Brief perinatal hypoxia increases severity of pulmonary hypertension after reexposure to hypoxia in infant rats

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Clinical observations have shown that exposure to adverse stimuli such as high blood flow or hypoxia from birth through infancy and childhood can accelerate the development of pulmonary hypertension in early adulthood (7, 39). Moreover, transient hypoxic pulmonary hypertension during the first week of life predisposes to exaggerated pulmonary hypertension in young adults exposed to high altitude despite having normal pulmonary artery pressure at low altitude (35). The link between hypoxic exposure in the early period of life and susceptibility for pulmonary vascular dysfunction has been suggested by several experimental studies (7, 15, 16). Adult rats exposed to hypoxia during the first days of life and then raised in room air have elevated pulmonary vascular resistance (PVR) and increased vascular reactivity to hypoxia (15). Exposure to hypoxia during the perinatal period and infancy augments the effect of monocrotaline-induced pulmonary hypertension in adult rats (7). Furthermore, perinatal hypoxia increases pulmonary vascular reactivity to acute hypoxia in adult rats recovering from chronic exposure to hypoxia (16). Despite these data, mechanisms underlying the potential effects of perinatal hypoxia on late sequelae are poorly understood.

Past studies have shown that hypoxia can impair lung alveolar growth (23), but less is known regarding effects on pulmonary vascular growth. Therefore, we hypothesized that disrupted pulmonary alveolar and vascular growth caused by brief perinatal hypoxia would predispose rats to a higher risk for developing pulmonary hypertension when reexposed to hypoxia in infancy. The present experiments were designed to determine whether 1) brief perinatal hypoxia causes persistent abnormalities of pulmonary alveolar and vascular growth throughout infancy despite a recovery period in normoxia, 2) brief perinatal hypoxia increases the severity of pulmonary hypertension when infant rats are reexposed to hypoxia, and 3) the increased severity of pulmonary hypertension after exposure to late hypoxia in infancy correlates with the degree of disrupted lung growth caused by brief perinatal hypoxia.

METHODS

Animals

All procedures and protocols were approved by the Animal Care and Use Committee at the University of Colorado.

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Health Sciences Center. Pregnant Sprague-Dawley rats (SDR) were purchased (Harlan Laboratories, Indianapolis, IN) and maintained at Denver’s altitude [1,600 meters; barometric pressure (Pb), 630 mmHg; inspired oxygen pressure (PIO2), 122 mmHg] for at least 1 wk before giving birth. Animals were fed ad libitum and exposed to day-night cycles alternatively every 12 h.

Study Design

Four groups of animals were used in these experiments as illustrated in Fig. 1. These groups included the following treatments: rats maintained in normoxia throughout the perinatal and postnatal periods (NN); rats maintained in normoxia in the perinatal period and then exposed to hypoxia at 2 wk (NH); rats exposed to hypoxia in the perinatal period and at 2 wk (HH); and rats exposed to perinatal hypoxia but then maintained in room air (HN). In the rats exposed to perinatal hypoxia, pregnant SDR were placed in a hypobaric chamber 3 days before the expected delivery (30). There were no differences in mortality between the perinatal hypoxia and control groups. The chamber was maintained to simulate high-altitude conditions at 17,000 feet [Pb, 410 mmHg; PIO2, 76 mmHg; and inspired oxygen fraction (FIO2), 0.11]. Temperature was 22–23°C, and humidity ranged from 20 to 70%. After birth, mothers and newborns were kept in the hypobaric chamber for an additional 3 days. There was a single, brief (less than 10-min) interruption of the hypoxic exposure for cage cleaning and animal care. In the room air controls, pregnant SDR were maintained in room air throughout the perinatal period. In rats reexposed to hypoxia (NH and HH groups), animals were placed in the hypobaric chamber at 2 wk of age for 1 wk. Rats were studied at 3 days and 2 and 3 wk of age (Fig. 1). When they were killed, rats were euthanized with an intraperitoneal injection of pentobarbital sodium (0.3 mg/g body wt).

