Inhaled NO preadministration modulates local and remote ischemia-reperfusion organ injury in a rat model

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Guery, Benoit, Remy Neviere, Nathalie Viget, Claude Foucher, Patrice Fialdes, Francis Wattel, and Gilles Beaucaire. Inhaled NO preadministration modulates local and remote ischemia-reperfusion organ injury in a rat model. J. Appl. Physiol. 87(1): 47–53, 1999.—Inhaled nitric oxide (iNO) has been shown to have a protective effect in lung ischemia-reperfusion (I/R)-induced injuries. We studied the role of NO (10 parts/million for 4 h) administered before I/R. In an isolated perfused lung preparation, iNO decreased the extravascular albumin accumulation from 2,059 ± 522 to 615 ± 105 µl and prevented the increase in lung wet-to-dry weight ratio. To study the mechanisms of this prevention, we evaluated the role of nitric oxide (NO) transport and lung exposure with matched experiments by using either lungs or blood of animals exposed to iNO and blood or lungs of naive animals. iNO-exposed blood with naive lungs did not limit the extravascular albumin accumulation (2,561 ± 397 µl), but iNO-exposed lungs showed a leak not significantly different from the group in which both lungs and blood were iNO exposed (855 ± 224 vs. 615 ± 105 µl). An improvement in heart I/R left ventricular developed pressure in the animals exposed to iNO showed that blood-transported NO was, however, sufficient to trigger remote organ endothelium and reduce the consequences of a delayed injury. In conclusion, preventive iNO reduces the consequences of lung I/R injuries by a mechanism based on tissue or endothelium triggering.

Endothelium triggering; lung permeability; heart ischemia-reperfusion-induced injury

NITRIC OXIDE (NO) PLAYS an important role in regulating vascular tone, platelet aggregation, white blood cell adhesion to endothelial cells, and host response to infection (22). Inhaled NO was proposed as a therapy to reduce pulmonary artery pressure (PAP) and improve arterial oxygenation in acute respiratory distress syndrome (ARDS), even when those results were recently challenged (21, 31). However, there is a growing body of evidence suggesting that inhaled NO may have effects in addition to pulmonary vascular dilatation. For example, inhaled NO prevented edema formation in isolated rabbit lungs perfused with the superoxide-generating system of xanthine and xanthine oxidase, suggesting that inhaled NO could reduce oxidant-mediated lung vascular injury (11). In ischemia-reperfusion (I/R) lung models, beneficial effects of inhaled NO have also been demonstrated with an improvement in both microvascular injury and endothelial dysfunction and a reduction in pulmonary neutrophil accumulation (1–3, 24). Proposed mechanisms for this beneficial effect include the direct compensatory for the decrease in endogenous NO production (19). However, there is a growing body of evidence suggesting a potential beneficial role for the administration of NO before ischemia. In a recent paper, Bacha et al. (1) evaluated the effect of inhaled NO in non-heartbeating-donor lung transplantation. In the NO group, cadavers and recipients were ventilated with NO for 5 h before a left lung allotransplantation. NO was also administered for 9 h after the transplantation. Consistent with the previous reports, inhaled NO attenuated I/R injury. This result raises, however, several concerns about the mechanisms that could be involved.

When inhaled NO reaches the pulmonary vasculature, it is rapidly bound to hemoglobin, which in turn limits systemic effects. In vitro and in vivo studies have shown that a biologically active form of NO can circulate in the plasma while reversibly bound to hemoglobin and serum proteins (12, 29). Thus hemoglobin and protein thiols can serve as NO adducts to preserve bioactivity and increase NO half-life in biological systems. Therefore, if, after inhalation, NO can be distributed in the systemic circulation through hemoglobin and various proteins, it can cause changes in the vasculature control mechanism and potentially in the endothelial response to the injury (28). Moreover, it has recently been demonstrated that, after inhalation, NO could be delivered to the peripheral microvasculature and show efficacy in NO-depleted tissues, such as the postsischemic mesenteric venule (4). Therefore, the objectives of our study were constructed on those bases. Using the isolated blood-perfused lung model, we first confirmed the hypothesis that inhaled NO administered before ischemia and reperfusion could ameliorate lung-induced injury. In a second series of lung experiments, we explored whether the beneficial effects of inhaled NO were more likely due to the exposure of the lung to inhaled NO or to a NO transport-associated mechanism. To do so, we performed cross-matched experiments in which either the lung or the blood of animals was exposed to inhaled NO before the I/R injury. Finally, we used a salt solution-perfused heart preparation to test the hypothesis that inhaled NO could prevent I/R-induced myocardial dysfunction by a

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mechanism independent of NO transport at the time of the injury.

