Responses of chronically hypoxic rat hearts to ischemia:

K<sub>ATP</sub> channel blockade does not abolish increased RV tolerance to ischemia

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Forkel, Joerg, Xiaochao Chen, Susanne Wandinger, Florian Keser, Alexey Duschin, Uwe Schwanke, Stilla Frede, Parwis Massoudy, Rainer Schulz, Heinz Jakob, and Gerd Heusch. Responses of chronically hypoxic rat hearts to ischemia: K<sub>ATP</sub> channel blockade does not abolish increased RV tolerance to ischemia. Am J Physiol Heart Circ Physiol 286: H545–H551, 2004. First published October 9, 2003; 10.1152/ajpheart.00022.2003.—Chronic hypoxia may precondition the myocardium and protect from ischemia-reperfusion damage. We therefore examined the recovery of left and right ventricular function after ischemia and reperfusion (15 min each) in isolated blood-perfused working hearts from normoxic (Norm) and hypoxic (Hypo; 14 days, 10.5% O<sub>2</sub>) adult rats. In addition, the mRNA expression of hypoxia-inducible factor (HIF)-1α and the protein expression of endothelial nitric oxide synthase (eNOS) were measured. Postischemic left ventricular function recovered to 66 ± 6% and 67 ± 5% of baseline in Norm and Hypo, respectively. In contrast, postischemic right ventricular function was 93 ± 2% of baseline in Hypo vs. 67 ± 3% in Norm (P < 0.05). Improved postischemic right ventricular function in Hypo (93 ± 2% and 96 ± 2% of baseline) was observed with 95% O<sub>2</sub> or 21% O<sub>2</sub> in the perfusate, and it was not attenuated by glibenclamide (5 and 10 µmol/l) (86 ± 4% and 106 ± 6% recovery). HIF-1α mRNA and eNOS protein expression were increased in both left and right hypoxic ventricles. In conclusion, postischemic right, but not left, ventricular function was improved by preceding chronic hypoxia. ATP-sensitive K<sup>+</sup> channels are not responsible for the increased right ventricular tolerance to ischemia after chronic hypoxia in adult rat hearts.

chronic hypoxia: glibenclamide; hypoxia-inducible factor-1α; endothelial nitric oxide synthase

ISCHEMIC PRECONDITIONING protects myocardium against subsequent ischemia-reperfusion injury (19). Protection is evident by limitation of infarct size (19, 22), better recovery of myocardial contractile function in some models (8), preservation of high-energy phosphate levels (13), and attenuation of arrhythmias (23). ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels are crucially involved in the protection of ischemic preconditioning (10).

Chronic hypoxic preconditioning also increases the tolerance of the myocardium to ischemia. In an isolated adult rat heart model, chronic hypoxia improved posts ischemic systolic function and protected against prolonged periods of ischemia (27). Although similarities of the phenomena of hypoxic and ischemic preconditioning have been found in protecting the myocardium, the additive cardioprotective effects of hypoxic and ischemic preconditioning suggest different mechanisms of protection (27).

Baker et al. (3, 4) reported that the increased tolerance to ischemia in isolated crystalloid-perfused immature rabbit hearts exposed to chronic hypoxia is associated with an activation of the K<sub>ATP</sub> channels. The beneficial effects of chronic hypoxic preconditioning on left and right ventricular (RV) developed pressure were abolished by the K<sub>ATP</sub> channel antagonist glibenclamide. The K<sub>ATP</sub> channel agonist bimakalim enhanced posts ischemic left ventricular (LV) function in normoxic hearts similar to that of hypoxic hearts (3). As in ischemic preconditioning, these results suggested a role for the K<sub>ATP</sub> channel in hypoxic preconditioning.

Because the protection by ischemic preconditioning and by chronic hypoxia is discussed to be age dependent (2), we now tested the protection afforded by chronic hypoxia in blood-perfused working adult rat hearts. The role of K<sub>ATP</sub> channels was investigated using the K<sub>ATP</sub> channel antagonist glibenclamide.

Hypoxia-inducible factor (HIF)-1 mediates a compensatory response to decreases in oxygen tension (24) leading to a characteristic pathophysiological phenotype with pulmonary polycythemia, hypertension, and RV hypertrophy (14). LV function of isolated working hearts from HIF-1α wild-type mice was preserved after ischemia-reperfusion after chronic hypoxia, whereas no cardioprotection after chronic hypoxia and ischemia-reperfusion was seen in HIF-1α<sup>+/-</sup> heterozygous mice. These results suggested a role for HIF-1α in hypoxic preconditioning (6). We expected the expression of HIF-1α to relate to the different responses of right and left ventricles to chronic hypoxia and therefore measured HIF-1α mRNA.