Study Measurements

Right ventricular systolic pressure. Rats were anesthetized with methoxyflurane by inhalation (Schering-Plough Animal Health, Union, NJ). After an adequate level of sedation was achieved, the rats were placed in a supine position while spontaneously breathing room air. After calibration of the zero point of the pressure transducer to the midanterioroposterior diameter of the chest, a 26-gauge needle was introduced percutaneously into the thorax via a subxyphoid approach. Right and left ventricular pressures were measured using a pressure transducer (Gould-Statham, Costa Mesa, CA) and recorded on a multichannel recorder (Grass Institute, Quinen, MA). Heart rate under these conditions was between 300 and 500 beats/min. If the heart rate fell below 300 beats/min, it was assumed that the level of anesthesia or trauma was inhibiting cardiac function, and measurements were not included in the analysis. This occurred in less than 20% of study animals. After hemodynamic measurements were made, the animals were euthanized for histology and measurement of heart weight.

Right ventricular hypertrophy. After a rat was killed, the heart was resected through a midline sternotomy. The right ventricle (RV) and left ventricle plus septum (LV+S) were dissected and weighed, and the ratio of RV to LV+S weights, RV to body weight, and LV+S to body weight were determined.

Hematocrit. Blood was collected from carotid arteries in capillary tubes immediately after euthanasia by direct puncture for determination of hematocrit.

Wet-to-dry lung weight ratio. Freshly harvested lungs were weighed (wet lung weight) and then dried in an oven at 70°C until the weight was constant (dry lung weight). Wet-to-dry lung weight ratios were calculated.

Barium-gelatin infusion and fixation of lung tissue. Rat lungs were prepared and fixed in situ. Phosphate-buffered saline was infused through a main pulmonary artery catheter to flush the pulmonary circulation free of blood. A barium sulfate-gelatin mixture was heated to 70°C and was infused through the pulmonary artery catheter at 73 mmHg pressure based on standard methodologies (13). Pressure was maintained for 5 min to ensure penetration of the barium mixture. The lungs were fixed by tracheal installation of 10% buffered Formalin at constant pressure (20 cmH2O). The trachea was ligated under pressure and after sustained inflation for 5 min. The lungs were removed and submersed in fixative for 24 h at 4°C. Lung volume was measured by water displacement. To obtain arteriograms, the left lung was placed on X-ray film and imaged using X-ray radiography.

Radial alveolar counts, pulmonary artery density, and morphometric analysis. For microscopic analysis, one transverse section was taken from the midplane of the upper, middle, and lower lobes of the Formalin-fixed right lung. Sections from each animal were processed and embedded in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Room Air</th>
<th>10%O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>Room Air</td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>10%O2</td>
<td>Room Air</td>
</tr>
<tr>
<td>HH</td>
<td>Room Air</td>
<td>10%O2</td>
</tr>
<tr>
<td>HN</td>
<td>10%O2</td>
<td>Room Air</td>
</tr>
</tbody>
</table>

Fig. 1. Study design of 4 study groups and treatment protocols. NN, exposure to normoxia throughout perinatal and postnatal study periods. NH, exposure to normoxia during perinatal period and hypoxia at 2 wk of age. HH, exposure to hypoxia in perinatal period and at 2 wk. HN, exposure to hypoxia during perinatal period only. d, Day.
paraffin wax. Paraffin sections (5 µm thick) were cut from each block and stained with hematoxylin and eosin. Analysis of each section was carried out in a blinded fashion. Alveolarization was assessed by the radial alveolar count (RAC) method of Emery and Mithal as described (10). Respiratory bronchioles were identified as bronchioles lined by epithelium in one part of the wall (32). From the center of the respiratory bronchiole, a perpendicular was dropped to the edge of the acinus (connective tissue septum or pleura), and the number of septa intersected by this line was counted. At least 15 counts were performed for each animal. Pulmonary artery density was determined by counting barium-filled arteries per high-power field (×200 magnification). Vascular wall thickness of small pulmonary arteries was measured on pulmonary arteries accompanying the terminal or respiratory bronchioles. At least 10 pulmonary arteries were measured using a Zeiss Interactive Digital Analysis System on hematoxylin and eosin-stained lung sections as previously described (2). The percent medial thickness of an artery was calculated by the following formula: (2 x medial thickness x 100)/external diameter.