MATERIALS AND METHODS

Animals

Specific pathogen-free Sprague-Dawley rats (280–300 g; Centre d’Elevage Dépré, St. Doulchard, France) were housed in the Lille University Animal Care Facility and allowed food and water ad libitum. All experiments were performed with approval of the Lille Institutional Animal Care and Use Committee.

NO Administration

Rats were kept in a hermetic chamber, which was continuously flushed with room air containing a fixed concentration of NO. NO was added at the inlet of the chamber to produce 10 parts/million (ppm) in the chamber. Gas flow was adjusted by using flowmeters calibrated before the study. NO and NO2 concentrations at the chamber outlet were continuously monitored with electrochemical sensors (Polytron, Dräger, Strasbourg, France).

Lung Study

Isolated perfused lung preparation. The isolated perfused lung model has been previously described (7, 8). Briefly, animals were anesthetized with ketamine (50 mg/kg; Parke Davis, Courbevoie, France) and xylazine (5 mg/kg; Sanofi, Libourne, France), both injected intramuscularly. The trachea was cannulated and connected to a small animal ventilator (Harvard Apparatus, South Natick, MA). The lungs were ventilated with a constant tidal volume of 10 ml/kg of 95% O2-5% CO2 at 60 breaths/min, with 2 cmH2O positive end-expiratory pressure. A catheter was inserted into the left carotid artery, and, after the administration of 1.0 ml of sodium heparin solution (5,000 U/ml) in this catheter, the animal was exsanguinated. The chest was then opened, and the main pulmonary artery was exposed. The pulmonary artery was cannulated through a right ventriculotomy, and the lungs were perfused with Krebs-Henseleit buffer. Next, the left atrial appendage was also cannulated through the left ventricle. The lungs and heart were removed en bloc from the chest and placed in a heated water-jacketed organ chamber. The lungs were then perfused with a mixture of one part Krebs-Henseleit buffer to three parts heparinized blood at 4.5 ml/min with a roller pump (Masterflex, Cole-Parmer Instrument, Barrington, IL). The perfusate was circulated through a 37°C heat exchanger. The effluent from the left atrial cannula was directed to the perfusate reservoir so that all drainage from the preparation returned to the reservoir. The reservoir was constantly stirred magnetically to provide adequate mixing. Airway pressure, respiratory air flow, PAP, and the pressure in the blood-buffer reservoir were recorded every 10 min for a 30-s period, and data were stored and analyzed in a personal computer (Targa, RESDIAG analysis system, Institut National de la Santé et de la Recherche Médicale U279) (26). The pulmonary capillary pressure was measured by using the double occlusion method described by Townsley et al. (30). Pulmonary artery flow and left atrial effluent were simultaneously occluded; the double occlusion capillary pressure was the pressure reached at equilibration of these two pressures under a no-flow state. This pressure was recorded every 10 min.

Evaluation of lung injury. Extravascular albumin accumulation in lung tissue and bloodless lung wet-to-dry weight (W/D) ratio were used to evaluate lung injury. These methods were previously described to measure lung injury in an experimental setting (19, 23). Briefly, extravascular albumin volume was determined by adding 125I-labeled human serum albumin (125I-HSA, 1 µCi/lung; CIS Biointernational, Gif sur Yvette, France) after 15 min of perfusion during an initial 15-min equilibrium period before ischemia. To measure intravascular volume, 131I-labeled HSA (1 µCi/lung) was added to the reservoir 5 min before the end of each experiment. 131I-HSA was prepared in our institution according to a standardized technique (9). At the end of each experiment, 2 ml of blood perfusate were centrifuged, and three plasma samples, 100 µl each, were collected to estimate radioactivity expressed as counts/min (cpm) (LKB 1282, Wallach). Corrections were made for the overlap between channels. Each lung was homogenized in 2 ml of sterile water, and the radioactivity was also determined in duplicate samples of 300 µl. Whole lung radioactivity was derived from the homogenate weight and the cpm of the samples. The albumin volume (in µl, plasma equivalent) for each isotope was determined by the ratio of whole lung cpm to the cpm per microliter of plasma. The volume of extravascular albumin accumulated in lung tissue was then calculated as follows.

