Protective effects of intravascular pressure and nitric oxide in ischemic lung injury

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Becker, Patrice M., Wendy Buchanan, and J. T. Sylvester. Protective effects of intravascular pressure and nitric oxide in ischemic lung injury. J. Appl. Physiol. 84(3): 803–808, 1998.—Cessation of blood flow during ischemia will decrease both distending and shear forces exerted on endothelium and may worsen ischemic lung injury by decreasing production of nitric oxide (NO), which influences vascular barrier function. We hypothesized that increased intravascular pressure (Piv) during ventilated ischemia might maintain NO production by increasing endothelial stretch or shear forces, thereby attenuating ischemic lung injury. Injury was assessed by measuring the filtration coefficient (Kf) and the osmotic reflection coefficient for albumin (σabl) after 3 h of ventilated ischemia; hence, both distending and shear forces on the vascular endothelium can be decreased during an ischemic injury. The absence of flow is a defining characteristic of pulmonary ischemia during ventilated pulmonary ischemia. The role of NO in ischemia-reperfusion injury of systemic organs is controversial. Both detrimental and beneficial effects of NO inhibition during systemic ischemia have been described (5, 9).

The absence of flow is a defining characteristic of ischemia; hence, both distending and shear forces on vascular endothelium can be decreased during an ischemic period. In previous experiments, we found that maintaining intravascular pressure (Piv) at physiological levels during ventilated ischemia attenuated the injury we had seen in lungs in which Piv during ischemia was low (4). Because this observation was made in a comparison of noncontemporaneous series of experiments, we decided to test the hypothesis that increased Piv protected against ischemic lung injury.

Higher Piv during ischemia might be protective in several ways. First, it would increase the degree of static and circumferential hoop stretch to which the vascular endothelium was exposed. Alterations in vascular and endothelial stretch have been shown to affect production of nitric oxide (NO) (1, 4), prostacyclin (11), and second messengers involved in signal transduction (22), as well as altering cytoskeletal organization (35), all of which might modify vascular barrier function. Second, if Piv was higher than airway pressure during ischemia, there might be rhythmic movement of fluid between alveolar and extra-alveolar vessels with ventilation, thus generating shear forces on the vascular endothelium. Changes in intravascular shear stress affect many of the same mediators as do changes in stretch (8, 23), as well as concentration of intracellular ions (27) and cytoskeletal organization (35). Third, increased Piv might affect ischemic injury indirectly, by increasing intravascular volume and diluting the effect of a toxic mediator released into the vasculature during the ischemic period.

To further explore potential mechanisms of a protective effect of increased Piv, we hypothesized that the absence of flow during ventilated pulmonary ischemia might lead to decreased production of NO. Increased Piv might restore a basal level of NO production by increasing either endothelial stretch or shear forces in the ischemic lung. The role of NO in ischemia-reperfusion injury of systemic organs is controversial. Both detrimental and beneficial effects of NO inhibition during systemic ischemia have been described (13, 29).

Thus this study was designed to evaluate the role of Piv on the generation of ischemic injury in the isolated ferret lung and to determine whether the effects of Piv in this preparation are mediated by NO.

METHODS

Preparation. Adult male ferrets were anesthetized with pentobarbital sodium (50 mg/kg ip). After tracheostomy, ventilation was maintained with 28% O2-balance N2 at a frequency of 20 breaths/min and a tidal volume of 12 ml/kg. An abdominal aortic cannula was placed through a midline incision, heparin was administered, and the animals were rapidly exsanguinated. After exsanguination, ventilation was maintained at 10 breaths/min and an end-expiratory pressure of 3 mmHg, with a warmed humidified gas mixture containing 95% O2-5% CO2. Cannulas were inserted into the left atrium and the pulmonary artery, and the lungs were
Residual blood was flushed from the lungs (pulmonary arterial pressure > left atrial pressure > airway pressure) with physiological salt (PSS) solution containing 3 g/dl albumin and 2 g/dl Ficoll, but no glucose, as previously described (3).

