Effect of ventilation on vascular permeability and cyclic nucleotide concentrations in ischemic sheep lungs

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Pearse, David B., Elizabeth M. Wagner, and Solbert Permutt. Effect of ventilation on vascular permeability and cyclic nucleotide concentrations in ischemic sheep lungs. J. Appl. Physiol. 86(1): 123–132, 1999.—Ventilation during ischemia attenuates ischemia-reperfusion lung injury, but the mechanism is unknown. Increasing tissue cyclic nucleotide levels has been shown to attenuate lung ischemia-reperfusion injury. We hypothesized that ventilation prevented increased pulmonary vascular permeability during ischemia by increasing lung cyclic nucleotide concentrations. To test this hypothesis, we measured vascular permeability and cGMP and cAMP concentrations in ischemic (75 min) sheep lungs that were ventilated (12 ml/kg tidal volume) or statically inflated with the same positive end-expiratory pressure (5 Torr). The reflection coefficient for albumin ($r_{AB}$) was 0.54 ± 0.07 and 0.74 ± 0.02 (SE) in nonventilated and ventilated lungs, respectively ($n = 5$, $P < 0.05$). Filtration coefficients and capillary blood gas tensions were not different. The effect of ventilation was not mediated by cyclic compression of alveolar capillaries, because negative-pressure ventilation ($n = 4$) also was protective ($r_{AB} = 0.78 ± 0.09$). The final cGMP concentration was less in nonventilated than in ventilated lungs (0.02 ± 0.02 and 0.49 ± 0.18 nmol/g blood-free dry wt, respectively, $n = 5$, $P < 0.05$). cAMP concentrations were not different between groups or over time. Sodium nitroprusside increased cGMP (1.97 ± 0.35 nmol/g blood-free dry wt) and $r_{AB}$ (0.81 ± 0.09) in nonventilated lungs ($n = 5$, $P < 0.05$) and $r_{AB}$ had no effect on $r_{AB}$. The nitric oxide synthase inhibitor N′-nitro-L-arginine methyl ester had no effect on lung cGMP ($n = 9$) or $r_{AB}$ ($n = 16$) in ventilated lungs but did increase pulmonary vascular resistance threefold ($P < 0.05$) in perfused sheep lungs ($n = 3$). These results suggest that ventilation during ischemia prevented an increase in pulmonary vascular protein permeability, possibly through maintenance of lung cGMP by a nitric oxide-independent mechanism.

Cyclic nucleotide concentrations and vascular permeability, but the effect of ventilation was not examined. In the present study we focused on the ischemic period and hypothesized that ventilation attenuates ischemia-reperfusion injury by preventing the increase in vascular permeability associated with ischemia before reperfusion. We further hypothesized that this effect was mediated by a ventilation-induced increase in the concentration of one or both cyclic nucleotides in the lung. To address these hypotheses, we measured lung cyclic nucleotide concentrations and vascular permeability in ventilated and nonventilated lungs subjected to ischemia without reperfusion.

METHODS

Studies were performed in excised sheep lungs subjected to 75 min of ischemia. Ventilated lungs were ventilated (28% O2-5% CO2-balance N2; 5 Torr positive end-expiratory pressure) with positive ($n = 26$) or negative ($n = 4$) pressure to achieve a tidal volume of 12 ml/kg and a rate of 10 breaths/min. Nonventilated lungs ($n = 32$) were statically inflated with the same gas mixture to a continuous positive airway pressure of 5 Torr.

Vented Lungs

Positive-pressure ventilation. Young sheep (20–37 kg) were anesthetized with intramuscular ketamine (30 mg/kg) and subsequently maintained by intravenous pentobarbital sodium (15 mg/kg loading dose, 20 mg·kg$^{-1}$·h$^{-1}$ infusion). After tracheostomy, mechanical ventilation was begun with O2-supplemented room air at a tidal volume of 12 ml/kg body wt and respiratory rate of 15 breaths/min. A sternotomy was performed.
performed, intravenous heparin (10,000 U) was administered, and the left atrium was cannulated. The sheep were exsanguinated into a closed reservoir, which was stirred and pressurized. Ventilation was adjusted to 10 breaths/min with 28% O₂-5% CO₂-balance N₂. The pulmonary artery was cannulated, and ventilation was briefly interrupted to allow excision of the lungs. The bronchoesophageal artery and heart were clamped, and the right upper lobe was removed for baseline measurements of cyclic nucleotides. The lungs were suspended from a force transducer and hyperinflated to 30 Torr to reverse atelectasis, and ventilation was resumed with the same gas mixture. At 30 min of ischemia, pulmonary vascular pressure was increased to 20 Torr and the vasculature was flushed antegrade with 400 ml of a mixture of blood and 3% Dextran 70-Ringer lactate mixture to achieve a known baseline hematocrit (Hct, 20%) and albumin concentration in the lung. The pulmonary arterial and left atrial cannulas were connected to a single reservoir, and intravascular pressure (Piv) was adjusted to 20, 30, 35, and 40 mmHg at 10-min intervals. The rate of lung weight gain over the last 5 min at each Piv was plotted against Piv, and the slope of this relationship was used to estimate the filtration coefficient (Kf) (11), as previously described (38).