Cardioprotective effects after ischemia-reperfusion have also been described for nitric oxide (NO), with posts ischemic application of a NO donor in isolated guinea pig hearts (16). In chronic hypoxic isolated immature rabbit hearts, increased production of NO and limited superoxide anion generation have been found and correlated with a better LV tolerance to ischemia (25). We therefore also assessed the endothelial NO synthase (eNOS) expression in normoxic and hypoxic left and right ventricles.

MATERIALS AND METHODS

Animals. Male Wistar rats (80 to 90 days old) weighing 230 to 460 g were used. Experimental protocols used in this study were approved by the Bioethical Committee of the district of Düsseldorf and adhere to the guiding principles of the American Physiological Society. To make them hypoxic, the rats were housed in a chamber for...
and hypoxic (n = 3 each) conditions (Table 1). O2 and CO2 tension were measured by an automatic detector (ABL System 615, Radiometer; Copenhagen, Denmark). Furthermore, immediately before cannulation for isolated heart experiments, blood samples were drawn from the aorta with a small needle, and hematocrit and hemoglobin content were assessed by the same automatic detector (Table 2). In additional experiments, the hearts of animals exposed to chronic hypoxia or normoxia were isolated, and right and left ventricles were dissected and weighed separately.

Perfusion solutions. A red blood cell perfusate was prepared, consisting of human red blood cells at a final hematocrit of 30%. The perfusion buffer used was a modified Krebs-Henseleit bicarbonate solution (in mmol/l: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 2.2 CaCl2, 10 glucose, and 5.0 pyruvate). Human serum albumin was added at a final concentration of 50 mg/ml. White cell-depleted human donor blood was centrifuged at 4°C with 3,000 revolutions/min for 10 min. The supernatant was removed and the resulting packed cells were mixed 1:1 with Krebs-Henseleit buffer. The centrifugation and resuspension steps were repeated three times. After the red blood cells were prepared, the perfusate was immediately used for the experiment.

Isolated rat heart preparation. The rats were anesthetized with enflurane inhalation. Heparin sodium (1,000 U, Braun; Melsungen, Germany) was administrated intraperitoneally 1 h before anesthesia. The chest was opened, the ascending aorta was cannulated, and retrograde Langendorff perfusion (15) of the nonworking heart was initiated with a red blood cell solution maintained at 37°C and at a constant pressure of 80 mmHg. Thereafter, the hearts were rapidly excised and perfused in a closed recirculating system at 37°C with a membrane oxygenator (Lilliput 1 D901, Dideco; Mirandola, Italy) in contact with 95% O2 -5% CO2 or 21% O2 -5% CO2 -74% N2 gas mixtures to achieve a PO2 of the red cell perfusate between 500 and 600 mmHg (for 95% O2) or 100 and 130 mmHg (for 21% O2). Two different O2 concentrations were used to investigate a potential influence of normoxic as opposed to hyperoxic reoxygenation.

During the initial retrograde Langendorff perfusion, hearts were trimmed of excess tissue, and superior and inferior caval veins were ligated. In left heart preparations, the left atrium was cannulated via the pulmonary venous orifices. A cannula was placed into the main pulmonary artery to permit drainage of the coronary sinus effluents. The hearts were switched to the working mode (20) after 15 min of aerobic, nonworking, and retrograde aortic perfusion and were perfused at a left atrial preload of 10 cmH2O and an aortic afterload of 75 mmHg. During the working mode, heart rate and peak systolic pressure were recorded with the use of a physiological recording system with CORDAT II software (26) and a pressure transducer (model SP 2202, Statham). The flowmeter was placed around the pulmonary artery to determine coronary flow throughout the experiment.

For right heart preparations, openings of the superior vena cava and the thoracic veins were ligated. The inferior vena cava was cannulated. This catheter was connected to a fluid-filled column and the preload was adjusted to 10 cmH2O. A cannula was placed into the main pulmonary artery and tied securely. The pulmonary cannula was connected to a fluid-filled column, where pulmonary afterload was adjusted to 15 mmHg. RV cardiac output and coronary flow were measured using flowmeters (model SP 2202, Statham) placed around the main pulmonary and aortic canulas. After 15 min of Langendorff perfusion, the RV working mode (28) was initiated.