Effects of Piv during ischemia. After the lungs were flushed of residual blood, the pulmonary arterial and left atrial cannulas were connected to a common reservoir, and levels of Piv were adjusted so that it was maintained at a level less than (Low Piv) or greater than end-expiratory pressure (High Piv) during ischemia. For the Low Piv group, this resulted in zone I conditions throughout the experiments. In the High Piv groups, the pressure chosen was physiological, resulting in zone III conditions at end expiration. In additional groups of lungs subjected to High Piv during ischemia, the NO inhibitor \(N^\text{G}-\text{nitro-L-arginine methyl ester (L-NAME; 10}^{-5}\text{M, n = 10, its inactive enantiomer }N^\text{G}-\text{nitro-D-arginine methyl ester (d-NAME; 10}^{-5}\text{M, n = 11, or L-NAME (10}^{-3}\text{M) plus an excess of L-arginine (5} \times 10^{-4}\text{M; L-NAME + L-Arg, n = 6) was added to the PSS solution instilled into the lungs at the start of ischemia.}\) L-NAME, d-NAME, and L-Arg were obtained from Sigma Chemical and were prepared fresh on the day of the experiment. Piv was adjusted in initial experiments by pressurizing the reservoir and then clamping the tubing to the lungs when the desired pressure was reached. By using this method, Piv in the High Piv groups declined during ischemia. Experiments were excluded if the lungs did not remain in zone III throughout the ischemic period, and, for later experiments, the reservoir was pressurized throughout ischemia without clamping the tubing to maintain Piv constant throughout the ischemic period. There were equal numbers of experiments performed by using each of these methods in all but one group (L-NAME + L-Arg). In the L-NAME + L-Arg group, five of six experiments were performed in which Piv was maintained by pressurizing the reservoir throughout ischemia. No significant differences in results were found in a comparison of experiments performed by using the two different methods for adjusting Piv. Results were also analyzed by using only data obtained when the reservoir was pressurized throughout ischemia because the number of experiments in each group by using this method was >5. This analysis did not differ from that used when all data were pooled; therefore, the data presented represent all experiments performed. Temperature during ischemia was maintained at 37°C by enclosing the lungs in a plastic bag and submerging it in a water bath for the duration of the ischemic period.

After 180 min of ischemia, the lungs were wrapped in plastic and suspended from a force transducer for measurement of lung weight. Drainage from the lung surface was collected in a small beaker suspended from a second force transducer. Pulmonary arterial, left atrial, and airway pressures were continuously monitored with Statham P-50 transducers referenced to the top of the lung. Weights and pressures were continuously recorded (Grass model 7). The pulmonary arterial cannula was connected to a reservoir containing PSS with washed red blood cells (RBCs; mean hematocrit (Hct) 23.3 ± 0.3%) preoxygenated with the ventilatory gas. After the lungs were flushed with 15–20 ml of this mixture, the left atrial cannula was connected to the same reservoir for measurement of \(K_r\) and \(\sigma_{ab}\) by using methods described previously (3).

Briefly, Piv was raised incrementally from 15 to 30 mmHg in 5-mmHg increments at 10- to 20-min intervals. Constant rate of weight gain during the last 5 min at each pressure was plotted against Piv, and the slope of this relationship to Piv of between 20 and 30 mmHg was used to estimate \(K_r\). After 10 min at the highest Piv, the PSS-RBC mixture was pumped from the left atrial cannula (Gison Minipuls) to a fraction collector (Gison 203) and then set to collect 1-ml samples. Hct and albumin concentration (bromcresol green) were determined in duplicate for each sample, and \(\sigma_{ab}\) was estimated iteratively from the relationship

\[
\frac{C}{C_i} = \left(1 - \sigma \right) (1 - Hct_i) (1 - Hct_d)^n (1 - Hct_e - \sigma)
\]

where \(x = (1 - Hct_i - \sigma) / Hct_i\), \(C\) represents albumin concentration, \(\sigma\) is osmotic reflection coefficient; and \(i\) represents initial reservoir value.

For comparison, permeability was assessed by the same method in two separate groups of control lungs ventilated with 16% O\(_2\)-5% CO\(_2\) to simulate in vivo conditions. After isolation, the lungs were flushed with PSS containing 5 mM glucose (n = 5) or PSS + 5 mM glucose + L-NAME (10\(^{-5}\)M; n = 4) and then filled with PSS-RBC mixture, and permeability was measured as described above. Ischemic time in these lungs was minimized and averaged 19 ± 1 min.

Statistical analysis. Effects of Piv and NO inhibition during ischemia were compared by using one-way analysis of variance, taking into account repeated measures where appropriate. When significant variance ratios were obtained, least significant differences were calculated to allow comparison of means. Values presented in the text are means ± SE. Differences were considered significant at \(P < 0.05\).

**RESULTS**

Piv during ischemia is shown in Fig. 1 and averaged 0.83 ± 0.4 mmHg (range: 0–2.3 mmHg) in the Low Piv group (n = 10), compared with an average Piv of 6.7 ± 0.4 mmHg (range: 4.5–8.7 mmHg) in the High Piv group (n = 15). Piv did not differ between the High Piv group and the L-NAME, d-NAME, and L-NAME + L-Arg groups, in which Piv during ischemia averaged 6.7 ± 0.4, 6.8 ± 0.4, and 7.1 ± 0.5 mmHg, respectively.

