NO and prostaglandin interactions during hemodynamic stress in the fetal ovine pulmonary circulation

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Zenge, Jeanne P., Robyn L. Rairigh, Theresa R. Grover, Laurent Storme, Thomas A. Parker, John P. Kinsella, and Steven H. Abman. NO and prostaglandin interactions during hemodynamic stress in the fetal ovine pulmonary circulation. Am J Physiol Lung Cell Mol Physiol 281: L1157–L1163, 2001.—Nitric oxide (NO) and prostacyclin (PGI2) are potent fetal pulmonary vasodilators, but their relative roles and interactions in the regulation of the perinatal pulmonary circulation are poorly understood. We compared the separate and combined effects of nitric oxide synthase (NOS) and cyclooxygenase (COX) inhibition during acute hemodynamic stress caused by brief mechanical compression of the ductus arteriosus (DA) in chronically prepared fetal lambs. Nitro-L-arginine (L-NNA; NOS antagonist), meclofenamate (Mec; COX inhibitor), combined drugs (L-NNA-Mec), or saline (control) was infused into the left pulmonary artery (LPA) before DA compression. In controls, DA compression decreased pulmonary vascular resistance (PVR) by 43% (P < 0.01). L-NNA, but not Mec, treatment completely blocked vasodilation and caused a paradoxical increase in PVR (+31%; P < 0.05). The effects of L-NNA-Mec and L-NNA on PVR were similar. To determine if the vasodilator effect of PGI2 is partly mediated by NO release, we studied PGI2-induced vasodilation before and after NOS inhibition. L-NNA treatment blocked the PGI2-induced rise in LPA blood flow by 73% (P < 0.0001). We conclude that NO has a greater role than PGs in fetal pulmonary vasoregulation during acute hemodynamic stress and that PGI2-induced pulmonary vasodilation is largely mediated by NO release in the fetal lung.

Pulmonary vascular resistance (PVR) decreases dramatically during the normal transition from the fetal to neonatal circulation at birth. During this transition, several physiological stimuli, including drainage of fetal lung fluid, rhythmic distension of the lung, and increased oxygen, contribute to the fall in PVR (3, 12, 18, 19, 22). Past studies have shown that these stimuli act in part by the release of nitric oxide (NO) and vasodilator prostaglandins (PGs) (3, 18, 20). Although nitric oxide synthase (NOS) inhibition blocks vasodilation resulting from increased oxygen and rhythmic lung distension, cyclooxygenase (COX) inhibitors attenuate pulmonary vasodilation caused by rhythmic distension but not increased oxygen (33).

Sudden changes in hemodynamic forces, including marked increases in shear stress, also contribute to the drop in PVR at birth (10). Vasoactive products, like NO and prostacyclin (PGI2), modulate the pulmonary vascular response to hemodynamic stress (3, 9, 18). In the chronically prepared fetal lamb, acute compression of the ductus arteriosus (DA) increases pulmonary blood flow, but this response is transient, and blood flow returns to baseline after several hours (1). Thus acute hemodynamic stress induced by mechanical compression of the DA causes pulmonary vasodilation, which is most likely the result of shear stress, and vasoconstriction, which may be the result of potent stretch stress or the myogogenic response (20).

It has been presumed that PGs and NO have additive effects on mediating pulmonary vasodilation at birth; however, the combined or interactive roles of NO and PGs in the fetal pulmonary vascular response to birth-related stimuli and acute hemodynamic changes have not been studied. NO and PGs cause vasodilation by increasing cGMP and cAMP production in the smooth muscle cell, respectively. Several studies have examined cross talk between NO and PGs in the systemic circulation, particularly at the level of cyclic nucleotide production (23). However, NO and PG interaction has not been studied in the perinatal pulmonary circulation.

To determine the relative contributions of PGs and NO to the regulation of the fetal pulmonary circulation, we studied the response to acute hemodynamic stress with a series of studies using the chronically prepared fetal lamb. We first examined the separate and combined effects of inhibition of NOS and COX during acute compression of the DA. To determine the relative potency of the NO and PG pathways in causing fetal pulmonary vasodilation, we then compared the direct effects of exogenous cGMP and cAMP on pulmonary...
vascular tone. To further evaluate the interactions between NO and PGs, particularly PGI₂, we examined whether PGI₂-induced pulmonary vasodilation is partly mediated by NO.

