Chronic hypoxia alters nitric oxide-dependent pulmonary vascular responses in lungs of newborn pigs

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Fike, Candice D., and Mark R. Kaplowitz. Chronic hypoxia alters nitric oxide-dependent pulmonary vascular responses in lungs of newborn pigs. J. Appl. Physiol. 81(5): 2078–2087, 1996.—Almost all of the studies evaluating the effect of chronic hypoxia on lung nitric oxide production have been performed in adult animals. Because results of studies in adult lungs should not be extrapolated to represent the newborn lung, we performed studies to determine whether decreased nitric oxide production might be involved in the pathogenesis of chronic hypoxia-induced pulmonary hypertension in newborns. We kept newborn pigs in chambers filled with room air (control) or 11–12% O₂ for either 3–5 (short) or 10–12 (long) days. Using isolated lungs, we measured pulmonary vascular responses to agents that either stimulate or inhibit the synthesis of nitric oxide. To define the vascular sites of altered production of nitric oxide, we applied the micropuncture technique and measured small venular pressures before and after treatment with a nitric oxide synthesis inhibitor. Pulmonary vascular responses to acetylcholine were blunted in chronically hypoxic piglets of both the short and long groups. The nitric oxide synthesis inhibitor had a different effect in the lungs of control piglets than in those of chronically hypoxic piglets of the long but not of the short group. For the long group, the nitric oxide synthesis inhibitors caused constriction of both arteries and veins in lungs of control but not of chronically hypoxic piglets. These findings support the idea that decreased pulmonary vasodilation in newborn piglets exposed to chronic hypoxia in newborn pigs and might therefore contribute to the pathogenesis of pulmonary hypertension in newborns.

PREVIOUSLY, WE (8) showed that chronic hypoxia causes pulmonary hypertension in newborn piglets and provided evidence for at least two different phases of the disease process. An early phase occurs over the first 3 days of exposure to hypoxia and is characterized by a pulmonary circulation with augmented constriction to acute hypoxia. A later phase occurs if hypoxia is maintained for 10–12 days and is characterized by a more remodeled pulmonary circulation that no longer exhibits greater acute hypoxic vasoconstriction than does the pulmonary circulation of control piglets (8).

Altered production of endogenously produced vasoactive agents could contribute to either one or both phases of the development of pulmonary hypertension. An early loss of the vasodilatory effect from an agent such as nitric oxide could increase pulmonary vascular resistance and initiate pulmonary hypertension (19). Alternatively or concurrently, loss of the antimitogenic effect of nitric oxide could lead to structural alterations and thereby contribute to the maintenance of pulmonary hypertension through vascular remodeling (19).

Evidence has recently been provided both for and against decreased nitric oxide production in chronic hypoxia-induced pulmonary hypertension in adult humans and animals. Pulmonary vascular responses to acetylcholine, a vasodilatory agent thought to act by stimulating nitric oxide synthesis by the pulmonary vascular endothelium, have been found to be blunted in adult humans with end-stage chronic obstructive lung disease (6) and in lungs of mature rats exposed to chronic hypoxia (2, 5, 17, 25). In addition, nitric oxide inhaled during chronic hypoxia prevented pulmonary hypertension in adult rats (15). Thus whether decreased nitric oxide production plays a role in the development of hypoxia-induced pulmonary hypertension in adults remains unclear.

Because the regulation of pulmonary vasomotor tone differs between adult and newborn animals (4, 27, 32), results of studies in adults cannot be extrapolated to represent the effect of chronic hypoxia on nitric oxide production in newborns. Therefore, we wanted to determine whether altered production of nitric oxide is involved in the pathogenesis of chronic hypoxia-induced pulmonary hypertension in newborns. To do this, we measured pulmonary vascular responses to agents that either stimulate or inhibit nitric oxide synthesis in control piglets and piglets exposed to chronic hypoxia. To investigate whether the duration of exposure to hypoxia affected these responses, we exposed the newborn piglets to either 3–5 or 10–12 days of hypoxia. We applied the micropuncture technique and measured pressures in small veins before and after treatment with nitric oxide synthesis inhibitors in an effort to define the vascular sites affected. Finally, to determine whether the impaired vascular responses are limited to agents that are endothelium dependent, we measured pulmonary arterial responses to an endothelium-independent agent, sodium nitroprusside, that may act similarly to nitric oxide and cause pulmonary vasodilation by increasing pulmonary vascular smooth muscle guanosine 3’,5’-cyclic monophosphate (cGMP).