Experimental protocols. Right and left heart preparations underwent the same perfusion protocol. Two periods of perfusion in working mode, W1 (15 min) and W2 (15 min), were separated by 15 min of global warm ischemia, with hearts bathed in 37°C Krebs-Henseleit buffer, and 15 min of reperfusion in a nonworking Langendorff mode.

The rat hearts were assigned to the following 11 groups: group 1, normoxic hearts performing LV work, gassed with 95% O2 (n = 7); group 2, hypoxic hearts performing LV work, gassed with 95% O2 (n = 8); group 3, normoxic hearts performing RV work, gassed with 95% O2 (n = 8); group 4, hypoxic hearts performing RV work, gassed with 95% O2 (n = 6); group 5, normoxic hearts performing RV work, gassed with 21% O2 (n = 6); group 6, hypoxic hearts performing LV work, gassed with 21% O2 (n = 4); group 7, normoxic hearts performing RV work, gassed with 21% O2 (n = 6); group 8, hypoxic hearts performing RV work, gassed with 21% O2 (n = 6); group 9, normoxic hearts performing RV work + 5 μmol/l glibenclamide, gassed with 21% O2 (n = 6); group 10, hypoxic hearts performing RV work + 5 μmol/l glibenclamide, gassed with 21% O2 (n = 12); and group 11, hypoxic hearts performing RV work + 10 μmol/l glibenclamide, gassed with 21% O2 (n = 4).

In groups 9–11, glibenclamide (5 and 10 μmol/l) was put into the perfusate 15 min before ischemia. Hemodynamic variables were determined at the end of W1 and at the end of W2 (coronary flow, aortic and pulmonary flow, and heart rate). Preischemic cardiac output of left hearts ranged between 31 to 85 ml/min (average 51 ± 3 ml/min). Preischemic cardiac output of right hearts ranged between 22 and 99 ml/min (average 57 ± 3 ml/min). Pre- and posts ischemic external heart work was computed as the product of mean aortic or pulmonary pressure, total output, and a conversion factor (1.33322 × 10−4) for expression in Joules per minute (9). Posts ischemic recovery was defined as posts ischemic external heart work over preischemic external heart work and expressed in percent.

Extraction and quantification of HIF-1α mRNA. Hearts from control (n = 10) and chronically hypoxic animals (n = 10) were removed, and the RVs and LVs were separated and immediately frozen in liquid nitrogen. Frozen myocardial tissue was homogenized in 4 M guanidine thiocyanate containing 0.1% β-mercaptoethanol. Total RNA was isolated by acid phenol-chloroform extraction (7) and redissolved in water. RNA concentration was determined by measurement of optical density at 260 nm. Total RNA (1 μg) was reversely transcribed into cDNA using oligo(dT)15 as a primer for RT (AMV RT, Promega; Heidelberg, Germany). Quantification of HIF-1α cDNA was carried out by real time PCR (Gene Amp 5700, PE Applied Biosystems;
Table 3. Left and right ventricular heart weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Normoxic LV</th>
<th>Normoxic RV</th>
<th>Hypoxic LV</th>
<th>Hypoxic RV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>80.8±0.7</td>
<td>19.2±0.7</td>
<td>77.8±0.5*</td>
<td>22.1±0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. LV, left ventricle; RV, right ventricle. Left and right ventricular heart weight in percentage of total heart weight under normoxic and hypoxic conditions (n = 7) are shown. *P < 0.05 vs. normoxic group (Student’s t-test for unpaired samples).

Chronic hypoxic and postischemic therapy.

Hypoxic conditions were created by incubation of rats in hypoxic chambers (10% O2, 90% N2) with continuous monitoring of oxygen saturation. The hypoxic condition was maintained for 2 weeks before ischemic and/or reperfusion experiments were performed.

