia (IH) on heart's sensitivity to infarction. A recent study in mice has shown that only 1 h of IH (5 cycles of 6% $O_2$ for 6 min followed by 21% $O_2$ for 6 min) is able to prevent myocardial dysfunction and infarct injury produced 24 h later by an ischemia-reperfusion sequence (8). This study suggests that IH is more efficient than CH as a PC trigger.

Nitric oxide (NO) is today well known as a mediator of various forms of delayed PC (6). NO formation mediates the delayed ischemic PC against both myocardial stunning (7) and infarction in the rabbit (12, 24). Indeed, administration of NO synthase (NOS) inhibitor 24 h after ischemic PC abolishes the delayed cardioprotection. The mechanism of hypoxic PC could be similar to the ischemic one and also implicate NO. Indeed, the cardioprotective effects of hypoxic and ischemic PC were described to be not additive, suggesting that they may share the same signaling pathway (18).

Many studies have shown that high-altitude hypoxia, another model of IH, might have cardioprotective effects against ischemic injury similar to those observed in ischemic PC. Indeed, this sort of IH protects myocardium by increasing coronary circulation and angiogenesis (29) and reduces incidence of arrythmias (2). Among mechanisms underlying this phenomenon, ATP-sensitive $K^+$ ($K_{ATP}$) channels play a critical role (2, 19). Those channels seem also to mediate the cardioprotective effect induced by CH in rabbit (3, 4). $K_{ATP}$ channels could also play a role in the acute IH-induced cardioprotection.

The aim of the present study was to examine the influence of *1*) two depths of IH, *2*) two durations of IH, and *3*) the role of repeated reoxygenation on infarction susceptibility assessed in isolated rat hearts, 24 h after the hypoxic stress. We also investigated the role of NOS and $K_{ATP}$ channel in the mechanism underlying IH-induced cardioprotection. Thus, 24 h after IH, we tested the effect of the nonselective NOS inhibitor $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) and the mitochondrial $K_{ATP}$ channel blocker 5-hydroxydecanoic acid (5-HD) on infarct size development in the isolated rat heart.

## METHODS

This investigation conforms with French law and local ethical committee guidelines for animal research. The protocol received approval from the Direction des Services Vétérinaires de l'Isère, France. Experiments were conducted on adult male Wistar rats (weight range 330–380 g) from Elevage Janvier (Le Genest-Saint-Isle, France) housed in controlled conditions and provided with standard rat chow.

*IH protocol.* During IH, the animals were housed in custom-made identical cylindrical Plexiglas chambers (length 28 cm, diameter 10 cm, volume 2.2 liters) with tightly fitted lids. A timed solenoid valve was used to distribute pure nitrogen to each chamber at a flow that was adjusted to reduce the ambient inspired oxygen fraction ($FI_{O_2}$) to 5 or

Address for reprint requests and other correspondence: C. Ribuot, Laboratoire HP2, Hypoxie Physiopathologie Respiratoire et Cardiovasculaire, EA3745, ESPRI INSERM, Faculté de Médecine-Pharmacie, Université Grenoble I, Domaine de la Merci, 38706 La Tronche Cedex, France (E-mail: Christophe.Ribuot@ujf-grenoble.fr).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

  

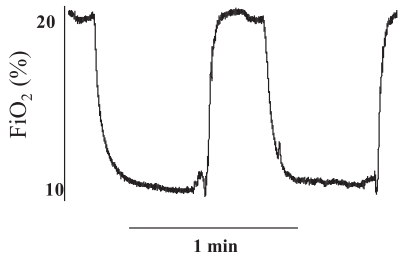


Fig. 1. Representative trace of the atmospheric changes that occurred during exposure to intermittent hypoxia (IH). Inspired oxygen fraction ($F_{I_{O_2}}$) was determined by continuous sampling from the chamber using an $O_2$ analyzer. IH was achieved by flushing nitrogen into the chamber until the $O_2$ concentration reached 10%. Hypoxia was maintained for 40 s, after which oxygen was flushed into the chamber to reestablish normoxia.

10% for 40 s. This was followed by infusion of compressed air, allowing a gradual return (over 20 s) of ambient air to a $F_{I_{O_2}}$ of 21%. The 5% depth was chosen to correspond with the $O_2$ desaturation observed in patients with obstructive sleep apnea syndrome. The 10% depth was chosen arbitrarily to investigate a less severe stress. This 1-min cycle was repeated for 30 min or 4 h. In parallel to nitrogen distribution, compressed air at the same flow rate was distributed to sham chambers to submit sham animals to similar noise and airflow disturbances. A dampening device at the chamber intake was used to dissipate the airstream, avoiding direct gas jets.

The level of $F_{I_{O_2}}$ in the chambers was controlled throughout the hypoxia protocol by using a Beckman OM11 $O_2$ analyzer (Fullerton, CA). Figure 1 provides an illustration of the $F_{I_{O_2}}$ profile produced by our IH protocol.

*Experimental groups.* Ten groups were subjected to a protocol summarized in Fig. 2. Twenty-four hours before the ischemia-reperfusion sequence, the rats were placed in the Plexiglas chambers and exposed to the following stimuli.

The following three groups were exposed to 4 h of normoxia or IH: *1*) S 4h group (sham rats; $n = 10$), normoxic cycles (21% $F_{I_{O_2}}$); *2*) IH5 4h group ($n = 8$), IH cycles with a minimal $F_{I_{O_2}}$ of 5% during hypoxia; *3*) IH10 4h group ($n = 11$), IH cycles with a minimal $F_{I_{O_2}}$ of 10% during hypoxia.

The following four groups infused with different pharmacological antagonists were previously submitted to 4 h of normoxia or IH. Two groups were infused with the NOS inhibitor L-NAME [3 μM (28)] during 10 min before no-flow global ischemia: *1*) S + L-NAME group ($n = 7$), normoxic cycles (21% $F_{I_{O_2}}$); *2*) IH10 + L-NAME group ($n = 7$), IH with a minimal $F_{I_{O_2}}$ of 10% during hypoxia. Two groups were infused with the mitochondrial $K_{ATP}$ channel blocker 5-HD [100 μM, (11)] during 10 min before no-flow global ischemia: *3*) S + 5-HD group ($n = 6$); *4*) IH10 + 5-HD group ($n = 6$).

The two following groups were exposed to a 30-min IH: *1*) S 30min group ($n = 6$), normoxic cycles during 30 min (21% $F_{I_{O_2}}$); *2*) IH10 30min group ($n = 7$), IH cycles during 30 min with a minimal $F_{I_{O_2}}$ of 10% during hypoxia.

One last group was exposed to CH during 4 h with a 10% $F_{I_{O_2}}$ (CH group, $n = 7$).

*Ischemia-reperfusion protocol.* Twenty-four hours after IH, the rats were anesthetized with 60 mg/kg ip pentobarbital sodium and treated with heparin (500 IU/kg iv). Hearts were rapidly excised and immersed in 4°C Krebs-Henseleit buffer solution (in mM: 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $KH_2PO_4$, 1.2 $MgSO_4$, 25.2 $NaHCO_3$, and 11.0 glucose). The aortic stump was cannulated, and the heart was perfused retrogradely using the Langendorff technique at a constant pressure (75 mmHg) with oxygenated Krebs-Henseleit buffer, as previously described (14). A water-filled latex balloon (Hugo Sachs, no. 4), coupled to a pressure transducer, was inserted into the left ventricular cavity via the left atrium for pressure recording. Left ventricular end-diastolic pressure was adjusted between 5 and 10 mmHg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and was maintained constant close to 37°C. After 20 min of stabilization, no-flow global ischemia was induced by stopping the perfusion for 30 min. Thereafter, the heart was reperfused for 120 min. Coronary flow (CF) was measured periodically throughout the ischemia-reperfusion procedure, by collecting the effluent; it was then expressed in milliliters per minute per gram. Heart rate (HR), left ventricular developed pressure (LVDP = difference between left ventricular systolic pressure and left ventricular end-diastolic pressure), and maximal rate of left ventricular pressure rise ($dP/dt_{max}$) and fall ($dP/dt_{min}$) were continuously recorded (PC Lab 4S, AD Instruments). At the end of the ischemia-reperfusion protocol, the atria were removed, and the heart was frozen at −20°C for 10 min. It was then cut into 2-mm transverse sections from apex to base (6–7 slices/heart). Once thawed, the slices were incubated at 37°C with 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4) for 10 min, fixed in 10% formaldehyde solution, and photographed. Incubation avoids a possible overestimation of infarct size due to no-reflow phenomenon. Area of infarcted tissue was measured by using a computerized planimetric technique (Image Tool for Windows) and expressed as a percentage of both right and left ventricular area. According to this method, the necrosis area corresponds to the zone not stained by the triphenyltetrazolium chloride.

*Exclusion criteria.* Only hearts with CF ≤ 20 ml/min and LVDP > 70 mmHg at the end of the stabilization period were included in this study.

Moreover, because of a recording problem for one rat in the S 4h group, the hemodynamic data represent a mean of 9 points, and the infarct size data a mean of 10 points.

*Materials.* All reagents were from Sigma-Aldrich (Saint Quentin Fallavier, France).

*Statistical analysis.* Hemodynamic and infarct size data are presented as means ± SE. Infarct size values were compared by using one-way ANOVA. Hemodynamic data were analyzed by using a two-way repeated-measures ANOVA. Post hoc multiple comparisons were performed by using Tukey tests. Statistical significance was set at $P < 0.05$.

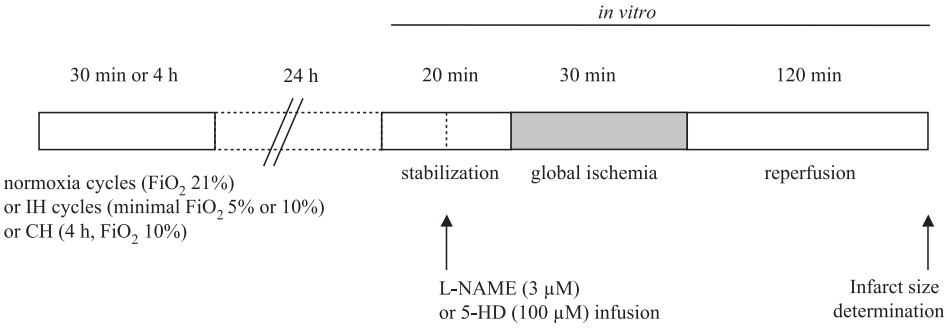


Fig. 2. Experimental protocol. Rats were submitted to either normoxia, IH during 30 min or 4 h, or continuous hypoxia (CH). Subsequently, all animals were allowed to recover for 24 h. Then, after a 20-min stabilization period, a 30-min no-flow global ischemia followed by a 120-min reperfusion was performed in vitro. Some hearts were infused with either $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME; 3 μM), a nonselective inhibitor of nitric oxide synthase (NOS), or 5-hydroxydecanoic acid (5-HD; 100 μM), a selective mitochondria ATP-sensitive $K^+$ ($K_{ATP}$) blocker for 10 min before ischemia.

Table 1. *Hemodynamic parameters of hearts from rats submitted to two durations of intermittent hypoxia (10% inspired oxygen fraction)*

| | S 30min | IH10 30min | S 4h | IH10 4h |
|---|---|---|---|---|
| *n* | 6 | 7 | 9 | 11 |
| CF, % | | | | |
| R15 | 62±2 | 66±5 | 62±3 | 70±6 |
| R60 | 48±2 | 50±5 | 52±4 | 51±5 |
| R120 | 38±5 | 45±9 | 41±3 | 44±4 |
| HR, % | | | | |
| R15 | 75±2 | 68±6 | 81±6 | 67±5 |
| R60 | 106±5 | 73±5 | 89±6 | 76±4 |
| R120 | 93±5 | 75±6 | 85±7 | 76±5 |
| LVDP, % | | | | |
| R15 | 56±11 | 77±20 | 83±13 | 90±10 |
| R60 | 61±8 | 59±11 | 74±13 | 97±10 |
| R120 | 42±8 | 53±12 | 50±6 | 66±7 |
| RPP, % | | | | |
| R15 | 42±8 | 52±15 | 65±9 | 62±9 |
| R60 | 65±10 | 46±12 | 63±9 | 64±9 |
| R120 | 40±9 | 40±10 | 43±9 | 52±7 |
| $dP/dt_{max}$, % | | | | |
| R15 | 52±10 | 70±20 | 61±10 | 73±8 |
| R60 | 69±14 | 62±14 | 62±10 | 77±8 |
| R120 | 52±12 | 56±13 | 43±5 | 63±6 |
| $dP/dt_{min}$, % | | | | |
| R15 | 60±10 | 76±31 | 70±11 | 77±10 |
| R60 | 71±14 | 68±18 | 58±8 | 77±9 |
| R120 | 55±11 | 45±10 | 39±4 | 56±6 |

Values are means ± SE, expressed as percentage of values at 20 min of stabilization; *n,* no. of rats. CF, coronary flow; HR, heart rate; LVDP, left ventricular developed pressure; RPP, rate-pressure product; $dP/dt_{max}$ and $dP/dt_{min}$: maximal rate of left ventricular rise and fall, respectively, expressed as percentage of stabilization values; R15, R60, and R120: reperfusion at 15, 60, and 120 min, respectively. See METHODS for explanation of groups.

## RESULTS

*Effects of the duration of IH.* Hemodynamic data summarized in Table 1 showed no statistically significant difference in CF, HR, LVDP, rate-pressure product (RPP), and $dP/dt_{max}$ during stabilization and ischemia-reperfusion periods, whatever the group observed. Likely, as observed in Fig. 3, the infarct size was similar in groups exposed to a 30-min IH (35.3 ± 6.7%) or normoxia (36.5 ± 4.0%). However, following 4 h of IH, the infarct size was significantly lower in hearts from rats exposed to 10% $F_{I_{O_2}}$ (21.8 ± 3.1%) compared with those from their respective normoxic controls (33.5 ± 2.5%).

*Effects of the depth of IH.* As shown in Table 2 and Fig. 4, there was no statistically significant difference in all of the studied hemodynamic parameters 24 h after 4 h of normoxia or IH (10% $O_2$) or CH (10% $O_2$). In contrast, IH with a minimal $F_{I_{O_2}}$ of 5% induced a decrease in LVDP (Fig. 4*A*) and RPP (Fig. 4*B*) and an increase in the infarct size (Fig. 5) (45.1 ± 3.6%) compared with the normoxic group (33.5 ± 2.5%). Four hours of CH at 10% $F_{I_{O_2}}$ did not modify infarct size (33.4 ± 7.0% in CH group) compared with normoxic exposure (Fig. 5).

*Effects of pharmacological antagonists' infusion.* Infusion of L-NAME had no effect on hemodynamic parameters (CF, HR, LVDP, RPP, $dP/dt_{max}$, and $dP/dt_{min}$), as summarized in Table 3, nor on infarct size, as illustrated in Fig. 6. However, 5-HD infusion decreased LVDP and $dP/dt_{max}$ (Table 3) in the IH10 + 5-HD group compared with the IH10 4h group at 15 min of reperfusion without altering infarct size (Fig. 6). Beneficial effect of PC by 4 h of IH ($F_{I_{O_2}}$ of 10%) was abolished by L-NAME (33.3 ± 4.0%) or 5-HD (35.5 ± 4.3%) infusion.