METHODS

Animals

For most studies, newborn pigs were placed in either a normoxic (control) or a hypoxic normobaric environment at age 24–72 h and maintained for either 3–5 (short group) or 10–12 days (long group). Some control animals were raised on
the farm and studied on the day of arrival at either 4–8 (short group) or 11–15 days of age (long group). Normobaric hypoxia was produced by delivering compressed air and N₂ to an incubator (ThermaCare). The O₂ content was regulated at 11–12% O₂ (PO₂ 60–70 Torr); CO₂ was absorbed with soda lime, and PCO₂ was maintained at 3–6 Torr. The chamber was opened approximately three times per day for cleaning and to weigh the animals. The animals were fed ad libitum with an artificial sow milk replacer from a feeding device attached to the chamber. On the day of study, the animal was weighed and anesthetized with ketamine (120 mg im) and pentobarbital sodium (25 mg iv). Before lung excision and perfusion, some animals had catheters placed for the measurement of pulmonary vascular resistance.

In Vivo Hemodynamic Measurements

Additional intravenous pentobarbital sodium was given as needed to maintain anesthesia during placement of the catheters. First, the trachea of the piglet was cannulated so that the animal could be ventilated if necessary. Next, a catheter was placed into the right femoral artery for monitoring systemic blood pressure and arterial blood gases. To measure cardiac output by the thermodilution technique, a thermistor was threaded through the left femoral artery into the aortic arch and a catheter that served as an injection port was threaded through the left carotid artery into the left ventricle. A catheter was then threaded through the right external jugular vein and right ventricle into the pulmonary artery and distal pulmonary vessel (pulmonary wedge pressure). The zero reference for all catheters was the mid-thorax. Cardiac output was measured at end expiration by the thermodilution technique as the mean of at least three determinations after injecting 3 ml of 0°C 0.9% saline. After blood gases, pulmonary arterial pressure, pulmonary wedge pressure, left ventricular end-diastolic pressure, and cardiac output were measured, the animal was given additional anesthesia and its lungs were excised for perfusion.

Lung Excision and Perfusion

A cannula was placed into the trachea, and the lungs were ventilated with room air. After a midline sternotomy was performed, the duc tus arteriosus was either ligated (short group) or a clamp was placed across it (long group). Through incisions in the right and left ventricles of each piglet, saline-filled cannulas were placed into the pulmonary artery and left atrium. The diaphragm and all abdominal contents were removed. The vascular cannulas were connected to the perfusion circuit.

Perfusate

Before the lung isolation described in Lung Excision and Perfusion, a femoral or carotid arterial cannula was placed in each animal. The animal was given heparin (1,000 IU/kg iv) and exsanguinated. Approximately 100–200 ml of blood from each animal were mixed with 50–100 ml of a 3% albumin-saline mixture for use in the perfusion circuit.

Perfusion Circuit

A rotary pump (model 7523-00, Cole Palmer Masterflex) continuously circulated the blood from a reservoir through a bubble trap into the pulmonary artery, through the lungs into the left atrium, and back to the reservoir. Pulmonary arterial, left atrial, and airway pressures were continuously monitored with strain-gauge transducers (Statham PD23 XL) and a recorder (Gould TA 2000). The most dependent edge of the diaphragmatic surface of the lung was used as the zero reference for vascular pressures and was also the surface used for micropuncture. After connection to the perfusion circuit, the lungs were perfused for 0.5–1 h to establish stability of the pulmonary arterial pressure.