Table 4. Preischemic and postischemic hemodynamic values

<table>
<thead>
<tr>
<th>Group</th>
<th>CF, ml/min</th>
<th>CO, ml/min</th>
<th>HR, beats/min</th>
<th>EHW, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Norm LV + 95% O2</td>
<td>7</td>
<td>5.2±0.8</td>
<td>45.7±5.6</td>
<td>208±32a</td>
</tr>
<tr>
<td>2: Hyp LV + 95% O2</td>
<td>8</td>
<td>8.5±0.9</td>
<td>51.9±6.6</td>
<td>232±10</td>
</tr>
<tr>
<td>3: Norm RV + 95% O2</td>
<td>4</td>
<td>8.7±0.1</td>
<td>47.9±8.9</td>
<td>285±21</td>
</tr>
<tr>
<td>4: Hyp RV + 95% O2</td>
<td>6</td>
<td>6.4±0.9</td>
<td>47.2±3.6</td>
<td>263±13</td>
</tr>
<tr>
<td>5: Norm LV + 21% O2</td>
<td>6</td>
<td>8.8±1.0</td>
<td>58.4±7.5</td>
<td>227±16</td>
</tr>
<tr>
<td>6: Hyp LV + 21% O2</td>
<td>4</td>
<td>8.7±1.5</td>
<td>45.6±5.2</td>
<td>187±20</td>
</tr>
<tr>
<td>7: Norm RV + 21% O2</td>
<td>6</td>
<td>5.4±1.0</td>
<td>59.0±6.3</td>
<td>291±13</td>
</tr>
<tr>
<td>8: Hyp RV + 21% O2</td>
<td>6</td>
<td>9.6±1.6</td>
<td>72.3±2.4</td>
<td>290±11</td>
</tr>
<tr>
<td>9: Norm RV, 5 μmol/l Glib, 21% O2</td>
<td>6</td>
<td>9.0±1.8</td>
<td>32.7±5.4b</td>
<td>255±20</td>
</tr>
<tr>
<td>10: Hyp LV, 5 μmol/l Glib, 21% O2</td>
<td>12</td>
<td>9.3±2.0</td>
<td>37.6±4.2</td>
<td>272±10</td>
</tr>
<tr>
<td>11: Hyp LV, 10 μmol/l Glib, 21% O2</td>
<td>4</td>
<td>5.0±1.5</td>
<td>66.3±10.2</td>
<td>289±16</td>
</tr>
</tbody>
</table>

Postischemic treatment and terror biomechanical.

Postischemic treatment was performed in all experimental groups. The animals were anesthetized with sodium pentobarbital (60 mg/kg), and the thoracic cavity was opened. The hearts were perfused ex vivo with a modified Krebs-Henseleit buffer containing 85 mmol/l NaCl, 1.25 mmol/l KCl, 1.2 mmol/l CaCl2, 1.25 mmol/l MgCl2, 10 mmol/l NaHCO3, and 5.5 mmol/l glucose, adjusted to pH 7.4 with NaOH. The hearts were perfused at a constant pressure of 80 mmHg for 30 min, and the heart rate, coronary flow, and external heart work were measured.

RESULTS

In samples of arterial blood gases (n = 3) obtained after 2 wk of hypoxia, P02 was lower compared with normoxic controls. PC02 did not differ between hypoxic and normoxic groups (Table 1). Hematocrit and hemoglobin were higher in hypoxic animals (Table 2). In chronically hypoxic rats, the RV weight in the percentage of total heart weight was increased over that in normoxic rats (Table 3). Total body weight was not different between normoxic and hypoxic rats (361 ± 30 vs. 318 ± 11 g; P = not significant).

Preischemic hemodynamic data. Cardiac output was similar in all groups, except for group 9, where cardiac output was lower than that of group 8. The heart rate in group 1 was lower than in groups 3, 7, 8, 10, and 11. LV external heart work in groups 1, 2, 5, and 6 and RV external heart work in the other groups were not different from each other (Table 4). Baseline hemodynamic values before administration of the KATP channel antagonist glibenclamide were similar in groups 9–11 (Table 5). Glibenclamide (5 μmol/l) decreased preischemic cardiac output and external heart work in normoxic right ventricles, whereas coronary flow and heart rate remained unchanged. There were no hemodynamic changes when hypoxic right ventricles were treated with glibenclamide (5 and 10 μmol/l). Coronary flows expressed as percentage of total cardiac output were between 10% and 17% in untreated normoxic and hypoxic left and right ventricles [LV norm 14 ± 1%, LV hyp 17 ± 2% (P = 0.5), RV norm 10 ± 1, and RV hypo 15 ± 3 (P = 0.08)].

