NO causes perinatal pulmonary vasodilation through K\(^+\)-channel activation and intracellular Ca\(^{2+}\) release

CONNIE B. SAQUETON, ROBERT B. MILLER, VALERIE A. PORTER, CARLOS E. MILLA, AND DAVID N. CORNFIELD
Division of Pediatric Pulmonary and Critical Care Medicine, Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455

Saqueton, Connie B., Robert B. Miller, Valerie A. Porter, Carlos E. Milla, and David N. Cornfield. NO causes perinatal pulmonary vasodilation through K\(^+\)-channel activation and intracellular Ca\(^{2+}\) release. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L925–L932, 1999.—Evidence suggests that nitric oxide (NO) causes perinatal pulmonary vasodilation through K\(^+\)-channel activation. We hypothesized that this effect worked through cGMP-dependent kinase-mediated activation of Ca\(^{2+}\)-activated K\(^+\) channel that requires release of intracellular Ca\(^{2+}\) from a ryanodinesensitive store. We studied the effects of 1) K\(^+\)-channel blockade with tetraethylammonium, 4-aminopyridine, a voltage-dependent K\(^+\)-channel blocker, or glibenclamide, an ATP-sensitive K\(^+\)-channel blocker; 2) cyclic nucleotide-sensitive kinase blockade with either KT-5823, a guanylate-sensitive kinase blocker, or H-89, an adenylate-sensitive kinase blocker; and 3) blockade of intracellular Ca\(^{2+}\) release with ryanodine on NO-induced pulmonary vasodilation in acutely prepared late-gestation fetal lambs. N-nitro-arginine, a competitive inhibitor of endothelium-derived NO synthase, was infused into the left pulmonary artery, and tracheotomy was placed. The animals were ventilated with 100% oxygen for 20 min, followed by ventilation with 100% oxygen and inhaled NO at 20 parts/million (ppm) for 20 min. This represents the control period. In separate protocols, the animals received an intrapulmonary infusion of the different blockers and were ventilated as above. Tetraethylammonium (n = 6 animals) and KT-5823 (n = 4 animals) attenuated the response, whereas ryanodine (n = 5 animals) blocked NO-induced perinatal pulmonary vasodilation. 4-Aminopyridine (n = 5 animals), glibenclamide (n = 5 animals), and H-89 (n = 4 animals) did not affect NO-induced pulmonary vasodilation. We conclude that NO causes perinatal pulmonary vasodilation through a cGMP-dependent kinase-mediated activation of Ca\(^{2+}\)-activated K\(^+\) channels and release of Ca\(^{2+}\) from ryanodine-sensitive stores.

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HIGH RESISTANCE AND LOW BLOOD FLOW characterize the fetal pulmonary circulation. At birth, pulmonary arterial blood flow increases 8- to 10-fold (8, 13, 14) and pulmonary arterial pressure decreases by 50% within the first 24 h of life (15). Factors that contribute to the postnatal adaptation of the pulmonary circulation include establishment of an air-fluid interface, ventilation of the lung, an increase in blood oxygen content, and elaboration of vasoactive substances from the endothelium (2, 10, 13, 14, 16, 23).

Endothelium-derived nitric oxide (EDNO) modulates pulmonary vascular tone under basal conditions in the fetus and during transition of the pulmonary circulation at birth (2, 10). Inhibition of EDNO synthesis elevates pulmonary vascular resistance in utero and attenuates postnatal adaptation of the pulmonary circulation (2, 10, 17). Furthermore, EDNO mediates the increase in pulmonary blood flow that results from several birth-related stimuli, including ventilation, elevation of oxygen tension, and increased shear stress (2, 10, 21, 22).

Previous studies from our laboratory provide support for the notion that K\(^+\)-channel activation plays a key role in perinatal pulmonary vasodilation and that EDNO may act through K\(^+\)-channel activation in the perinatal pulmonary circulation. First, oxygen causes fetal pulmonary vasodilation through activation of a protein kinase-sensitive K\(^+\) channel (12). Second, the observation that tetraethylammonium (TEA), a K\(^+\)-channel antagonist, attenuated, whereas glibenclamide (Glib), a blocker of the ATP-sensitive K\(^+\) channel, had no effect on perinatal pulmonary vasodilation (32) further supports the hypothesis that TEA-sensitive K\(^+\) channels modulate the transition from fetal to neonatal pulmonary circulation. Interestingly, K\(^+\)-channel blockade and EDNO synthase inhibition attenuated pulmonary vasodilation in response to sequential ventilation with low and high inspired oxygen fractions (F\(_{\text{I}}\text{O}_2\)) (10) in remarkably similar patterns.

