Chronic and Intermittent Hypoxia Induce Different Degrees of Myocardial Tolerance to Hypoxia-Induced Dysfunction

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Chronic hypoxia (CH) is believed to induce myocardial protection, but this is in contrast with clinical evidence. Here, we test the hypothesis that repeated brief reoxygenation episodes during prolonged CH improve myocardial tolerance to hypoxia-induced dysfunction. Male 5-week-old Sprague-Dawley rats (n = 7–9/group) were exposed for 2 weeks to CH (F_IO_2 = 0.10), intermittent hypoxia (IH, same as CH, but 1 hr/day exposure to room air), or normoxia (N, F_IO_2 = 0.21). Hearts were isolated, Langendorff perfused for 30 min with hypoxic medium (Krebs-Henseleit, PO_2 = 67 mmHg), and exposed to hyperoxia (PO_2 = 670 mmHg). CH hearts displayed higher end-diastolic pressure, lower rate-pressure product, and higher vascular resistance than IH. During hypoxic perfusion, anaerobic mechanisms recruitment was similar in CH and IH hearts, but less than in N. Thus, despite differing only for 1 hr daily exposure to room air, CH and IH induced different responses in animal homeostasis, markers of oxidative stress, and myocardial tolerance to reoxygenation. We conclude that the protection in animals exposed to CH appears conferred by the hypoxic preconditioning due to the reoxygenation rather than by hypoxia per se.

[Exp Biol Med Vol. 227(6):389–397, 2002]

Key words: chronic hypoxia; intermittent hypoxia; hypoxic preconditioning; isolated perfused heart; reoxygenation

Myocardial hypoxia is a common denominator in several human disorders, including coronary artery, cyanotic congenital heart, and obstructive pulmonary diseases, as well as stroke. All of these disorders jeopardize the heart's ability to provide the needed supply of O_2 and to adjust this supply to the changing needs of O_2, thereby entering a vicious cycle and exacerbating hypoxia. For example, despite extensive improvement, outcome of heart surgery in hypoxic neonates is often associated with high morbidity and mortality (1). As cyanotic infants or children have worse outcomes and more reperfusion injuries compared with non-cyanotic controls (2), previous exposure to chronic hypoxia (CH) is believed to be a risk factor. On an experimental ground, this view has been supported by a few studies (3–5). Yet at present, the chronically hypoxic heart is generally believed to be more tolerant to ischemia than the normal heart (6–12). Although the discrepancy between experimental and clinical evidences has been attributed from time to time to elusive factors such as different degrees of hypoxic compensation, different calcium handling properties, or varying extents of anaerobic metabolism recruitment, no clear explanation has been provided.

At present, no universal model is available to adequately mimic chronic heart perfusion with hypoxic blood. The most commonly used approach includes animal raising in hypoxic or hypobaric chambers for extended periods of time followed by sacrifice, heart excision and perfusion with oxygenated media, and application of ischemia as required. In this approach, there are at least two events that potentially lead to heart reoxygenation before it is intended. First, the design of the hypoxic or hypobaric chambers usually allows exposure of the animal to room air whenever the chamber is opened for maintenance. Second, to obtain the baseline values necessary to assess ischemia tolerance, hearts from hypoxic animals are often perfused with oxygenated media. Both of these events may induce some degree of reoxygenation injury before baseline values are

Funds were budgeted for the experimental research laboratory by the Department of Cardiovascular Surgery (Centre Hospitalier Universitaire Vaudois). This work was also supported in part by the Ministero dell' Istrvzibue, Università e Ricerca grant "Molecular mechanisms of the protection of the ischemic heart."

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Received November 5, 2001.
Accepted February 15, 2002.

1535-3702/02/2276-0389$15.00

taken. The reoxygenation injury, which is more severe in hypoxic than ischemic hearts (13), is a major factor that induces preconditioning, thereby potentially leading to apparently improved ischemia tolerance. However, as far as we know, the question of whether repeated *in vivo* reoxygenation episodes affect ischemia tolerance has not yet been addressed.

The aim of this study was to test the hypothesis that CH represents a risk factor with respect to post-hypoxia dysfunction, and that repeated reoxygenation episodes *in vivo* precondition the heart. For these purposes, we used a hypoxic chamber that prevents any exposure of the animal to room air during maintenance. The experimental protocol included the removal of the heart under hypoxia, and heart perfusion with a hypoxic medium, followed by hyperoxic perfusion. With this design, we monitored the myocardial changes during either the first oxygenation after continuous 2-week exposure to hypoxia, or the reoxygenation after hypoxia with repeated reoxygenation episodes (intermittent hypoxia, IH). In addition, we measured some markers of oxidative stress. We will show that although they differed by only 1 hr of daily exposure to room air, CH and IH induced different patterns of animal homeostasis and myocardial resistance to the reoxygenation-induced injury.

Materials and Methods

Animals. Male 5-week-old Sprague-Dawley rats (Lyon, France) were caged in the chambers described below for 2 weeks and were divided into three groups (n = 7–9 each). Control normoxic (N) rats breathed room air (F_IO_2 = 0.21). Rats exposed to CH breathed in a normobaric hypoxic (F_IO_2 = 0.10) atmosphere. Rats exposed to IH were subjected to the same treatment as CH rats, but were exposed to room air for 1 hr/day.