Postischemic hemodynamic data. Postischemic coronary flow did not differ significantly between any of the groups and there was no difference compared with preischemic coronary flow within groups (Table 4). Postischemic recovery of coronary flow is shown in Fig. 1. There was no significant difference between groups, although postischemic recovery of cor...
Coronary flow in hypoxic LV tended to be improved compared with normoxic LV. Postischemic cardiac output of untreated and glibenclamide-treated normoxic right ventricles (groups 7 and 9) was significantly lower than preischemic values of the respective groups. Hypoxic right ventricles (gassed with 21% O₂) had higher postischemic cardiac outputs than the respective normoxic controls both with and without the addition of glibenclamide. Except for group 1, postischemic heart rate was similar in all groups. Postischemic external heart work in groups 1 and 2 was significantly lower than the respective values before ischemia.

Recovery of function. Postischemic recovery of external heart work did not differ between hypoxic and normoxic LVs gassed with 95% O₂. However, hypoxic right ventricles recovered significantly better than normoxic right ventricles both when gassed with 95% or 21% O₂ (Fig. 2). The lowest postischemic functional recovery was observed when normoxic right ventricles were treated with 5 μmol/l glibenclamide. On the other hand, glibenclamide (5 and 10 μmol/l) did not influence postischemic recovery of hypoxic right ventricles (Fig. 2).

Expression of HIF-1α mRNA and eNOS. HIF-1α mRNA expression in hypoxic LV and RV myocardium over that in normoxic controls. Postischemic recovery of coronary flow did not significantly differ between the groups.

A prior study (3) in immature rabbit hearts demonstrated postischemic recoveries of hypoxic RVs and LVs that were similarly improved over those of the respective normoxic controls. In contrast, in the present study using adult rat hearts, chronic hypoxia improved postischemic RV but not LV func-

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**DISCUSSION**

In the present study, adult rats exposed to 2 wk of hypoxia had improved postischemic RV function, whereas recovery of LV function of hypoxic hearts was not different from that of normoxic controls. The KATP channel antagonist glibenclamide did not prevent the better RV recovery of function in the hypoxic hearts, whereas in normoxic hearts, glibenclamide resulted in a decrease of RV recovery. HIF-1α mRNA and eNOS expression were increased in hypoxic LV and RV myocardium over that in normoxic controls. Postischemic recovery of coronary flow did not significantly differ between the groups.

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**Table 5. Preischemic hemodynamic values before and after glibenclamide administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Preischemic: Before Drug Administration</th>
<th>Preischemic: After Drug Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CF, ml/min</td>
<td>CO, ml/min</td>
</tr>
<tr>
<td>9: Norm RV, 5 μmol/l Glib, 21% O₂</td>
<td>4</td>
<td>7.5 ± 1.1</td>
<td>54.8 ± 6.3</td>
</tr>
<tr>
<td>10: Hyp RV, 5 μmol/l Glib, 21% O₂</td>
<td>5</td>
<td>7.6 ± 1.0</td>
<td>67.2 ± 5.5</td>
</tr>
<tr>
<td>11: Hyp RV, 10 μmol/l Glib, 21% O₂</td>
<td>4</td>
<td>5.4 ± 1.6</td>
<td>66.5 ± 9.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. *P < 0.05 vs. predrug baseline data of the same group; †P < 0.05 vs. postdrug groups 10 and 11 (statistical comparison with two-way ANOVA, followed by Student-Newman-Keuls test).
tion. Indeed, the effect of chronic hypoxia on ischemic tolerance has been shown to be age dependent in the LV. In a Langendorff model, LVs of immature rabbit hearts (hypoxic from birth) were more tolerant to ischemia than LVs of adult rabbits subjected to chronic hypoxia (2). Differences concerning postischemic LV functional recovery between our and earlier investigations (3) could thus be explained by different responses of immature and adult LVs to chronic hypoxia. Also, the respective models differed in that we used blood perfusion and a normoxic PO₂, whereas the cited study used crystalloid perfusion and a PO₂ of 500–600 mmHg (3).

To the best of our knowledge, no prior study has looked at the effects of chronic hypoxia on RV recovery in adult hearts. The role of RV hypertrophy in the observed improved tolerance to ischemia remains uncertain. In principle, similar kinase pathways are activated during hypertrophy and preconditioning (18). Other mechanisms to induce RV hypertrophy, such as pulmonary artery banding, could help to find out in the future whether the development of RV hypertrophy is an independent mediator of ischemic tolerance in the right ventricle.