![Intravascular pressure during ischemia](http://jap.physiology.org/Downloaded-on-October-30,2017)

**Fig. 1.** Mean intravascular pressure (Piv) during 180 min of ventilated normothermic ischemia. Values are means ± SE. L-NAME, NG-nitro-L-arginine methyl ester; d-NAME, NG-nitro-o-arginine methyl ester; L-Arg, L-arginine.
Piv remained greater than end-expiratory airway pressure throughout the ischemic period in the High Piv groups. In contrast, Piv remained below both peak and end-expiratory airway pressure throughout ischemia in the Low Piv group. Peak airway pressure during ischemia averaged 6.8 ± 0.2 mmHg and did not vary among any of the groups of lungs studied (data not shown).

Differences in \( \sigma_{\text{ab}} \) are shown in Fig. 2. The \( \sigma_{\text{ab}} \) was significantly decreased in the Low Piv group compared with the High Piv group (0.35 ± 0.04 vs. 0.67 ± 0.04, respectively). The addition of L-NAME during ischemia attenuated the protective effect of High Piv (\( \sigma_{\text{ab}} \) 0.37 ± 0.04), whereas its inactive enantiomer, D-NAME, had no effect (\( \sigma_{\text{ab}} \) 0.63 ± 0.07). The deleterious effects of L-NAME could be overcome by an excess of L-Arg (\( \sigma_{\text{ab}} \) 0.56 ± 0.05). For comparison, \( \sigma_{\text{ab}} \) values in minimally ischemic, normoxic, normoglycemic control lungs with or without L-NAME is shown in Table 1. Mean \( \sigma_{\text{ab}} \) in control lungs averaged 0.68 ± 0.06 (Table 1), consistent with values previously measured in our laboratory (5) and those measured in vivo in other species by using different methods (18, 19). The effects of L-NAME were ischemia dependent because the concentration of L-NAME that increased permeability in ischemic lungs had no effect on permeability in control lungs (\( \sigma_{\text{ab}} \) 0.62 ± 0.02, Table 1).

Figure 3 shows the rate of lung weight gain during the estimation of \( K_f \). Lungs subjected to low Piv during ischemia gained significantly more weight at any given Piv than did lungs in the High Piv group. \( K_f \), estimated from the slope of the relationship between Piv and rate of lung weight gain at Piv \( =20 \) mmHg also tended to be higher in the Low Piv group, but this difference did not reach statistical significance (\( P = 0.11 \), Table 2). Neither rate of lung weight gain nor estimated \( K_f \) differed with the addition of L-NAME, D-NAME, or L-NAME + L-Arg during ischemia compared with High Piv alone (Fig. 3, Table 2). Fluid filtered but draining from the surface of the lungs would result in an underestimation.

Table 1. Effects of L-NAME on vascular permeability in control ferret lungs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \sigma_{\text{ab}} )</th>
<th>( K_f ), g·min(^{-1})·100 g(^{-1})·mmHg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.68 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td>Control + L-NAME, 10(^{-5}) M</td>
<td>0.62 ± 0.02</td>
<td>0.054 ± 0.014</td>
</tr>
<tr>
<td>Historic control</td>
<td>0.69 ± 0.07</td>
<td>0.033 ± 0.004</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 control and n = 4 control + N\(^{G}\)-nitro-L-arginine methyl ester (L-NAME) lungs. \( \sigma_{\text{ab}} \), Osmotic reflection coefficient for albumin; \( K_f \), filtration coefficient; NS, not significant; ND, not done. Historic control refers to results found in Ref. 3.

Table 2. Effects of intravascular pressure and nitric oxide inhibition on \( K_f \) during ischemia

<table>
<thead>
<tr>
<th>Intravascular Pressure During Ischemia, mmHg</th>
<th>( K_f ), g·min(^{-1})·100 g(^{-1})·mmHg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0.324 ± 0.127</td>
</tr>
<tr>
<td>High</td>
<td>0.136 ± 0.042</td>
</tr>
<tr>
<td>L-NAME</td>
<td>0.167 ± 0.049</td>
</tr>
<tr>
<td>D-NAME</td>
<td>0.238 ± 0.081</td>
</tr>
<tr>
<td>L-NAME + L-Arg</td>
<td>0.178 ± 0.061</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of lungs; D-NAME, N\(^{G}\)-nitro-D-arginine methyl ester; Arg, arginine.
of \( K_r \) as measured by rate of lung weight gain alone. We therefore collected fluid draining from the surface of the preparation during the estimation of \( K_r \). The Hct of this fluid was always equal to or greater than the reservoir Hct, suggesting that this leak reflected vascular anastomotic channel drainage, rather than filtered fluid. Neither rate of drainage from the surface of the preparation nor airway pressure during the estimation of \( K_r \) differed among the groups (data not shown).