Statistical Analysis

Statistical comparison was made using analysis of variance and Fisher’s protected least significant difference test and the Statview software package (Abacus Concepts, Berkeley, CA). Differences were considered significant at P < 0.05 vs. normoxic rats. *P < 0.05 vs. normoxic rats. †P < 0.05 vs. HN rats. ‡P < 0.05 vs. HH rats. *P < 0.05 vs. NH, HN, and NN groups.

RESULTS

Body, Cardiac, and Lung Weights

Body weight was reduced at 3 days in the perinatal hypoxic rats but was normal at 14 days (Table 1). The rats exposed to hypoxia from 14 to 21 days of age (NH and HH groups) had reduced body weight at 21 days compared with normoxic control (NN) and perinatal hypoxic (HN) rats. In comparison to controls, the perinatal hypoxic rats had higher RV-to-LV+S and RV-to-body weight ratios at 3 and 14 days of age (Fig. 2; Table 1). After later exposure to hypoxia during 14–21 days of age, rats that had been exposed previously to perinatal hypoxia (HH group) had a greater RV-to-

Table 1. Effects of perinatal and late hypoxia on heart weights in infant rats

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Group</th>
<th>Body Weight, g</th>
<th>RV/Body Weight</th>
<th>LV+S/Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Normoxic (7)</td>
<td>9.3 ± 0.4</td>
<td>0.13 ± 0.1</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Perinatal (8)</td>
<td>6.5 ± 0.3*</td>
<td>0.18 ± 0.01*</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>14</td>
<td>Normoxic (8)</td>
<td>28.4 ± 1.4</td>
<td>0.11 ± 0.01</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Perinatal</td>
<td>30.9 ± 1.2</td>
<td>0.14 ± 0.01*</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>hypoxia (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>NN (6)</td>
<td>5.1 ± 0.2</td>
<td>0.11 ± 0.08</td>
<td>0.33 ± 0.02†</td>
</tr>
<tr>
<td></td>
<td>HH (9)</td>
<td>44.5 ± 1.2†</td>
<td>0.22 ± 0.02†</td>
<td>0.43 ± 0.03†</td>
</tr>
<tr>
<td></td>
<td>HN (5)</td>
<td>52.1 ± 1.3</td>
<td>0.15 ± 0.01</td>
<td>0.43 ± 0.02†</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of rats in parentheses. RV, right ventricle; LV+S, left ventricle plus septum; NN, control rats; NH, rats exposed to late hypoxia; HH, rats exposed to perinatal and late hypoxia; HN, rats exposed to perinatal hypoxia. *P < 0.05 vs. normoxic rats. †P < 0.05 vs. NN rats. #P < 0.05 vs. HN rats.

Table 2. Effects of perinatal and late hypoxia on lung weight and volume in infant rats