Fig. 2. Postischemic recovery of LV and RV external heart work in groups 1–4 (gassed with 95% O₂) and in groups 5–11 (gassed with 21% O₂). Data are means ± SE. #P < 0.05 vs. all other groups; *P < 0.05 vs. normoxic control (one-way ANOVA, followed by Student-Newman-Keuls test).

Fig. 3. RT-PCR analysis of hypoxia-inducible factor-1α (HIF-1α) gene expression in the LV and RV of normoxic and hypoxic rats (n = 10 each). The values of HIF-1α mRNA are expressed in picograms per microgram of total RNA. norm, hearts from normoxic rats; hyp, hearts from chronically hypoxic rats. Data are means ± SE. #P < 0.05 vs. all other groups; *P < 0.05 vs. normoxic groups (one-way ANOVA analysis plus Student-Newman-Keuls test).

Fig. 4. Densitometric data from endothelial nitric oxide synthase (eNOS) immunoblots of LV and RV form normoxic and hypoxic rats (n = 6 each). The values of eNOS are expressed in densitometric units. norm, determination under normoxic conditions; hyp, determination after chronic hypoxia. Data are means ± SE. *P < 0.05 vs. normoxic groups (Student’s t-test for unpaired samples).
The fact that postischemic recovery of coronary flow did not correlate with postischemic recovery of function is in accordance with our earlier studies in isolated hearts (16). However, the conclusions drawn from coronary flow measurements in this model are limited because in the working right heart preparation a large portion of flow running through the aortic cannula perfuses the left anterior descending and the circumflex artery of the nonworking LV. We cannot determine the right coronary artery flow, which certainly is a limitation of this working heart model. The same limitations apply for the working left heart preparation.

K_A TP channel inhibition using glibenclamide was only used in right heart preparations because no cardioprotective effect of hypoxic preconditioning was observed in left hearts. Hypoxic preconditioning was not inhibited by glibenclamide in our model, but glibenclamide also depressed preischemic function in normoxic, but not in hypoxic hearts. This is in contrast to earlier studies, in which glibenclamide was reported to abolish the protective effects of hypoxic preconditioning (3, 4). As mentioned earlier, the models used in the respective studies were different. Our results may suggest a different sensitivity to K_A TP channel modulation in preischemic and posts ischemic normoxic and hypoxic hearts. This notion is supported by the triggering of arrhythmias by the K_A TP channel agonist diazoxide in normoxic, but not in chronically hypoxic adult rat hearts (1).

The increase in HIF-1α mRNA expression in hypoxic left and right ventricles obviously does not support an involvement of HIF-1α in the observed hypoxic preconditioning of right ventricles. However, the sensitivity for a preconditioning effect mediated by HIF-1α may be different in hypoxic RV and LV, and this has not yet been investigated. A recent report (11) describes HIF-3α to represent a rapidly reacting component of the HIF system. The heart, however, contained the lowest amounts. Nevertheless, the fact that only one HIF-subunit was measured may be a limitation of the present study. A further limitation is that HIF-1α mRNA levels do not indicate function or activity of the protein, which could not be measured because it is degraded very rapidly.

The cardioprotective effects of NO have been reported for different species (5, 16, 17). Again, the elevation of eNOS in both right and left hypoxic ventricles in our model suggests that the observed protection is probably not mediated by NO. However, again, the NO effect on a hypertrophied chronically hypoxic ventricle may be different from the effect on a nonhypertrophied normoxic ventricle. A recent review (21) suggests a role for both the constitutive and the inducible forms of NOS in cardioprotection. The effect of cell cycle regulators, such as G1 cyclins and associated cyclin-dependent kinases, activated during cardiac hypertrophy and thought to mediate apoptosis in hypoxic cells, are, on the other hand, attenuated by NO (21), possibly relating to the effects observed in the present study. Future experiments with direct inhibition of NOS may help to identify the functional sequences of endogenous NO blockade in hypoxic right ventricles.

In conclusion, chronic hypoxia improved RV functional recovery in adult rat hearts, whereas postischemic LV function remained unchanged. K_A TP channel blockade with glibenclamide did not abolish the cardioprotection afforded by chronic hypoxia. The relation of our experimental data to the clinical results in children with cyanotic heart disease undergoing surgery is not clear in detail. Cyanosis did not influence the surgical outcome in infants (0–12 mo), but in children >12 mo old, cyanosis was associated with greater troponin I release and worse outcome (12).

REFERENCES


21. Pignatti C and Stefanelli C. Ischemia/reperfusion-induced apoptosis:


