Nitric oxide decreases lung liquid production via guanosine 3′,5′-cyclic monophosphate

JAMES J. CUMMINGS1 AND HUAMEI WANG2
1Departments of Pediatrics and Physiology, East Carolina University School of Medicine, Greenville, North Carolina 27858; and 2Department of Pediatrics, State University of New York, Buffalo, New York 14222

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Cummings, James J., and Huamei Wang. Nitric oxide decreases lung liquid production via guanosine 3′,5′-cyclic monophosphate. Am J Physiol Lung Cell Mol Physiol 280: L923–L929, 2001.—We studied the role of cGMP in nitric oxide (NO)-induced changes in lung liquid production (Jv) in chronically instrumented fetal sheep. Forty-five studies were done in which Jv was measured by a tracer dilution technique. Left pulmonary arterial flow (Qlpa) was measured by a Doppler flow probe. There were two series of experiments. In the first, we gave 8-bromo-cGMP, a cGMP analog, either by the pulmonary vascular or intraluminal route; in the second, we used agents to inhibit or enhance endogenous cGMP activity. When infused directly into the pulmonary circulation, 8-bromo-cGMP significantly increased Qlpa but had no effect on Jv. Conversely, when instilled into the lung liquid, 8-bromo-cGMP had no effect on Qlpa but significantly reduced Jv. Inhibition of guanylate cyclase activity with methylene blue totally blocked, whereas phosphodiesterase inhibition with Zaprinast significantly enhanced, the effect of instilled NO on Jv. Thus the reduction in lung liquid caused by NO appears to be mediated by cGMP, perhaps through a direct effect on the pulmonary epithelium.

At the time of birth, dramatic changes must also occur within the pulmonary circulation to enable the lung to function as the organ of gas exchange. In the fetus, pulmonary blood flow is minimal because of high vascular resistance (36). The transition from fetal to newborn life is accompanied by a decrease in pulmonary vascular resistance that results in a severalfold increase in pulmonary blood flow and a decrease in pulmonary arterial pressure (17, 21). One or more mediators, including prostaglandins and nitric oxide (NO), may be involved (2, 8, 11, 18, 30, 31, 37, 39). Interestingly, these same mediators also decrease net lung liquid production (Jv) (13, 14, 29), suggesting a relationship between these two critical perinatal events.

NO mediates pulmonary vasodilation, in part, by stimulating soluble guanylate cyclase, thereby increasing cGMP levels in vascular smooth muscle (22, 32). Abman and Accurso (1) were the first to show that direct-acting cGMP agents increased pulmonary blood flow in the fetal lamb, suggesting an important role for the NO-cGMP pathway in the transition of the pulmonary circulation at birth. However, studies (15, 25, 26) of the effects of cGMP on fetal Jv have led to conflicting results.

In the present series of studies, we sought to better delineate the effects of the NO-cGMP pathway in the fetal lung. We measured Jv and pulmonary hemodynamics in chronically instrumented fetal lambs from 122 to 138 days of gestation. We compared the effects of a cGMP analog, either infused into the pulmonary circulation or instilled directly into the lung liquid, and found that we could separate the effects of cGMP on pulmonary vasodilation and lung liquid resorption. We also studied the effects of cGMP inhibition and enhancement on NO-induced lung liquid resorption and found support for the notion that NO reduces fetal lung liquid via cGMP stimulation.

METHODS

All operative procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee at the State University of New York (Buffalo, NY).

Surgical preparation. By methods previously described (13, 14), we prepared 25 singleton fetal lambs for chronic vascular access and measurement of net Jv. Time-dated pregnant ewes (mixed breeds) were operated on at 122 ± 3 days gestation (term is 147 days). The sheep received 750–1,000 mg of thiamylal sodium intravenously followed by general anesthesia with 1% halothane and nitrous oxide.