All animals had free access to water and conventional laboratory diet containing 90 mg/kg α-tocopherol until 24 hr before the experiment. Water and food consumption was assessed every 2 days. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Hypoxic Chambers. The design of the cage used in this study prevents exposure of the animals to room air during feeding, cleaning operations, and sacrifice (Fig. 1). The assembly is composed of two types of chambers. The hypoxic chamber, a 350 × 350 × 200-mm transparent plastic box for three animals each, is equipped with a single 165-mm diameter window with a plastic sleeve (Iris Sleeve; Nufer Medical, Gumligen, Switzerland). The compensation chamber, with the same dimensions of the hypoxic chamber, has two windows in the facing walls. Both chambers are independently flushed with gas containing 0.100 ± 0.001 O_2 (Carbagas, Lausanne, Switzerland). The O_2 tension inside the chambers is continuously monitored by an O_2 electrode (Servomex Oxygen Analyzer 570 A, Zurich, Switzerland) through a vent hole in the wall.

Before opening the hypoxic chamber to clean the cage, replenish food and water, or sacrifice an animal, the com-

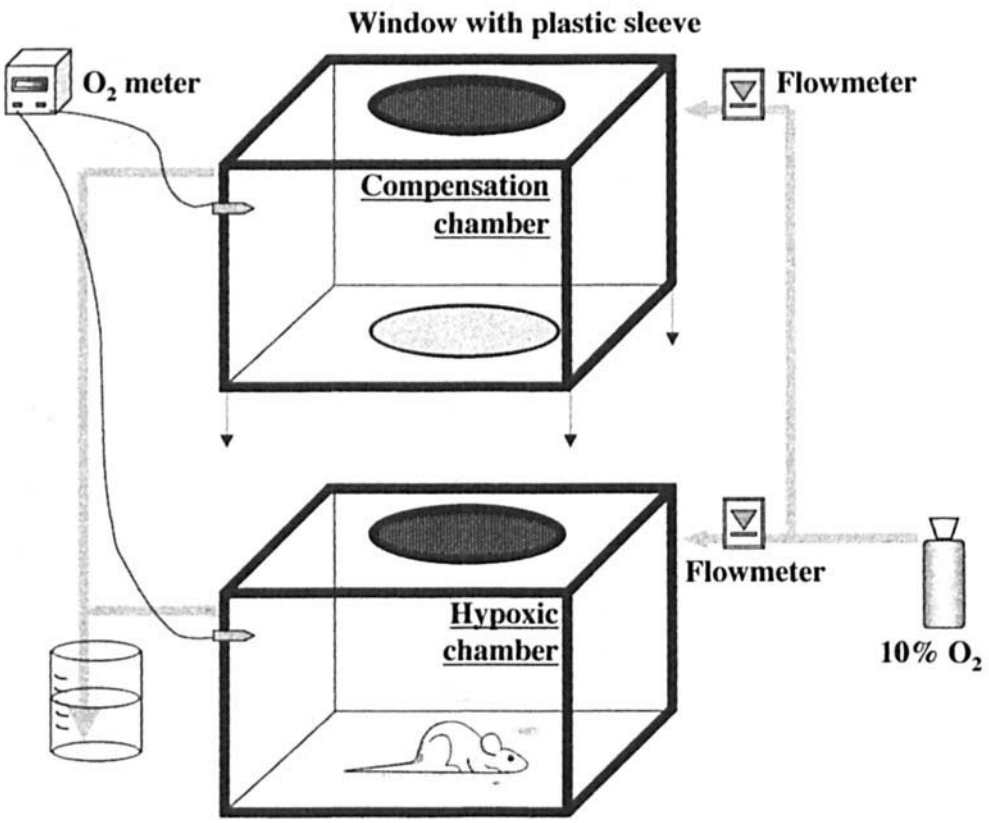

Figure 1. Sketch of the hypoxic and compensation chambers. The chambers (350 × 350 × 200 mm) were equipped with a 165-mm diameter window with a plastic sleeve. Before opening the hypoxic chamber for maintenance or operating the animal, the compensation chamber, previously flushed for 1 hr with the hypoxic gas, was placed over the hypoxic chamber with the windows adjacent one to the other. For sacrifice, the animal was transferred into the compensation chamber, the windows were closed, and the compensation chamber was moved near the perfusion apparatus.

pensation chamber is flushed for 1 hr with the hypoxic gas, and is then placed over the hypoxic chamber. The presence of guides enables positioning of the two chambers in order to get the windows exactly adjacent to each other. To access the animal, the operator inserts her/his arm through three sleeves in sequence (two in the compensating chamber and one in the hypoxic chamber). For sacrifice, the animal is transferred into the compensation chamber, the windows are closed, and the compensation chamber is moved near the perfusion apparatus. The presence of two sleeves in the facing walls, as well as the possibility to set the compensation chamber in the upright position over one of the walls, allows the operator to perform all of the following operations in the compensation chamber, which is continuously flushed with the hypoxic gas. The animal is first anesthetized, then the surgical instruments, a small dissection table, and a beaker containing saline previously bubbled with the hypoxic gas are introduced inside the chamber through one of the windows. The rat is immobilized over the dissection table, the chest is opened, a ligature is passed around the aorta, and the heart is excised and dropped into the beaker. The beaker is rapidly taken outside the chamber through one of the windows, the aorta is immediately mounted on the apparatus, and the heart is perfused with the hypoxic medium. The time required from heart excision through mounting on the perfusion apparatus is about 90 sec. With the described technique, the animals remain continuously exposed to 10% O_2, either in the hypoxic or compensation chamber, thereby avoiding any unwanted exposure to room air.