Although nitric oxide (NO) causes vasodilation through activation of soluble guanylate cyclase (4), the mechanism whereby increased cGMP levels cause vasorelaxation remains incompletely understood. There are reports that NO acts on K\(^+\) channels in arterial smooth muscle cells (SMCs) either directly (5) or through a cGMP-sensitive kinase (3, 29). Recent evidence (7, 24, 27) suggested that NO causes vasodilation in part through activation of a Ca\(^{2+}\)-activated K\(^+\) (K\(_{\text{Ca}}\)) channel by local release of Ca\(^{2+}\) from an intracellular Ca\(^{2+}\) store.

In SMCs, free cytosolic Ca\(^{2+}\) is the major determinant of the contractile state (19, 33). The primary source of Ca\(^{2+}\) is influx across the plasma membrane. Ca\(^{2+}\) can also be released from the intracellular sarcoplasmic reticulum (SR) Ca\(^{2+}\) store through ryanodine receptors (7, 24). Ryanodine receptors are Ca\(^{2+}\)-activated release channels named for their ability to be blocked by the plant alkaloid ryanodine. The Ca\(^{2+}\) released from the intracellular SR stores in SMCs is not sufficient to cause contraction. Rather, the Ca\(^{2+}\) re-
leased can cause activation of $K_{Ca}$ channels, which, in turn, causes membrane hyperpolarization and closure of voltage-operated $Ca^{2+}$ channels (7, 24, 27). This prevents entry of extracellular $Ca^{2+}$ into the cytosol, resulting in a decrease in cytosolic $Ca^{2+}$ concentration and relaxation of SMCs (33). Therefore, we hypothesized that NO causes perinatal pulmonary vasodilation through 1) cGMP-dependent kinase-mediated activation of a $K_{Ca}$ channel and 2) release of intracellular $Ca^{2+}$ from a ryanodine-sensitive store.

To test these hypotheses, we studied the effect of $K^{+}$-channel inhibition, cyclic nucleotide-dependent kinase inhibition, and $Ca^{2+}$-release inhibition on inhaled NO (I$_{NO}$)-induced perinatal pulmonary vasodilation. Acutely prepared, late-gestation fetal lambs were treated with N-nitro-$l$-arginine (L-NNA), a competitive inhibitor of NO synthase, to prevent the production of endogenous NO. In separate experimental protocols, TEA, a preferential $K_{Ca}$-channel antagonist (12, 28, 32), 4-aminopyridine (4-AP), a voltage-dependent $K^{+}$ ($K_v$)-channel antagonist (28), Glib, an ATP-sensitive $K^{+}$ ($K_{ATP}$)-channel blocker (11), KT-5823, a guanylate kinase blocker (12), H-89, an adenylate kinase antagonist (12), ryanodine, a blocker of intracellular $Ca^{2+}$ release (27), or saline was infused continuously into the left pulmonary artery (LPA). Pulmonary and systemic hemodynamics were then monitored in response to sequential ventilation with an F$_{IO2}$ of 1.00, followed by ventilation with an F$_{IO2}$ of 1.00 and I$_{NO}$ at 20 parts/million (ppm) for 20 min.

MATERIALS AND METHODS

Surgical Preparation

All procedures and protocols performed in this study conformed with the Guide for the Care and Use of Laboratory Animals [DH$\text{E}W$ Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892] and were approved by the Animal Care and Use Committee of the University of Minnesota (Minneapolis, MN) and the Veterans Affairs Medical Center (Minneapolis). Eleven mixed-breed pregnant ewes between 136 and 141 days gestation (term = 147 days) were obtained. One ewe and its fetus were used as time controls, leaving 10 fetuses available for the experimental protocols. The ewes were fasted for 24 h before surgery and sedated with intravenous pentobarbital sodium (800–1,000 mg), which crosses the placenta to induce fetal anesthesia. The ewes were further anesthetized with 1% sodium (800–1,000 mg), which crosses the placenta to induce fetal anesthesia. The ewes were further anesthetized with 1% lidocaine (800–1,000 mg), which crosses the placenta to induce fetal anesthesia. The ewes were further anesthetized with 1% lidocaine (800–1,000 mg), which crosses the placenta to induce fetal anesthesia. The ewes were further anesthetized with 1% lidocaine (800–1,000 mg), which crosses the placenta to induce fetal anesthesia.