Extravascular albumin volume (µl) = [125I-HSA lung (cpm)/125I-HSA plasma (cpm/µl)] – [131I-HSA lung (cpm)/131I-HSA plasma (cpm/µl)]

The amount of free tracer was checked every week by using a Whatman paper chromatography with methanol-water (3:1); a percentage of free activity <5% was considered satisfactory for both albumin preparations.

GAVIMETRY. A bloodless lung W/D ratio was performed for each group. For these experiments, lungs were prepared as described in isolated perfused lung preparation and set up in the isolated perfused lung model. At the end of the experiment, the lungs were removed, and the wet weight was recorded. The lungs were then placed in a 37°C incubator for 7 days, at which time the dry weight was recorded.

The fraction of blood in the lung measured by the gravimetric method was subtracted from both the wet and the dry lung weights. To do so, homogenates of the lungs were centrifuged (3,500 rpm for 8 min), and the hemoglobin content was performed on a Coulter counter (Gen S, Miami, FL). The automated counts were routinely verified by manual countings. The counts were expressed as cells per microliter. The white blood cell counts were utilized to calculate the percentage of white blood cell decrease expressed as the ratio between white blood cell count after 60 min of reperfusion (B) and the count before ischemia (A), or (B × 100)/A.

Heart Study

Isolated perfused heart preparation. Four hours after randomization, animals were lightly anesthetized with ether. After the animal was decapitated, the heart was rapidly excised and perfused through a Langendorff apparatus at 37°C with Krebs-Henseleit solution of the following composition (in mM): 120 NaCl, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.25 CaCl2, 25 NaHCO3, and 11 glucose. The perfusion buffer was equilibrated with a 95% O2-5% CO2 gas mixture, resulting in a buffer PO2 >600 Torr, and perfused at a constant flow rate of
10 ml/min. A latex balloon (compliant volume >150 µl) connected to a pressure transducer (Inflow, Baxter, Edwards Laboratory, Irvine, CA) was inserted into the left ventricular cavity and secured in position with a ligature placed around the aortic cannulae. The balloon was then inflated to achieve a left ventricular end-diastolic pressure (LVEDP) of 5 mmHg. Left ventricular developed pressure (LVDP), its first derivative (LVdP/dt), and perfusion pressure (PP) were monitored and recorded on a chart recorder (recorder and modules, Kontron Supermon, Basel, Switzerland). After 5 min of equilibration, the heart was paced at 300 beats/min and allowed to equilibrate for 30 min. At this time, baseline measurements were collected.

Experimental Protocol

Effects of NO inhalation on I/R-induced lung injury. To test the effects of a 4-h period of NO inhalation (10 ppm) before I/R, the following three groups of animals were constituted. 1) Con: The control group did not undergo any ischemia and was perfused for 105 min. 2) IR: After 15 min of equilibration in the isolated lung setup, the lungs were submitted to 45 min of ischemia without ventilation, making sure, to avoid any bias, that the vascular as well as the alveolar compartment did not have any residual positive pressure (27). The lungs were then reperfused for a 60-min period. 3) NOIR: The animals were exposed for 4 h to 10 ppm of NO. Their lungs were then harvested in the isolated preparation and submitted to the same protocol as the IR group.

Effect of inhaled NO: respective role of lung-triggering and NO transporter-associated mechanisms. To isolate a NO transporter-associated mechanism from a tissue-triggering effect, cross-matched experiments were performed in two groups of animals. 1) NOLG: The animals were exposed for 4 h to 10 ppm of NO. Their lungs were then harvested in the isolated preparation and submitted to the same protocol as the IR group.

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Results

Hemodynamic Parameters

PAPs were comparable for the five groups at the different times of the protocol. Even if the groups exposed to NO seemed to present lower levels of PAP, this trend did not reach statistical significance. The same patterns were observed for left atrial pressure and capillary pressure with no difference among and within groups. The pulmonary airway pressure remained stable for the NOIR group throughout the entire experiment, comparable to the Con group. For the three remaining groups, IR, NOLG, and NOBD, there was a trend toward an increase in the airway pressure with time, which did not, however, reach statistical significance. These results are summarized in Table 1.