Heart Perfusion. Rats were anesthetized with an i.p. injection of sodium thiopental (10 mg/100 g body weight) and heparin (500 units). Hearts were rapidly excised and immersed in isotonic saline at ambient temperature (25°C). These operations were performed in the compensation chamber. The aorta was immediately cannulated and the heart was perfused at 37°C with hypoxic Krebs-Henseleit containing 2.0 m*M*/L free Ca^{2+} and 11 m*M*/L glucose, pH 7.33 ± 0.01. A roller pump (Ismatec SA; Labortechnik-Analytik, Glattbrugg-Zurich, Switzerland) delivered the medium (flow = 15 ml/min) to a 8-μm pore size, 47-mm diameter filter (MSI, Westboro, MA), a membrane oxygenator (Dideco, Mirandola, Italy), a pre-heater, and the cannula. The gas was provided from cylinders (Carbagas) containing O_2, CO_2, and N_2 in the following proportions: 0.10/0.06/0.84 or 0.94/0.06/0.00 for the hypoxic and hyperoxic perfusions (nominal accuracy 0.001), respectively. The temperature of the heart and of the perfusion medium was maintained at 37°C by an external water bath. A latex balloon filled with saline was introduced into the left ventricle and was connected to a pressure transducer (MPC-500; Millar Instruments Inc., Houston, TX) to monitor performance. An additional transducer was inserted above the aortic cannula to monitor the coronary perfusion pressure. A cannula was inserted into the pulmonary artery to collect the venous return and to monitor venous PO_2 by an O_2-sensing electrode (model 5300 Oxygen Monitor; Yellow Springs Inc., Yellow Springs, OH).

Measurements of Myocardial and Metabolic Function. Myocardial performance was recorded by a LabView system (National Instruments, Austin, TX) running on a PC. The measured parameters included the end-diastolic pressure (EDP), the heart rate (HR), the left-ventricle developed pressure (LVDP), the coronary perfusion pressure (CPP), and the venous PO_2. As the membrane oxygenator used in this study allows complete equilibration of the liquid phase with the gas (14), the arterial PO_2 was inferred from the nominal O_2 content in the gas cylinder. The O_2 consumption was calculated from this value, the measured venous PO_2, the coronary flow, and the O_2 solubility coefficient in water (15). The rate · pressure product (RPP, i.e., LVDP·HR) is an integrated index of myocardial performance. We did not pace hearts because pacing induces biochemical changes that interfere in this study (16). The vascular resistance was calculated as (CPP − EDP)/flow/(ventricle weight) (17). Samples of the venous effluent were taken at the end of the hypoxic and hyperoxic perfusions and were frozen at −80°C for lactate assay by enzymatic methods (COBAS FARA II; Hoffman-La Roche, Basel, Switzerland). When glucose is the only oxidizable substrate, the total turnover of ATP is calculated as (lactate release) + $(6 \cdot VO_2)$ (14).

Experimental Protocol. Hearts were subjected to a 30-min hypoxic perfusion (coronary flow = 15 ml/min, PO_2 = 67 mmHg). During this period, we measured the intraventricular balloon volume needed to increase EDP from 0 to 10 mmHg ($V_{0\rightarrow10}$), an index of ventricular volume. Afterward, the balloon volume was kept constant. After the hypoxic perfusion, hearts were oxygenated for 30 min (hyperoxic perfusion and coronary flow = 15 ml/min, PO_2 = 670 mmHg).

Blood. Immediately after heart mounting on the perfusion apparatus, a blood sample was withdrawn into a 5-ml heparinized tube. After mixing, the sample was divided into two aliquots. One was centrifuged (2500 rpm × 10 min) and plasma was stored at −80°C until use. In the other, hemoglobin concentration, hematocrit, and red blood cell count were measured (Abbott Cell-dyn 3500 R System, Baar, Switzerland).

In separate experiments, blood gases (ABL 700 analyzer; Radiometer, Copenhagen, Denmark) from femoral artery blood were measured in anesthetized rats.

Plasma Levels of Coenzyme Q and α-Tocopherol. The thawed plasma sample (0.5 ml) was mixed with 0.1 *M* Na-dodecilsulphate (0.5 ml), added with ethanol-isopropanol (2 ml, 95/5, v/v), and was extracted with n-hexane (4 ml) for 5 min while being continuously shaken in a vortex mixer. The mixture was centrifuged (1000 rpm × 2 min), and the upper phase was recovered and evaporated to dryness at 40°C under N_2. The residue was dissolved in ethanol (200 μl), and 40 μl of this solution was injected into a HPLC apparatus (Beckmann Gold, Fullerton, CA)

equipped with Chrompack column (ODS, 4.6 × 10 mm; Varian, Appleton, WI). The eluent (ethanol-methanol, 70/30, v/v) was flushed at 0.5 ml/min under isocratic conditions. Coenzyme Q_9 and α-tocopherol were detected photometrically at $\lambda = 275$ nm. α-Tocopherylacetate (Merck, Darmstadt, Germany) was the internal standard. The plasma levels of coenzyme Q_9 and α-tocopherol were calculated from the areas of the respective peaks by a four-point calibration curve obtained by injecting the standard mixture at known concentrations. The within-run and between-run coefficients of variation for this determination are 0.8% and 3.8%, respectively.