METHODS

Animal Preparation

The Animal Care Committee at the University of Colorado Health Sciences Center reviewed and approved all procedures and protocols used in these studies. Pregnant Columbia-Rambouillet ewes between 125 and 130 days gestation (147 days = term) were fasted for at least 24–48 h before surgery. Each animal was sedated with pentobarbital sodium (2–4 g for total dose) and anesthetized with a lumbar injection of 1% tetracaine hydrochloride (3 mg). Animals received prophylactic antibiotics (penicillin and gentamicin) before surgery. During anesthesia, the ewes breathed spontaneously. A hysteroscopy was performed using the sterile technique, and the fetal left forelimb was exteriorized. After local infiltration with 1% lidocaine, a skin incision was made under the left forelimb. Polyvinyl catheters (20 gauge) were placed in the ascending aorta and superior vena cava via the axillary artery and vein, respectively. Through a left thoracotomy and pericardiotomy, the left pulmonary artery (LPA), the main pulmonary artery (MPA), and left atrium were cannulated by direct puncture through purse-string sutures as previously described (3). An ultrasonic flow transducer (Transonic Systems, Ithaca, NY) was placed around the LPA during baseline periods, as previously described (20). The fetal left forelimb was gently placed back in the uterus. Ampicillin (500 mg) was infused into the amniotic cavity before closure of the hysterotomy. Polyvinyl catheters (20 gauge) were placed in the ascending aorta and superior vena cava via the axillary artery and vein, respectively. Through a left thoracotomy and pericardiotomy, the left pulmonary artery (LPA), the main pulmonary artery (MPA), and left atrium were cannulated by direct puncture through purse-string sutures as previously described (3). An ultrasonic flow transducer (Transonic Systems, Ithaca, NY) was placed around the LPA to measure blood flow to the left lung. To maintain patency of the DA, the wall of the DA was injected with 10% buffered formalin solution during a continuous infusion of PGE₁ (0.1 μg/min) and during DA compression until the compression was released (4). In another group, L-NNA and Mec were both infused into the LPA (31, 33).

Physiological Measurements

The flow transducer cable was connected to an internally calibrated flowmeter (Transonic Systems) for continuous measurements of the LPA blood flow. The absolute values of flow were determined from phasic blood flow signals obtained during the baseline periods, as previously described (20). The MPA, left atrial, aortic, and amniotic catheters were attached to blood pressure transducers (TSD 104; Biopac, Santa Barbara, CA) that were calibrated with a mercury manometer. Pressures and flow signals were recorded continuously on a Power Mac 7100/80 AV using an analog-to-digital converter system (Biopac). The data were sampled at a rate of 200 samples/s. Pressures were referenced to the amniotic pressure. Heart rate (HR) was determined from the phasic pulmonary blood flow signal. PVR in the left lung was calculated as the difference between mean pulmonary arterial pressure (PAP) and left atrial pressure (LAP) divided by mean LAP blood flow. Blood samples from the MPA catheter were used for blood gas analysis and oxygen saturation measurements (OSM 3 hemoximeter and ABL 520; Radiometer, Copenhagen, Denmark). All blood gases were measured at 39.5°C.

Drug Preparation

Fresh solutions of nitro-l-arginine hydrochloride (l-NNA; Sigma Chemical, St. Louis, MO) were made immediately before each study. l-NNA (30 mg) was dissolved with HCl and diluted with 1 ml of normal saline. NaOH was used to correct the solution to physiological pH (~7.40). Meclofenamate (Mec; Sigma Chemical) was prepared by dissolving 60 mg in 20 ml of normal saline. Before each study, a fresh sample of a 1:10 dilution with normal saline was prepared. PGI₂ (1 mg) was dissolved in 1 ml of 100% ethanol and was diluted with 1 ml of normal saline. 8-Bromo-cGMP and 8-bromo-cAMP (8-BrcAMP; 0.5, 1.5, or 3 mg; Sigma) were dissolved in 1 ml of normal saline under light-sensitive conditions. Study drugs were infused at a rate of 0.1 ml/min into the LPA. The dose of l-NNA was chosen from extensive published studies that have demonstrated effective blockade of NOS activity (9, 30). The dose of Mec was chosen from previously published studies from our laboratory (1) and others (31, 33).

We studied the hemodynamic response to acute DA compression after treatment with different doses of Mec (3, 30, and 60 μg/min). Compared with the 3 μg/min dose, we found a greater reduction in pulmonary blood flow during DA compression with the 30 and 60 μg/min doses. However, there was no difference observed between these two doses. Thus the minimum Mec dose used in this study was 30 μg/min (see below).

Experimental Design

The following four main experimental protocols are included in this study: 1) the separate and combined pulmonary vascular effects of NOS and COX inhibition during acute compression of the DA; 2) the separate and combined pulmonary vascular effects of NOS and COX inhibition during rapid incremental increases in PAP; 3) the effects of exogenous 8-BrcGMP and 8-BrcAMP on fetal pulmonary vasodilation; and 4) the effects of NOS inhibition on PGI₂-induced pulmonary vasodilation.

Protocol 1: Separate and combined pulmonary vascular effects of NOS and COX inhibition during acute compression of the DA. To study the separate and combined effects of NOS and COX inhibition on the hemodynamic response to DA compression, we partially inflated the DA occluder after treatment with l-NNA, Mec, or both agents. Control studies (n = 7) were performed using a normal saline infusion at 6 ml/h starting 10 min before occlusion until the occluder was deflated. l-NNA (30 mg over 10 min) was infused into the LPA 30 min before DA compression (n = 7). Mec (30 or 60 μg/min) was infused into the LPA 10 min before DA compression until the compression was released (n = 4). In another group, l-NNA and Mec were both infused into the LPA (n = 7). After stable hemodynamic measurements, the DA occluder was partially inflated for 30 min. The degree of DA compression was determined by targeting an increase in the mean PAP 35% above baseline values. Elevated mean PAP was maintained at a constant value throughout the compression period (Fig. 1). For each study, serial hemodynamic measurements were made at 10-min intervals throughout the baseline period (30 min), during drug infusion, and during DA compression. Arterial blood gas tensions
and pH were measured 10–20 min before occlusion and at 0, 30, and 60 min of the experiment.

Protocol 2: Separate and combined pulmonary vascular effects of NOS and COX inhibition during rapid incremental increases in PAP. To study the hemodynamic effects of NOS and COX inhibition on the myogenic response, we performed a series of rapid partial compressions of the DA after infusion of L-NNA and/or Mec. Four different treatment groups were studied. The control group \( (n = 4) \) received a continuous normal saline infusion at 6 ml/h (the same rate as the Mec infusion) starting 20 min before DA compression. A second group \( (n = 3) \) was treated with L-NNA (30 mg) 30 min before DA compression. A third group \( (n = 4) \) received a continuous infusion of Mec (30–60 \( \mu \)g/min) starting 20 min before DA compression. The fourth group \( (n = 3) \) was treated with L-NNA-Mec and Mec. The DA was compressed successively to increase mean pulmonary artery pressure (PAP) by 10, 20, or 40\% above baseline values in random order. Each compression was maintained for 10 min and was repeated after 10 min of recovery.

Protocol 3: Effects of exogenous 8-BrcGMP and 8-BrcAMP on fetal pulmonary vasodilation. To further evaluate the contributions of NO and PGI₂ on pulmonary vasodilation, we compared the effects of exogenous 8-BrcGMP and 8-BrcAMP on fetal pulmonary blood flow. After 30 min of stable baseline hemodynamic measurements, 8-BrcAMP or 8-BrcGMP \( (n = 4) \) was infused into the LPA at 10-min intervals serially. Hemodynamic measurements were recorded at 10-min intervals throughout the experiment. Arterial blood gases were measured at baseline and after the last drug infusion.

Protocol 4: Effects of NOS inhibition on PGI₂-induced fetal pulmonary vasodilation. To determine if PGI₂-induced vasodilation is partly mediated by NO release, we infused PGI₂ before and after treatment with the NOS antagonist L-NNA. After 30 min of stable baseline measurements, PGI₂ \( (400–500 \mu g; n = 4) \) was infused over 10 min into the LPA. A second dose of the study drug was repeated 30 min after the L-NNA infusion \( (30 \mu g \times 10 \) min). To determine whether the effects of L-NNA were specifically the result of NOS inhibition, we compared the response to 8-BrcGMP \( (n = 5) \), an endothelium-independent vasodilator that acts downstream to NOS blockade. Hemodynamic measurements were recorded at 10-min intervals throughout the experiment. Arterial blood gases and pH were measured at baseline and after the last drug infusion.

Data Analysis

Data are presented as means \( \pm \) SE. Statistical analysis was performed with the Prism software package. A one-way ANOVA for repeated measures was used to compare between-group or between-time points. One-way ANOVA with the Newman-Keuls post hoc multiple comparison test was used for comparison between groups at different time points. \( P < 0.05 \) was considered significant.

RESULTS

Protocol 1: Separate and Combined Effects of NOS and COX Inhibition on the Hemodynamic Response to Acute Compression of the DA

In control animals, DA occlusion increased LPA blood flow by 128\% above baseline values during the 30-min compression period (Fig. 1). PVR decreased by 42\% \( (P < 0.05 \) vs. baseline; Fig. 2). COX inhibition reduced the effects of DA compression seen in the control group by only 14\% and attenuated the fall in PVR by 33\%. The effects of COX inhibition were not different from those in control studies. After L-NNA treatment, the rise in LPA blood flow was blocked, and PVR increased by 31\% \( (P < 0.05) \). After combined L-NNA-Mec infusions, LPA blood flow did not increase during DA compression. This response was not different from that observed after L-NNA infusion alone. Mean aortic pressure (AoP), HR, LAP, pH, and blood gas parameters did not change during the study period or between study groups (Tables 1 and 2).

Protocol 2: Separate and Combined Effects of NOS and COX Inhibition on PVR During Serial Increases in DA Compression (Myogenic Response)

In control and Mec-treated animals, LPA blood flow progressively increased with each incremental change in MPA pressure (Fig. 3A). After treatment with L-NNA or L-NNA-Mec, LPA blood flow did not change despite progressive increases in MPA pressure (Fig. 3A).
In contrast to the fall of PVR in control and in Mec-treated animals, PVR actually increased with increasing MPA pressure after L-NNA and L-NNA-Mec treatment (Fig. 3B). Arterial blood gas tensions and pH, mean AoP, HR, and LAP did not change from baseline values during brief DA occlusions.

**Protocol 3: Effects of Exogenous 8-BrcGMP and 8-BrcAMP on Fetal Pulmonary Vasodilation**

Infusion of 8-BrcGMP increased pulmonary blood flow by 188% (P < 0.01; Fig. 4) and decreased PVR by 65% (P < 0.01; Table 3). In contrast to the vasodilator effects of 8-BrcGMP, 8-BrcAMP (0.5–3 mg) did not increase pulmonary blood flow (Fig. 4) or PVR (Table 3). Arterial blood gas tensions, pH, and HR did not differ between either treatment group (Table 3).

**Protocol 4: Hemodynamic Effects of PGI2 Before and After NOS Inhibition**

In control animals, PGI2 infusion increased pulmonary blood flow by more than twofold (85 ± 30 to 183 ± 47; P < 0.001) without changes in MPA pressures. After recovery, a repeat infusion of PGI2 caused the same magnitude of vasodilation. PGI2-induced pulmonary vasodilation was attenuated by 74% after NOS inhibition (P < 0.01; Fig. 5A). In contrast, the pulmonary vasodilator response to 8-BrcGMP remained intact after L-NNA (Fig. 5B), demonstrating selectivity.

| Table 1. Serial measurements of arterial blood gas tensions and pH at baseline and after 30 min of DA compression in each study group |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Control         | Mec             | l-NNA           | l-NNA-Mec       |
| pH              | 7.38 ± 0.01     | 7.37 ± 0.02     | 7.36 ± 0.01     | 7.37 ± 0.01     |
| 30 Min          | 7.33 ± 0.01     | 7.36 ± 0.01     | 7.35 ± 0.01     | 7.35 ± 0.01     |
| PCO2, mmHg      | 46 ± 0.9        | 47 ± 1.0        | 48 ± 1.3        | 46 ± 2.2        |
| 30 Min          | 47 ± 0.9        | 48 ± 0.5        | 49 ± 1.6        | 46 ± 2.0        |
| PAO2, mmHg      | 19 ± 1.2        | 23 ± 1.2        | 21 ± 0.8        | 22 ± 0.5        |
| 30 Min          | 20 ± 0.9        | 23 ± 1.0        | 20 ± 0.8        | 22 ± 0.6        |

Values are means ± SE. Mec, meclofenamate; l-NNA, nitro-L-arginine; PAO2, arterial Pao2; DA, ductus arteriosus.

| Table 2. Comparison of hemodynamic variables at baseline and after 30 min of DA compression between study groups |
|-----------------|-----------------|-----------------|-----------------|
|                 | Control         | Mec             | l-NNA           | l-NNA-Mec       |
| AoP, mmHg       | 42 ± 1.8        | 41 ± 1.3        | 40 ± 1.9        | 44 ± 1.2        |
| 30 Min          | 44 ± 3.5        | 41 ± 1.4        | 41 ± 1.3        | 48 ± 2.6        |
| LAP, mmHg       | 2.9 ± 0.3       | 2.3 ± 0.5       | 3.1 ± 0.9       | 2.7 ± 0.6       |
| 30 Min          | 3.6 ± 0.6       | 3.5 ± 0.7       | 2.8 ± 0.1       | 3.1 ± 1.0       |
| HR, beats/min   | 173 ± 7.0       | 140 ± 6.0       | 162 ± 9.0       | 175 ± 14.0      |
| 30 Min          | 207 ± 14*       | 149 ± 12        | 165 ± 8.0       | 198 ± 16.0      |

Values are means ± SE. AoP, aortic pressure; LAP, left arterial pressure; HR, heart rate. *P < 0.001 vs. baseline.
for NOS inhibition. Arterial blood gas tensions, pH, and HR did not change throughout the different treatments, whereas PAP and AoP did increase after L-NNA infusion (Table 4).

DISCUSSION

Although NO and vasodilator PGs modulate the pulmonary vascular tone in the developing lung, their relative roles in vasoregulation and potential interactions have not been studied. Therefore, we performed a series of experiments to investigate the separate and combined effects of NOS and COX inhibition on pulmonary vasoreactivity during brief DA compression in the chronically prepared fetal lamb. We report that NOS inhibition completely blocked the rise in pulmonary blood flow during DA occlusion and caused a paradoxical increase in PVR. In contrast, COX inhibition did not attenuate the rise in pulmonary blood flow and fall in PVR during acute DA compression. The combination of NOS and COX inhibition did not cause a further increase in PVR during DA compression from that achieved during NOS blockade alone. In addition, we found that NOS, but not COX, inhibition during incremental increases in partial DA compression unmasked a vasoconstrictor, or myogenic, response. Combined NOS and COX inhibition did not further augment the myogenic response beyond that observed with NOS inhibition alone.

To better understand the lack of an additive effect of combined COX and NOS inhibition during hemody-

Table 3. Comparison of hemodynamic variables and arterial blood gas tensions at baseline and after 30 min of 8-BrcGMP and 8-BrcAMP infusions

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<tr>
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<th>8-BrcGMP</th>
<th>8-BrcAMP</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 Min</td>
</tr>
<tr>
<td>PVR, mmHg·ml⁻¹·min</td>
<td>0.98±0.2</td>
<td>0.34±0.1*</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>47±2</td>
<td>44±2</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>46±0.5</td>
<td>43±2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>177±15</td>
<td>199±14</td>
</tr>
<tr>
<td>pH</td>
<td>7.35±0.01</td>
<td>7.35±0.01</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>19±0.5</td>
<td>20±0.6</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>46±2</td>
<td>49±3</td>
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</table>

Values are means ± SE. 8-BrcGMP, 8-bromo-cGMP; 8-BrcAMP, 8-bromo-cAMP; PVR, pulmonary vascular resistance; PAP, pulmonary artery pressure. *P < 0.05 vs. other treatment groups.

Table 4. Comparison of hemodynamic variables during PGI₂ treatment before and after L-NNA infusion

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<tr>
<th></th>
<th>Control</th>
<th>1-NNA</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>PGI₂</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>43±2</td>
<td>42±2</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>41±1</td>
<td>42±1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>160±8</td>
<td>169±11</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>21±1</td>
<td>20±1</td>
</tr>
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Values are means ± SE. *P < 0.05 vs. control and 1-NNA baseline values.
dynamic stress, we studied the effects of NOS inhibition on PGI2-induced fetal pulmonary vasodilation. We report that NOS inhibition blocked the vasodilator effects of PGI2 by 73%, suggesting that NO mediates most of the PGI2 response in the fetal lung. In addition, 8-BrcGMP, but not 8-BrcAMP, caused marked pulmonary vasodilation. Overall, these findings demonstrate that the NO-cGMP cascade is a more potent modulator of pulmonary vascular tone during acute hemodynamic stress than dilator PGs in the fetus.

Previous studies that have examined the roles of NO and PGs in modulating the response to shear stress in the postnatal circulation are conflicting. Abrupt increases in flow stimulate both NO and PG release in isolated endothelial cells in culture (5, 8, 11, 13, 24, 32). In isolated neonatal lamb lungs, NOS inhibition during normoxia increased PVR more than COX inhibition, but during hypoxia, COX inhibition augmented PVR more than NOS inhibition (15). Barnard et al. (6) compared the effects of NO and PG inhibition on the pulmonary vascular response to shear stress in isolated rat and dog lungs. They found that NO plays a greater role than PGs in mediating vasodilation during shear stress in the isolated perfused adult rat lung, but there is a greater role for PGs than NO in isolated dog lungs (6). Both NO and vasodilator PG inhibition increased vasoconstriction during shear stress in isolated porcine small pulmonary arteries (21). Thus the roles of NO and PGs in the modulation of vascular tone during acute hemodynamic stress appear to be dependent upon species, the age of the animal, and study conditions.

We have previously shown that the fetal pulmonary vasodilator response during acute DA compression is transient and that blood flow returns toward baseline values despite continued DA compression (1). Prolonged exposure to other vasodilator stimuli, such as increased oxygen tension and several pharmacological agents, also causes only transient pulmonary vasodilation (1, 2, 4). These findings suggest that, in the normal fetal pulmonary circulation, mechanisms exist that oppose vasodilation and maintain high PVR, which may reflect a potent myogenic response in the fetal lung (1, 7, 17). The myogenic response is partly defined as the ability of the vasculature to constrict in response to an increase in transmural pressure (7). Recently, we have shown that the myogenic response is normally present in the high-resistance fetal pulmonary circulation in vivo and is unmasked by NOS inhibition (30). Our current study extends these observations by demonstrating that COX inhibition did not cause a similar response and did not alter the effects of NOS inhibition alone.

Several potential mechanisms may underlie our observations that combined treatment with COX and NOS inhibitors does not have additive effects on shear stress-induced pulmonary vasodilation or on the vasoconstrictor response during increases in DA compression. First, dilator PGs, especially PGI2, may act in part through stimulation of NOS and increased NO production. Second, fetal vascular smooth muscle cells may be more responsive to cGMP than to cAMP, as suggested by our findings that 8-BrcGMP, but not 8-BrcAMP, causes fetal pulmonary vasodilation. Third, “cross talk” between these cyclic nucleotides can modulate the effects of the two dilator systems in the smooth muscle cell. For example, in the systemic circulation, interactions between the NO and PG pathways have been demonstrated at the level of cyclic nucleotides (23). In the cerebral circulation, cAMP may increase cGMP levels directly by inhibiting cGMP hydrolysis or indirectly through the activation of cGMP-dependent protein kinases (23). Fourth, PG-induced vasodilation may be partly mediated by the stimulation of NO release. We found that PGI2-induced vasodilation is blocked by nearly 75% after NOS inhibition. Other published reports, including studies of pig coronary arteries and rat Kuppfer cells (14, 28), have also observed that PGs stimulate endothelial release of NO. Finally, past studies have shown that NO may directly modulate PGI2 synthesis, but these findings have been conflicting. L-Arginine, a key substrate for NO production, increases PGI2 synthesis in isolated neonatal rat lungs (27) and in other models (10, 16, 26, 29, 34). Alternatively, NOS inhibition increases PG synthesis in isolated neonatal ovine lungs, suggesting that NO attenuates COX activity (15).

In conclusion, we found that NOS inhibition completely blocked shear stress-induced pulmonary vasodilation, whereas COX inhibition had no effect. NOS, but not COX, inhibition uncovered a myogenic response during hemodynamic stress in the fetal pulmonary circulation. In addition, NOS inhibition attenuated PGI2-induced pulmonary vasodilation, suggesting that PGI2 acts largely by NO release, and 8-BrcGMP, but not 8-BrcAMP, caused fetal pulmonary vasodilation. Thus the NO-cGMP cascade appears to play a more critical role than dilator PGs in the modulation of the vascular response to acute hemodynamic stress in the fetal lung, and NO may mediate PGI2 pulmonary vasodilation.

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