Effect of time and vascular pressure on permeability and cyclic nucleotides in ischemic lungs

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Pearse, David B., and Patrice M. Becker. Effect of time and vascular pressure on permeability and cyclic nucleotides in ischemic lungs. Am J Physiol Heart Circ Physiol 279: H2077–H2084, 2000.—We previously found that increased intravascular pressure decreased ischemic lung injury by a nitric oxide (NO)-dependent mechanism (Becker PM, Buchanan W, and Sylvester JT. J Appl Physiol 84: 803–808, 1998). To determine the role of cyclic nucleotides in this response, we measured the reflection coefficient for albumin ($\sigma_{abl}$), fluid flux (J), cGMP, and cAMP in ferret lungs subjected to either 45 min (“short”; $n = 7$) or 180 min (“long”) of ventilated ischemia. Long ischemic lungs had “low” (1–2 mmHg, $n = 5$) or “high” (7–8 mmHg, $n = 6$) vascular pressure. Other long low lungs were treated with the NO donor (Z)-1-[(3-ammoniopropyl)-N-(n-propyl)amino]diazen-1-ium-1,2-diolate (PAPA-NONOate; $5 \times 10^{-4}$ M, $n = 6$) or 8-bromo-cGMP ($5 \times 10^{-4}$ M, $n = 6$). Compared with short ischemia, long low ischemia decreased $\sigma_{abl}$ (0.23 ± 0.04 vs. 0.73 ± 0.08; $P < 0.05$) and increased J (1.93 ± 0.26 vs. 0.58 ± 0.22 ml·min$^{-1}$·100 g$^{-1}$; $P < 0.05$). High pressure prevented these changes. Lung cGMP decreased by 66% in long compared with short ischemia. Lung cAMP did not change. PAPA-NONOate and 8-bromo-cGMP increased lung cGMP, but only 8-bromo-cGMP decreased permeability. These results suggest that ischemic vascular injury was, in part, mediated by a decrease in cGMP. Increased vascular pressure prevented injury by a cGMP-independent mechanism that could not be mimicked by administration of exogenous NO.

cGMP; cAMP; lung injury; reflection coefficient; filtration coefficient

ISCHEMIA-REPERFUSION LUNG injury is characterized by increased pulmonary vascular permeability and edema (28) and occurs following medical (44) and surgical (18) thrombolysis, cardiopulmonary bypass (35), and lung transplantation (2). Although the clinical manifestations of lung dysfunction are observed following reperfusion, experimental evidence suggests that the injury begins during the ischemic phase (6, 7). For example, we demonstrated increased pulmonary vascular permeability to water and protein in isolated ferret (6) and sheep (30) lungs following 180 and 75 min of ischemia, respectively. The mechanism of the increased vascular permeability in ischemic lungs is unclear, but the detection of reactive oxygen species (ROS) (1) and lipid peroxidation products (7, 14) and a protective effect of ROS scavengers (7) suggests that a component of the injury may be oxidant mediated.

Several investigators have found that subjecting the ischemic pulmonary vasculature to mechanical strains can have an ameliorating effect on the injury following reperfusion (3, 26, 39). For example, either ventilatory stretch (with or without oxygen) or increased static intravascular pressure during ischemia attenuated the increased vascular permeability after reperfusion (39). We extended these findings by demonstrating that these mechanical stimuli attenuated the increased vascular permeability caused by pulmonary ischemia before reperfusion. Specifically, ventilation for 75 min of ischemia prevented the decrease in the reflection coefficient for albumin ($\sigma_{abl}$) that occurred in statically inflated ischemic lungs (30). Statically inflated ischemic lungs had a marked decrease in peripheral lung cGMP concentration, whereas ventilated lungs did not, suggesting that the protective effect of ventilatory stretch may have been mediated by maintenance of endothelial cGMP concentrations (30). Interestingly, the protective effects of ventilation on $\sigma_{abl}$ and lung cGMP were not blocked by inhibition of nitric oxide (NO) synthase, suggesting that NO-induced stimulation of soluble guanylate cyclase (GC) was not involved (30). Ventilatory lung stretch was not protective after 180 min in ischemic ferret lungs, although the combination of ventilation and increased static vascular pressure blocked the increased vascular permeability at this time point (5). Unlike the effect of ventilatory stretch, the protective effect of increased vascular pressure was prevented by the administration of a NO synthase inhibitor, suggesting that hoop stretch of the vasculature attenuated injury via NO release (5).