Micro puncture Technique

For micropuncture, glass micropipettes were made with a pipette puller (model 720, David Kopf Instruments), and the tips were beveled to a 2- to 5-μm diameter with a rotating diamond stone. The pipette was mounted on a micromanipulator and connected to a servo-nulling pressure-measuring system (model 5A, Instruments for Physiology and Medicine). A fiber-optic light source illuminated the lung surface, and subpleural vessels were classified as venules by observing the direction of blood flow through a stereomicroscope. Vessel diameter was measured with a calibrated scale. To establish the zero reference, the pipette was advanced and immersed in a pool of normal saline overlying the vessel. The pipette was advanced to puncture the pleural membrane and vessel wall. Criteria for a valid microvascular pressure measurement were stable pressure recordings for at least 1 min, no changes in pressure associated with a small adjustment of the gain in the servo-nulling system (an indicator that the pipette tip is in a free liquid pool), an immediate and similar change in microvascular pressure in response to a transient 0.5- to 1-cmH₂O adjustment in left atrial pressure, and an unaltered zero reference when the pipette tip was withdrawn into the saline pool. For all micropuncture measurements, the lung was inflated to a constant airway pressure of 5 cmH₂O with a normoxic mixture of 21% O₂-5–10% CO₂-balance N₂, and left atrial pressure was set at 10 cmH₂O (zone 3 conditions) by adjusting the height of the blood reservoir.

Study Protocols

Three series of experiments were performed. For all series, the lungs were ventilated with a normoxic mixture of 21% O₂-5–7% CO₂-balance N₂ with a large-animal ventilator, a tidal volume of 15 ml/kg, and a respiratory rate of 12–16 breaths/min. Except during micropuncture, the left atrial pressure was set to zero. Unless indicated otherwise below, blood flow was adjusted to 50 ml·min⁻¹·kg⁻¹ and maintained constant for the remainder of each study.

Series 1. For series 1, changes in pulmonary vascular responses to acetylcholine were measured in control and chronically hypoxic lungs of both the short and long groups. Because baseline pulmonary arterial pressure is greater in chronically hypoxic than in control animals (see Ref. 8; results), the pulmonary vascular pressure of some control animals was elevated to a level comparable to that of chronically hypoxic animals by adding 1 M KCl to the perfusate of the control lungs of both groups. Acetylcholine was added cumulatively to the reservoir to achieve concentrations of 10⁻⁶ to 10⁻⁴ M. The acetylcholine response was transient so that the pulmonary arterial pressure was allowed to return to baseline between doses.

Series 2. For series 2, changes in pulmonary vascular responses to the endothelium-independent agent sodium nitroprusside were measured in control and chronically hypoxic lungs of both groups. Some of these lungs had also been used for series 1. Before sodium nitroprusside was added, either 1 M KCl or angiotensin was used to raise the pulmonary arterial pressure, as described in Series 1. Sodium nitroprusside was then added cumulatively to the reservoir to achieve concentrations of 10⁻⁸ to 10⁻³ M. Pulmonary arterial pressure decreased with each dose of sodium nitroprusside and
remained decreased for over 10 min so that the pulmonary arterial pressure was not allowed to return to baseline between doses.

Series 3. For series 3, changes in pulmonary vascular pressure were measured in response to an agent that competitively inhibits nitric oxide synthesis, N-nitro-L-arginine methyl ester (L-NAME). In preliminary studies, the dose of L-NAME that caused the largest change in pulmonary arterial pressure was determined. To do this, for some lungs from each group, cumulative small volumes of L-NAME (0.5, 2.7, and 27 mg) were added to the reservoir to achieve concentrations of $1 \times 10^{-5}$, $7 \times 10^{-5}$, and $7 \times 10^{-4}$ M, respectively. For all groups, the highest pulmonary arterial pressure was achieved with $7 \times 10^{-5}$ M L-NAME. Therefore, for lungs from each group, pulmonary arterial pressures were measured under baseline conditions and with $7 \times 10^{-5}$ M L-NAME. In some of these lungs from each group, the micropuncture technique was used to measure small venular pressures under baseline conditions and with $7 \times 10^{-5}$ M L-NAME.