The reflection coefficient for albumin (ra Alb) was determined by the filtered-volumes method (25) modified for a nonflowing system (4). After 10 min at the highest Piv, the intravascular blood was removed from the lung at 200 ml/min (Gilson Minipuls 2) via the pulmonary artery and collected in 20 sequential 12-ml samples to measure blood gases and calculate ra Alb as previously described (38). Hct and albumin concentration were determined in duplicate for each blood sample and in a baseline sample. The ra Alb value calculated from the intravascular fluid sample with the greatest difference in Hct from the baseline value was defined as the ra Alb for that lung. Pulmonary capillary blood gases were estimated by measuring Po2, Pco2, and pH in the blood sample with the greatest Hct change (n = 4). Immediately after collection of the blood samples, biopsies were obtained from the left lung for determination of cyclic nucleotide concentrations.

In three lungs the pulmonary arteries were separately cannulated to allow measurement of ra Alb in each lung. The pulmonary arteries in these lungs were flushed with 200 ml from separate pressurized reservoirs. Blood samples for the measurement of ra Alb were simultaneously collected from each pulmonary artery by the same pump. Separate values of Kf could not be determined, however, because the lungs were suspended together.

Negative-pressure ventilation. Positive-pressure ventilation phasically decreases pulmonary capillary transmural pressure, because alveolar pressure increases relative to vascular pressure with each inspiration (39). To determine whether cyclic compression of alveolar vessels played a role in the ventilation-induced changes in vascular permeability, lungs were ventilated by decreasing lung surface pressure (n = 4). Under these conditions, alveolar pressure remained constant relative to vascular pressure throughout the respiratory cycle so that alveolar vessels were subjected to longitudinal stretch but not cyclic compression (39). To accomplish this, lungs were suspended in a Plexiglas box equipped with ports for the tracheal and vascular cannulas. After hyperinflation of the lung with positive airway pressure as described above, the external tracheal cannula was connected to a spirometer filled with 28% O₂-5% CO₂-balance N₂. The spirometer bell was weighted to provide a constant airway pressure of 5 Torr relative to atmospheric pressure. Negative-pressure ventilation was initiated by cycling box pressure with gas containing 28% O₂-5% CO₂-balance N₂ to prevent diffusive gas loss across the lung. The box pressure oscillations were adjusted to provide a tidal volume of 12 ml/kg. Lung weight and, therefore, Kf were not measured.

Nonventilated Lungs

To determine the role of ventilation, 32 lungs were subjected to the protocol described above, except airway pressure was kept constant at 5 Torr after the hyperinflation maneuver. One nonventilated lung had separate ra Alb for the right and left lung, as described above. A subset of nonventilated lungs (n = 4) was studied in the Plexiglas box to control for the conditions present for negative-pressure ventilation. Blood gases were obtained in nonventilated lungs, which were studied outside (n = 5) and inside (n = 4) the Plexiglas box.

Cyclic Nucleotide Measurements

Lung biopsies of the right upper lobe (baseline) and left lung (final) were immediately frozen in liquid nitrogen for determination of cAMP and cGMP by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) by the method of Pradelles and Grassi (43). Blood samples were also frozen and processed to allow correction for the blood contribution of cyclic nucleotides. A portion of each lung and blood sample was used for determination of tissue blood content, extravascular lung water, and blood-free dry weight (bfdw) by the method of Pearce et al. (36).

Pharmacological Protocols

Figure 1 shows the distribution of ischemic lungs in the various treatment groups. Two groups of ventilated lungs were treated with 1 mM Nω-nitro-L-arginine methyl ester...
(L-NAME, n = 16), a nitric oxide (NO) synthase inhibitor, or 1 mM N\textsuperscript{$\text{O}$-nitro-D-arginine methyl ester (D-NAME, n = 5), its inactive isomer. Three lungs in each group were paired comparisons consisting of single right and left lungs from three sheep. In these lungs, L-NAME was administered to one lung and D-NAME to the other before all cannulas were connected to the same reservoir containing D-NAME. In 10 of the lungs treated with L-NAME and all the lungs treated with D-NAME, the drug was added to the vascular flush and reservoir solutions. In five of the L-NAME-treated ventilated lungs, an additional dose of L-NAME (33 mg/kg iv) was administered to the animal 10 min before exsanguination to ensure inhibition of NO synthase during the entire ischemic period.