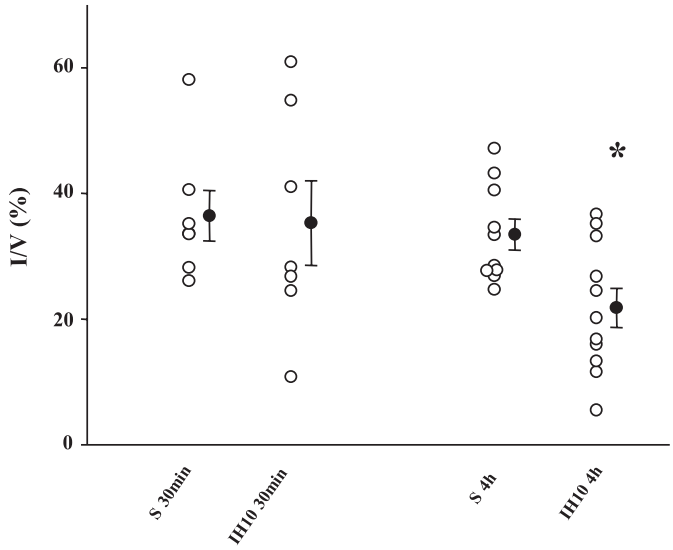


Fig. 3. Infarct size (I) expressed as a percentage of ventricles (V) assessed after a no-flow global ischemia (30 min)-reperfusion (120 min) sequence, in groups exposed for 30 min or 4 h to IH (10% $F_{I_{O_2}}$) or normoxia. ○, Individual values; ●, means ± SE. See METHODS for explanation of groups. *$P < 0.05$ vs. the other groups using a one-way ANOVA.

## DISCUSSION

*IH as a PC stimulus.* We have demonstrated for the first time that exposure of rats to brief episodes of IH with 10% $O_2$

Table 2. *Hemodynamic parameters of hearts from rats submitted to two depths of hypoxia and to continuous hypoxia during 4 h*

| | S 4h | IH10 4h | IH5 4h | CH |
|---|---|---|---|---|
| *n* | 9 | 11 | 8 | 7 |
| CF, % | | | | |
| R15 | 62±3 | 70±6 | 60±3 | 70±9 |
| R60 | 52±4 | 51±5 | 49±3 | 61±9 |
| R120 | 41±3 | 44±4 | 45±7 | 48±7 |
| HR, % | | | | |
| R15 | 81±6 | 67±5 | 69±8 | 74±3 |
| R60 | 89±6 | 76±4 | 87±3 | 82±5 |
| R120 | 85±7 | 76±5 | 87±6 | 76±4 |
| LVDP, % | | | | |
| R15 | 83±13 | 90±10 | 59±4* | 62±9 |
| R60 | 74±13 | 97±10 | 46±6* | 67±8 |
| R120 | 50±6 | 66±7 | 28±4* | 46±6 |
| RPP, % | | | | |
| R15 | 65±9 | 62±9 | 41±6* | 44±5 |
| R60 | 63±9 | 64±9 | 40±6* | 55±6 |
| R120 | 43±9 | 52±7 | 24±4* | 34±3 |
| $dP/dt_{max}$, % | | | | |
| R15 | 61±10 | 73±8 | 46±4† | 47±8 |
| R60 | 62±10 | 77±8 | 42±6† | 57±5 |
| R120 | 43±5 | 63±6 | 26±3† | 39±5 |
| $dP/dt_{min}$, % | | | | |
| R15 | 70±11 | 77±10 | 54±6 | 49±8 |
| R60 | 58±8 | 77±9 | 41±5† | 49±4 |
| R120 | 39±4 | 56±6 | 24±3† | 34±4 |

Values are means ± SE, expressed as percentage of values at 20 min of stabilization; *n,* no. of animals. *$P \leq 0.05$ vs. S 4h and IH10 4h. †$P \leq 0.05$ vs. IH10 4h.