To evaluate whether L-NAME inhibited the vascular responses to acetylcholine, a protocol similar to series 1 was followed with other lungs from each group. Vascular responses to acetylcholine were measured before and after the addition of L-NAME under baseline conditions and with L-NAME. In the long group, the pH, PO$_2$, and PCO$_2$ values obtained during hemodynamic measurements did not differ between control and chronically hypoxic pigs. In the short group, pH values of the control and chronically hypoxic pigs were the same, but blood PO$_2$ and PCO$_2$ values were slightly lower in the long-group hypoxic pigs (Table 1). Measurements of pulmonary arterial pressure, pulmonary wedge pressure, left ventricular end-diastolic pressure, cardiac output, aortic pressure, and calculated pulmonary vascular resistance [(pulmonary arterial pressure - wedge pressure)/cardiac output] are summarized in Table 2. All the preceding measurements and calculated pulmonary vascular resistances were similar for chamber-raised and farm-raised pigs of both the short and long groups, so they are combined in Table 2. Measurements of left ventricular end-diastolic pressure were not significantly different from those of wedge pressure (Table 2).

### RESULTS

#### In Vivo Hemodynamic Measurements

On the day of study, the chronically hypoxic piglets from both groups weighed less and had higher Hct than the corresponding control piglets (Table 1). In the short group, the pH, PO$_2$, and PCO$_2$ values obtained during hemodynamic measurements did not differ between control and chronically hypoxic piglets. In the long group, pH values of the control and chronically hypoxic piglets were the same, but blood PO$_2$ and PCO$_2$ values were slightly lower in the long-group hypoxic piglets (Table 1).

### Table 1. Data for short and long groups of piglets

<table>
<thead>
<tr>
<th>Study Wt, g</th>
<th>In Vivo</th>
<th>Isolated Lung</th>
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<tbody>
<tr>
<td>Hct, %</td>
<td>pH</td>
<td>PO$_2$, Torr</td>
</tr>
<tr>
<td>Short control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,377 ± 532 (30)</td>
<td>25 ± 5</td>
<td>7.41 ± 0.05</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Short hypoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,037 ± 316* (25)</td>
<td>30 ± 7*</td>
<td>7.43 ± 0.05</td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>Long control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,838 ± 762 (35)</td>
<td>27 ± 6</td>
<td>7.41 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long hypoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,672 ± 520* (25)</td>
<td>42 ± 5*</td>
<td>7.42 ± 0.04</td>
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</tbody>
</table>

Values are means ± SD; nos. in parentheses, no. of animals. Short, 3–5 days; long, 10–12 days; Hct, hematocrit. *Significantly different from corresponding control, P < 0.05 by unpaired t-test.
2. Thus, if we were unable to obtain a wedge pressure, left ventricular end-diastolic pressure was used for the calculation of pulmonary vascular resistance. For both the short and long groups, pulmonary arterial pressures and calculated pulmonary vascular resistances were greater in chronically hypoxic than in control piglets (Table 2). Furthermore, pulmonary vascular resistance was greater in hypoxic piglets from the long group than from the short group. Hence the magnitude of pulmonary hypertension increased with the length of exposure of newborn pigs to chronic hypoxia.

In isolated lungs perfused with homologous blood and 3% albumin-saline, Hct values were similar in control and chronically hypoxic animals of the short group but were slightly greater in chronically hypoxic than in control animals of the long group (Table 1). The pH, PO2, and PCO2 values obtained during perfusion were similar in all lungs (Table 1).

Series 1

For both the short and long groups, responses to acetylcholine differed between chronically hypoxic and control lungs (Table 3, Fig. 1). Similar differences in responses were found between chronically hypoxic and their corresponding control lungs with all doses of acetylcholine, but for illustration purposes, Table 3 presents only the responses to the largest dose of acetylcholine (10−6 M).

When pulmonary vascular tone was not elevated with KCl, baseline pulmonary arterial pressure was greater in isolated perfused lungs of chronically hypoxic piglets than in control lungs for both the short and long groups (Table 3). When comparing acetylcholine responses between lungs without comparable baseline pulmonary arterial pressures (Table 3), the percent change but not the absolute change from baseline pulmonary arterial pressure was less in chronically hypoxic than in control lungs of the long group. In contrast, the absolute change but not the percent change was greater in chronically hypoxic than in control lungs of the short group (Table 3). On the basis of these findings, it would appear that the shorter period of exposure to hypoxia enhanced dilatory responses to acetylcholine, whereas the longer exposure blunted the acetylcholine-induced dilation.