On the basis of these observations, we hypothesized that 1) the increased vascular permeability observed after 180 min of ventilated ischemia with low vascular pressure would be accompanied by a decrease in lung...
cGMP concentration, 2) treatments designed to increase lung cGMP concentration would attenuate the increase in vascular permeability, and 3) the protective effect of increased static vascular pressure would be associated with sustained levels of lung cGMP concentration. To address these hypotheses, we measured lung cyclic nucleotide concentrations, \( \sigma_{alb} \), and fluid flux (\( J \)) in ventilated ferret lungs subjected to 180 min of ischemia in the presence or absence of increased static vascular pressure or an NO donor compound. These results were compared with measurements made after 45 min of ischemia. An additional group of 180 min ischemic lungs was treated with 8-bromo-cGMP, a cell-permeant cGMP analog, to determine whether directly increasing lung cGMP concentration could also mimic the effect of increased intravascular pressure.

**METHODS.** Adult male ferrets were anesthetized with pentobarbital sodium (50 mg/kg ip). After tracheotomy, mechanical ventilation was begun with room air at a tidal volume of 12 ml/kg body wt and respiratory rate of 20 breaths/min. An abdominal catheter incision was placed through a midline incision, heparin (1,000 U/kg iv) was administered, and the ferrets were rapidly exsanguinated. Ventilation was adjusted to 10 breaths/min with 95% O_2-5% CO_2 and positive end-expiratory pressure of 3 mmHg. These settings were constant for the remainder of the experiment. The pulmonary artery and left atrium were cannulated, and the lungs were excised. The pulmonary vasculature was flushed with 50 ml of PSS containing 3 g/dl albumin, 2 g/dl Ficoll, and no glucose as previously described (6).

**Effects of ischemic time and intravascular pressure.** After the lungs were flushed of residual blood, intravascular pressure (\( P_{iv} \)) was controlled by connecting the vascular cannulas to a pressurized reservoir containing the same flush solution, and airway and vascular pressures were measured by Statham model P50 transducers referenced to the level of the lung hilum. The temperature was maintained at 37°C by enclosing the lungs in plastic and submerging them in a water bath. The lungs were then subjected to either 45 min ("short"; \( n = 7 \)) or 180 min ("long"; \( n = 26 \)) of ischemia while maintaining vascular pressure at either 1–2 mmHg ("low" \( P_{iv} \)) or 7–8 mmHg ("high" \( P_{iv} \)). Lungs subjected to short \( P_{iv} \) ischemia (\( n = 7 \)) were compared with groups of long low \( P_{iv} \) (\( n = 8 \)) and long high \( P_{iv} \) (\( n = 6 \)) lungs. In additional groups of long low \( P_{iv} \) lungs, the NO donor (Z)-1-[N-(3-ammoniopropyl)-N-(n-propyl)amino]diazene-1-ium-1,2-diolate (PAPA-NONOate; 5 × 10^{-4} M, \( n = 6 \)) or the cell-permeant analog of cGMP, 8-bromo-cGMP (5 × 10^{-4} M, \( n = 6 \), was added to the PSS solution instilled into the lungs at the start of ischemia. The PAPA-NONOate and 8-bromo-cGMP were obtained from Sigma Chemical (St. Louis, MO) and prepared fresh daily.