Ventilation was initiated with a volume ventilator (Siemens model 900B) with the following initial settings: rate, 20 breaths/min; tidal volume, 40 ml; positive end-expiratory pressure, 4 cmH$_2$O; inspiratory time, 0.5 s; and F$_{IO2}$, 1.00. Tidal volume was adjusted to maintain a peak inspiratory pressure of 35 cmH$_2$O. Ventilator rate and inspired oxygen concentration were adjusted to maintain fetal pH and carbon dioxide tension at prevention values.

Drug Preparation

L-NNA (Sigma) was suspended in HCl, and the pH was corrected to 7.4 with NaOH. TEA (Sigma), H-89 (Calbiochem), ryanodine (Alomone), and 4-AP (Sigma) were dissolved in saline. Glib (Calbiochem) was dissolved in 50% dextrose and 0.1 N NaOH, heated, stirred, and pH balanced. KT-5823 (LC Services) was dissolved in <0.1% DMSO, with the balance saline.

Experimental Design

After a 60-min recovery period (Fig. 1), serial MPA blood gas tensions and pH were monitored at 20-min intervals. L-NNA (10 mg/ml) was continuously infused into the LPA at a rate of 0.1 ml/min for 30 min (total dose 30 mg) immediately after the recovery period. To ensure that endogenous NO release was blocked, acetylcholine was administered via the MPA at 2.5 μg/min for 5 min (total 12.5 μg). This dose generally increases pulmonary blood flow by 20–25 ml/min (1). If there were any pulmonary vasodilation in response to acetylcholine (i.e., pulmonary blood flow > 5 ml/min), the same dose of L-NNA was repeated. Generally, a single dose of L-NNA was sufficient to inhibit pulmonary vasodilation. After 20 min of L-NNA infusion, pannucronium bromide (0.5 mg; Gensia Laboratories, Irvine, CA) was administered into the superior vena cava to prevent spontaneous fetal respiration. The head was removed from the uterus, a tracheostomy was performed, and a 4.5-mm endotracheal tube was placed. Ventilation was initiated with a volume ventilator (Siemens model 900B) with the following initial settings: rate, 20 breaths/min; tidal volume, 40 ml; positive end-expiratory pressure, 4 cmH$_2$O; inspiratory time, 0.5 s; and F$_{IO2}$, 1.00. Tidal volume was adjusted to maintain a peak inspiratory pressure of 35 cmH$_2$O. Ventilator rate and inspired oxygen concentration were adjusted to maintain fetal pH and carbon dioxide tension at prevention values.
Tracheostomy

L-NNa
1 mg/min
via LPA
for 30 min

Vent + 1.00 FiO2

Table 2. Effect of kinase blockers and ryanodine on fetal blood gases and hemodynamics in response to sequential ventilation

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Pco2, Torr</th>
<th>Po2, Torr</th>
<th>MPAP, mmHg</th>
<th>AoP, mmHg</th>
<th>HR, beats/min</th>
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<tr>
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<tr>
<td>Control</td>
<td>7.32 ± 0.02</td>
<td>43 ± 2</td>
<td>17 ± 1</td>
<td>52 ± 3</td>
<td>50 ± 3</td>
<td>156 ± 3</td>
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<tr>
<td>TEA</td>
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<td>13 ± 1</td>
<td>56 ± 3</td>
<td>55 ± 2</td>
<td>152 ± 3</td>
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<tr>
<td>Glib</td>
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<td>48 ± 2</td>
<td>17 ± 2</td>
<td>54 ± 4</td>
<td>47 ± 2</td>
<td>148 ± 2</td>
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<tr>
<td>Fio2 of 1.0</td>
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<tr>
<td>Control</td>
<td>7.33 ± 0.01</td>
<td>43 ± 2</td>
<td>24 ± 1†</td>
<td>53 ± 3</td>
<td>52 ± 3</td>
<td>158 ± 1</td>
</tr>
<tr>
<td>TEA</td>
<td>7.32 ± 0.02</td>
<td>42 ± 3</td>
<td>24 ± 2†</td>
<td>55 ± 3</td>
<td>52 ± 2</td>
<td>150 ± 2</td>
</tr>
<tr>
<td>4-AP</td>
<td>7.35 ± 0.01</td>
<td>44 ± 3</td>
<td>24 ± 2†</td>
<td>56 ± 3</td>
<td>56 ± 3</td>
<td>142 ± 2</td>
</tr>
<tr>
<td>Glib</td>
<td>7.33 ± 0.01</td>
<td>48 ± 2</td>
<td>21 ± 2</td>
<td>50 ± 3</td>
<td>55 ± 3</td>
<td>160 ± 1</td>
</tr>
<tr>
<td>Fio2 of 1.0 and 20 ppm lN0</td>
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<tr>
<td>Control</td>
<td>7.34 ± 0.01</td>
<td>43 ± 2</td>
<td>27 ± 1†</td>
<td>49 ± 2</td>
<td>51 ± 2</td>
<td>153 ± 2</td>
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<tr>
<td>TEA</td>
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<td>43 ± 3</td>
<td>27 ± 3†</td>
<td>52 ± 3</td>
<td>52 ± 3</td>
<td>155 ± 4</td>
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<tr>
<td>4-AP</td>
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<td>40 ± 4</td>
<td>26 ± 3†</td>
<td>53 ± 3</td>
<td>56 ± 4</td>
<td>150 ± 5</td>
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<tr>
<td>Glib</td>
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<td>45 ± 7</td>
<td>26 ± 4†</td>
<td>45 ± 8</td>
<td>48 ± 3</td>
<td>160 ± 6</td>
</tr>
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</table>

Values are means ± SE. MPAP, main pulmonary artery pressure; AoP, aortic pressure; HR, heart rate; TEA, tetraethylammonium; 4-AP, 4-aminopyridine; Glib, glibenclamide; Fio2, inspired O2 fraction; ppm, parts/million; lN0, inhaled nitric oxide. Preventation is time period after study drug infusion but immediately before ventilation. Significantly different (P < 0.05) from: *control value under identical conditions; †preventation; ‡ventilation with Fio2 alone.
weighed because fixed dosages of the drugs (listed under each protocol) were given as intrapulmonary infusions, thus minimizing systemic effects. However, the average weight of the fetus at this gestational age was $\sim4$ kg (32).

Experimental Protocols

Protocol 1: $K^+$-channel blockade

TEA (20 mg at 10 mg/ml; n = 6 animals), 4-AP (17 mg at 8.5 mg/ml; n = 5 animals), or Glib (30 mg at 15 mg/ml; n = 5 animals) was administered via the LPA over 20 min. Ventilation was then initiated as described in Experimental Design. LPA blood flow, hemodynamic values, and fetal blood gas tensions were obtained during each study condition. Previous work (11, 12) from our laboratory confirmed that this dose of Glib was sufficient to block the pulmonary vasodilation caused by lemakalim, an ATP-sensitive $K^+$-channel opener. The effects of TEA and 4-AP on lemakalim-induced pulmonary vasodilation were not tested because lemakalim can cause prolonged perinatal pulmonary vasodilation (11).

Protocol 2: Kinase inhibition

The guanylate-sensitive kinase-specific blocker KT-5823 (220 µg at 110 µg/ml; n = 4 animals) or the adenylly-sensitive kinase-specific blocker H-89 (110 µg at 55 µg/ml; n = 4 animals) was administered via the LPA over 20 min. Ventilation was then initiated as previously described. LPA blood flow, hemodynamic values, and fetal blood gas tensions were obtained during each study condition. Previous work from our laboratory (12) confirmed that these doses of KT-5823 and H-89 were sufficient to cause inhibition of the perinatal pulmonary vasodilation caused by 8-bromo-cGMP. Vehicle controls were performed for KT-5823.

Protocol 3: Intracellular Ca$^{2+}$-release blockade with ryanodine

Ryanodine (150 µg at 75 µg/ml; n = 5 animals) was administered via the LPA over 20 min. Ventilation was then initiated as previously described. LPA blood flow, hemodynamic values, and fetal blood gas tensions were obtained during each study condition.

Data Analysis

The data on the variables measured are expressed as means ± SE. To evaluate the individual effects of each of the drugs studied and because of the repeated measures in the study protocol, the data were analyzed by ANOVA for repeated measures.

Contrasts between the least-squares means observed during each treatment period among the study drugs were obtained with Scheffé’s method so as to identify significant differences between the drugs under a given condition. In addition, similar methodology was used to contrast the responses observed for each of the drugs under the different treatment conditions. An $\alpha$ value of 0.05 was used as the cutoff for significance. All analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Control. Measurements were performed after L-NNA infusion and immediately before initiation of ventilation (n = 10 animals). Under these conditions, LPA flow was $30 \pm 4$ ml/min (Fig. 2), and MPAP and AoP were $52 \pm 3$ and $50 \pm 3$ mmHg, respectively (Table 1).

After ventilation with an $F_{\text{IO}_2}$ of 1.00, LPA flow significantly increased to $215 \pm 24$ ml/min ($P < 0.0001$ vs. prevention; Fig. 2). MPAP, AoP, and heart rate did not change. The $P_{\text{O}_2}$ increased from prevention level ($P < 0.05$; Table 1), whereas pH and $P_{\text{CO}_2}$ remained unchanged.

With administration of $I_{\text{NO}}$ at 20 ppm and continued ventilation with an $F_{\text{IO}_2}$ of 1.00, LPA flow increased further (to $365 \pm 29$ ml/min; $P < 0.0001$ vs. ventilation with $F_{\text{IO}_2}$-alone value; Fig. 2). MPAP, AoP, and heart rate did not change. The $P_{\text{O}_2}$ increased further, but this increase was not significantly different from ventilation with an $F_{\text{IO}_2}$ of 1.00 alone. The $P_{\text{CO}_2}$ and pH remained unchanged.

Protocol 1: $K^+$-Channel Blockade

Under prevention conditions, LPA flows and fetal blood gas tensions in the $K^+$-channel blockade-treated animals were not different from control levels. Administration of TEA caused an increase in MPAP and AoP (P < 0.05 vs. control level; Table 1). Hemodynamic parameters were not significantly different from the control levels in the Glib- and 4-AP-treated animals. With ventilation and an $F_{\text{IO}_2}$ of 1.00, LPA flow increased from prevention values in all groups (P < 0.001; Fig. 2). MPAP, AoP, heart rate, and fetal blood gas tensions were not significantly different from control levels (P > 0.1; Table 1). MPAP and AoP in the TEA-treated animals were not different from prevention values. $P_{\text{O}_2}$ increased significantly from prevention levels in the TEA- and 4-AP-treated animals (P < 0.01) but not in the Glib-treated groups (P = 0.1; Table 1).

With administration of $I_{\text{NO}}$ at 20 ppm and continued ventilation with an $F_{\text{IO}_2}$ of 1.00, LPA flow increased further in the Glib- and 4-AP-treated animals (P < 0.01) but not in the TEA-treated animals (P = 0.3 vs. ventilation with $F_{\text{IO}_2}$-alone level; Fig. 2). Moreover, LPA flow was significantly lower (P = 0.0003) compared with the control level under this condition. MPAP
decreased significantly from the preventilation level in the TEA-treated animals (P = 0.05; Table 1), whereas AoP, heart rate, and PCO2 did not significantly change. The pH increased in the 4-AP group compared with the preventilation level (P < 0.05). Compared with ventilation with F1O2-alone level, PO2 did not increase significantly (P > 0.10) in all groups except the Glib group where the increase was significant (P = 0.05; Table 1).

Protocol 2: Kinase Inhibition

Under preventilation conditions, LPA flow, hemodynamics, pH, and PO2 in the KT-5823- and H-89-treated animals were not significantly different from control values. PCO2, however, was higher in the kinase-inhibited animals (P < 0.05 vs. control value; Table 2).

With ventilation at an F1O2 of 1.00, LPA flow increased in all groups (P < 0.03 vs. preventilation level; Fig 3). However, LPA flow in the KT-5823-treated animals was significantly lower than the control value (P = 0.03; Fig 3) compared with that in the H-89 group where the flow was comparable to the control value (P = 0.9). MPAP, AoP, heart rate, pH, and PCO2 were not different from the control or preventilation values. PO2 increased from preventilation values in the KT-5823- and H-89-treated animals (P < 0.01; Table 2).

With administration of L-NNA at 20 ppm and continued ventilation at an F1O2 of 1.00, LPA flow increased further in the H-89-treated animals (P = 0.006 vs. ventilation with F1O2-alone level; Fig 3). In the KT-5823-treated animals, LPA flow was not significantly different from the LPA flow with ventilation with F1O2-alone value (P = 0.4; Fig 3). LPA flow was significantly lower in the KT-5823-treated animals compared with the control value (P < 0.0001; Fig 3). Hemodynamic parameters were not different from the control or ventilation with F1O2-alone values. pH increased in the H-89-treated animals compared with preventilation value (P = 0.01; Table 2). PO2 significantly increased in all groups (P < 0.05 vs. preventilation value) but was not significantly increased compared with ventilation with F1O2-alone value (P > 0.1; Table 2).

Protocol 3: Intracellular Ca2+-Release Blockade With Ryanodine

Under preventilation conditions, LPA flow, hemodynamic parameters, and fetal blood gas tensions in the ryanodine-treated animals were not different from the control values (Table 2, Fig 4).

With ventilation at an F1O2 of 1.00, LPA flow significantly increased from the preventilation level in the ryanodine-treated animals (P < 0.0001; Fig 4). MPAP, AoP, heart rate, pH, and PCO2 did not change significantly from preventilation values nor were they significantly different from control values (P > 0.1; Table 2). PO2 in the ryanodine-treated animals was significantly lower than the control value (P < 0.01; Table 2).

With administration of L-NNA at 20 ppm and continued ventilation at an F1O2 of 1.00, LPA flow in the ryanodine-treated animals did not change (P = 0.9 vs. ventilation with F1O2-alone value; Fig 4). This was significantly lower than what was observed in the control group (P < 0.0001). Hemodynamic parameters, pH, and PCO2 did not significantly change and were not different from control values (Table 2). PO2 in the ryanodine-treated animals was significantly lower than the control value.
NO derived from the pulmonary endothelium plays a key role in perinatal pulmonary vasodilation. Although NO causes vasodilation through an increase in cytosolic cGMP (4), the subcellular mechanism whereby cGMP causes relaxation of the pulmonary vascular SMCs remains incompletely understood. In this study, we tested the hypothesis that NO causes perinatal pulmonary vasodilation through activation of a KCa channel by local release of Ca2+ from an intracellular Ca2+ store.

Although endogenous NO production was pharmacologically inhibited, the effect of either K+ channel blockade; ryanodine, a blocker of Ca2+ release from the SR; or kinase inhibition on the perinatal pulmonary vasodilation caused by INO was studied. We report that blockade of the KCa channel attenuated the NO-induced pulmonary vasodilation, whereas ryanodine and KT-5823, a guanylate kinase inhibitor, blocked NO-induced pulmonary vasodilation. Inhibition of either the KATP or Kv channels or adenylate kinase had no effect on NO-induced perinatal pulmonary dilation. These results support the hypothesis that NO causes perinatal pulmonary vasodilation by activating KCa channels through a cGMP-sensitive protein kinase-mediated pathway that requires release of Ca2+ from a ryanodine-sensitive store.

These results provide details into the subcellular mechanisms whereby NO causes perinatal pulmonary vasodilation. Although previous studies (3, 5, 26, 29) have demonstrated that NO causes K+ channel activation, this is the first report demonstrating that NO causes perinatal pulmonary vasodilation through activation of a KCa but not a Kv channel. Further mechanistic details derive from the observation that guanylate kinase activity, but not adenylate kinase activity, is necessary for NO to cause perinatal pulmonary vasodilation. These results provide support for the notion that NO causes KCa-channel activation through a cGMP-sensitive kinase-mediated pathway and not through direct action on the KCa channel.

The present study also provides evidence that subcellular Ca2+ release from ryanodine-sensitive pools in the SR is required for NO-induced perinatal pulmonary vasodilation. After treatment with ryanodine, NO did not cause perinatal pulmonary vasodilation. This observation fits well with previous studies (7, 24, 25, 27) that demonstrated that KCa channels are activated by subcellular Ca2+ release from ryanodine-sensitive pools in the SR. The data reported in these studies suggested that in the perinatal pulmonary circulation, NO acts to increase cGMP concentration in the cytosol, which activates cGMP-dependent kinase to phosphorylate the KCa channels and/or the ryanodine-sensitive Ca2+ pool. Phosphorylation of the ryanodine-sensitive Ca2+ stores results in a local increase in Ca2+ in the region of the KCa channel and activates nearby sarcolemmal KCa channels, resulting in K+ efflux, membrane hyperpolarization, dosing of voltage-operated Ca2+ channels, and vasodilation. Activation of only a small number of high-conductance KCa channels would be necessary to effect a significant change in pulmonary arterial SMC membrane potential (6, 7, 24, 27).