Separate groups of nonventilated lungs were treated with 1 mM sodium nitroprusside (SNP, n = 5) to increase cGMP, 1 mM isoproterenol (Iso, n = 4) to increase cAMP, 1 mM L-NAME (n = 6), or 1 mM D-NAME (n = 8). One lung in the L-NAME-treated group and one lung in the D-NAME-treated group were single lungs from the sheep described above. All drugs were added to the vascular flush and reservoir solutions.

To determine whether 1 mM L-NAME was sufficient to block NO synthase in the sheep pulmonary vasculature, the effect of L-NAME on pulmonary arterial pressure-flow curves was studied in three isolated perfused lungs. The lungs were perfused in situ with autologous blood (38) treated with indomethacin (100 mg in 20 ml of 1 N NaHCO\textsubscript{3}) to inhibit thromboxane-induced pulmonary hypertension and prostacyclin production (38). Ventilatory parameters were as described above for the ischemic lung protocol. Steady-state pulmonary arterial pressure-flow curves were obtained after treatment with saline diluent, 1 mM L-NAME, 1 mM D-NAME, and 30 mM L-arginine. All drugs were obtained from Sigma Chemical (St. Louis, MO).

Statistics

The pulmonary vascular permeability and blood-gas data were analyzed by one-factor ANOVA. The cyclic nucleotide data were analyzed by two-factor (group and time), split-plot ANOVA (50). The pressure-flow curves were compared by two-factor ANOVA with two repeated measures. The fractional changes in Hct and albumin in ventilated and nonventilated lungs were compared by unpaired t-test. The cyclic nucleotide data were not normally distributed and thus were transformed to logarithms before statistical analysis. When significant (P < 0.05) variance ratios were obtained, least significant differences were calculated to allow comparison of individual means. Values are means ± SE. Differences were considered significant when P < 0.05.

RESULTS

Effect of Ventilation on Pulmonary Vascular Permeability and Tissue Cyclic Nucleotide Concentrations

Lungs subjected to positive- or negative-pressure ventilation had increased σ\textsubscript{lab} values compared with nonventilated lungs (Fig. 2). For example, σ\textsubscript{lab} averaged 0.74 ± 0.02 in lungs ventilated with positive pressure compared with 0.54 ± 0.07 in nonventilated lungs. There were no differences in K\textsubscript{a} between ventilated and nonventilated lungs: 0.03 ± 0.02 and 0.02 ± 0.01 g·

\text{min}^{-1}·\text{mmHg}^{-1}·100 \text{g}^{-1}, respectively (data not shown). Extravascular lung water normalized to blood-free dry weight was also not different: 4.78 ± 0.23 and 4.89 ± 0.23 g/g bfdw in ventilated and nonventilated lungs, respectively (n = 5). Pulmonary capillary blood gases differed only in that the arterial PaCO\textsubscript{2} in the nonventilated lungs studied outside the Plexiglas box was less. The blood gases in the ventilated and nonventilated lungs studied inside the box (Fig. 2, right) were not different, despite the persistent difference in σ\textsubscript{lab}.

The effect of ventilation on lung cyclic nucleotide concentrations is shown in Figs. 3 and 4. The cGMP concentration decreased from 1.49 ± 0.56 to 0.02 ± 0.02 nmol/g bfdw (P < 0.05) in the nonventilated lungs. The final cGMP concentration in the ventilated lungs was not significantly different (P = 0.18) from the baseline value (0.49 ± 0.18 and 1.08 ± 0.30 nmol/g bfdw, respectively) but was greater than the final cGMP concentration in the nonventilated lungs (P < 0.05, ANOVA interaction term). As shown in Fig. 4, the cAMP concentrations were not different over time or between groups: 6.13 ± 0.82 and 5.30 ± 0.90 nmol/g bfdw in the baseline and final lung biopsies, respectively.
Effect of SNP and Iso on Lung Cyclic Nucleotide Concentration and Pulmonary Vascular Permeability in Nonventilated Lungs

Treatment of nonventilated lungs with SNP caused a significant increase in cGMP concentration from 0.74 ± 0.14 to 1.97 ± 0.35 nmol/g bfdw (Fig. 3). The cAMP concentrations in the SNP-treated lungs were not different from those in the untreated nonventilated lungs (Fig. 4). In the nonventilated lungs treated with Iso, cAMP concentration increased from 8.99 ± 2.26 to 100.0 ± 50.3 nmol/g bfdw (Fig. 4). Iso did not affect cGMP levels, which decreased to 0.06 ± 0.05 nmol/g bfdw in the Iso-treated group (Fig. 3).