After the desired ischemic time, the right lung was freeze-clamped for measurement of lung cAMP and cGMP concentrations. The left lung was weighed and suspended from a force transducer. The pulmonary artery cannula was connected to a pressurized stirred reservoir containing a mixture of PSS and autologous washed erythrocytes (hematocrit (Hct) 20%). After the vasculature of the left lung was flushed with 10 ml, the left atrial cannula was connected to the same reservoir and intravascular pressure was increased from 15 to 30 mmHg in 5-min intervals to allow assessment of vascular leaks. \( P_{iv} \) was maintained at 30 mmHg for 20–30 min to allow convective fluid filtration. In six lungs (one short, two long low \( P_{iv} \), one long NO, and two long high \( P_{iv} \)), \( P_{iv} \) was increased in equal steps of 10 min each. After the increase in \( P_{iv} \), the intravascular PSS-erythrocyte mixture was pumped at 17 ml/min from the left atrial cannula to a fraction collector adjusted to obtain 1-ml samples.

Protein permeability was measured by determining \( \sigma_{alb} \), which was estimated by the filtered volumes method modified for a nonflowing system as previously described (6). Hct and albumin concentration were determined in duplicate for each sample, and \( \sigma_{alb} \) was estimated iteratively from the relationship

\[
\frac{C}{C_0} = \frac{1 - Hct_0 - \sigma (1 - Hct_0)}{1 - Hct_0 - \sigma}
\]

where \( x = (1 - Hct_0 - \sigma)/Hct_0 \), \( C \) represents albumin concentration, and \( C_0 \) and \( Hct_0 \) represent initial reservoir values.

To measure water permeability, we performed an additional analysis of the Hct vs. vascular volume relationship obtained for the measurement of \( \sigma_{alb} \). The amount of erythrocyte-free fluid that left the lung per unit period of time can be determined by

\[
J = \frac{\sum_{n=1}^{n-s} \frac{V_n(Hct_n - Hct_0)}{Hct_0}}{t}
\]

where \( n \) is the sample number, \( x \) is the last sample, \( V_n \) is the sample volume, and \( t \) is the duration of increased \( P_{iv} \). Lin et al. (19) described a similar technique to measure the distribution of fluid filtration in isolated rat lungs.

Figure 1 is a representative plot from a single experiment of filtered fluid vs. withdrawn vascular volume sample number after 20 min at \( P_{iv} \) of 30 mmHg. The shaded area in Fig. 1 represents the volume of fluid that had left the seventh
sample; \( J \) for the entire lung is the area under the curve. A complete Hct curve was present in 13 of 27 experiments. In the remainder, the down slope of the curve failed to intersect the x-axis. Under these circumstances, the curve was extrapolated on a semi-log plot. The average extrapolated sample number necessary to complete these curves was 4.3 \( \pm \) 0.8. The filtration time used in the calculation of \( J \) was chosen as the time spent at \( P_{iv} \) of 30 mmHg, because the contribution to filtration made by the short times (\( \leq 5 \) min) at the lower levels of \( P_{iv} \) was trivial, based on our previous measurements of filtration coefficient (\( K_f \)) in this preparation (5). An estimate of filtration time, and therefore \( J \), could not be determined in the six lungs that had a \( P_{iv} \) of 30 mmHg for less than 10 min, because of the significant contribution of the lower \( P_{iv} \) levels to filtration.

Cyclic nucleotide measurements. Lung biopsies were immediately frozen in liquid nitrogen for later determination of cAMP and cGMP by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI). Cyclic nucleotides were measured in an additional group of lungs \((n = 3)\) subjected to minimal ischemia (<15 min) to estimate in vivo lung concentrations.

Statistical analysis. The pulmonary vascular permeability and cyclic nucleotide data were analyzed by a one-factor ANOVA. The cyclic nucleotide data were not normally distributed and thus were transformed to logarithms before statistical analysis. When significant \((P \leq 0.05)\) variance ratios were obtained, least-significant differences were calculated to allow comparison of individual means. Values presented in the text are means \( \pm \) SE. Differences were considered significant when \( P \leq 0.05\).