Interestingly, the frequency of the local elevations in Ca2+ concentration, termed Ca2+ sparks (6), has been shown to be inversely proportional to the contractile state of the vasculature (24, 27). Decreasing spark frequency or blocking the sparks results in vasconstriction (34), whereas increasing the frequency of sparks results in vasodilation. Thus NO may cause vasodilation via an increase in spark frequency. Such a construct is strengthened by the observation of Porter et al. (27) that cyclic nucleotides modulate the frequency of these sparks. If this mechanism is operative, then fetal pulmonary arterial SMCs would possess a spontaneous transient outward current, which Reeve et al. (28) have previously demonstrated. Taken together, the clear evidence that NO causes vasodilation by increasing cGMP concentration, it seems reasonable to conclude that NO causes perinatal pulmonary vasodilation through a pathway that entails a cyclic nucleotide-mediated increase in local Ca2+ release.

These findings fit well with previous reports (12, 28) that demonstrated a maturational shift in the K+ channel setting of the resting membrane potential in pulmonary arterial SMCs from the KCa channel in the fetus and newborn to the Kv channel in the adult. In the perinatal pulmonary circulation, when the response of the pulmonary circulation to an acute increase in oxygen tension and NO is biologically imperative, the KCa channel sets the resting membrane potential (12). In the adult pulmonary circulation, several reports (35, 36) indicated that the Kv channel regulates the resting membrane potential and is inactivated by an acute decrease in oxygen tension, allowing for hypoxic pulmonary vasoconstriction to prevent hypoxemia. Such a developmental shift in the ion channel that responds to a change in oxygen tension in the pulmonary circulation might represent the mechanism whereby the pulmonary circulation of the normal newborn infant is adapted to respond to an acute increase in oxygen tension and NO, whereas the pulmonary circulation of the adult is adapted to respond to an acute decrease in oxygen tension.

This represents the first effort to address these questions with an integrative physiological approach. The current findings demonstrate that in vitro findings are applicable in vivo. However, the findings are limited by the specificity of the pharmacological probes used. In the absence of specific K+ channel agonists, we were obliged to assume that the effects of the pharmacological K+ channel antagonists are directly applicable in vivo. To mitigate against this concern, we tested whether the drugs were acting as predicted whenever the pharmacological tools were available. Moreover, the concentrations used in these studies were based on extrapolations from in vitro work. It is not clear that either the effects or concentrations of these drugs can be directly applied. With regard to the use of TEA...
rather than a more specific \(K_{Ca}\)-channel blocker such as iberiotoxin or charybdotoxin, previous reports (25, 28, 31) have shown that the dose of TEA used worked specifically at the \(K_{Ca}\) channels. Moreover, because of the technical difficulty of the surgical preparation and the need to use more than one study drug per protocol, it was important to use agents such as TEA with a shorter duration of action than the toxins (18, 28). Furthermore, these agents may possess nonspecific effects that confound our interpretation of the data. For example, there is increasing evidence that endothelial cells possess \(K_{Ca}\) channels (30). Use of \(K\)-channel agonists may have affected the endothelial cells in an unanticipated manner. With respect to ryanodine administration, there are no pharmacological probes currently available to specifically test whether the ryanodine-sensitive stores had been blocked. However, the lack of a response to the addition of \(I_{NO}\) does suggest biological relevance.

In summary, we report that NO causes perinatal pulmonary vasodilation through activation of a \(K_{Ca}\) channel. The present study provides data that the \(K_{Ca}\) channel is activated through cGMP-sensitive kinase and requires \(Ca^{2+}\) release from a ryanodine-sensitive intracellular store. We speculate that if \(K_{Ca}\)-channel activation does play a central role in the postnatal adaptation of the pulmonary circulation, then an alteration in \(K_{Ca}\)-channel activity may lead to the altered perinatal pulmonary vascular reactivity that is the hallmark of persistent pulmonary hypertension of the newborn. The implication of the present study is that in addition to alterations in endothelium-derived vasoactive products such as NO and endothelin, infants with persistent pulmonary hypertension of the newborn may have alterations in \(K_{Ca}\)-channel activity or in the signal transduction pathway that leads to \(K_{Ca}\)-channel activation.

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Address for reprint requests and other correspondence: D. N. Cornfield, Box 742, Division of Pediatric Pulmonology and Critical Care, Univ. of Minnesota Medical School, 420 Delaware St. S.E., Minneapolis, MN 55455 (E-mail: cornf001@tc.umn.edu).

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