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Groups</th>
<th>Dry Lung Weight/Body Weight</th>
<th>Wet/Dry Weight</th>
<th>Lung Volume/Body Weight, ml/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Normoxic (7)</td>
<td>0.32 ± 0.02 (4)</td>
<td>5.80 ± 0.04 (4)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Perinatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hypoxia (8)</td>
<td>0.38 ± 0.04 (5)</td>
<td>6.16 ± 0.34 (5)</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>Normoxic (7)</td>
<td>0.28 ± 0.01 (7)</td>
<td>5.01 ± 0.06 (7)</td>
<td>0.021 ± 0.004 (5)</td>
</tr>
<tr>
<td></td>
<td>Perinatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hypoxia (5)</td>
<td>0.25 ± 0.01(6)*</td>
<td>5.24 ± 0.04(6)*</td>
<td>0.022 ± 0.002 (5)</td>
</tr>
<tr>
<td>21</td>
<td>NN (6)</td>
<td>0.18 ± 0.01 (6)</td>
<td>4.97 ± 0.06 (6)</td>
<td>0.023 ± 0.001 (4)</td>
</tr>
<tr>
<td></td>
<td>NH (7)</td>
<td>0.23 ± 0.01 (6)</td>
<td>4.84 ± 0.07 (6)</td>
<td>0.021 ± 0.001 (5)</td>
</tr>
<tr>
<td></td>
<td>HH (9)</td>
<td>0.22 ± 0.01 (4)</td>
<td>4.87 ± 0.09 (4)</td>
<td>0.020 ± 0.001 (7)</td>
</tr>
<tr>
<td></td>
<td>HN (5)</td>
<td>0.21 ± 0.01(4)</td>
<td>4.85 ± 0.07 (4)</td>
<td>0.015 ± 0.001(5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of rats in parentheses. NA, data not available. *P < 0.05 vs. normoxic rats. †P < 0.05 vs. NH rats.
and HH groups) had increased dry lung weight-to-body weight ratio at 21 days compared with that in normoxic control (NN) and perinatal hypoxic (HN) rats. There was no difference in lung wet-to-dry weight ratio among these four groups at this age. The perinatal hypoxic rats (HN group) had reduced lung volume-to-body weight ratio compared with the other three groups at 21 days.

**Right Ventricular Systolic Pressure**

Right ventricular systolic pressure (RVSP) was measured in 2- and 3-wk-old rats including normoxic control rats and those exposed to perinatal and/or late hypoxia (Fig. 3). As shown in Fig. 3, there was no difference in RVSP between normoxic and perinatal hypoxic rats at 2 wk of age. After exposure to hypoxia during 2–3 wk of age, the rats that had previously been exposed to brief perinatal hypoxia (HH group) had a greater increase (77% above normoxic control rats (NN)) in RVSP than the rats born in room air (NH group, 27% increase; P < 0.05, HH vs. NH). There was no difference in RVSP between normoxic control (NN) and perinatal hypoxic (HN) rats at 3 wk of age.

**Hematocrit**

At 14 days of age, hematocrit was increased in the perinatal hypoxic rats (P < 0.05; Fig. 4). After late exposure to hypoxia during 14–21 days of age, rats previously exposed to brief perinatal hypoxia (HH group) demonstrated a greater increase in hematocrit in comparison to the hematocrit of the rats born in room air (NH group; P < 0.05). There was no difference in hematocrit between NN and NH groups at 3 wk of age (P > 0.05).

**Lung Histology and RACs**

Figure 5 shows lung histology of normoxic and perinatal hypoxic rats at 2 wk of age. Although the RACs were not different between these two groups at 2 wk (Fig. 6), pulmonary artery density was reduced in perinatal hypoxic rats compared with normoxic control rats (P < 0.05; Figs. 7 and 8). Figure 9 shows the lung histology of normoxic control (NN), NH, HN, and HH groups at 3 wk of age. Rats from the HH group have fewer and larger alveoli and fewer arteries compared with the other study groups. RAC revealed no difference between normoxic and perinatal hypoxic groups at 2 wk of age (Fig. 6). After late exposure to hypoxia during 2–3 wk of age, the HH group demonstrated a more marked reduction (30%; P < 0.05) in RAC in comparison with control rats (NN group; Fig. 6). There were no differences in RAC among the NN, NH, and HN groups at 3 wk of age (Fig. 6).