RESULTS

Compared to short ischemic lungs, long low \( P_{iv} \) ischemia caused a decrease in \( \sigma_{alb} \) \((0.23 \pm 0.04 \) vs. \( 0.73 \pm 0.08\) \) and an increase in \( J \) \((1.93 \pm 0.26 \) vs. \( 0.58 \pm 0.22 \) ml\( \cdot \)min\(^{-1}\cdot\)100 g\(^{-1}\); \( P < 0.05\)), indicating increases \((P < 0.05)\) in vascular permeability to protein and water, respectively (Fig. 2). Treatment with 8-bromo-cGMP attenuated the increase in protein permeability as evidenced by a \( \sigma_{alb} \) value of 0.36 \( \pm \) 0.04, which was greater \((P < 0.05)\) than long low \( P_{iv} \) ischemia. 8-Bromo-cGMP treatment did not decrease \( J \) compared with untreated long low \( P_{iv} \) lungs, although there was a trend in this direction \((P = 0.09)\). The NO donor PAPA-NONOate had no effect on vascular permeability (Fig. 2), whereas the increased static vascular pressure in the long high \( P_{iv} \) lungs prevented the increases in protein and water permeability \((\sigma_{alb} = 0.65 \pm 0.07, J = 0.89 \pm 0.32 \) ml\( \cdot \)min\(^{-1}\cdot\)100 g\(^{-1}\); \( P < 0.05\)).

As shown in Fig. 3, lung cGMP concentration in short ischemic lungs \((0.21 \pm 0.06 \) ng/g\) fell within the 95% confidence intervals for lungs subjected to minimal ischemia, whereas lung cGMP concentration was significantly decreased by long low \( P_{iv} \) and long high \( P_{iv} \) ischemia \((0.07 \pm 0.01 \) and \( 0.04 \pm 0.01 \) ng/g, respectively) compared with short ischemic lungs. Both NO- and 8-bromo-cGMP-treated lungs had increased lung cGMP concentration compared with long low \( P_{iv} \) lungs. Lung cAMP concentration, which averaged \( 1.28 \pm 0.11 \) ng/g, did not differ between groups (Fig. 3).

DISCUSSION

Increasing evidence suggests that mechanical perturbations of the pulmonary vasculature during ischemia have a protective effect on the vascular permeability changes following ischemia and reperfusion of the lung (37, 39). We have been interested in the increased vascular permeability that occurs during ischemia before reperfusion (6). As shown in Fig. 2, subjecting ferret lungs to 180 min of warm, ventilated ischemia (long ischemia) decreased \( \sigma_{alb} \) compared with 45 min of ischemia (short ischemia), indicating an increase in protein permeability. The \( \sigma_{alb} \) is a dimensionless index that describes the ability of the vascular endothelium to maintain an oncotic pressure gradient during convective fluid movement; a \( \sigma_{alb} \) value of 0 indicates free movement of albumin across the vessel wall, whereas a \( \sigma_{alb} \) value of 1 indicates impermeabil-

![Fig. 2. Effect of short ischemia (\( n = 7 \)), long low \( P_{iv} \) ischemia (\( n = 8 \)), and long ischemia treated with either (Z)-1-[N-(3-ammoniopropyl)-N-(n-propyl)amino]diazen-1-ium-1,2-diolate (PAPA-NONOate), a nitric oxide (NO) donor \((n = 6)\), 8-bromo-cGMP \((n = 6)\), or high \( P_{iv} \) \((n = 6)\) on the reflection coefficient for albumin (top) and the fluid flux (bottom) \((n = 4, 6, 5, 6, \) and \( 4, \) respectively). Data are means \( \pm \) SE. \# \( P < 0.05 \) vs. short ischemic lungs. * \( P < 0.05 \) vs. long ischemia.
68% increase in vascular permeability (change in ventilated ischemic ferret lung. As shown in Fig. 3, the otide concentration and vascular permeability in the determine the relationship between lung cyclic nucle-

trations.