More importantly, however, when baseline pulmonary arterial pressures in control lungs were elevated to levels comparable to the pulmonary arterial pressure in the chronically hypoxic lungs (Table 3, Fig. 1), both the absolute and percent changes in acetylcholine responses were significantly less for all but the lowest dose of acetylcholine in the chronically hypoxic compared with the control lungs of both the short and long groups (Table 3, Fig. 1). In addition, the dilatory response to acetylcholine tended to be even more blunted in the lungs of piglets from the long hypoxia group than from the short hypoxia group (Table 3, Fig. 1). The conclusions from these findings are that both short and long periods of hypoxia blunt dilation to acetylcholine and that the blunting appears to be greater with longer exposure to chronic hypoxia.

The percent change in pulmonary arterial pressure tended to be greater with all doses of acetylcholine in control lungs of the short compared with the long group (Table 3, Fig. 1B). This latter finding supports the
notion that there is a maturational decrease in the pulmonary vascular response to acetylcholine in control piglets. Of note, the combined effects of maturation and the longer exposure to chronic hypoxia resulted in the greatest blunting of acetylcholine responses (Table 3, Fig. 1).

**Series 2**

Before addition of sodium nitroprusside, either KCl or angiotensin was used to elevate pulmonary arterial pressures of all control lungs to levels similar to the baseline pulmonary arterial pressures of chronically hypoxic animals (Table 4). For both the long and short groups and with all doses of sodium nitroprusside, absolute and percent decreases in pulmonary vascular pressure were similar in chronically hypoxic and corresponding control animals (Table 4, Fig. 2). However, the absolute but not the percent change in pulmonary arterial pressure tended to be greater in lungs of hypoxic animals from the long group than from the short group (Table 4, Figs. 2). This latter finding probably reflects the higher baseline pulmonary arterial pressure in the hypoxic animals of the long group (Table 4). More importantly, in contrast to the findings with acetylcholine, these results indicate that neither long nor short periods of exposure to hypoxia altered the ability of the pulmonary artery to dilate to sodium nitroprusside (Table 4, Fig. 2).

There was no difference in vascular responses to sodium nitroprusside between control lungs of the short and long groups (Table 4, Fig. 2). This indicates that, unlike the responses to acetylcholine, there are no maturational differences in the pulmonary vascular responses to sodium nitroprusside between the control piglets of the age groups studied.

**Series 3**

With left atrial pressure constant, pulmonary arterial and small venular pressures increased with addition of L-NAME (7 × 10⁻³ M) to the perfusate of lungs of control and chronically hypoxic piglets of the short group (Fig. 3A). These vascular pressures were used to calculate total and segmental pressure gradients across the pulmonary circulation, as summarized in Fig. 4A. With L-NAME, the pressure gradient increased across the total pulmonary circulation and across both the arterial and venous segments in lungs of control and chronically hypoxic piglets of the short group (Fig. 4A). However, the magnitude of the increase in the total pressure gradient did not differ between lungs of control and chronically hypoxic piglets either when expressed as an absolute change from the baseline pressure gradient (increase in total pressure gradient: control, 6 ± 3 mm Hg; hypoxia, 10 ± 3 mm Hg).

**Table 4. Responses to sodium nitroprusside in lungs in which baseline pulmonary arterial pressure in control piglets was elevated to a level comparable to that in chronically hypoxic piglets**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Elevated Pulmonary Arterial Pressure, cm H₂O</th>
<th>Absolute Change in Pulmonary Arterial Pressure, cm H₂O</th>
<th>Change in Pulmonary Arterial Pressure, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short control</td>
<td>6 ± 3</td>
<td>−21 ± 10</td>
<td>−56 ± 9</td>
</tr>
<tr>
<td>Short hypoxia</td>
<td>10 ± 10</td>
<td>−17 ± 8</td>
<td>−46 ± 10</td>
</tr>
<tr>
<td>Long control</td>
<td>8 ± 6</td>
<td>−27 ± 6</td>
<td>−60 ± 7</td>
</tr>
<tr>
<td>Long hypoxia</td>
<td>6 ± 8†</td>
<td>−33 ± 24†</td>
<td>−54 ± 17†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. Dose of sodium nitroprusside was 10⁻³ M. Significantly different (P < 0.05, 1-way analysis of variance with multiple comparison test) from: *short control; †short hypoxia.
sure gradient of 21.8 ± 20 and 25 ± 22 cmH₂O in 12 control and 12 chronically hypoxic lungs, respectively) or when expressed as a percent change from the baseline pressure gradient (percent increase in total pressure gradient of 103 ± 96 and 53 ± 32% in 12 control and 12 chronically hypoxic lungs, respectively). The lack of a difference in the effect of the nitric oxide synthesis inhibitor between control and chronically hypoxic lungs of the short group suggests that the short exposure to hypoxia did not alter the basal synthesis of nitric oxide.