Preadministration of Inhaled NO Protects From I/R-induced Lung Injury

Compared with the Con group, I/R induces an important extravascular accumulation of albumin, with values increasing from 125 ± 61 to 2,059 ± 522 µl (P <
Administration of NO before the injury significantly decreased this leak of albumin to 615 ± 105 µl. The values observed in the NOIR group were, however, higher than those in the Con group, suggesting a decrease in, more than a prevention of, the injury (Fig. 1). Consistent with these results, the lung W/D ratios were significantly decreased when the animals were exposed to NO, compared with the IR group (Fig. 2). There was no statistical difference in the number of leukocytes remaining in the perfusate at the end of the experiment even if there was a trend toward a lower number for the animals exposed to NO (Table 2).

Lung Triggering Is More Important Than NO Transporter-Associated Mechanisms in the Preventive Effect of Inhaled NO

The measurement of extravascular albumin showed a limitation of albumin leak in the group in which the lungs, but not the blood, were exposed to NO. The values obtained were comparable to those of the NOIR group and significantly lower than those of the IR group (Fig. 1). The lung W/D ratios were comparable to the lower values observed in the NOLG group and higher values in the NOBD group (Fig. 2). The leukocyte count in the perfusate followed the same pattern observed in the first series of experiments, i.e., a lower percent in the groups exposed to NO but no statistical threshold reached (Table 2).

Inhaled NO Prevents Myocardial-Induced I/R Injury Independently of NO Transport

Baseline LVDP, its first derivatives (+dP/dt and −dP/dt), and PP (cardiac PP) were similar in the NOIR and IR groups. During ischemia, the magnitude of peak ischemic contracture decreased in the NOIR group compared with the IR group (35 ± 4 vs. 20 ± 3 mmHg; P = 0.01). After 40 min of reperfusion, LVDP recovery (%initial LVDP) was higher in NOIR animals (52 ± 3 vs. 31 ± 4% P = 0.004). LVEDP, on reperfusion, was reduced in NOIR animals compared with the IR group (15 ± 2 vs. 20 ± 4 mmHg; P = 0.04) (Fig. 3).

DISCUSSION

The important findings of this study are that, first, inhaled NO preadministration protects from I/R-induced lung injury, and this prevention is mainly based on lung triggering more than on the NO transporter-associated mechanism. Second, inhaled NO prevents myocardial-induced I/R injury independently of NO transport. Inhaled NO has been shown in experimental as well as clinical settings to have beneficial effects and was, therefore, evaluated in different pathologies, such as ARDS (21, 31). In addition, NO was studied in lung I/R injury because of the resurgence of lung transplantation for end-stage lung disease. Different mechanisms are known to be responsible for reperfusion injury: one of the most important is a dysfunction of pulmonary vascular endothelium, which is manifested by pulmonary hypertension and increased vascular permeability (19).

As NO is a key mediator produced by the endothelium, different authors tried to evaluate this molecule in I/R-induced injury. Eppinger et al. (3) showed that the administration of inhaled NO was protective at 4 h of reperfusion by reversing lung hypoperfusion and lung neutrophil sequestration. Comparable results were found by Barbotin-Larrieu et al. (2) in a pig model. In an isolated buffer-perfused rat lung model, Moore et al. (24) studied the effects of NO synthase inhibitors and NO donors after 45 min of ischemia followed by 30 min

Table 2. Initial number of PMN in perfusate before ischemia-reperfusion and percentage of PMN remaining in perfusate at end of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Initial PMN, 10³/ml</th>
<th>PMN Ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>1.4 ± 0.1</td>
<td>68.5 ± 11.4</td>
</tr>
<tr>
<td>IR</td>
<td>1.6 ± 0.3</td>
<td>57.9 ± 4.5</td>
</tr>
<tr>
<td>NOIR</td>
<td>1.6 ± 0.3</td>
<td>33.5 ± 2.2</td>
</tr>
<tr>
<td>NOLG</td>
<td>1.8 ± 0.5</td>
<td>47.2 ± 5.2</td>
</tr>
<tr>
<td>NOBD</td>
<td>1.4 ± 0.1</td>
<td>46.7 ± 10.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. PMN, polymorphonuclear cells. PMN ratio is calculated by the ratio of no. of cells at end of reperfusion to no. of cells before ischemia.
of reperfusion. They could prevent the increase in microvascular permeability associated with I/R in the presence of NO donors, but three different NO synthase inhibitors failed to prevent I/R-induced injury and permeability increase, suggesting a mechanism independent of the production of peroxynitrite anion. The authors do not, however, rule out the formation of peroxynitrite anion and its potential related damage, especially when alveolar oxygen tension remains elevated during the time of ischemia. In fact, there was, in the study of Moore et al. as well as in our study, no flow or ventilation during the ischemic period. On the contrary, Ischiropoulos et al. (10), with use of a ventilation of 95% O2-5% CO2 during this period, showed biochemical evidence of peroxynitrite formation. These authors concluded that peroxynitrite reactivity could contribute to the oxidative injury of the lung during I/R. Fukahara et al. (5) showed that superoxide dismutase activity was less depressed after NO inhalation, suggesting that suppression of oxygen free radical production would limit lung injury. Taken together, all of these studies show a beneficial effect of NO in lung I/R.