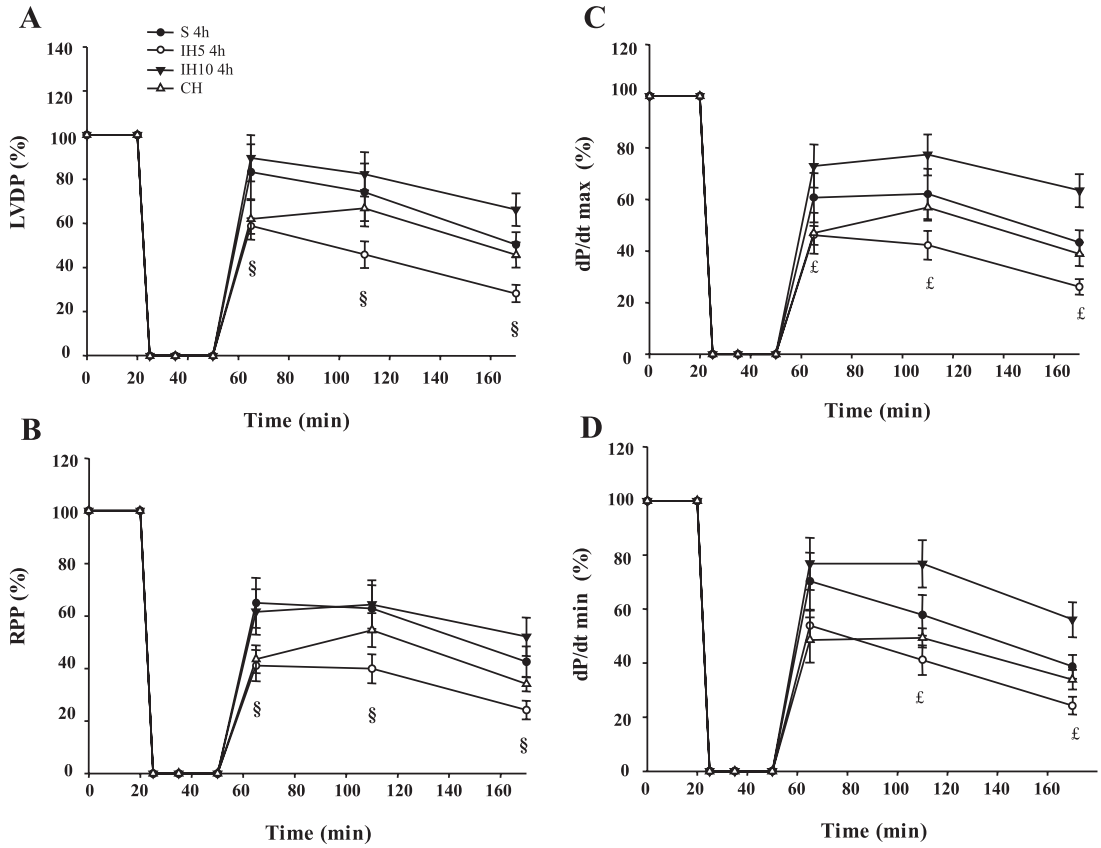


Fig. 4. Effect of the depth of IH on hemodynamic parameters. Hearts from rats submitted to either normoxia, IH (5 or 10% $F_{I_{O_2}}$), or CH during 4 h were perfused in Langendorff mode with Krebs-Henseleit buffer. After 20 min of stabilization, hearts were subjected to 30 min of no-flow global ischemia followed by 120 min of reperfusion. Left ventricular developed pressure (LVDP; *A*), rate-pressure product (RPP; *B*), and maximal rate of left ventricular pressure rise ($dP/dt_{max}$; *C*) and fall ($dP/dt_{min}$; *D*), expressed as percentage of values at 20 min of stabilization, were monitored continuously. §$P < 0.05$, IH5 4h group vs. S 4h group and IH10 4h group. £$P \leq 0.05$ vs. IH10 4h group.

induced protection against myocardial infarction, induced by total and global ischemia and reperfusion in isolated perfused heart, 24 h later. We have also demonstrated that exposure to CH during the same duration and depth had no effect. This second observation is in contrast with previous studies in rats (22) and mice (26), reporting that continuous exposure to 10% $O_2$ for 4 h resulted in delayed cardioprotection. This discrepancy could be explained in the first case by the ischemic model used (permanent left anterior descending coronary artery occlusion) to produce myocardial infarction and, in the second case, by species specificity in infarction susceptibility. Our study showed that intermittence of hypoxia, and hence reoxygenation, was essential to protect rat myocardium against infarction. One potential mechanism underlying this delayed cardioprotection could be the formation of reactive oxygen species (ROS), during the hypoxia-reoxygenation alternation. Indeed, ROS generated during alternation of hypoxia and reoxygenation appear to play a key role in the effects of IH on carotid body function and gene expression (21). Moreover, ROS are possibly involved in the mediation of cardioprotection conferred by many other forms of delayed PC, such as ischemic PC (23, 27) or heat stress (1).

*Role of the duration of IH.* We also showed that the delayed cardioprotection induced by IH is time dependent. After 4-h exposure, we reported a significant infarct size reduction, whereas a shorter 30-min exposure was ineffective. We can

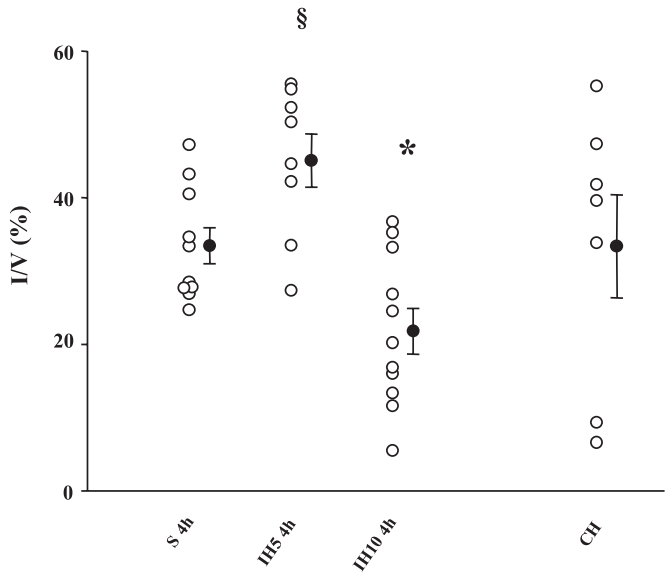


Fig. 5. Infarct size (I/V) assessed after a no-flow global ischemia (30 min)-reperfusion (120 min) sequence, in groups submitted to 4 h of normoxia, IH (5 or 10% $F_{I_{O_2}}$), or CH. ○, Individual values; ●, means ± SE. *$P < 0.05$ vs. the other groups. §$P < 0.05$ vs. S 4h and IH10 4h groups, using a one-way ANOVA.

Table 3. *Hemodynamic parameters after L-NAME or 5-HD infusion in groups exposed to 4 h of normoxia or hypoxia (10% inspired oxygen fraction)*

| | S 4h | IH10 4h | S + L-NAME | IH10 + L-NAME | S + 5-HD | IH10 + 5-HD |
|---|---|---|---|---|---|---|
| *n* | 9 | 11 | 6 | 7 | 6 | 6 |
| CF, % | | | | | | |
| R15 | 62±3 | 70±6 | 50±4 | 52±6 | 64±9 | 66±4 |
| R60 | 52±4 | 51±5 | 35±4 | 36±5 | 58±6 | 51±2 |
| R120 | 41±3 | 44±4 | 30±2 | 28±4 | 48±6 | 41±3 |
| HR, % | | | | | | |
| R15 | 81±6 | 67±5 | 58±11 | 67±7 | 67±10 | 77±7 |
| R60 | 89±6 | 76±4 | 68±9 | 85±7 | 83±3 | 88±5 |
| R120 | 85±7 | 76±5 | 72±10 | 81±7 | 85±2 | 86±9 |
| LVDP, % | | | | | | |
| R15 | 83±13 | 90±10 | 116±11 | 74±9 | 50±7 | 41±5* |
| R60 | 74±13 | 97±10 | 97±11 | 62±6 | 61±6 | 54±5 |
| R120 | 50±6 | 66±7 | 61±10 | 42±6 | 48±5 | 41±5 |
| RPP, % | | | | | | |
| R15 | 65±9 | 62±9 | 64±11 | 52±9 | 33±7 | 30±4 |
| R60 | 63±9 | 64±9 | 62±8 | 53±7 | 50±5 | 46±4 |
| R120 | 43±9 | 52±7 | 42±7 | 34±5 | 41±4 | 34±2 |
| $dP/dt_{max}$, % | | | | | | |
| R15 | 61±10 | 73±8 | 86±6 | 59±9 | 40±6 | 33±4* |
| R60 | 62±10 | 77±8 | 84±6 | 58±6 | 60±5 | 52±5 |
| R120 | 43±5 | 63±6 | 55±9 | 42±6 | 48±4 | 39±5 |
| $dP/dt_{min}$, % | | | | | | |
| R15 | 70±11 | 77±10 | 95±8 | 66±11 | 38±6 | 32±4* |
| R60 | 58±8 | 77±9 | 82±8 | 56±6 | 51±6 | 44±4 |
| R120 | 39±4 | 56±6 | 50±8 | 37±5 | 41±4 | 32±4 |

Values are means ± SE, expressed as percentage of values at 20 min of stabilization; *n,* no. of animals. L-NAME, $N^{\omega}$-nitro-L-arginine; 5-HD, 5-hydroxydecanoic acid. *$P \leq 0.05$ vs. IH10 4h.

hypothesize that, with a short duration of hypoxia, biochemical triggers were not produced in sufficient amount to initiate PC and to allow myocardial protection. This is also seen with ischemic PC in which a minimum duration (25) and number of events (17) are required for cardioprotection to occur.

*Role of the depth of IH.* Interestingly, we observed that a 4-h IH with a minimal $FI_{O_2}$ of 5% was able to render the heart more sensitive to ischemic injury, thus increasing infarct size, 24 h later. As a result, LVDP and RPP were significantly lower in the IH5 4h group than in the other groups. Thus, according to the depth of the hypoxic phase, either limitation or aggravation of infarction can be reached. This is, to our knowledge, the first demonstration of a heart's increased sensitivity to infarction 24 h after an acute IH exposure. We have recently described that chronic exposure (8 h during daytime, for 35 days) to the same intermittent hypoxic stimulus (5% $FI_{O_2}$) resulted in increased sensitivity to myocardial infarction (13). The novel, important contribution of the present paper is the rapid onset of this event. Because patients with obstructive sleep apnea syndrome have higher rates of coronary heart disease (20), we could hypothesize that they are also more vulnerable than others to infarction and that this vulnerability is rapidly present in the natural history of the pathology.

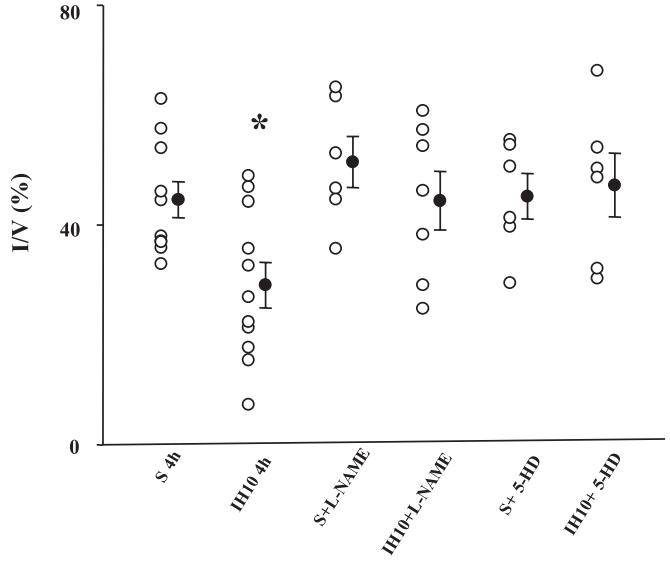


Fig. 6. Infarct size (I/V) assessed after a no-flow global ischemia (30 min)-reperfusion (120 min) sequence, in hearts infused with L-NAME and 5-HD. ○, Individual values; ●, means ± SE. *$P < 0.05$ vs. the other groups, using a one-way ANOVA.

*Role of NO in the IH-induced cardioprotection.* L-NAME, a NOS inhibitor, completely abrogated the IH-induced reduction in infarct size. The fact that NO could be a mediator of the IH response is in accordance with previous studies showing that protection conferred by other PC are also mediated by NO (for review, see Ref. 5). For example, heat stress-induced reduction in infarct size was abolished by L-NAME infusion before and during ischemia, in the isolated rat heart (14).

*Role of mitochondrial $K_{ATP}$ channel in the IH-induced cardioprotection.* It has been shown that $K_{ATP}$ channels are involved in mediating IH-induced cardioprotection in the rat using the sarcolemmal $K_{ATP}$ channel blocker glibenclamide (30). This study used a high-altitude IH model. Another study demonstrated that both mitochondrial $K_{ATP}$ and sarcolemmal $K_{ATP}$ channels contribute to the cardioprotection in a chronic hypoxia model (3). Here, we showed an involvement of mitochondrial-type $K_{ATP}$ in the cardioprotective effect conferred by

acute IH, as this effect was abolished by the selective mitochondrial $K_{ATP}$ channel blocker 5-HD.

*Limitation of our study.* The major end point of our study being infarct size, we used the more reliable model, i.e., the retrograde perfusion one. Therefore, the infarct size limitation that we observed with 10% IH is only accompanied by a tendency to improve LVDP. In contrast, the increase in infarct size observed with 5% IH is accompanied by a significant decrease in LVDP.

The fact that infarct size was significantly modified without ventricular function variation is not surprising, as ventricular stunning should be similar even with different infarct sizes. This apparent discrepancy was previously observed (13).

In summary, this study showed that acute IH for 4 h with 10% $O_2$ induced a delayed PC against myocardial infarction in the rat. We have demonstrated the influence of the IH reoxygenation, the duration and the depth of IH exposure on the degree of cardioprotection. We showed here the implication of NOS and mitochondrial $K_{ATP}$ channels in the cardioprotection afforded by acute IH.

## GRANTS

This study was supported, in part, by a grant from the Conseil Scientifique of Association Grenobloise des Insuffisauts Respiratoire à dom (France).