The effects of these drugs on $\sigma_{aab}$ are shown in Fig. 5. The $\sigma_{aab}$ of nonventilated lungs treated with SNP (0.81 ± 0.09) was not different from that measured in ventilated lungs but greater than that in untreated nonventilated lungs. The $\sigma_{aab}$ in the Iso-treated nonventilated lungs (0.41 ± 0.13) was different from that in the untreated nonventilated group. The $K_f$ values in the SNP- and Iso-treated nonventilated lungs were not different from those in the non-drug-treated groups: 0.03 ± 0.004 g·min⁻¹·mmHg⁻¹·100 g⁻¹ (data not shown).

Effect of L-NAME on Lung Cyclic Nucleotide Concentration and Pulmonary Vascular Permeability in Ventilated Lungs

Treatment with L-NAME did not affect cGMP or cAMP concentrations compared with untreated ventilated lungs (Figs. 3 and 4). The final cGMP concentration in the L-NAME-treated ventilated lungs was not significantly different (P = 0.34) from the baseline value (0.98 ± 0.27 and 1.49 ± 0.48 nmol/g bfdw, respectively) but was greater than the final cGMP concentration in the nonventilated lungs (P < 0.05, ANOVA interaction term). The five lungs treated with an intravenous dose of L-NAME before exsanguination were not different from the four lungs that were treated with L-NAME in the reservoir blood only (final cGMP 0.96 ± 0.40 and 1.01 ± 0.36 nmol/g bfdw, respectively; data not shown).

Neither L-NAME nor D-NAME altered the effect of ventilation on $\sigma_{aab}$ (Fig. 6). The data in Fig. 6 include the experiments in which a paired lung comparison was performed, because these results did not differ from the unpaired data derived from separate animals. The $K_f$ was also unaffected (data not shown). Moreover, similar to the cGMP results, the additional intravenous dose of L-NAME administered to five of the L-NAME-treated ventilated lungs had no effect on $\sigma_{aab}$ or $K_f$ (data not shown).

Effect of L-NAME on Pulmonary Vascular Resistance

L-NAME, but not D-NAME, caused a threefold increase (P < 0.01) in the slope of the pressure-flow relationship (0.09 ± 0.03 vs. 0.28 ± 0.05 mmHg·ml⁻¹·min⁻¹·kg) without affecting the extrapolated zero-flow intercept (Fig. 7). Addition of L-arginine returned the slope to control levels.
Effect of Ventilation on Maximal Fractional Change in Hct and Albumin Concentration

The measurement of $\sigma_{ab}$ in this study is based on the relative effect of convective fluid filtration on the concentrations of erythrocytes and albumin in the pulmonary circulation. The maximal fractional change in Hct ($\Delta Hct_{\text{max}}$) is determined by the amount of water filtration in the portion of the vascular bed with the greatest water permeability. To compare the relative changes in Hct and albumin concentration between ventilated and nonventilated lungs, $\Delta Hct_{\text{max}}$ and the maximal fractional change in albumin concentration ($\Delta Alb_{\text{max}}$) were determined for nonventilated ($n = 19$) and ventilated ($n = 24$) lungs (Fig. 8). Experiments in which $\sigma_{ab}$ was determined separately in each lung and nonventilated lungs treated with Iso or SNP were excluded from this analysis, because the Hct-vascular volume curves were truncated in the split lung experiments, and neither Iso nor SNP was administered to ventilated lungs.

The initial Hct and albumin concentration did not differ between groups: $21.1 \pm 0.6\%$ and $0.0223 \pm 0.006$ g/dl for nonventilated and $21.7 \pm 0.4\%$ and $0.0214 \pm 0.006$ g/dl for ventilated lungs. In the nonventilated lungs the $\Delta Hct_{\text{max}}$ ($0.300 \pm 0.016$) was greater than ($P < 0.0001$) the $\Delta Alb_{\text{max}}$ ($0.213 \pm 0.012$), whereas there was no difference between the corresponding values in the ventilated group. The $\Delta Hct_{\text{max}}$ was also greater ($P < 0.0001$) in nonventilated than in ventilated lungs.