and $K_{n}$ ventilated sheep lungs where 75 min of low $P_{iv}$ ischemia (6). These data are similar to our experience in injury occurred at a time between 45 and 180 min of continued ventilation and low $P_{iv}$, ischemic vascular chemic ferret lungs, suggesting that, in the presence of not different from a separate group of minimally is-

s $K_{n}$ (Fig. 2) and $s_{alb}$ to be a useful vascular injury index, because it is sensitive to small changes in vascular permeability and is not affected by changes in vascular surface area (43). Long low $P_{iv}$ ischemia also increased vascular permeability to water as evidenced by an increase in $J_{w}$ in the current study (Fig. 2) and $K_{w}$ in a previous study (6). We previously showed that $s_{alb}$ and $K_{w}$ following short ischemia were not different from a separate group of minimally ischemic ferret lungs, suggesting that, in the presence of continued ventilation and low $P_{iv}$, ischemic vascular injury occurred at a time between 45 and 180 min of ischemia (6). These data are similar to our experience with the effect of ischemia on vascular permeability in ventilated sheep lungs where 75 min of low $P_{iv}$ ischemia was also associated with normal values of $s_{alb}$ and $K_{w}$ (29, 30).

Effect of ischemia on lung cyclic nucleotide concentrations. The first objective of the current study was to determine the relationship between lung cyclic nucleotide concentration and vascular permeability in the ventilated ischemic ferret lung. As shown in Fig. 3, the 68% increase in vascular permeability (change in $s_{alb}$) in the long ischemic lungs was associated with a 66% decrease in lung cGMP concentration compared with short ischemic lungs which, in turn, were not different from minimally ischemic lungs. Ischemia had no effect on lung cAMP concentration, suggesting that the effect of ischemia on lung cGMP concentration was not a manifestation of nonspecific cellular injury. We recently reported a selective decrease in lung cGMP concentration in ischemic sheep lungs (30). In that study, 75 min of ischemia under conditions of static inflation and low $P_{iv}$ caused a 99% decrease in lung cGMP concentration without altering lung cAMP concentration. The decrease in cGMP was associated with an increase in vascular permeability to protein and water. Ventilation attenuated both the decrease in cGMP and the increase in vascular permeability without altering pulmonary capillary blood gas tensions, suggesting that ventilatory lung stretch delayed ischemic vascular injury possibly by maintaining pulmonary endothelial concentrations of cGMP. In support of this hypothesis, treatment of statically inflated ischemic lungs with sodium nitroprusside, an NO donor, restored normal levels of both cGMP concentration and vascular permeability (30). The current data extend these findings by suggesting that the protective effect of ventilation on lung cGMP concentration in low $P_{iv}$ ischemic lungs becomes ineffective between 75 and 180 min of ischemia, coincident with the time that vascular permeability increases.

The mechanism behind the ischemia-induced decrease of lung cGMP is unknown. GC, the enzyme responsible for cGMP synthesis, has two isoforms, soluble (sGC) and particulate (pGC), designated by their location in the cell (38). Both enzymes convert GTP to cGMP and are present in conduit (45) and microvascular pulmonary endothelium (34, 46), but they respond to different agonists. sGC is activated by low-molecular weight monoxides of NO, oxygen (CO), and hydrogen (OH), whereas pGC is stimulated by natriuretic peptides and the peptide guanylin (38). The steady-state cGMP concentration is determined by the balance between production by GC and metabolism by phosphodiesterase (PDE) enzymes. Thus the ischemia-induced decrease in cGMP concentration occurred from decreased GC agonist stimulation, decreased GTP, decreased GC activity or enhanced PDE function.

Assuming that sGC is a major determinate of endothelial cGMP concentration, there is substantial indirect evidence to suggest that the decreased lung cGMP content observed in the present study could have resulted from decreased NO production. For example, mechanical stimuli have been shown to increase lung NO production including lung distension (32), pulmonary vascular distension (4), and pulmonary vascular shear stress (4), raising the possibility that the lack of these mechanical stimuli could lead to decreased NO production. In fact, lung surface NO production decreased 70% over 6 h of nonventilated ischemia in excised rat lungs (33). Unfortunately, the effect of ischemia on lung cGMP concentration was not determined in that study. On the other hand, NO synthase inhibition did not decrease cGMP concentration in ventilated sheep lungs after 75 min of ischemia, suggest-

Fig. 3. Effect of short ischemia ($n = 7$), long low $P_{iv}$ ischemia ($n = 8$), and long ischemia treated with either PAPA-NONOate, an NO donor ($n = 6$), 8-bromo-cGMP ($n = 6$), or high $P_{iv}$ ($n = 6$) on lung cGMP and cAMP concentrations. Solid and dashed lines are mean and 95% confidence limits for cyclic nucleotide concentrations from 3 minimally ischemic (<15 min) lungs. Data are means ± SE. *$P < 0.05$ vs. short ischemic lungs. **$P < 0.05$ vs. all other long ischemic groups.
ing that either the pGC system was involved or other pathways of sGC stimulation were more important than NO in maintaining cGMP levels (30). We could not find any published reports regarding the effect of pulmonary ischemia on GTP production or the activities of GC or PDE.

Although pharmacological interventions designed to increase cellular cGMP concentration decreased protein permeability in pulmonary endothelial monolayers (45) and intact lungs following a variety of injuries, including ischemia-reperfusion lung injury (22, 23, 33), less is known about the effects of a selective decrease in cGMP concentration. Garthwaite and Garthwaite (15) found that inhibition of GC (by any of 5 unrelated inhibitors) induced cell necrosis in rat cerebral slices incubated in 95% O₂. Cell death was attenuated by reducing the O₂ concentration, administering antioxidants, or treating with 8-bromo-cGMP, suggesting that a decrease in cGMP predisposed to oxidant injury. The mechanism of this effect was not determined, but subsequent studies by Munkres (24) in strains of mutant yeast suggested that a defect of cGMP synthesis caused multiple antioxidant enzyme deficiencies including deficits in mitochondrial and cytosolic superoxide dismutase. Supplementing cGMP or antioxidant enzymes, but not cAMP, reversed the antioxidant enzyme deficiencies and defects in growth and development attributed to excessive oxidant injury in the mutant strains. The mechanism for cGMP regulation of antioxidant enzyme function was not determined, although an inhibitory effect of actinomycin D indicated that gene transcription was a necessary step. These data suggest that a reduction in cGMP could interfere with cellular antioxidant function and therefore predispose to cellular dysfunction or injury under conditions of increased oxidant stress.

**Effect of NO and 8-bromo-cGMP on vascular permeability and lung cGMP concentration.** To determine whether the decrease in cGMP observed in the long low Pᵢᵥ lungs was responsible for the increase in vascular permeability, we attempted to restore cGMP levels back to the normal range by administering the NO donor compound PAPA-NONOate. PAPA-NONOate spontaneously releases NO in a first order process with a half-life of ~15 min at physiological pH and temperature (17). As shown in Figs. 2 and 3, generation of NO within the pulmonary vasculature restored normal lung cGMP concentration without ameliorating the increased pulmonary vascular permeability. There are several possible explanations for this result, specifically: 1) the decreased lung cGMP concentration was a marker of ischemic injury but was not a cause of increased vascular permeability; 2) NO failed to increase cGMP in critical endothelial or subendothelial compartments despite an increase in whole lung cGMP concentration; 3) the duration of the cGMP response was inadequate to sustain protection to the end of the protocol because NO production stopped or PDE activity increased; and 4) a separate injurious effect of NO occurred countering cGMP-mediated protection. When NO concentrations exceed a critical level, protective antioxidant effects or cGMP-mediated actions may be overwhelmed by NO toxicity (17). The toxic effects of excess NO may be mediated by the generation of additional ROS (25) such as peroxynitrite or may occur through oxidant-independent pathways (17). To address this possibility, we treated two additional long low Pᵢᵥ lungs with 10 μM PAPA-NONOate. Vascular permeability in these lungs remained elevated as evidenced by an average σᵢᵱₑ of 0.16 (data not shown), suggesting that the lack of protection conferred by PAPA-NONOate was not due to NO toxicity.

To bypass the potential problems associated with NO, we treated a separate group of long low Pᵢᵥ lungs with 8-bromo-cGMP, a cell-permeant cGMP analog resistant to PDE. We used the concentration of 8-bromo-cGMP that conferred 100% survival in a rat model of lung transplantation described by Pinsky et al. (33). As shown in Figs. 2 and 3, 8-bromo-cGMP produced a small but significant increase in σᵢᵱₑ accompanied by an increase in lung cGMP concentration, indicating a cGMP-induced decrease in protein permeability. Treatment with 8-bromo-cGMP did not decrease J, however, suggesting that water permeability was unaffected. A change in protein but not water permeability is consistent with the theory that different-sized pores are responsible for the movement of water and protein across the microvascular barrier of the lung (41). An increase in the number of large pores responsible for protein conductance could theoretically decrease σᵢᵱₑ without affecting J (41, 43).

As mentioned above, cGMP administration has been shown to attenuate several forms of oxidant injury including ischemia-reperfusion lung injury (33), hydrogen peroxide-induced liver injury (8), and hydrogen peroxide-induced protein permeability in pulmonary artery (40) and aortic endothelial monolayers (21). Administration of cGMP has been less successful in preventing increases in pulmonary endothelial permeability that were not mediated by ROS such as thrombin (27), phorbol myristate acetate (9), and protamine (13), perhaps suggesting that cGMP interferes at a relatively proximal, ROS-dependent step in the pathway mediating the increase in permeability. In this regard, it was interesting to note that the effect of 8-bromo-cGMP on vascular permeability in the long low Pᵢᵥ group in the current study (Fig. 2) was nearly identical to the previously reported effect of adding superoxide dismutase and catalase to the vascular flush solution of long low Pᵢᵥ lungs which caused σᵢᵱₑ to increase from 0.19 to 0.32 (7). One explanation for this result was that only a small component of the ischemic injury present at 180 min was oxidant-mediated and thus responsive to antioxidants or cGMP. The absence of a correlation between vascular permeability and lipid peroxidation in lungs subjected to varying ischemic times (7) supports this conclusion.

Potential downstream targets for cGMP include cGMP-dependent protein kinases (PKG), cGMP-regulated ion channels, and cGMP-regulated PDE (20). The precise mechanisms underlying the antioxidant properties of cGMP remain poorly understood, but the abil-
ility to reverse the protective effects of 8-bromo-cGMP on reperfusion lung injury with an inhibitor of PKG (33) suggests that this may be the most important downstream pathway in ischemia-reperfusion lung injury. Moreover, PKG activity was recently reported in pulmonary microvascular endothelial cells (11). The potential targets and effects of PKG are numerous (11), but the specific mechanisms mediating the protection conferred by cGMP remain poorly understood. For example, one of the proteins phosphorylated by PKG, vasodilator-stimulated phosphoprotein (VASP), is associated with focal adhesions, where it interacts with cytoskeletal structures (12). Although the role of VASP in regulating endothelial barrier function is not known, phosphorylation of VASP or other cytoskeletal-associated proteins could explain the ability of cGMP to inhibit hydrogen peroxide-induced actin stress fiber formation in cultured endothelial cells (21). PKG modulates ion channels (42), ion pumps (42), and other second messenger pathways (36), however, so many possible protective mechanisms exist. Finally, the studies demonstrating a predisposition toward oxidant injury in cells with decreased cGMP (15, 24) suggest that PKG may be an important regulator of cellular antioxidant enzyme function.