**DISCUSSION**

The finding that \( \sigma_{alb} \) was significantly higher in lungs subjected to increased levels of Piv during 3 h of ventilated ischemia suggests that maintenance of Piv at physiological levels was protective in this model of ischemic injury. The rate of lung weight gain was higher at any given Piv in the Low Piv group, and \( K_r \), estimated from the slope of this relationship, tended to decrease, although this change did not reach statistical significance. Because \( K_r \) depends on both surface area and permeability raising Piv during ischemia may decrease permeability but increase vascular surface area. These two effects will cause \( K_r \) to change in opposite directions and might therefore make \( K_r \) a less sensitive indicator of vascular permeability than \( \sigma \). Other possible explanations for a discrepancy in the magnitude of the change of \( \sigma \) and \( K_r \) are discussed below.

The mechanism by which increasing Piv attenuates ischemic injury is unknown, although there are several possibilities. First, raising Piv will increase static and circumferential hoop stretch of vascular endothelium. The application of stretch has been shown to alter the endothelial cytoskeleton (35) and thus could affect permeability to macromolecules. In addition, NO production increased in endothelium and vascular rings subject to stretch (1, 14), and NO inhibition has been shown to increase permeability of systemic vasculature (12). Stretch may also increase vascular endothelial production of prostacyclin (11) and stimulate the generation of inositol phosphate and diacylglycerol (22). Prostacyclin may alter endothelial permeability (16), whereas inositol lipid metabolites may increase intracellular calcium and activate protein kinase C, thereby influencing vascular barrier function (26).

Second, in the absence of circulating flow, if Piv is maintained at levels greater than airway pressure during ischemia, there will be rhythmic movement of fluid between alveolar and extra-alveolar vessels with ventilation. This will not be the case in lungs in which Piv is lower than airway pressure during ischemia because, under these circumstances, alveolar vessels will be collapsed throughout ischemia. The maintenance of even low levels of shear stress may affect basal levels of NO (23) and prostacyclin (8) production by the pulmonary vascular endothelium and may also alter endothelial cytoskeletal organization, hence permeability (30).

Third, increased Piv may attenuate ischemic lung injury not by distention of the vasculature but by its effects on intravascular volume. Increased intravascular volume could lead to dilution of a toxic mediator released during the ischemic period, thus attenuating injury.

We were interested in the role NO production might play in the protective effects of Piv on vascular barrier function during pulmonary ischemia because NO production from endothelium increases with both mechanical deformation or intravascular shear stress (1, 14, 23). In our model, inhibition of NO during ischemia worsened injury with High Piv, as manifest by increased pulmonary vascular permeability. This deleterious effect of NO inhibition was not seen in lungs treated with D-NAME and could be overcome by the concurrent administration of L-Arg, suggesting that the effects of L-NAME occurred via its effects on NO synthase inhibition, rather than other mechanisms (6).

Although NO inhibition has been shown to increase microvascular protein permeability in uninjured systemic vasculature (12), this was not the case in our preparation, in which L-NAME had no effect on the permeability of normoxic, normoglycemic, minimally ischemic control lungs. On the other hand, similar to our findings, inhibition of NO also increased ischemic injury in systemic organs, as measured by cerebral infarct volume and hemorrhage after middle cerebral artery occlusion in rats (34). Similarly, several groups have shown that exogenously administered NO may protect against ischemia-reperfusion injury in systemic organs, including intestine and heart (20, 29). Conversely, studies have suggested that NO may exacerbate ischemia-reperfusion injury in systemic organs (15) by generating peroxynitrite anion (ONOO−), a potent OH−-like oxidant formed by the interaction of NO with superoxide anion (5). Because NO inhibition worsened injury in our model, it is unlikely that ONOO− mediates the increased vascular permeability seen with ventilated pulmonary ischemia.

Consistent with our hypothesis that decreased NO during pulmonary ischemia contributes to injury, Pinsky et al. (21) found that NO levels in rat lungs, measured by a porphyrin microsensor at the lung surface, decreased during lung preservation for orthotopic transplantation. Supplementing the preservation solution with 8-bromoadenosine 3′,5′-cyclic monophosphate attenuated the injury seen during reperfusion posttransplantation in this model, as measured by improved recipient survival, gas exchange, and decreased neutrophil infiltration in the allograft. Vascular permeability was not assessed directly in the above-mentioned study. Lungs were preserved inflated, although the investigators do not report measuring oxygen tension during the ischemic period, and the Piv of the ischemic lungs was not mentioned.