Morphological Measurements. At the end of the perfusion, hearts were removed from the perfusion apparatus, excess water was absorbed on tissue paper, and the heart mass was weighed. Atria were excised, and the free walls of the right and left ventricles as well as the septum were dissected free, dried at 90°C for 48 hr, and weighed separately. Right ventricular hypertrophy was assessed from the weight ratio (right ventricle)/(left ventricle + septum).

Statistics. Data are expressed as mean ± SE. The significance level was $P = 0.05$ (two-tailed). To detect significant differences among the three groups, we performed one-way analysis of variance (ANOVA). If significant, the differences between selected pairs of data were tested using the Fischer comparison procedure (StatView; Abacus Concepts, Berkeley, CA).

Results

Animal Homeostasis. Table I shows the changes induced by 2-week CH or IH. During normal maintenance, the O_2 level in the hypoxic chamber never increased by >1%. During sacrifice, the O_2 level in the compensation chamber never increased by >2%. The mortality rate was 3/12 and 2/9 for CH and IH, respectively. Arterial PO_2 ranged between 34 and 38 mmHg in both CH and IH rats vs. 60 and 70 mmHg in N rats.

Exposure to either CH or IH increased hematocrit, hemoglobin concentration, and red blood cell count by 70%. Hypoxia decreased food and water intake with respect to N. Food intake was not significantly different between IH and CH, but water intake was slightly higher in IH than CH. As a result, N rats experienced net weight gain in 2 weeks (+102 ± 11 g). In contrast, CH rats underwent weight loss (−51 ± 5 g), whereas IH rats neither gained nor lost weight (+4 ± 8 g). As the energy expenditure in caged rats can be assumed to be the same in all animals, and there was no significant point-by-point correlation between body weight changes and food or water intake (not shown), the efficiency in food assimilation should have been greater in IH rats.

Hearts weighed less in CH as compared with N and IH. The heart weight/body weight ratio, and thus hypertrophy, was higher in hypoxic animals (both CH and IH). The weight ratio (right ventricle)/(left ventricle + septum) increased significantly in all hypoxic animals, indicating right ventricular hypertrophy. The balloon volume needed to increase EDP from 0 to 10 mmHg ($V_{0\rightarrow10}$, an index of the left ventricular volume) was less in IH than CH and N hearts.

Myocardial Performance. We previously tested the apparatus by perfusing hearts ($n = 6$) under aerobic conditions ($PO_2 = 670$ mmHg, flow = 15 ml/min) for 110 min: the changes of LVDP, HR, and EDP accounted for less than 8.3 ± 4.3 mmHg, 22 ± 10 min^{-1}, and 1.2 ± 0.9 mmHg, respectively (18). Absolute values for those parameters were 113 ± 1 mmHg, 270 ± 4 min^{-1}, and 10.2 ± 0.1 mmHg, respectively.

Because the balloon volume was fixed at the start of the perfusion to yield EDP = 10 mmHg, EDP was the same in the three groups during the hypoxic perfusion (Fig. 2). The oxygenation of CH hearts did not immediately alter EDP; however, the changes became evident >10 min after the onset of hyperoxia. The reoxygenation of IH and N hearts decreased EDP more markedly in IH than N. At the end of the hyperoxic perfusion, the difference in EDP between N

Table I. Blood and Morphological Data in Animals at the End of the 2-Week Exposure to Either Chronic or Intermittent Hypoxia ($F_IO_2 = 0.10$)

	Normal	Chronic hypoxia	Intermittent hypoxia
n	9	9	7
Initial body weight (g)	249 ± 18	238 ± 2	245 ± 2
Hematocrit	0.40 ± 0.02	0.69 ± 0.02[a]	0.68 ± 0.01[a]
Hemoglobin (g/l)	124 ± 4	219 ± 5[a]	215 ± 2[a]
Red blood cell count (RBC/μl/1000)	6.32 ± 0.18	10.34 ± 0.24[a]	9.97 ± 0.30[a]
Food intake (g/day)	30.7 ± 1.3	21.3 ± 1.9[a]	17.9 ± 1.1[a]
Water intake (ml/day)	50.4 ± 4.9	19.9 ± 0.9[a]	22.5 ± 0.7[a,b]
Final body weight (g)	351 ± 13	187 ± 4[a]	250 ± 7[b]
Heart weight (mg)	1312 ± 43	1111 ± 50[a]	1407 ± 56[b]
Heart weight/body weight (mg/g)	3.74 ± 0.04	5.95 ± 0.28[a]	5.63 ± 0.10[a]
Balloon volume to increase EDP from 0 to 10 mmHg (μL)	75.65 ± 6.45	81.29 ± 7.63	56.38 ± 5.69[b]
Right ventricle/left ventricle + septum	0.33 ± 0.02	0.59 ± 0.03[a]	0.59 ± 0.02[a]

Note. Data are mean ± SE.
[a] Significant difference versus normal.
[b] Significant difference versus chronic hypoxia ($P < 0.05$, ANOVA and Fischer's post-test).