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delivered with supplemental oxygen by a piston-type ventilator (Harvard Apparatus, South Natick, MA). We opened the uterus through a midline abdominal incision and exposed the fetal head, neck, and left foreleg. A thoracotomy was performed in the left third intercostal space. The pericardium was opened, and an ultrasonic Doppler flow probe (Transonic Systems, Ithaca, NY) was placed around the left pulmonary artery just beyond the bifurcation from the main pulmonary artery. We then inserted polyvinyl catheters (0.40 mm ID, Performance Plastic, Akron, OH) into the left pulmonary artery and left atrium. The catheters and leads to the transducer were then secured to the exterior of the chest, and the chest was closed. Through an incision in the fetal neck, we inserted polyvinyl catheters into the carotid artery and jugular vein. We then made an incision along the side of the fetal trachea and inserted a 6-Fr Foley-type 1.5-ml balloon-tipped catheter (Fuji Systems, Tokyo, Japan) into the proximal trachea ~4–5 cm above the carina. The tracheal incision was closed around the catheter, isolating the distal 2–3 cm of the catheter within the tracheal lumen. With the balloon deflated, the diameter of the catheter was ~20% of the diameter of the trachea, thereby allowing free movement of liquid from the fetal lung into the upper airway. Through a second tracheal incision, distal to the first, a small polyvinyl catheter was inserted, and its tip was positioned well below the Foley balloon for later measurement of intratracheal pressure. After closing the neck incision, we sutured a catheter to the fetal skin for later monitoring of amniotic liquid pressure (to be used as a reference for all other pressure measurements). Skin incisions were closed, and all catheters were tunneled through the uterine and abdominal walls, which were doubly oversewn to prevent fluid leakage. A pouch was sewn to the maternal flank to prevent the ewe from damaging the catheters.

We injected antibiotics into the amniotic sac (1 million U of penicillin and 400 mg of gentamicin) and fetal vein (300,000 U of penicillin and 30 mg of gentamicin) at the time of surgery and daily thereafter. The ewe also received a daily intramuscular injection of a penicillin-dihydrostreptomycin mixture (1 million U of procaine penicillin G and 1,250 mg of dihydrostreptomycin sulfate; Combiotic, Pfizer, New York, NY) and 600 mg of gentamicin. Vascular catheters were flushed with isotonic saline and filled with a heparin solution (1,000 U/ml) daily.

**NO preparation.** On the day of each experiment, NO-saturated saline and vehicle were freshly and steriley prepared as follows. Fifty milliliters of saline were bubbled with pure nitrogen for 15 min in a rubber-capped flask. (It was previously determined that such a solution would become fully deoxygenated within 15 min.) This deoxygenated saline was then bubbled with a 10% NO-90% N₂ gas mixture (Matheson, Twinsburg, OH) that had been scrubbed with 1 M NaOH. During gas bubbling, the environmental temperature was maintained at 22–23°C. These steps were taken to avoid the production of nitrogen dioxide and peroxynitrite (35). Excess NO gas was withdrawn from the flask through an 18-gauge needle inserted into the rubber cap. The concentration of NO in the saturated saline solution was calculated from the chemical solubility data (42) as ~0.2 mM. In some preparations, the actual content of NO was measured by chemiluminescence (chemiluminescent NO analyzer model 42H, Thermo Environmental Instruments, Franklin, MA) and was consistent with our estimates made with solubility data. We also found that the NO content remained stable for up to 48 h if the solution remained capped and was kept in darkness. During each experiment, an aliquot of freshly prepared solution was instilled into the fetal trachea after an equal volume of lung liquid was removed. For control experiments, saline was similarly deoxygenated with 100% N₂ gas. 

**Measurement of pulmonary blood flow.** To ensure that no tracer was leaking out of the tracheal incision, we sampled amniotic fluid before inflating the tracheal balloon at the start of each experiment and compared it with amniotic fluid obtained at the end of each experiment just before deflating the balloon. Also, when the fetus was killed at the end of the study, we assessed the integrity of our tracheal drainage system by reinflating the Foley catheter, instilling methylene blue into the lung under pressure, and visually checking for leakage of dye into the oropharynx or around the tracheal suture site. In two cases, leakage of the label was detected, and data from these fetuses were excluded from analysis. Finally, to ensure the integrity of the radiolabel, we tested the stock solution of each radiolabel by precipitating the albumin with 10% trichloroacetic acid, spinning down the protein to a pellet, and measuring the supernatant for radioactivity. In all cases the amount of unbound radiolabel did not exceed 5%. When the 125I-labeled albumin was used, we also took plasma samples periodically to ensure that the radioactive tracer remained within the lung lumen over the time course of the experiments. In none of these cases was radioactivity detectable in the plasma samples.

Duplicate 100-μl aliquots from each sample were assayed to obtain the concentration of tracer present. For the radioactive tracer, 125I activity was measured in a gamma counter (Isomedic model 10-600, ICN, Cleveland, OH); for the dye tracer, absorption was measured at 240-nm wavelength in a spectrophotometer. Fetuses that were studied more than once had samples of lung liquid taken at the start of each subsequent experiment to measure background tracer concentration in the lung liquid. After the instillation of fresh tracer, activity or absorption in samples taken during the course of the experiment was then adjusted by subtracting this background amount.