Conversely, the $\Delta Alb_{\text{max}}$ was less ($P < 0.05$) in the nonventilated than in the ventilated lungs ($0.256 \pm 0.017$). Figure 8 also shows that the peak Hct and albumin values in the nonventilated lungs oc-
resulted in a significantly greater pulmonary vascular permeability mimicked in nonventilated lungs by increasing tissue inflation alone (Fig. 2). The effect of ventilation on ischemic vascular permeability was comparable to estimates of $\sigma_{\text{alb}}$ in isolated lung preparations (1, 34, 48), ventilatory lung motion during ischemia attenuated the increased pulmonary vascular permeability during reperfusion. This protective effect of ventilation was present in inspired gas. The more acidic pH in the ventilated lung could confer protection against ischemia (26). As shown in Fig. 2 (left), the Po2 values of toxic O2 products may be involved.

DISCUSSION

It has long been recognized that the injury observed after reperfusion of an ischemic lung can be attenuated by the maintenance of ventilation during ischemia (8, 15). In more recent studies in intact animals (17) and isolated lung preparations (1, 34, 48), ventilatory lung motion during ischemia attenuated the increased pulmonary vascular permeability measured during reperfusion. These studies did not address the potential effect of ventilation on pulmonary vascular permeability changes during ischemia or attempt to identify the mechanistic link between ventilatory lung motion and pulmonary vascular barrier function. Our data suggest that ventilation during ischemia prevents increased vascular permeability during ischemia, before reperfusion. This protective effect of ventilation was associated with maintenance of lung cGMP concentrations, could not be blocked by L-NAME, and could be mimicked in nonventilated lungs by increasing tissue cGMP concentrations with SNP, suggesting that ventilation protected by maintaining cGMP levels in the pulmonary vasculature.

Effect of Ventilation on Ischemic Vascular Permeability

We found that ventilation of the ischemic sheep lung resulted in a significantly greater $\sigma_{\text{alb}}$ than did static inflation alone (Fig. 2). The $\sigma_{\text{alb}}$ in the ventilated lungs was comparable to estimates of $\sigma_{\text{alb}}$ (0.84) in intact sheep lungs (35), suggesting that ventilation was necessary to maintain a normal level of vascular protein permeability over this period of ischemia.

Interestingly, Kf did not differ between the two groups and was similar to that in ischemic ferret (3, 4) and intact dog lungs (28). Decreases in $\sigma_{\text{alb}}$ without accompanying increases in Kf were recently reported in protocols utilizing ischemic ferret (3) and perfused sheep (38) lungs. Theoretically, $\sigma_{\text{alb}}$ can decrease in the absence of an increase in water conductance if different-sized pores are responsible for the movement of water and protein across the microvascular barrier of the lung (51). An increase in the number of large pores responsible for protein conductance could decrease $\sigma_{\text{alb}}$ without affecting Kf (51, 52). Alternatively, measurement of whole lung Kf could miss true increases in regional water conductance because of the small size of the affected region or because of the known dependence of Kf on vascular surface area (51). A decreased vascular surface area could offset increased water permeability, resulting in an unchanged Kf. These possibilities will be discussed below.

Lung ischemia is capable of causing vascular injury without reperfusion, but the effect of ventilation was not previously studied (4, 7, 44). Protein and water permeability increased after 3 h of ventilated ischemia in ferret lungs (4), whereas only 1 h of ischemia was required to increase protein permeability in statically inflated dog and rat lungs (44). Although the mechanism of this injury is unknown, the observation that ischemia without reperfusion caused lipid peroxidation in isolated rat lungs (14) suggests that the generation of toxic O2 products may be involved.

The protective effect of ventilation during ischemia on IR lung injury has been attributed to 1) enhanced preservation of alveolar Po2 (15), 2) ventilatory motion of pulmonary vascular blood (32), or 3) an uncharacterized protection conferred directly by cyclic lung distension (17, 34, 48). It is clear that, independent of ventilation, alveolar Po2 during ischemia can affect pulmonary vascular injury before (5) and after (12, 21, 48) reperfusion. The protective effect of ventilation, however, was shown to be independent of alveolar Po2, as evidenced by studies that demonstrated that anoxic and normoxic ventilation were equally protective compared with static inflation with anoxic (17) or normoxic gas (34). For example, Schutte et al. (48) compared ventilation with 95% N2-5% CO2 with static inflation with the same gas mixture during 180 min of ischemia. In the lungs that had been ventilated during ischemia, Kf was decreased after reperfusion compared with the static inflation group, confirming a Po2-independent protective effect of ventilation.