Recently, Bacha et al. (1) demonstrated a potential beneficial role of NO preexposure in lung transplantation. They administered NO before the transplantation to both cadavers and recipients and also for 9 h after the surgery (1). Inhaled NO attenuated I/R injury, but it was difficult to distinguish the role of preventive NO by itself from these results. This study led us to test the hypothesis that inhaled NO preadministration could prevent I/R-induced injuries. Our results are consistent with this previous work; in fact, we observed a decrease in the lung W/D ratio in the group exposed to NO before the injury compared with the Con group. The measurement of the leak of radiolabeled albumin also indicates a decrease in the injury. The maximum amount of leakage occurred in the group exposed to room air. NO inhalation decreases the extravascular albumin but does not completely avoid it. The values observed in the group submitted to I/R but exposed to NO are still higher than, and significantly different from, the values of the Con group (P < 0.01). However, we must emphasize some limitations in our study and particularly in our methodology. Concluding on the occurrence of a permeability increase with the measurement of albumin accumulation assumes that any increase in albumin clearance reflects an increase in endothelial permeability. However, albumin transcapillary flux can be forced through vascular pressure increase and, therefore, mimic a permeability-type leak. It has been shown that, during reperfusion, a transient and very early increase in PAP is possible (19) and may thus be responsible for an increased leakage of albumin in the group not treated with NO, artificially increasing albumin accumulation. For this reason, we calculated the ratio of albumin to lung water by dividing the extravascular albumin accumulation in each group by the lung water. Our results show a ratio of 23.62 in the Con group, 157.47 in the IR group, and 83.90 in the NOIR group. We, therefore, conclude that, even if we underestimated the role of a transient increase in PAP, the increase in albumin accumulation in the IR group compared with the NOIR group mainly reflects a decrease in the lung endothelial permeability. Uncoupling of water and albumin transport confirms a protective role of NO in I/R-induced lung injury. From this part of our study, we can conclude that preventive inhaled NO can limit the consequences of lung I/R injury, normalizing the lung W/D ratio and decreasing, without a return to the values of the Con group, the extravascular albumin.

The second part of the work was dedicated to studying the mechanisms responsible for this protective effect of NO. As NO was administered before the injury, two hypotheses had to be explored: NO could either induce a triggering of the endothelium, which would prepare the lung for the injury and influence its response, or the exposure to inhaled NO could allow a saturation of blood NO transporters, such as the hemoglobin or other proteins (28, 29), to increase NO availability at the time of the injury. To explore these hypotheses, we performed matched experiments using lungs of an exposed animal with blood of a control one and vice versa. Our results showed a significant decrease in lung W/D ratio as well as in extravascular lung albumin in the group in which lungs were exposed to NO and the perfusate used blood recovered from a naive animal. We can emphasize here that the exposure of the lung by itself is much more important for the beneficial effects of NO than the increase in NO availability at the time of the injury. The vascular endothelium is capable of responding to a variety of physiological stresses, leading to the modulation of different enzymatic activities. Motterlini et al. (25) have shown that NO donors could induce a concentration-dependent increase in heme oxygenase activity, a stress enzyme. They treated porcine endothelial cells for 6 h with different NO-releasing compounds and showed an increase in the heme oxygenase activity ranging from 5.7- to 8.5-fold. Inhibitors of NO synthase activity completely abolished this effect. We can, therefore, postulate that NO preadministration can activate the endothelium and prepare it for the I/R-induced response.
There is a large body of arguments in the literature that evokes leukocyte sequestration as a major player in I/R-induced injury in the lung, as well as in other organs such as the intestine and the myocardium. Kurose et al. (15) showed that inhibition of leukocyte adherence and emigration with NO donors in gut I/R significantly reduced microvascular dysfunction. Using intravital microscopy on a cat mesenteric preparation, Kubers et al. (14) studied the effects of the inhibition of NO production and showed an increase of more than 15-fold in leukocyte adherence, suggesting that endothelium-derived NO could be an important modulator of leukocyte adherence. The same group further studied the mechanisms responsible for NO-induced changes in leukocyte adherence and showed that NO did not affect the flux of rolling polymorphonuclear cells but decreased adhesion without a direct effect on CD18 expression (13). The antiadhesive properties of NO were also studied by Gaboury et al. (6), who showed, with intravital microscopy of the intestine, that this effect was related to the ability of NO to inactivate superoxide anion and prevent the proadhesive effect of the oxidant. Apparently contradicting these results, Fox-Robichaud et al. (4) recently showed that inhaled NO did not directly affect leukocyte adhesion. They used leukocytes from animals allowed to breathe NO for 6 h and examined leukocyte rolling and adhesion on immobilized platelet layers. Leukocytes from animals breathing NO had identical rolling and adhesive properties as control leukocytes. These results suggest that the NO effects are probably mainly directed toward the endothelium, a result that is consistent with our experiments.