**Potential loss of label.** To ensure that no tracer was leaking out of the tracheal incision, we sampled amniotic fluid before inflating the tracheal balloon at the start of each experiment and compared it with amniotic fluid obtained at the end of each experiment just before deflating the balloon. Also, when the fetus was killed at the end of the study, we assessed the integrity of our tracheal drainage system by reinflating the Foley catheter, instilling methylene blue into the lung under pressure, and visually checking for leakage of dye into the oropharynx or around the tracheal suture site. In two cases, leakage of the label was detected, and data from these fetuses were excluded from analysis. Finally, to ensure the integrity of the radiolabel, we tested the stock solution of each radiolabel by precipitating the albumin with 10% trichloroacetic acid, spinning down the protein to a pellet, and measuring the supernatant for radioactivity. In all cases the amount of unbound radiolabel did not exceed 5%. When the 125I-labeled albumin was used, we also took plasma samples periodically to ensure that the radioactive tracer remained within the lung lumen over the time course of the experiments. In none of these cases was radioactivity detectable in the plasma samples.

**Measurement of pulmonary blood flow.** The Doppler flowmeter measured the transit time for an ultrasonic signal directed alternately in the upstream and downstream directions along the left pulmonary artery. The difference between the upstream and downstream integrated transit times was a measure of volume flow. All flow probes were precalibrated in the factory with a gravity-fed constant-flow bench setup. Manufacturer’s specifications included measurement of flows from 0 to 250 ml/min, with a maximum error of ±15%. Each probe was rechecked (zero-flow reading in a beaker of sterile saline) before surgical placement.

**Experimental protocol.** Animals were allowed to recover from surgery for at least 3 days before experiments began.
Experiments were done on unanesthetized fetuses that had normal, stable arterial pH and blood gas tensions (pH range, 7.30–7.40; arterial Po2 range, 16–24 mmHg; and arterial Pco2 range, 44–56 mmHg) and pulmonary arterial blood flow. The fetuses were studied while their ewes stood upright in a cage with free access to food and water. Eighteen of the fetuses were studied more than once; a minimum rest period of 48 h between experiments was observed in those cases.

We measured arterial pH, Po2, and Pco2 in the fetuses hourly with a calibrated blood gas/acid-base analyzer (Acid-Base Laboratory 3, Radiometer Medical, Copenhagen, Denmark). We continuously measured vascular, tracheal, and amniotic liquid pressures with calibrated transducers connected to an eight-channel amplifier-recorder (Gould Electronics, Cleveland, OH). Vascular and tracheal pressures were referenced to liquid pressure within the amniotic sac. Both mean and phasic left pulmonary arterial blood flow (Qpaa) were recorded continuously. Variables were averaged every 10 min and then again within each experimental period.

Effect of 8-bromo-cGMP. We measured Jv, pulmonary blood flow, and vascular pressures during a 1- to 2-h baseline period for 1–2 h after administering 8-bromo-cGMP (treatment period) and then for 1–2 h after pulmonary blood flow and vascular pressure had returned to baseline (recovery period). In one series of experiments, we infused 8-bromo-cGMP into the pulmonary arterial catheter at a rate of 200 μg/min; this rate was chosen after dose-response experiments in which we infused 8-bromo-cGMP at rates of 50, 100, 200, and 400 μg/min and observed vascular responses. In a second, separate series of experiments, we instilled 5 mg of the cGMP analog directly into the lung liquid; this dose was chosen to achieve an estimated concentration in lung liquid of \(10^{-4}\) M. In all cases, the 8-bromo-cGMP (Sigma, St. Louis, MO) was prepared by dissolving the dry powder in normal saline; in the infusion experiments, we dissolved 24 mg in 10 ml and infused it at a rate of 5 ml/h.

Effect of cGMP inhibition or enhancement. We measured Jv, pulmonary blood flow, and vascular pressures for 1–2 h before, during, and after NO instillation in the presence of either methylene blue (to block cGMP production by guanylate cyclase) or Zaprinast (to block cGMP inactivation by phosphodiesterase). Each blocker (Sigma) was given twice, first to study its effects alone and then 5 min before NO instillation to study its effects on the NO instillation. Thus there were four experimental periods: baseline, block, block plus NO, and recovery. Both blockers were prepared by dissolving 3 mg in 3 ml of normal saline and were given as an intravenous bolus over 30–60 s. The 10% NO solution was instilled directly into the lung liquid (8 ml over 1–2 min); depending on the lung liquid volume at the time of instillation, the initial concentration of NO in the lung liquid was estimated to range from 0.01 to 0.02 mM. This represents an equivalent total dose of NO that would be achieved by breathing a 20 ppm gas mixture for \(\sim 1\) min (23).