Less attention has been paid to the effects of ventilation on Pco2 and pH in the ischemic lung. In isolated lung preparations, diffusive loss of CO2 across the lung could cause alveolar Pco2 to be lower in statically inflated than in ventilated ischemic lungs if 5% CO2 is present in inspired gas. The more acidic pH in the ventilated lung could confer protection against ischemic injury (26). As shown in Fig. 2 (left), the Po2 values
were not different but the P_{CO_2} was decreased in the nonventilated lungs, producing a more alkalotic pH. The difference in \( \sigma_{alb} \) remained, however, in the ventilated and nonventilated lungs sealed in the box (Fig. 2, right), suggesting that the protective effect of ventilation was not mediated by alterations of blood gases. Similarly, Hamvas et al. (17) compared different inspired fractions of CO\(_2\) and showed that the protective effect of ventilation during ischemia was not dependent on pH.

The effect of ventilation on the movement of pulmonary vascular blood and the potential delivery of nutrients during ischemia depends on the type of ventilation, the vascular pressure, and the patent of the pulmonary artery and vein (32). For example, Obermiller et al. (32) showed that positive-pressure ventilation caused reverse pulmonary venous blood flow in an in situ ischemic dog lung with patent pulmonary veins by cyclic ventilatory compression of alveolar capillaries. We reasoned that ventilating by lowering the surface pressure of the lung and keeping alveolar pressure constant relative to vascular pressure would minimize this effect, because the tidal emptying and filling of the alveolar vessels would be eliminated (39).

As shown in Fig. 2, this form of ventilation proved to be equally efficacious in preventing vascular injury, suggesting that ventilation was not serving as a surrogate pump to deliver nutrients to the alveolar capillary bed. This result is consistent with the observation that the protective effect of ischemic ventilation on reperfusion injury in intact dog lungs occurred after occlusion of all vascular connections to the ischemic lung (17). On the basis of these data, we concluded that the protective effect of ventilation was mediated by a direct effect of lung stretch that was independent of gas exchange and motion of the blood.

Effect of Lung Distension on Cyclic Nucleotide Concentrations

Ventilatory distension of the lung has been shown to trigger release of surfactant (45) and prostanoids (13), enhance lung parenchymal substrate utilization (45), and increase lung cyclic nucleotide levels (19, 46). To determine whether changes in tissue cyclic nucleotide concentrations could be involved in the protection conferred by ventilation, we measured cGMP and cAMP in lung biopsies obtained at the beginning and end of the ischemic period. Although our baseline biopsy was obtained \(-2\)–\(5\) min after death, the baseline cyclic nucleotide concentrations averaged across all groups \((n = 27)\) and expressed per gram wet weight \((0.28 \pm 0.05\) and \(1.54 \pm 0.22\) nmol/g wet wt for cGMP and cAMP, respectively) were similar to lung cyclic nucleotide concentrations averaged from six separate groups of normal intact rats \((0.42 \pm 0.24\) and \(1.13 \pm 0.17\) nmol/g wet wt for cGMP and cAMP, respectively) from previous studies (18, 19, 22), suggesting that significant changes in cyclic nucleotide concentration did not occur between death and the baseline biopsy. The time course of lung cGMP concentration differed as a function of ventilation, decreasing more in nonventilated than in ventilated lungs (Fig. 3). cAMP levels were not affected by ischemia or ventilatory status (Fig. 4). These data suggested that, in the absence of blood flow, ventilatory distension of the lung provided an important stimulus for the continued generation of cGMP or inhibited excessive cGMP degradation.

Whereas ventilation of ischemic sheep lungs served to maintain levels of cGMP, large tidal volume ventilation increased lung cGMP concentration in intact anesthetized rats (19) and perfuse cGMP concentration in perfused rat lungs (6). Similar to our results, ventilation did not affect cAMP concentrations in either study (6, 19). The cellular source of the ventilation-induced change in cGMP in these studies was unknown, but the large increase in perfuse but not parenchymal concentrations observed in the rat lung study (6) coupled with the poor diffusibility of cyclic nucleotides across cell membranes (10) suggest that the source may have been vascular endothelial cells. Cyclic stretch of systemic vessel endothelial cells in vitro (33) increased endothelial cGMP content, further supporting this notion. Unfortunately, there are no published measurements of cGMP after in vitro stretch of pulmonary arteries or pulmonary artery endothelium.

A large body of literature supports a causal relationship between the increased cGMP concentration and \( \sigma_{alb} \) in the ventilated lungs. Pharmacological interventions designed to increase cellular cGMP concentration, such as administration of cGMP analogs, atrial natriuretic peptide (ANP), or NO, prevented increased protein permeability in pulmonary endothelial monolayers (53) and intact lungs after a variety of injuries, including IR lung injury (24, 27, 42). Similar results have been obtained by treatments designed to increase lung cAMP concentrations (49, 53). The mechanisms behind the protective effects of cGMP and cAMP are uncertain but are hypothesized to involve inhibition of neutrophil adhesion (42, 49) as well as direct effects on the endothelium to reverse cytoskeletal contraction and intercellular gap formation through activation of cyclic nucleotide-dependent protein kinases (53).