Effect of increased $P_{iv}$ on vascular permeability and lung cGMP concentration. As shown previously (5), increasing static vascular pressure during 180 min of ischemia to a level above end-expiratory airway pressure prevented the increase in protein permeability observed in ischemic lungs with low vascular pressure (Fig. 2). Increased $P_{iv}$ did not attenuate the increase in $K_f$ in our previous study, although there was a trend for a decreased $K_f$ in the high $P_{iv}$ lungs. As shown in Fig. 2 in the current study, high $P_{iv}$ significantly decreased $J$, suggesting a protective effect on water permeability. Schutte et al. (39) recently showed in isolated rabbit lungs that an increased $P_{iv}$ (3–4 mmHg) during 120 min of ischemia prevented an increased $K_f$ following reperfusion. Although the mechanism of this effect was not determined, protection occurred in the presence or absence of ventilatory lung motion during ischemia (39), suggesting that the effect was mediated by vascular distention rather than enhanced ventilatory motion of blood between the alveolar and extra-alveolar vessels. We were able to block the protective effect of high $P_{iv}$ on ischemic vascular injury by $N^G$-nitro-$l$-arginine methyl ester ($l$-NAME), an NO synthase inhibitor, but not $d$-NAME, its inactive isomer, suggesting that vascular distension preserved barrier function through increased NO production (5). Moreover, the inhibition caused by $l$-NAME was reversed by adding excess $l$-arginine, confirming the specificity of the effect. On the basis of these data, we expected to see an increase in cGMP concentration in long high $P_{iv}$ lungs compared with long low $P_{iv}$ lungs. As shown in Fig. 3, however, lung cGMP concentration was decreased in both groups despite their differences in vascular permeability. The interpretation of this result depends on the ability of the whole lung measurement of cGMP to detect small changes within a single cellular compartment of the lung. Given that many investigators routinely use PDE inhibitors in their experimental systems to allow detection of small changes in cellular cyclic nucleotide concentrations, it may not be surprising that we were unable to see a difference in the absence of PDE inhibition. Moreover, pulmonary vascular endothelial cells were shown to transport cGMP into the vascular lumen of perfused lungs under conditions of increasing vascular distension at constant flow (4), suggesting an additional pathway for loss of endothelial cGMP. On the other hand, it is also possible that NO was generated in the alveolar capillary bed by vascular distension and prevented the adverse effects of ischemia without a concomitant change in cGMP concentration. When generated in small concentrations, NO has been shown to have potent antioxidant properties not dependent on the generation of cGMP (10, 17). Moreover, cultured rat pulmonary microvascular endothelial cells did not express sGC (34) and thus were not capable of an NO-induced increase in cGMP concentration. It is not known whether these cultured cells accurately reflected their in vivo counterparts or whether ferret lung is similar to rat lung in this regard.

Measurement of $J$. We used the profile of increased Hct values in the sequential vascular volume samples to calculate $J$. As an index of water conductance, this measurement has both advantages and disadvantages compared with a $K_f$ value determined from measuring lung weight. The advantages include the ability to measure an index of water conductance that is not affected by changes in vascular volume or loss of edema fluid from the lung surface. Both of these factors influence the gravimetric measurement of $K_f$ (16, 31). The disadvantages of the $J$ measurement include an underestimation of maximal fluid conductance if red blood cells remain trapped in the pulmonary vasculature from the most injured regions or significant hemorrhage occurs.

Summary. Three hours of low $P_{iv}$ ischemia in ventilated ferret lungs caused an increase in vascular permeability that was associated with a decrease in lung cGMP (but not cAMP) concentration. Lungs subjected to shorter ischemic times had no change in either permeability or cGMP concentration. Restoration of lung cGMP concentrations with 8-bromo-cGMP, but not an NO donor, significantly attenuated the increase in permeability. Increased static pulmonary vascular pressure prevented the increase in vascular permeability but did not affect the decrease in cGMP concentration. We conclude that the increase in vascular permeability caused by 180 min of ischemia in ventilated lungs was mediated in part by a decrease in lung cGMP. Increased $P_{iv}$ prevented injury by a cGMP-independent mechanism that could not be mimicked in low $P_{iv}$ lungs by exogenous NO.

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