## REFERENCES

1. **Arnaud C, Joyeux M, Garrel C, Godin-Ribuot D, Demenge P, and Ribuot C.** Free-radical production triggered by hyperthermia contributes to heat stress-induced cardioprotection in isolated rat hearts. *Br J Pharmacol* 135: 1776–1782, 2002.
2. **Asemu G, Papousek F, Ostadal B, and Kolar F.** Adaptation to high altitude hypoxia protects the rat heart against ischemia-induced arrhythmias. Involvement of mitochondrial K(ATP) channel. *J Mol Cell Cardiol* 31: 1821–1831, 1999.
3. **Baker JE, Contney SJ, Gross GJ, and Bosnjak ZJ.** KATP channel activation in a rabbit model of chronic myocardial hypoxia. *J Mol Cell Cardiol* 29: 845–848, 1997.
4. **Baker JE, Curry BD, Olinger GN, and Gross GJ.** Increased tolerance of the chronically hypoxic immature heart to ischemia. Contribution of the KATP channel. *Circulation* 95: 1278–1285, 1997.
5. **Bolli R.** Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. *J Mol Cell Cardiol* 33: 1897–1918, 2001.
6. **Bolli R.** The late phase of preconditioning. *Circ Res* 87: 972–983, 2000.
7. **Bolli R, Manchikalapudi S, Tang XL, Takano H, Qiu Y, Guo Y, Zhang Q, and Jadoon AK.** The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. Evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res* 81: 1094–1107, 1997.
8. **Cai Z, Manalo DJ, Wei G, Rodriguez ER, Fox-Talbot K, Lu H, Zweier JL, and Semenza GL.** Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. *Circulation* 108: 79–85, 2003.
9. **Engelman DT, Chen CZ, Watanabe M, Engelman RM, Rousou JA, Flack JE 3rd, Deaton DW, Maulik N, and Das DK.** Improved 4- and 6-hour myocardial preservation by hypoxic preconditioning. *Circulation* 92: II417–II422, 1995.
10. **Engelman DT, Watanabe M, Engelman RM, Rousou JA, Kisin E, Kagan VE, Maulik N, and Das DK.** Hypoxic preconditioning preserves antioxidant reserve in the working rat heart. *Cardiovasc Res* 29: 133–140, 1995.
11. **Grover GJ, Murray HN, Baird AJ, and Dzwonczyk S.** The KATP blocker sodium 5-hydroxydecanoate does not abolish preconditioning in isolated rat hearts. *Eur J Pharmacol* 277: 271–274, 1995.
12. **Imagawa J, Yellon DM, and Baxter GF.** Pharmacological evidence that inducible nitric oxide synthase is a mediator of delayed preconditioning. *Br J Pharmacol* 126: 701–708, 1999.
13. **Joyeux-Faure M, Stanke-Labesque F, Lefebvre B, Beguin P, Godin-Ribuot D, Ribuot C, Launois SH, Bessard G, and Lévy P.** Chronic intermittent hypoxia increases infarction in the isolated rat heart. *J Appl Physiol* 98: 1691–1696, 2005.
14. **Joyeux M, Arnaud C, Godin-Ribuot D, Demenge P, Lamontagne D, and Ribuot C.** Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts. *Cardiovasc Res* 55: 619–625, 2002.
15. **Karliner JS, Honbo N, Epstein CJ, Xian M, Lau YF, and Gray MO.** Neonatal mouse cardiac myocytes exhibit cardioprotection induced by hypoxic and pharmacologic preconditioning and by transgenic overexpression of human Cu/Zn superoxide dismutase. *J Mol Cell Cardiol* 32: 1779–1786, 2000.
16. **Ladilov Y, Maxeiner H, Wolf C, Schafer C, Meuter K, and Piper HM.** Role of protein phosphatases in hypoxic preconditioning. *Am J Physiol Heart Circ Physiol* 283: H1092–H1098, 2002.
17. **Miura T, Goto M, Urabe K, Endoh A, Shimamoto K, and Iimura O.** Does myocardial stunning contribute to infarct size limitation by ischemic preconditioning? *Circulation* 84: 2504–2512, 1991.
18. **Neckar J, Papousek F, Novakova O, Ost'adal B, and Kolar F.** Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. *Basic Res Cardiol* 97: 161–167, 2002.
19. **Neckar J, Szarszoi O, Koten L, Papousek F, Ost'adal B, Grover GJ, and Kolar F.** Effects of mitochondrial K(ATP) modulators on cardioprotection induced by chronic high altitude hypoxia in rats. *Cardiovasc Res* 55: 567–575, 2002.
20. **Peker Y, Hedner J, Kraiczi H, and Loth S.** Respiratory disturbance index: an independent predictor of mortality in coronary artery disease. *Am J Respir Crit Care Med* 162: 81–86, 2000.
21. **Prabhakar NR.** Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. *J Appl Physiol* 90: 1986–1994, 2001.
22. **Sasaki H, Fukuda S, Otani H, Zhu L, Yamaura G, Engelman RM, Das DK, and Maulik N.** Hypoxic preconditioning triggers myocardial angiogenesis: a novel approach to enhance contractile functional reserve in rat with myocardial infarction. *J Mol Cell Cardiol* 34: 335–348, 2002.
23. **Sun JZ, Tang XL, Park SW, Qiu Y, Turrens JF, and Bolli R.** Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pigs. *J Clin Invest* 97: 562–576, 1996.
24. **Takano H, Manchikalapudi S, Tang XL, Qiu Y, Rizvi A, Jadoon AK, Zhang Q, and Bolli R.** Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation* 98: 441–449, 1998.
25. **Takeshima S, Vaage J, and Valen G.** Preconditioning the globally ischaemic, isolated rat heart: the impact of the preconditioning model on post-ischaemic systolic and diastolic function. *Scand J Clin Lab Invest* 57: 637–646, 1997.
26. **Xi L, Tekin D, Gursoy E, Salloum F, Levasseur JE, and Kukreja RC.** Evidence that NOS2 acts as a trigger and mediator of late preconditioning induced by acute systemic hypoxia. *Am J Physiol Heart Circ Physiol* 283: H5–H12, 2002.
27. **Yamashita N, Hoshida S, Taniguchi N, Kuzuya T, and Hori M.** A "second window of protection" occurs 24 h after ischemic preconditioning in the rat heart. *J Mol Cell Cardiol* 30: 1181–1189, 1998.
28. **Yoshida K, Mizukami Y, and Kitakaze M.** Nitric oxide mediates protein kinase C isoform translocation in rat heart during postischemic reperfusion. *Biochim Biophys Acta* 1453: 230–238, 1999.
29. **Zhong N, Zhang Y, Zhu HF, Wang JC, Fang QZ, and Zhou ZN.** Myocardial capillary angiogenesis and coronary flow in ischemia tolerance rat by adaptation to intermittent high altitude hypoxia. *Acta Pharmacol Sin* 23: 305–310, 2002.
30. **Zhu HF, Dong JW, Zhu WZ, Ding HL, and Zhou ZN.** ATP-dependent potassium channels involved in the cardiac protection induced by intermittent hypoxia against ischemia/reperfusion injury. *Life Sci* 73: 1275–1287, 2003.