**Barium-Gelatin Arteriograms and Pulmonary Artery Density**

Barium-gelatin arteriograms showed decreased background haze in the perinatal hypoxic rats in comparison with normoxic rats at 2 wk of age (Fig. 7). This reduction in artery density was quantitated by direct histological measurements, demonstrating a 29% reduction in arteries per high-power field (P < 0.05; Fig. 8). At 3 wk of age, arteriograms showed a decrease in background haze and lumen diameters of axial arteries in rats exposed to later hypoxia during 2–3 wk of age (Fig. 10, NN vs. NH, HN vs. HH). Moreover, HH group exhibited a further decrease in comparison with NH group. After exposure to hypoxia during 2–3 wk of age, the rats previously exposed to brief perinatal hypoxia (HH group) exhibited a greater reduction (50%) in pulmonary artery density in comparison with that of the rats born in room air (NH group, 39% reduction; Fig. 8).

**Arterial Wall Thickness Percentage**

There was no difference in arterial wall thickness between normoxic and perinatal hypoxic rats at 2 wk of age (11.6% in normoxic rats and 12.1% in perinatal hypoxic rats). At 3 wk of age, there were no differences...
in arterial wall thickness among the four groups (11.7, 12.1, 13.7, and 12.7% in the NN, NH, HH, and HN groups, respectively).

DISCUSSION

We found that brief perinatal hypoxia increased the severity of pulmonary hypertension after reexposure to hypoxia after 2 wk of age. This response was manifested by greater increases in RVSP and higher RV-to-LV S ratio in comparison with rats that were not exposed to perinatal hypoxia. Moreover, the exaggerated pulmonary hypertension correlated with disrupted lung growth. In rats that have been exposed to brief perinatal hypoxia, pulmonary vascular growth was decreased, as reflected by a reduction of pulmonary artery density at 2 wk of age, despite a recovery period in normoxia. Exposure to hypoxia later during infancy also reduced pulmonary artery density, but previous exposure to brief perinatal hypoxia augmented this reduction. Perinatal hypoxia plus later hypoxia in infancy also decreased alveolarization as demonstrated by reduced RACs at 3 wk of age. Finally, brief perinatal hypoxia enhanced the subsequent increase in hematocrit when infant rats were exposed to hypoxia.

These results support our hypothesis that disruption of lung growth by brief perinatal hypoxia could predispose rats to higher risk for developing pulmonary hypertension later in infancy. Past studies have demonstrated that early hypoxia can reduce alveolarization and lung growth (22, 24). However, this is the first study suggesting that perinatal hypoxia contributes to high risk for the development of pulmonary hypertension later in infancy. These findings also suggest that persistent reduction of pulmonary vascular growth, as reflected by decreased pulmonary artery density, may contribute to the greater severity of pulmonary hypertension after reexposure to hypoxia. Interestingly, brief perinatal hypoxia reduced lung artery density but not alveolar number at 2 wk, suggesting a greater effect of perinatal hypoxia on the attenuation of vascular growth than alveolarization.

These results suggest that brief perinatal hypoxia increased the RV-to-LV+S ratio in infant rats as early as 3 days of age. A previous study showed that hypobaric hypoxia (380 mmHg) did not increase the RV-to-LV+S ratio in infant rats exposed from day 8 after birth until after 14 days of exposure (25). The faster response in our study suggests that the pulmonary vasculature during the perinatal period is extremely reactive to hypoxia (11, 32, 36). At birth, PVR falls rapidly from high fetal values due to vasodilatation (1, 9, 17). In the normal postnatal rat lung, PVR continues to fall at a much slower rate during infancy due to a decrease in arterial medial thickness and an increase in pulmonary arterial number (25). Hypoxic exposure during the late fetal and early postnatal period could interfere with vascular dilatation, remodeling, and growth of the developing lung circulation (14, 17, 26, 27). Failure to

Fig. 5. Lung histology of normoxic (a) and perinatal hypoxic (b) rats at 2 wk of age. Micrographs are representative and at same magnification, ×100. Arrows indicate barium-filled arteries.