Control studies. Lung liquid secretion and pulmonary hemodynamics were measured in similarly prepared fetuses, with saline used in place of either agent infusion or NO instillation. This resulted in four experimental periods, each lasting 1–2 h, similar to the NO experiments noted in Effect of cGMP inhibition or enhancement.

Data analysis. After adding a known quantity of tracer to the lung liquid, we calculated the volume of liquid within the lung by removing a 1- to 2-ml sample and measuring the tracer concentration. At each subsequent time point, we recalculated the volume of liquid within the lung by removing a sample of lung liquid, measuring the tracer concentration, and correcting for the amount of tracer removed in previous samples. We determined the cumulative lung liquid volume (actual volume plus cumulative volume removed for sampling), and this was plotted over time for each experiment. By least squares regression of the resulting linear plot, we calculated the initial lung volume (by extrapolating to time = 0) and \(Jv\), the rate of change of cumulative lung liquid volume over time (Fig. 1). \(Jv\) represents the sum of liquid secretion and absorption, processes that may coexist within the lung. A positive value for \(Jv\) indicates net liquid production, whereas a negative value indicated net absorption.

Results are expressed as means ± SD. ANOVA for repeated measures over time was used to assess changes in all variables; if a significant change was found by ANOVA, then mean values from the treatment and recovery periods were compared with the mean value from the baseline period with Scheffé’s test. Unpaired t-tests with the Bonferroni correction for multiple comparisons were used to compare control and experimental groups. A P value < 0.05 was taken as significant.

RESULTS

Control studies. In eight control experiments (7 fetuses, 130 ± 4 days gestation) that lasted at least 6 h, there was no change in pulmonary blood flow or \(Jv\) between the experimental periods (Figs. 2–5). There were also no significant changes in pulmonary or systemic arterial blood pressure, left atrial pressure, or heart rate (Tables 1 and 2) or in blood gas tensions or systemic arterial pH (data not shown).

cGMP infusion studies. When infused into the pulmonary circulation, 8-bromo-cGMP (200 μg/min) increased Qpaa but had no effect on \(Jv\) (Figs. 2 and 3). In 10 experiments in 7 fetuses (128 ± 3 days gestation), Qpaa increased from 49 ± 18 to 88 ± 38 ml/min (P = 0.009). With no significant changes in pulmonary arterial or left atrial pressures, calculated pulmonary vascular resistance decreased ~40%. Pulmonary arterial resistance returned to baseline values within 90 min. There were no significant changes in carotid arterial pressure (Table 1), arterial blood gas tensions, or pH.
after 8-bromo-cGMP infusion. There was a small but significant increase in fetal heart rate ($P < 0.003$) during 8-bromo-cGMP infusion (Table 1).

cGMP instillation studies. When instilled directly into the fetal lung liquid, 8-bromo-cGMP ($10^{-4}$ M) decreased $J_v$ but had no effect on $Q_{lpa}$ (Figs. 2 and 3). In 12 experiments in 8 fetuses (131 ± 5 days gestation), $J_v$ decreased from 22 ± 9 to 2 ± 16 ml/h ($P = 0.0002$). $J_v$ returned to baseline values within 90 min. There were no significant changes in pulmonary or carotid arterial pressures, left atrial pressure, or heart rate (Table 1) or in arterial blood gas tensions or pH (data not shown) after 8-bromo-cGMP instillation.

Methylene blue studies. Methylene blue injection (3 mg) caused no significant change in any variable and totally blocked the effect of NO on $J_v$. In six experiments in five fetuses (130 ± 4 days gestation), NO instillation after methylene blue pretreatment resulted in a modest increase in $Q_{lpa}$ from 61 ± 16 to 86 ± 33 ml/min ($P = 0.033$) but no change in $J_v$ from its baseline value of 19 ± 4 ml/h (Figs. 4 and 5). There were no significant changes in left atrial pressures, heart rate (Table 2), arterial blood gas tensions, or pH (data not shown) after either methylene blue injection or NO instillation. However, both pulmonary and carotid arterial pressures increased after methylene blue injection, returned to baseline during NO instillation, and again rose as the hemodynamic effects of NO wore off ($P < 0.05$; Table 2).