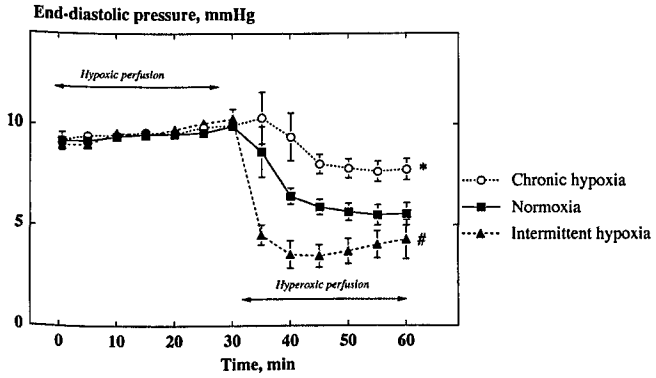

Figure 2. CH impairs the diastolic relaxation following (re)oxygenation. End-diastolic pressure in hearts from rats exposed to normal conditions (■), CH (○), and IH (▲), during the hypoxic and hyperoxic perfusions. An asterisk indicates significant difference versus normal; A pound symbol indicates significant difference versus CH ($P < 0.05$, ANOVA and Fischer' post-test). The statistics was evaluated at the end of the hypoxic and hyperoxic perfusion steps.

and IH hearts vanished ($P = 0.11$). Thus, EDP was higher in CH than IH and N hearts.

The rate · pressure product was the same in the three groups during the hypoxic perfusion (Fig. 3). HR and LVDP were 157 ± 8 min^{-1} and 52 ± 3 mmHg, respectively. During the hyperoxic perfusion, HR increased by 31.9% ± 2.2% in all groups. In contrast, LVDP increased by 22.2 ± 3.8 mmHg in CH hearts vs. 32.6 ± 5.7 and 39.7 ± 4.9 in N and IH, respectively. As a result of these changes, CH hearts appeared less protected than IH and N hearts with respect to the systolic performance.

Vascular resistance describes the vascular adjustments in hearts with various degrees of hypertrophy (Fig. 4). Resistance remained steady throughout the hypoxic perfusion in the order of N>CH>IH. The hyperoxic perfusion increased resistance to a larger extent in N and CH hearts than in IH hearts (inset of Fig. 4). Thus, at the end of the hyperoxic perfusion, the resistance ranked N>CH>IH as during the hypoxic perfusion, but with more marked differences.

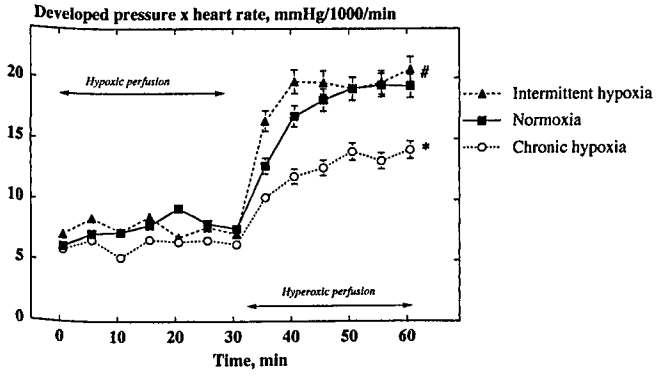

Figure 3. CH impairs the recovery of the rate · pressure product following (re)oxygenation. Myocardial contractility (rate · pressure product) in hearts from rats exposed to normal conditions (■), CH (○), and IH (▲) during the hypoxic and hyperoxic perfusions. See legend of Figure 2 for other details.

Myocardial Bioenergetics. Bioenergetics were evaluated only at the end of the hypoxic and hyperoxic perfusions when venous lactate was sampled (Fig. 5). The venous PO_2 at the end of the hypoxic perfusion ranged between 0 and 3 mmHg in all groups. After (re)oxygenation, venous PO_2 ranked CH>IH>N. Venous lactate concentration at the end of the hypoxic perfusion was the highest in N hearts, intermediate in IH, and lowest in CH hearts. After (re)oxygenation, lactate was undetectable in all groups.

Figure 6, which summarizes myocardial bioenergetics, shows the anaerobic, e.g., lactate release, and aerobic, e.g., O_2 consumption, contributions to total ATP production. During the hypoxic perfusion, the O_2 consumption was the same in all the groups. Thus, the higher ATP production in N hearts reflects their higher lactate release. After the (re)oxygenation, the lactate release was virtually zero for all the hearts. Although both the O_2 consumption and ATP turnover were the lowest for IH, in CH hearts, these parameters were ranked between N and IH.

Figure 7 summarizes the findings relative to coenzyme Q (Q_9 is the predominant form in rodents) and α-tocopherol. Coenzyme Q level in plasma was nearly doubled in CH than IH and N rats, whereas α-tocopherol remained constant in all the groups.

Discussion

Animal Changes. The F_IO_2 selected for hypoxia experiments, which is equivalent to 5500 m altitude (19), yields an arterial PO_2 not far from the trigger for surgical intervention in pediatric cardiac surgery (conventionally set at 30 mmHg). The increased hematocrit, hemoglobin concentration, and red blood cell count, common findings in hypoxic studies, are due to increased hypoxia-induced erythropoietin production (20). The blood parameters were similar in CH and IH, thus the erythropoietic response was not influenced by repeated reoxygenation episodes.

In IH rats, hypoxia-induced deterioration could offset the normally expected weight gain. In contrast, CH rats, despite unlimited access to food and water, underwent weight loss, as in humans exposed to high altitude (21). Hypoxia exposure induces right ventricular hypertrophy as a consequence of pulmonary hypertension (22). Table I shows that despite different body weights, right ventricular hypertrophy was the same in CH and IH animals.