Fig. 6. Decreased alveolar number in rats exposed to brief perinatal hypoxia and later hypoxia in infancy. Alveolar number, as determined by radial alveolar counts, was decreased in HH group at 3 wk of age in comparison with NN rats. *P < 0.05 for HH vs. NN.
achieve the normal fall in PVR during this critical period of lung growth despite the return to normoxia after 3 days of age was sufficient to cause persistent RVH at 2 wk of age. At 2 wk, however, RVSP and pulmonary artery wall thickness returned to normal values, but artery density was still decreased. These findings suggest that the higher RV/LV+S ratio after brief perinatal hypoxia at 14 days of age may not be due to sustained elevation of pulmonary artery pressure but likely reflect slower regression of RVH following perinatal hypoxia. However, the disrupted vascular growth following brief perinatal hypoxia as reflected by reduced pulmonary artery density at 14 days of age did not recover at that time point and may account for the high risk for pulmonary hypertension later during infancy.

The first 2–3 wk after birth is the critical period for rat lung development including rapid alveolarization and vascular growth (5, 25). Events in late fetal life could also be important for subsequent alveolar development in rats because a combination of prenatal and postnatal hypoxic exposure seems to be required to impair the postnatal increase of gas-exchange surface area (22, 28). Our results confirm the suggestion from previous studies that from perinatal through infancy in rats, lung development is especially susceptible to external stimuli including dexamethasone (20, 21, 37) and hyperoxia (12, 33, 34, 40).

It is remarkable that a brief period of perinatal hypoxia, 3 days before and 3 days after birth, resulted in an increased severity of pulmonary hypertension when infant rats were exposed to hypoxia during 2–3 wk of age. Several mechanisms may underlie this observation, including 1) disrupted vascular and alveolar growth, 2) augmented elevation in hematocrit level, and 3) increased vasoactivity or tone of pulmonary vessels. Based on our data, we suggest that disrupted vascular growth and reduced surface area, as reflected by the persistent reduction in pulmonary artery density following brief perinatal hypoxia, may be a significant factor contributing to exaggerated pulmonary hypertension when infant rats were exposed to hypoxia (3, 8). Decreased cross-sectional area of the pulmonary vascular bed may imply that the relatively high blood flow adds an additional hemodynamic stress that could further accentuate the hypertensive response to later hypoxia in infancy. Mechanisms that disrupt pulmonary vascular growth after a brief exposure to hypoxia during the critical period of development are uncertain. Brief perinatal hypoxia also disrupts alveolarization, as reflected by the trend of decreased RACs, and may also contribute to the severity of pulmonary hypertension in older rats.

Our data suggest that although early hypoxia can impair alveolar and vascular growth, inhibition of vessel density may be more critical for the risk of pulmonary hypertension later in life. The exact relationship between impaired alveolarization and angiogenesis is uncertain. The process of septation, which is essential for the formation of alveolar walls, involves alternate upfolding of one of the two capillary layers on both sides of the primary septa (6). This process suggests that during the critical period of alveolarization, disrupted development of pulmonary microcirculation could potentially cause lung hypoplasia by decreasing septation. Recent preliminary data suggest that inhibitors of angiogenesis, including thalidomide and fumagillin, can decrease alveolarization and cause lung hypoplasia in the developing rat lung (19).

Another factor that may contribute to the greater severity of pulmonary hypertension is the augmented rise in hematocrit. It is well known that elevated viscosity due to polycythemia increases pulmonary

![Fig. 7. Barium-gelatin arteriograms of normoxic (control) and perinatal hypoxic rats at 2 wk of age. Background haze was decreased in perinatal hypoxic rats.](http://ajplung.physiology.org/)

Fig. 8. Effects of hypoxia on pulmonary artery density. At 2 wk of age, pulmonary artery density was reduced in perinatal hypoxic rats. After exposure to hypoxia during 2–3 wk of age, NH group had lower pulmonary artery density than NN group and HH group had most marked reduction. *P < 0.05 vs. normoxic rats. **P < 0.05 in comparison of values between NH and HH groups. †P < 0.05 vs. NN.
artery pressure independent of hypoxia-induced vaso-constriction or structural changes of pulmonary vascular bed (29, 38). It is interesting that brief perinatal hypoxia resulted in such a marked "priming effect" on erythropoietic cells, and this effect persisted later in infancy despite an apparent recovery period in room air. As shown in a previous study, mean red cell volume and reticulocyte count were greater after only 3 days of

Fig. 9. Lung histology of study groups at 3 wk. a: NN, control rats. b: NH, exposed to late hypoxia. c: HN, exposed to perinatal hypoxia. d: HH, exposed to both perinatal and late hypoxia. Micrographs are representative and at same magnification, ×100. Arrows indicate barium-filled arteries.