In control lungs of the short group, L-NAME caused equal increases in the pressure gradients across the arterial and venous segments (increase in pressure gradients of 15 ± 6 and 14 ± 5 cmH₂O in arterial and venous segments, respectively). This latter finding suggests that basal synthesis of nitric oxide is important in maintaining a low pressure gradient across both the arterial and venous compartments in 4- to 8-day-old piglets.

In lungs from the long chronically hypoxic group, neither pulmonary arterial nor venular pressures increased significantly with L-NAME, but there was a significant increase in both pressures with L-NAME in
the lungs from the long control group (Fig. 3B). More specifically, adding the nitric oxide synthesis inhibitor to control lungs of the long group caused the pressure gradients to increase across both arterial and venous segments (increases in pressure gradients of 3.6 and 2.6 cmH2O in arteries and veins, respectively), whereas adding L-NAME to chronically hypoxic lungs did not change the pressure gradient across either vascular segment (Fig. 4B). The difference in the effect of the nitric oxide synthesis inhibitor between control and chronically hypoxic animals in the long group suggests that longer exposure to chronic hypoxia impairs basal synthesis of nitric oxide. The additional finding that the pressure gradients across both the arterial and venular compartments increased with the nitric oxide synthesis inhibitor in lungs of control but not in lungs of chronically hypoxic piglets of the long group indicates that the pulmonary vascular sites of impairment in nitric oxide synthesis include both pulmonary arteries and veins.

Pulmonary arterial pressure responses to acetylcholine (10−6 M) before and after L-NAME (7 × 10−4 M) show that for lungs of all groups treatment with L-NAME inhibited the vasodilatory effect of acetylcholine (Table 5). This finding suggests that L-NAME inhibits acetylcholine-stimulated synthesis of nitric oxide in lungs of newborn piglets.

**DISCUSSION**

The most important findings of this study are that pulmonary vascular responses to both the nitric oxide synthesis stimulator acetylcholine and the nitric oxide synthesis inhibitor L-NAME were blunted in the lungs of piglets exposed to 10–12 days of chronic hypoxia. These findings support the hypothesis that nitric oxide synthesis is impaired during chronic hypoxia in the lungs of newborn pigs. The additional finding that the pressure gradient across both arterial and venular segments increased with L-NAME in the lungs of control but not in the lungs of chronically hypoxic piglets suggests that the sites of impaired nitric oxide synthesis with chronic hypoxia are in both arteries and veins. All of the preceding findings are particularly important because little information is available regarding the effect of chronic hypoxia on nitric oxide production in newborn lungs. Results of studies in adult lungs should not be extrapolated to represent the newborn lungs because the regulation of pulmonary vascular tone differs between newborns and adults (4, 27, 32).

Our finding that pulmonary vascular responses to acetylcholine are blunted in the lungs of newborn piglets exposed to chronic hypoxia are similar to some (2, 5, 17, 25) but not all findings (14) in chronically hypoxic adult rat lungs. However, it is more appropriate to compare our findings with those with newborn animals. Consistent with our results, other investigators found that acetylcholine responses were decreased in conduit pulmonary arteries isolated from lungs of high-altitude (hypobaric hypoxic) calves compared with

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**Table 5.** Changes in pulmonary arterial pressure with ACh before and after L-NAME in lungs of control and chronically hypoxic animals of short and long groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline Pulmonary Arterial Pressure, cmH2O</th>
<th>Change in Pulmonary Arterial Pressure with ACh, cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n L-NAME</td>
<td>Before</td>
</tr>
<tr>
<td>Short control</td>
<td>5</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Short hypoxic</td>
<td>4</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>Long control</td>
<td>8</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Long hypoxic</td>
<td>3</td>
<td>28 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. L-NAME, Nω-nitro-L-arginine methyl ester. Dose of ACh is 10−6 M. * Significantly different from immediately preceding value, P < 0.05 by paired t-test.
age-matched control calves (22). Yet these same investigators found that the pulmonary vascular responses to acetylcholine were enhanced at the high altitude compared with control calves when in vivo studies were performed (22). Of note, in these latter in vivo studies, the pulmonary arterial pressures were significantly greater in the high-altitude compared with control calves (22). We also found larger responses to acetylcholine in chronically hypoxic than in control piglets of the short group when baseline pulmonary arterial pressures were greater in the former than in the latter animals (Table 3). Other investigators (12, 13) have reported that responses to acetylcholine are enhanced if baseline pulmonary arterial pressure is increased. In this light, the differences between findings in the calf conduit vessels and in intact calves might be attributed to the fact that the baseline pulmonary arterial pressures were not equalized in the control and hypoxic calves but were so in the conduit vessels (22). Thus these differences should not be interpreted as an inconsistency in the effect of chronic hypoxia on pulmonary vascular responses to acetylcholine in newborns. Instead, when comparisons are made between newborn control and chronically hypoxic animals with similar levels of pulmonary arterial pressure, unlike the inconsistency of results in adult animals, the pulmonary vascular responses to acetylcholine are consistently impaired. Furthermore, our findings show that this impairment occurs as early as 3–5 days after the exposure and seems to progress with length of chronic hypoxia.

One possible explanation for impaired responses to acetylcholine is that chronic hypoxia impairs the ability of the pulmonary circulation to respond to all vasodilators that increase smooth muscle cell cGMP. This explanation is not likely because the dilation to sodium nitroprusside was not affected by exposure to chronic hypoxia. Similarly, no differences in pulmonary vasodilatory response to sodium nitroprusside were found between high-altitude (hypobaric hypoxic) and control calves (22). And in adult rats, some (2) but not all (5) investigators have found no difference in the pulmonary vasodilatory response to sodium nitroprusside between control and chronically hypoxic animals. These unaltered responses to sodium nitroprusside suggest that chronic hypoxia does not affect the ability of the pulmonary circulation to produce cGMP. However, because sodium nitroprusside may cause pulmonary dilation by mechanisms other than by elevating cGMP (24), it remains possible that lower increases in pulmonary vascular smooth muscle cGMP explain the blunted pulmonary vascular responses to acetylcholine found in chronically hypoxic newborn animals.

Another explanation for blunted responses to acetylcholine could be that chronic hypoxia affects muscarinic receptors in the pulmonary vascular endothelium. Finding concurrent blunting of both receptor- and nonreceptor-mediated agents has been interpreted to indicate that impaired acetylcholine responses are not due to the effect of chronic hypoxia on muscarinic receptors (2, 6). To clearly evaluate this issue, the effect of chronic hypoxia on muscarinic-receptor numbers and subtypes needs to be determined.

It is also possible that dilatory responses to acetylcholine are blunted because chronic hypoxia decreases either basal and/or stimulated nitric oxide production by the pulmonary vascular endothelium. This possibility is supported by our observation that an inhibitor of nitric oxide synthesis caused pulmonary vasoconstriction in control but not in chronically hypoxic piglets of the long group. In comparison, nitric oxide synthesis inhibitors had either the same or a greater pulmonary vasoconstrictor effect in chronically hypoxic adult rats compared with control rats (21, 31). Maturational differences in the effect of chronic hypoxia on nitric oxide production most likely explain the difference between our findings in newborn piglets and findings in adult rat lungs (21, 31).