The oxidative stress was greater in IH than CH rats (Fig. 7). The total (oxidized and reduced) plasma level of coenzyme Q is under consideration as a potential biomarker of oxidative stress (23). Coenzyme Q is, among lipophylic antioxidants, the only one produced by endogenous biosynthesis. Its plasma level is the result of a balance between biosynthesis and destruction by peroxidative phenomena or elimination (24). Increased coenzyme Q level in CH rats is consistent with high values observed in hypoxemic fetuses (25) and the faster biosynthesis early during altitude adaptation (26). Coenzyme Q is known to reduce tocopheryl

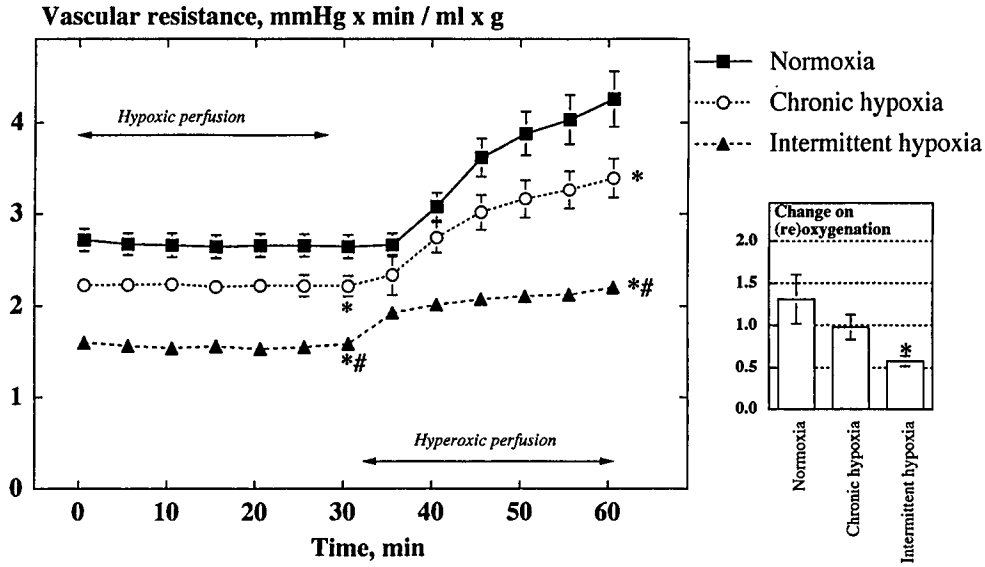

Figure 4. IH impairs the vascular resistance following (re)oxygenation. Vascular resistance in hearts from rats exposed to normal conditions (■), CH (○), and IH (▲) during the hypoxic and hyperoxic perfusions. See legend of Figure 2 for other details. The inset reports the change in vascular resistance upon (re)oxygenation.

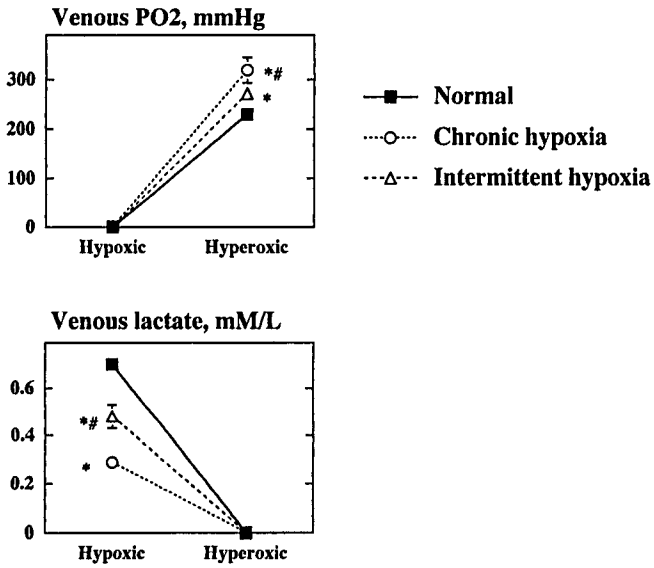

Figure 5. CH increases venous PO_2 during hyperoxia and decreases venous lactate during hypoxia. Venous PO_2 and venous lactate concentration in hearts from rats exposed to normal conditions (■), CH (○), and IH (△) at the end of the hypoxic and hyperoxic perfusions. See legend of Figure 2 for other details.

radical and semidehydroascorbate back to tocopherol and ascorbate, thereby acting as antioxidant (27). Its low level in IH rats may reflect either its utilization to maintain the plasma level of α-tocopherol or its internalization in the cells to contrast the reoxygenation (24). Being available only from dietary sources, α-tocopherol did not change appreciably among the groups in our study, despite reduced food intake in CH and IH rats, in agreement with the observed decrease in coenzyme Q, as opposed to maintained level of α-tocopherol, in CCl_4-treated rats (23).

Isolated Heart Perfusion. Hearts were isolated and Langendorff-perfused with a blood-free medium according to an established technique. The described set-up rules out all the potentially confusing blood-related responses, including the presence of hormones and coagulation factors, lymphocyte-mediated inflammatory processes, uneven spacing of red cells within capillaries, hematocrit heterogeneity, and hypoxia-induced changes in the hemoglobin-O_2 affinity. Absence of hemoglobin reduces the blood O_2 capacity, but high PO_2 and flow partially compensated for that reduction. The accurate determination of O_2 delivery to heart under the various conditions used in this study requires knowledge of variables that were not measured, i.e., hemoglobin-O_2 saturation in arterial and coronary sinus blood, or myocardial O_2 extraction. However, the selected experimental conditions represent a first approach to unravel the response of hypoxic hearts to abrupt increase in O_2 supply.