Fig. 10. Representative barium-gelatin arteriograms of left lungs of 2 rats from each study group at 3 wk of age.
hypoxia in the altitude-susceptible H strain of SDR in comparison with the resistant M strain, although hematocrit did not differ between the strains until 21 days of hypoxia (18). We speculate that brief perinatal hypoxia caused subtle alterations in the erythropoietic system, which did not lead to striking elevation in hematocrit until triggered by hypoxia later in infancy.

Increased pulmonary vasoreactivity and tone following brief perinatal hypoxia may also be an important factor. Adult rats that had been exposed to hypoxia from 1 to 6 days of life have elevated PVR and increased vasoreactivity to acute hypoxia (15). Another study demonstrated that perinatal hypoxia (1 wk before and 1 wk after birth) increases pulmonary vasoreactivity to acute hypoxia in adult rats during recovery from chronic hypoxia (16). These reports support our findings that exposure to brief perinatal hypoxia alters the pulmonary circulation, but these reports examined the effects on pulmonary vasoreactivity and/or vasoconstriction in adult rats after a recovery period in room air. The increased vasoreactivity and vasoconstriction may be due to altered endothelial or smooth muscle function, but further physiological studies are needed to clarify the links between perinatal hypoxia and altered vasoreactivity. Moreover, decreased nitric oxide (NO) synthesis may be related to exaggerated pulmonary vasoconstriction at high altitude in young adults who experienced transient pulmonary hypertension during early neonatal period (35). NO is important in perinatal pulmonary vasodilator responses to oxygen (10, 36) and may contribute to developmental changes in pulmonary vascular tone and reactivity (1, 9, 23). In addition, cell culture studies have reported that NO influences vascular endothelial and smooth muscle cell growth in vitro (26, 41). Further studies are needed to determine the effect of perinatal hypoxia on lung NO synthase expression and NO synthesis in the developing lung circulation and the relationship between NO and lung vascular growth following brief perinatal hypoxia. In addition, the increase in RVH after the second exposure to hypoxia may reflect incomplete and slower regression of RVH after exposure to perinatal hypoxia. The rate of rise in RV-to-LV+S ratio in the NH group appears to parallel the rise in RVH in the HH group.

Our findings showed that in rats, perinatal hypoxia increases the severity of pulmonary hypertension when the rats are reexposed to hypoxia later in infancy. More studies are needed to determine the applicability of the animal model to humans. Nevertheless, it may be worth attention for those infants and children who suffered from hypoxia during the critical period of lung development, for example, patients with bronchopulmonary dysplasia, persistent pulmonary hypertension of the newborn, and cyanotic congenital heart disease. Their early hypoxic experience might influence the susceptibility of pulmonary vasculature to adverse stimuli later in life.

We conclude that brief perinatal hypoxia increases the severity of pulmonary hypertension when infant rats are reexposed to hypoxia. We suggest that disrupted alveolarization and altered pulmonary vascular growth following brief perinatal hypoxia contribute to exaggerated pulmonary hypertension with exposure to hypoxia later in infancy. We speculate that brief hypoxia exposure during a critical period of lung growth alters the course of normal lung development and leaves persistent changes in lung structure and function that cause an exaggerated response to adverse stimuli later in life.

We thank Charles Ahrens for performing radiography for arteriograms and Malathi Jakkula for technical assistance. This work was supported by an American Heart Association (National) Grant-In-Aid to S. H. Abman.

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Received 29 June 1999; accepted in final form 10 September 1999.

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