With the protective effect of NO observed in the ex vivo perfused-lung model, we expected to observe in the group pretreated with NO an increase in the number of circulating neutrophils at the end of reperfusion, thus witnessing the decrease in leukocyte adhesion to the vascular endothelium. The trend of the numbers we obtained is not consistent with this hypothesis, even if statistical significance is not reached. In fact, the larger number of circulating cells is observed in the groups not exposed to NO. However, we used, to perfuse the lungs, a solution mixing blood and Krebs-Henseleit buffer; the numbers of leukocytes in the perfusate were, therefore, lower than what is obtained in vivo. At the beginning of the experiments, the number of leukocytes in the perfusate was between $1.4 \times 10^3$ and $1.8 \times 10^3$ cells/mm$^3$. These small numbers may explain the absence of differences between the groups. Lu et al. (19), using whole blood perfusion, found initial numbers of leukocytes between 25 and $40 \times 10^4$ cells/ml (19). Similarly, most of the models studying the role of leukocytes in the injury were using whole blood perfuse.

From this second part of our study, we can conclude that a triggering of the endothelium is necessary for inhaled NO to provide its beneficial effect; a transporter-mediated mechanism is not sufficient. The leukocyte does not seem in our model to have a prominent role.

The third part of our study was designed to test the hypothesis that exposure of a remote endothelium to NO carried in the blood is sufficient to reduce I/R-induced injury. We exposed animals to inhaled NO for 4 h and studied the response of the heart to I/R. The Langendorff isolated heart preparation has been widely used to study the effects of myocardial I/R (16, 17). With the use of this model, ischemic contracture and postischemic contractile dysfunction have been proposed as a reliable index of the various features of I/R-induced myocardial dysfunction. The link between ischemic contracture and postischemic contractile dysfunction and ischemic injury is strong enough that it became acceptable to use these functional parameters to compare the anti-ischemic efficacy of various interventions and to compare the susceptibility to ischemia of isolated hearts from animals of different species. In our study, rats exposed to inhaled NO displayed evidence of postischemic dysfunction compared with time-matched control rats. Improvement of postischemic myocardial dysfunction associated with NO inhalation manifested as a reduction in the magnitude of peak ischemic contracture pressure. In addition, on reperfusion, postischemic myocardial dysfunction was ameliorated in rats exposed to inhaled NO and was manifested as a reduction of postreperfusion LVDP and increased LVP recovery. As we have previously discussed in the lung, an endothelial dysfunction has also been described in the pathogenesis of the myocardial reperfusion injury after ischemia (18, 20). From these results, inhaled NO seems able to reach the myocardial endothelium and prevent I/R-induced injury.

To summarize our results, inhaled NO prevents I/R-induced lung injury. The mechanism responsible for this effect is based mainly on tissue or, more specifically, endothelium triggering; the role of blood-carried NO does not seem predominant. However, the exposure of a remote endothelium to NO carried by the hemoglobin or the proteins seems sufficient to reduce the consequences of I/R. The mechanisms of this prevention remain to be explored more accurately, but, nevertheless, administration of inhaled NO looks very promising in reperfusion-induced injuries.

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