The inter-group differences in cardiac weight, hypertrophy, ventricular volume, and body weight might mismatch the initial perfusion conditions. However, the employed perfusion protocol could reduce the impact of those variables. First, the balloon volume was set for each heart to a value that yielded the same EDP, irrespective of the group. Thus, myocardial load and performance were the same in all the groups during the initial phase, and the performance changes in response to abrupt (re)oxygenation could therefore be monitored. Second, other critical parameters such as vascular resistance, lactate release, O_2 consumption, and ATP turnover are expressed in relation to heart weight.

The lesser $V_{0\rightarrow10}$ in IH hearts might be explained as a result of increased diastolic stiffness or decreased left ventricular volume. As the ventricle volume/heart weight ratio was 65.9 ± 7.7 and 41.0 ± 4.5 μl/mg (P = 0.007) in CH and IH hearts, respectively, it is likely that CH hearts develop hypertrophy accompanied by dilatation, whereas IH hearts show signs of hypertrophy only.

Myocardial Performance. Although hypoxic perfusion did not reveal striking differences among the groups, the (re)oxygenation induced different responses in CH, IH, and N hearts. In the Langendorff heart with fixed-volume

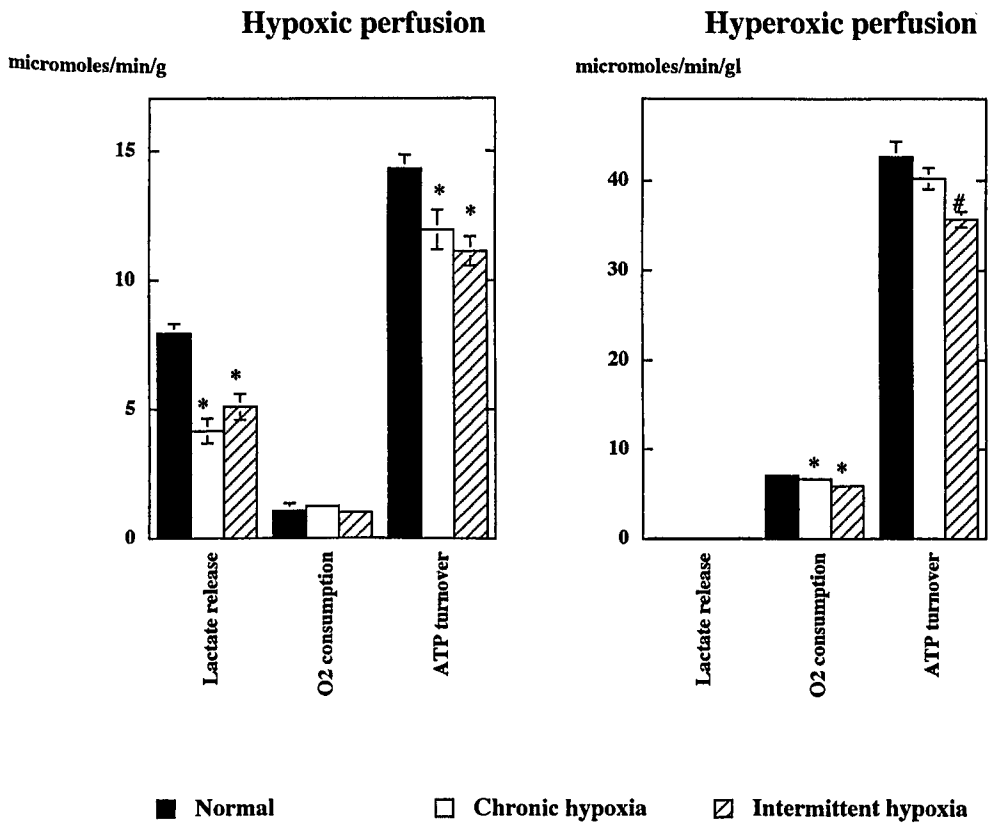

Figure 6. The aerobic and anaerobic contributions to ATP turnover. Lactate release (proportional to the anaerobic contribution), O_2 consumption (proportional to the aerobic contribution), and total ATP turnover in the various groups during the hypoxic baseline and (re)oxygenation. An asterisk indicates significant difference versus normal; a pound symbol indicates significant difference versus CH ($P < 0.05$, ANOVA and Fischer's post-test).

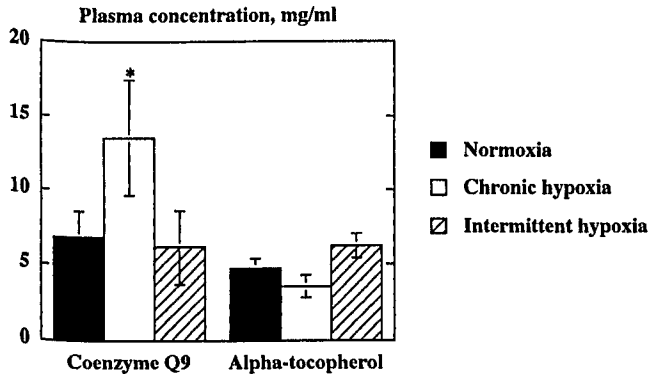

Figure 7. Plasma levels of coenzyme Q and α-tocopherol. Exposure to CH increases plasma coenzyme Q_9, but α-tocopherol remains constant. An asterisk indicates significant difference versus normal ($P < 0.05$, ANOVA and Fischer's post-test).

intraventricular balloon, higher EDP indicates impaired diastolic compliance. Thus, diastolic performance was more protected in IH than N and CH hearts. The EDP change during (re)oxygenation represents a compromise between two contrasting effects. On one hand, reoxygenation of hypoxic hearts reverts the hypoxia-induced Ca^{2+} load (28), thereby leading to EDP decrease. On the other hand, the oxidative stress associated with hypoxia reoxygenation leads to diastolic contracture in this model (14). It is difficult to assess the relative contribution of these effects, but in IH and N hearts, the deleterious effect of the oxidative stress was apparently less than in CH hearts. Perhaps the repeated reoxygenation episodes induced a greater antioxidant protection in the IH hearts.