Our findings also indicate that the pulmonary vascular sites in which the altered responses to nitric oxide inhibitors occur include both arteries and veins. Furthermore, the difference in effect of nitric oxide synthesis inhibitors on pulmonary vascular pressures is not apparent until after 3–5 days of hypoxia. This latter finding might seem inconsistent with our finding that responses to acetylcholine were impaired after only 3–5 days of hypoxia. It is therefore possible that chronic hypoxia has different effects on the stimulated and basal syntheses of nitric oxide (30). In addition, both stimulators and inhibitors of nitric oxide synthesis may have pulmonary vascular effects that are not related to nitric oxide. Nonetheless, our findings of altered pulmonary vascular responses to acetylcholine and L-NAME support the idea that nitric oxide production has been decreased with chronic hypoxia in newborn pig lungs.

Decreased production of nitric oxide by chronic hypoxia could also be due to impairments in the activity and/or amount of the enzyme endothelial nitric oxide synthase (eNOS). Reduced eNOS expression was noted in the pulmonary circulation of adults with pulmonary hypertension due to a variety of causes, including chronic obstructive lung disease (10). The amount of eNOS was decreased in mature bovine pulmonary arterial endothelial cells cultured under hypoxic conditions for 48 h (18). In contrast, another group of investigators found that both the activity and amount of eNOS were increased in the lungs of chronically hypoxic adult rats (28). Because of the discrepancies in the preceding findings, no consistent conclusion can be made regarding the effect of chronic hypoxia on eNOS production or activity in adult lungs. The effect of chronic hypoxia on eNOS expression in the lungs of newborns remains to be evaluated.

Although not the original intent of the study, maturational differences in nitric oxide synthesis can be made by comparing responses to acetylcholine and nitric oxide synthesis inhibitors between control piglets of the long and short groups. Although not consistently statistically significant (Fig. 1), the vasodilatory effect of acetylcholine tended to be greater in the younger (4- to 8-day-old) than in older (11- to 15-day-old) piglets. In addition, nitric oxide synthesis inhibitors caused greater
pulmonary vasoconstriction in the lungs of the short control piglets compared with the lungs of the long control piglets (Fig. 3). Both of these findings indicate that basal synthesis of nitric oxide is more important in regulating pulmonary vasomotor tone in the younger (4- to 8-day-old) than in the older (11- to 15-day-old) piglets. Like us, other investigators also found a greater pulmonary vasoconstrictor effect from nitric oxide synthesis inhibitors in the lungs of younger (1-day-old) than in older (7-day-old) piglets (23). Our findings also agree with the finding of greater responses to acetylcholine in pulmonary arterial rings of 3- to 10-day-old piglets than in pulmonary arterial rings of 3- to 10-wk-old piglets (16). However, others have found that responses to acetylcholine increased with postnatal age in piglets (32). Studies with lamb lungs have also provided evidence for both increases and decreases in nitric oxide synthesis with maturation (1, 11, 12, 29).

Some of the variability in the above results may be due to differences in pulmonary vascular tone and animal age.

There has been some agreement regarding the sites regulated by nitric oxide in the neonatal pulmonary circulation. Specifically, our findings and those of other investigators using vessels (9, 29) or lungs (12) from lambs or piglets (20) indicate that nitric oxide plays an important role in maintaining low pulmonary venous tone. The primary difference among the results of the investigators using vessels (9, 29) or lungs (12) from lambs or piglets (20) is that nitric oxide is almost equally important in maintaining low tone across arteries and veins. Some of the variability in the above results may be due to differences in pulmonary vascular tone and animal age.

In summary, our findings show that pulmonary vascular responses to nitric oxide synthesis inhibitors and stimulators are altered when newborn pigs are exposed to chronic hypoxia. The nature of these alterations supports the hypothesis that pulmonary vascular nitric oxide production is decreased when newborn pigs are exposed to chronic hypoxia. Moreover, the decrease in nitric oxide production appears to occur in both pulmonary arteries and veins. In addition, our findings that blunting of pulmonary vascular responses to acetylcholine is present after only 3–5 days of hypoxia, persists, and progresses with longer exposure to hypoxia suggest that decreased production of nitric oxide is involved in the pathogenesis of both phases of pulmonary hypertension that we have described in the lungs of newborn piglets. Further experiments are necessary to determine the mechanism for the decreased production of pulmonary vascular nitric oxide that occurs when newborn piglets are exposed to chronic hypoxia.

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