Effect of SNP and Iso on Vascular Permeability and Cyclic Nucleotide Concentrations in Nonventilated Lungs

If the increase in pulmonary vascular permeability in nonventilated lungs was related to the marked decrease in tissue cGMP concentration, we reasoned that increasing the cGMP content in nonventilated lungs into the normal range should decrease vascular permeability. As discussed below, there are multiple pathways for stimulating cGMP production. We chose activation of soluble guanylate cyclase (sGC) by administration of SNP, an NO donor compound, because SNP was shown to increase the cGMP concentration in nonventilated, ischemic dog lung slices (9) and pulmonary endothelial cells in vitro (53). Therefore, an inability to increase cGMP concentration in the nonventilated lungs would suggest that sGC activity was depressed or adequate substrate concentrations for sGC were lacking. As shown in Fig. 3, this was not the
case; the SNP-treated nonventilated lungs had a final cGMP concentration that was not different from the ventilated group, indicating that the sGC pathway was intact. Moreover, SNP treatment increased the nonventilated \( \sigma_{ab} \) into the normal range, suggesting that the effect of ventilation on vascular permeability may have been mediated through its effect on cGMP concentration.

The results in the Iso-treated nonventilated group were consistent with the potential importance of cGMP. Although Iso markedly increased tissue cAMP levels, \( \sigma_{ab} \) remained low (Fig. 4) accompanied by a decrease in the cGMP concentration similar to that seen in the untreated nonventilated lungs (Fig. 3). When combined with the cAMP time course data in the untreated lungs, these data strongly suggest that an endothelial deficiency of cAMP was not the cause of the increased vascular permeability in nonventilated ischemic lungs. Moreover, the inability to correct the \( \sigma_{ab} \) by increasing tissue cAMP concentrations suggests that the effect of SNP was not mediated through a cGMP-inhibited cAMP phosphodiesterase, one of the proposed mechanisms for the beneficial effect of cGMP on endothelial barrier function (53).

As mentioned above, other studies of IR lung injury have found a potential role for cAMP in the generation and treatment of the vascular injury (30, 49). For example, Naka et al. (30) reported that the cAMP concentration decreased 33% in nonventilated rat lungs subjected to 6 h of ischemia before transplantation. Treatments designed to increase lung cAMP content attenuated reperfusion injury after transplantation (30), but these data are not directly comparable to the current study because of the differences in ischemic time and the focus on reperfusion (30) rather than ischemic injury.

Role of NO in Ventilation-Induced Effect on cGMP and Vascular Permeability

Guanylate cyclase, the enzyme responsible for cGMP synthesis, has two isoforms, sGC and particulate guanylate cyclase (pGC), designated by their location in the cell (47). Both enzymes are found ubiquitously in the lung, including pulmonary endothelium (40, 53), but they respond to different agonists. sGC is activated by low-molecular-weight monoxides of nitrogen (NO), oxygen (O\(_2\)), and hydrogen (OH), whereas pGC is stimulated by natriuretic peptides and the peptide guanylin (47).

We considered NO to be a likely candidate for ventilation-induced cGMP production, because 1) NO synthase was widely present in normal rat and human lung by immunohistochemistry, including pulmonary vascular endothelium (20); 2) mechanical stimuli have been shown to amplify lung NO production, including lung distension (41), pulmonary vascular distension (2), and pulmonary vascular shear stress (2); and 3) lung surface NO production decreased 70% over 6 h of nonventilated ischemia in excised rat lungs (42). If NO was responsible for the effect of ventilation in our study, we reasoned that administration of L-NAME to a ventilated lung should mimic the nonventilated state. As shown in Figs. 3 and 6, L-NAME had no effect on vascular permeability or lung cGMP concentration in ventilated lungs, even when administered before ischemia began, suggesting that NO was not involved. The same dose of L-NAME caused a threefold increase in pulmonary vascular resistance in perfused sheep lungs, suggesting that, unlike the pulmonary vasculature of the dog (2), the NO pathway is an important modulator of pulmonary vascular tone in the sheep (Fig. 7). D-NAME had no effect, and the administration of L-arginine reversed the effect of L-NAME, demonstrating that the increased resistance was secondary to NO synthase inhibition. These data suggest that the biomechanical stimuli of flow-induced shear stress and ventilatory lung stretch altered vascular function through separate biochemical pathways.