In analogy to diastolic performance, myocardial contractility too was more protected upon (re)oxygenation in IH and N than CH hearts. Again, performance improvement due to restoration of aerobic mechanisms with highly efficient energy-yielding paths is challenged by the oxidative stress derived from hypoxia reoxygenation. Apparently, this compromise was more favorable in IH and N than CH hearts.

Low resistance in IH and CH hearts shows that the effect of hypoxia-induced vasodilation, probably led by increased NO release (29), persisted in isolated hearts. Rapid switch from *in vivo* high-hematocrit blood perfusion to *in vitro* blood-free perfusion might have induced reactive hyperemia that potentially disturbs vascular resistance data. However, hematocrit and blood viscosity as well as hypertrophy were the same in CH and IH rats. Yet, the response of IH and CH hearts was different. Therefore, such differences cannot be ascribed to abrupt changes in perfusion conditions, but must be due to inherent differences between CH and IH hearts. As above, we believe that oxidative stress following repeated reoxygenation episodes in IH hearts may induce protection.

Bioenergetic Metabolism. The very low venous PO_2 in all groups during hypoxic perfusion indicates that hearts extracted all available O_2, in agreement with previous data obtained under acute hypoxia (14). We calculated the relative contributions of aerobic and anaerobic mechanisms

to the ATP production during hypoxic perfusion from lactate release and O_2 consumption, assuming ATP/lactate ratio = 1.0 (glucose as substrate without significant glycogenolysis), and ATP/O_2 = 6 (no mitochondrial uncoupling). In the isolated rat heart, and in the absence of triglycerides in the perfusion medium, the contribution of intracellular triglyceride stores to ATP production is very small (30). With these assumptions, the contribution of aerobic mechanisms to total ATP turnover was 45% ± 1%, 66% ± 2%, and 55% ± 2% in N, CH, and IH hearts, respectively. Thus, IH and CH induced differential patterns of hypoxic adaptation. Yet, the performance of N, CH, and IH hearts was the same (Fig. 3). Further studies are needed to understand this feature, but the increased mitochondrial oxidative mechanisms in hypoxic hearts might result from hypoxia adaptation, which increases size and capacity of mitochondria (31). In contrast, the lower contribution of aerobic mechanisms in IH versus CH hearts might be a consequence of mitochondrial damage secondary to increased oxidative stress. As a matter of fact, during the hyperoxic perfusion, when hearts rely almost entirely on aerobic mechanisms, the ATP turnover is slightly less in IH than CH and N hearts, probably reflecting the mitochondrial damage led by repeated reoxygenation episodes.

Clinical Implications. In this study, we show that repeated reoxygenation episodes protect hearts against hypoxia-reoxygenation, whereas continuous CH does not. This result is related to the hypoxia effect on ischemia tolerance. Most of the studies on this issue support a protective role for hypoxia. In a few of them, however, it was reported that animals were repeatedly reoxygenated during hypoxia due to chamber opening for maintenance (6, 7). In another study, it was declared that hearts were perfused with hyperoxic media, thereby undergoing reoxygenation, before baseline measurements (8). Still in other studies, both operations were performed (9–12). In the light of the present data, the improved tolerance to ischemia, a common finding in these studies, can be attributed to hypoxic preconditioning due to reoxygenation rather than to hypoxia per se. As a matter of fact, in the experimental studies supporting the deleterious effects of hypoxia, heart reoxygenation before baseline was prevented by surgically installing a cardiac bypass (3–5).

Hypoxia-induced erythropoietin was recently proposed as a local protective factor (32, 33). Although we have not measured erythropoietin in this study, Table I shows that the erythropoietic response was the same in CH and IH rats. In addition, there is no proof that myocardial tissue produces erythropoietin. Therefore, it is difficult to support a protective function of erythropoietin in hearts (34). Rather, differential response to CH and IH of mechanisms known to protect hearts, as activation of K_{ATP} channels (35) and recruitment of signaling transduction pathways (36) should be critically evaluated. It was also recently reviewed (37) that IH, although with different times and modes than those employed in this study, is more effective than CH in activating activator protein-1 and hypoxia-inducible factor-1.

Conclusion. Despite differing by only a 1-hr daily exposure to room air, CH and IH induced different responses both at the level of whole animal homeostasis and myocardial tolerance to reoxygenation-induced injury. The tolerance to hyperoxic perfusion was impaired in CH hearts, but improved in IH hearts. This behavior is consistent with preconditioning exerted by *in vivo* repeated reoxygenation episodes.

The authors acknowledge Prof. L. B. Gladden (Auburn University, Auburn, AL) for his useful suggestions in the preparation of the manuscript, and Dr. L. Colacicco (Universita' Cattolica del S. Cuore, Rome, Italy) for his skilful technical help in the assay of coenzyme Q and α-tocopherol. The authors are grateful to Dideco (Mirandola, Italy) for providing the membrane micro-oxygenators.

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