The mechanism by which NO attenuated ischemic injury in our model is not known. NO may decrease injury due to oxygen radical generation by acting as an oxygen radical scavenger. Low concentrations of inhaled NO have been shown to attenuate hyperoxic or oxidant-mediated injury in adult rat lungs and fetal rat lung epithelial cells (10) and Chinese hamster lung fibroblasts (33). It is unknown whether the effects of NO inhibition in our preparation are due to antioxidant
effects because we have not yet demonstrated evidence of oxygen radical generation in our model.

Other possible mechanisms by which NO may help maintain barrier function are by direct effects on second messengers such as guanosine 3',5'-cyclic monophosphate, which caused relaxation of endothelial cells and decreased paracellular permeability in vitro (17). NO also is an important inflammatory mediator and may decrease leukocyte adhesion (13). There are no circulating neutrophils in our preparation, although it is likely that resident neutrophils are present and could mediate an anti-inflammatory effect of NO.

NO may also influence prostacyclin generation. Administration of the NO donors 3'-morpholinosydnonimine, sodium nitroprusside, and nitroglycerin, were shown to activate cyclooxygenase, leading to increased prostaglandin (PG) I2 production, in bovine aortic endothelial cells in culture, as well as in plasma from conscious adult rats (25). PG12 decreased transcellular transport of fluorescent across cultured porcine arterial endothelial cell monolayers, probably via a adenosine 3',5'-cyclic monophosphate-dependent mechanism (16). We have not yet tested whether the protective effects of NO in our model are related to altered prostacyclin production.

Interestingly, L-NAME caused a significant decrease in $\sigma$ in our preparation, without a significant increase in $K_t$. There are several possible explanations for this result. First, the effects of both increased hydrostatic pressure (31) and agents causing nonhydrostatic pulmonary edema, such as arachidonic acid (32), have been shown to have nonuniform effects on $K_t$ and $\sigma$. The model proposed to explain this discrepancy suggests that the pulmonary vascular bed contains both numerous small pores, which account for the majority of hydraulic conductivity, and fewer larger pores, through which protein flux occurs (18). An injury affecting even a small percentage of the large pores would be predicted to affect protein flux to a much greater extent than total hydraulic conductivity (32). Conversely, an injury primarily affecting the small pores would affect $K_t$ to a greater degree than $\sigma$ (31). Second, vascular surface area at any given Piv may decrease with administration of L-NAME in our study, thus decreasing the rate of lung weight gain at any Piv, and hydraulic conductivity estimated from this relationship. In support of this possibility, it has been shown that pulmonary vasoconstrictor agents administered to isolated dog lobes exposed to a constant hydrostatic pressure decreased the size of the perfused microvascular bed (7) and that administration of L-NAME to isolated rat lungs increased basal pulmonary vascular resistance (2). Furthermore, flow in these L-NAME-treated lungs decreased when Piv was raised and the arteriovenous pressure gradient was held constant, compared with untreated lungs (2). An effect of L-NAME on surface area would have no influence on the estimation of $\sigma$ because our modification of the filtered volumes technique assumes only that the volume of the filtering compartment in the lung is not changing when the measurement is made (3). Third, others have shown that inhibition of NO synthase decreased hydraulic conductivity of isolated perfused mesenteric capillaries (24), although the mechanism of this effect is not known. Protein permeability was not measured in the above study. Fourth, increased edema formation as Piv is elevated may decrease both the hydrostatic and oncotic pressure gradients driving filtration, thereby causing deracruitment of the pulmonary vascular bed, and decreased surface area and $K_t$ (28). Because $\sigma$ decreased to a comparable extent in Low Piv and L-NAME-treated lungs, the extent of edema formation should have been similar in both groups if vascular surface area for filtration did not differ between them; thus we do not think this is the sole explanation for our findings.

In summary, increasing Piv during ischemia attenuated injury of isolated ventilated ferret lungs. This protective effect may be mediated by NO because the effect could be abolished by administration of the NO inhibitor L-NAME, but not by d-NAME. Additionally, the effects of L-NAME could be overcome by the administration of an excess of L-Arg. We speculate that maintaining intravascular distention, or a minimal level of shear stress, during ischemia is responsible for maintaining basal levels of NO production and that NO maintains vascular barrier function in this model by scavenging superoxide anion, altering PG12 metabolism, or by causing direct relaxation of endothelial cells.

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