Interestingly, L-NAME blocked the protective effect of increased vascular volume on ischemic vascular injury in ferret lungs (3, 5), suggesting that vascular distension may have increased cGMP through NO release (3). Similarly, in isolated rabbit lungs, Schutte et al. (48) showed that increased vascular volume during ischemia decreased reperfusion injury. This study nicely demonstrated that vascular distension and ventilatory stretch had separate protective effects on the increase in K\(_t\) that occurred after reperfusion, but the mechanisms were not determined (48).

The mechanism for the effect of ventilation on lung cGMP concentration is unknown. Although we have not ruled out the possibility that ventilation stimulated sGC through a non-NO pathway, such as CO, the characteristics of the pGC axis make it an appealing possibility. The natriuretic peptide hormone ANP is synthesized in the lung, and ANP receptors are present on smooth muscle, epithelial, and endothelial cells (40). Muramatsu et al. (29) found increased concentrations of perfusate and tissue cGMP in isolated lungs from chronically hypoxic rats that could be decreased by treatment with an antibody to ANP but not by NO synthase inhibition, whereas inhibition of NO synthase but not ANP antibody treatment increased pulmonary vascular resistance, suggesting that, under these conditions, ANP increased cGMP in a non-smooth-muscle cell compartment, possibly endothelium. Moreover, ANP decreased pulmonary vascular resistance (40) and protected pulmonary endothelial barrier function from thrombin- and oxidant-induced injury in monolayers (23, 53) and isolated lungs (23) by increasing cGMP. ANP is released from the atria by stretch, but no published studies have examined the effect of ventilation on AnP release.

The relationship between the changes in lung cGMP concentration and vascular permeability observed in this study has two potential caveats. 1) We do not know which cells in the lung altered their cGMP concentration as a function of ventilation or SNP treatment. Changes in individual cellular compartments, such as the pulmonary endothelium, could have been missed by measuring the signal from whole lung homogenate. 2) The restoration of normal lung cGMP and vascular
permeability in nonventilated lungs does not prove a causal relationship between the two events. As discussed above, NO attenuates increased endothelial permeability from many causes. Thus SNP could have been protective, even if the vascular insult in nonventilated lungs was unrelated to the decrease in whole lung cGMP concentration. If the protective effect of SNP was nonspecific, however, we might have expected ISO to also attenuate the injury given the number of studies that demonstrate the coprotective effects of cGMP and cAMP in the same injurious processes (30, 42, 53). Further studies examining the effect of stretch on cyclic nucleotides and permeability in isolated vessels and endothelial monolayers may help resolve these issues.

Effect of Ventilation on $\Delta Hct_{\text{max}}$ and $\Delta AIB_{\text{max}}$

As discussed above, the lack of a difference in $K_r$ between nonventilated and ventilated lungs suggested that water permeability was not affected by ventilation or the $K_r$ measurement was unable to detect a regional increase in fluid conductivity. To distinguish between these possibilities, we calculated the mean $\Delta Hct_{\text{max}}$, which is an index of maximal regional water permeability in the pulmonary vasculature. If ventilation had truly independent effects on water and protein permeability, we would have expected to see similar $\Delta Hct_{\text{max}}$ values between the two groups. Under these circumstances, the difference in $\sigma_{\text{diff}}$ would have occurred solely because of differences in $\Delta AIB_{\text{max}}$. The $\Delta Hct_{\text{max}}$ was significantly greater in nonventilated lungs (Fig. 8), however, suggesting that the lack of ventilation was also associated with an increase in water permeability. We can think of only two ways to reconcile this result with the whole lung $K_r$ and extravascular lung water data: 1) total vascular surface area was reduced in the nonventilated lungs, or 2) the region of increased water permeability in the nonventilated lungs occurred in such a focal region that it was not detected by $K_r$. In the former scenario the leftward shift of $\Delta Hct_{\text{max}}$ and $\Delta AIB_{\text{max}}$ in the nonventilated lungs could be secondary to decreased erythrocyte and plasma transit times because of decreased vascular volume, whereas in the latter it may represent an anatomic shift in the locus of maximal permeability in nonventilated lungs toward (or within) the arterial extra-alveolar vessels. On the basis of our current data, we are unable to distinguish between these two possibilities. A reduction in vascular surface area in nonventilated lungs could contribute to the increased pulmonary vascular resistance observed during reperfusion after nonventilated ischemia (16, 31). The recent observation (34) that ventilation during ischemia was associated with a lower pulmonary vascular resistance during reperfusion than with static inflation during ischemia lends credence to this theory.

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