Zaprinast studies. Zaprinast injection (3 mg) resulted in a slight but significant decrease in $J_v$, with no change in pulmonary hemodynamics. NO instillation (0.01–0.02 mM) after Zaprinast injection resulted in a further decrease in $J_v$ and significantly increased pulmonary arterial blood flow (Figs. 4 and 5). In nine experiments in nine fetuses (131 ± 3 days gestation), Zaprinast injection alone decreased $J_v$ from 23 ± 6 to 17 ± 4 ml/h ($P = 0.01$). After Zaprinast pretreatment, NO instillation further decreased $J_v$ to 4 ± 11 ml/h ($P = 0.0005$) and also significantly increased $Q_{lpa}$ from 54 ± 18 to 108 ± 16 ml/min ($P < 0.0001$). With no
significant changes in pulmonary arterial or left atrial pressures, the calculated pulmonary vascular resistance decreased ~60%. Pulmonary arterial resistance returned to baseline values within 90 min. There was also a small but significant decrease in carotid arterial pressure from 42 ± 3 to 40 ± 3 mmHg after NO instillation (P = 0.008). There were no significant changes in heart rate (Table 2), arterial blood gas tensions, or pH (data not shown) after either Zaprinast injection or NO instillation.

**DISCUSSION**

These studies confirm a previous finding by our laboratory (14) that NO instilled into the lung liquid of fetal sheep caused significant decreases in both $J_v$ and pulmonary vascular resistance. They also confirm previous work in which Iwamoto and Morin (23) documented the effects of instilled NO on the fetal pulmonary circulation.

Because NO mediates pulmonary vasodilation, in part by stimulating soluble guanylate cyclase, we asked whether this was also the mechanism by which it reduces $J_v$. We first reported (16) that cGMP instilled into fetal lung liquid results in a reduction in net $J_v$ without changing pulmonary hemodynamics. Junor et al. (25) recently reported similar findings in fetal sheep, although the reduction in net $J_v$ was more modest. Pulmonary hemodynamics were not measured in that study.

Because cGMP is a potent vasodilator, the lack of pulmonary hemodynamic changes after cGMP instillation suggests that the effects may be localized to the pulmonary epithelium. The separation of epithelial and endothelial effects was further demonstrated by our reverse findings when cGMP was infused directly into the pulmonary circulation; this caused a significant increase in pulmonary blood flow but no change in luminal liquid secretion.

A temporal separation of the epithelial and endothelial effects of cGMP was noted by Kabbani and Cassin (26), who performed similar infusions in seven fetal sheep. During a 1-h infusion of 8-bromo-cGMP, they found an immediate increase in pulmonary hemodynamic changes after cGMP instillation. Because cGMP is a potent vasodilator, the lack of pulmonary hemodynamic changes after cGMP instillation suggests that the effects may be localized to the pulmonary epithelium. The separation of epithelial and endothelial effects was further demonstrated by our reverse findings when cGMP was infused directly into the pulmonary circulation; this caused a significant increase in pulmonary blood flow but no change in luminal liquid secretion.

**Table 1. Data from control and cGMP groups by experimental period**

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental Period</th>
<th>Days Gestation</th>
<th>n</th>
<th>Carotid Arterial Pressure, mmHg</th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Left Atrial Pressure, mmHg</th>
<th>Heart Rate, min⁻¹</th>
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<tbody>
<tr>
<td>Control (saline)</td>
<td>Baseline</td>
<td>130 ± 4</td>
<td>8</td>
<td>44 ± 5</td>
<td>45 ± 5</td>
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<tr>
<td>Vascular cGMP</td>
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Values are means ± SD; n = no. of experiments. *Significantly different from the preceding period within the same group, P < 0.05. †Significantly different from control value for the same period, P < 0.05.

**Table 2. Data from control and nitric oxide studies by experimental period**

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<th>Group</th>
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<th>Days Gestation</th>
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<th>Carotid Arterial Pressure, mmHg</th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Left Atrial Pressure, mmHg</th>
<th>Heart Rate, min⁻¹</th>
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<td>Methylene blue/nitric oxide</td>
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<td>46 ± 3*</td>
<td>3 ± 2</td>
<td>166 ± 22</td>
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<td>Zaprinast/nitric oxide</td>
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<td></td>
<td>Nitric oxide</td>
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<td>40 ± 3†</td>
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<td>158 ± 13</td>
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Values are means ± SD; n = no. of experiments. *Significantly different from the preceding period within the same group, P < 0.05. †Significantly different from control value for the same period, P < 0.05.
Interestingly, in contrast to Kabbani and Cassin (26), we could not demonstrate a delayed response of \( J_v \) to the vascular infusion of cGMP despite monitoring the parameters for up to 2 h after the infusion. There are several possible explanations for this difference. First, the infusion rate of 8-bromo-cGMP they used was between 50 and 150% higher than that in our study; it is possible that at higher vascular concentrations, sufficient amounts could reach the pulmonary epithelium and exert a direct effect. Second, the fetuses used in our study were generally younger (range, 124–135 vs. 129–140 days). However, although it is conceivable that the effect of cGMP on \( J_v \) might be upregulated with advancing gestational age, we would expect to have seen some effect in our older fetuses, and we did not. Third, there may have been an indirect effect of cGMP infusion, particularly considering the much higher doses used in that study. Indeed, the authors themselves suggest that the sustained increase in heart rate they saw might have been indicative of epinephrine release, and epinephrine is known to elicit dramatic resorption of lung liquid.

Further evidence for the role of cGMP in NO-induced changes in \( J_v \) was provided by our next series of experiments in which we used agents known to enhance or inhibit cGMP activity. Methylene blue, an inhibitor of cGMP production, totally blocked the effect of NO on \( J_v \). Interestingly, methylene blue did not block the pulmonary hemodynamic effects of NO, even though it was infused directly into the pulmonary circulation. One possible explanation is that methylene blue does not inhibit cGMP production in the fetal pulmonary epithelium; another is that there is a guanylate cyclase-independent pathway of NO-induced pulmonary relaxation in the fetal lamb. However, these intriguing possibilities could not be further explored within the context of the present study.

Finally, we examined the effects of Zaprinast, a phosphodiesterase inhibitor, and found that it significantly enhanced the effect of NO on \( J_v \). In previous work (14), our laboratory showed that 10% NO instilled directly into the fetal lung liquid led to a 48% reduction in net \( J_v \). In the present study, we found that phosphodiesterase inhibition with Zaprinast not only caused a significant reduction in net \( J_v \) alone but also enhanced the effects of NO by causing a further 78% reduction in net \( J_v \). Taken together, these findings strongly support a cGMP-mediated pathway for the effects of NO on \( J_v \).

Our experiments with Zaprinast also strengthen our speculation as to the localization of the effect of cGMP. When Zaprinast alone was infused, we found a significant fall in \( J_v \) yet no change in pulmonary hemodynamics. This was similar to what we observed when we instilled cGMP directly into the lung liquid but not when we infused it directly into the pulmonary circulation. These observations suggest that significant cGMP activity was present at a site other than the pulmonary endothelium and support the notion that the pulmonary epithelium may have significant local production of cGMP.

The mechanism by which cGMP causes a reduction in \( J_v \) is unclear. Because net \( J_v \) is the result of secretory Cl\(^-\) and absorptive Na\(^+\) processes (33), a reduction in net \( J_v \) such as the one we found when we instilled the cGMP analog could be due to a decrease in Cl\(^-\) secretion, an increase in Na\(^+\) absorption, or both. However, numerous in vitro studies in pulmonary epithelium have shown that cGMP stimulates Cl\(^-\) secretion (27, 38) and inhibits amiloride-sensitive Na\(^+\) absorption (24, 28). If the predominant effects of cGMP were through these channels, it should lead to increased not decreased luminal liquid. Thus there must be an alternate mechanism by which cGMP leads to \( J_v \). One intriguing possibility is that cGMP might inhibit Na\(^+\)-K\(^+\)-ATPase in the pulmonary epithelium as it does in renal and brain tissue; however, a recent study (20) in cultured alveolar type II cells was not able to show such an effect.

In this study, we instilled NO in aqueous form into the lung liquid. That the epithelial surface may be the best route of exogenous NO administration has been suggested by Frostell et al. (19), who noted that this route would prevent immediate scavenging by oxyhemoglobin. The dose chosen was based on our previous work in which we showed that NO concentrations in the range of 10\(^{-5}\) to 10\(^{-4}\) M in the lung liquid led to measurable effects on the pulmonary circulation.

This study extends our previous work (14) and shows that the reduction in \( J_v \) caused by NO is mediated by cGMP, independent of its effects on the pulmonary endothelium.

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