Three-week neonatal hypoxia reduces blood CGRP and causes persistent pulmonary hypertension in rats

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Keith, I. M., S. Tjen-A-Looi, H. Kraicz, and R. Ekman R. Three-week neonatal hypoxia reduces blood CGRP and causes persistent pulmonary hypertension in rats. Am J Physiol Heart Circ Physiol 279: H1571–H1578, 2000.—To increase understanding of persistent pulmonary hypertension, we examined chronic pulmonary effects of hypoxia at birth and their relationships with immunoreactive levels of the potent vasodilator, calcitonin gene-related peptide (CGRP). Rats were born in 10% hypobaric hypoxia, where they remained for 1–2 days, or in 15% hypoxia, where they remained for 21 days. All were then reared in normoxia for 3 mo followed by reexposure to 10% hypoxia for 7 days (H→H) or continued normoxia (H→N); age-matched normoxic rats were hypoxic for the last 7 days (N→H) or normoxic throughout (N→N). Results are as follows. Pulmonary arterial pressure (P\textsubscript{PA}) in 10% H→N rats was normal at the end of the experiment (13 wk), but in rats reexposed to hypoxia (H→H), pressure rose to 19% above N→H controls. In 15% H→N rats, P\textsubscript{PA} remained high, similar to that of N→H rats, and increased further by 40% on reexposure (H→H). Medial thickness of small pulmonary arteries in 10% H→H rats also increased by 40% over N→H controls and was equally high in 15% H→N and H→H rats. In N→H rats from both experiments, right ventricular hypertrophy index (RVH) was increased after hypoxia at 15–16 wk. Also, in the 15% study, RVH remained elevated in H→N rats and increased in H→H rats by 19% above N→H controls. Blood CGRP was reduced by neonate and adult hypoxia, and hypoxic reexposure (H→H) further lowered blood CGRP in the 15% but not 10% study. Declining left ventricular blood CGRP correlated highly with logarithmically increasing P\textsubscript{PA} in the 15% study ($r = -0.81$, $P = 0.000$). In conclusion, 1) short perinatal exposure to 10% O\textsubscript{2}, exacerbated pulmonary hypertension with hypoxia later in life, 2) 15% O\textsubscript{2} at birth and for 21 days caused persistent pulmonary hypertension and exacerbation with reexposure, and 3) P\textsubscript{PA} correlated highly with declining blood CGRP levels in the 15% study.

hypoxic sensitization; persistent hyperventilation; right ventricular hypertrophy; calcitonin gene-related peptide

PERSISTENT PULMONARY HYPERTENSION (PPH) of the newborn is a clinically challenging lung condition, and its etiology is poorly understood. These patients are usually full-term or postterm infants who have had perinatal asphyxia, meconium aspiration, diaphragmatic hernia, pneumonia, or sepsis (1, 30), all of which could interfere with pulmonary oxygenation or gas exchange. In the United States the incidence of PPH varies between 0.07 and 2.3% of live births, accounting for ~1% of admissions to neonatal intensive care units (42). Despite aggressive management with hyperventilation, fluids, and vasodilators, mortality is as high as 34–60% (1, 9, 10, 21). These lung disorders together constitute a group in great need of further understanding of the mechanism(s) associated with pulmonary hypertension (PH) and subsequent development of effective treatment.

In addition to newborns, PH affects animals and humans of all ages. Aside from pathologically elevated pulmonary arterial pressure (17), PH is frequently associated with pulmonary vascular remodeling and tissue edema and right ventricular hypertrophy (16). Airway hypoxia is probably the most common cause of PH. It occurs at high altitude and can also result from hypventilation (e.g., sleep apnea) and restrictive lung disorders or inflammatory processes that interfere with airway oxygenation. Among these disorders are respiratory distress syndrome among infants, adult respiratory distress syndrome, and chronic obstructive pulmonary disease among adults. Moreover, infants who have died from sudden infant death syndrome carry markers suggestive of airway hypoxia and PH (40).

Although the pulmonary vasculature is relatively responsive to a variety of vasoactive agents, it appears that an imbalance between constrictor and dilator peptides may contribute to the pulmonary vasoconstriction and hypertension that occurs under hypoxic airway conditions. For example, the neuropeptide calcitonin gene-related peptide (CGRP) effectively dilates precontracted systemic and pulmonary arteries in vitro (26, 27) utilizing CGRP-1 receptors (2, 13, 38, 40); it is one of the most potent endogenous vasodilators known to date (41, 43). CGRP has one endotheli-um-dependent mode of action (6) and also dilates some systemic arteries and the pulmonary circulation, inde-
dependent of endothelial factors such as nitric oxide (27, 35). Our laboratory previously shown that endogenous CGRP has an essential protective role in hypoxia-induced PH (HPH) (37, 38) and that circulating levels of immunoreactive CGRP are reduced in rats with HPH, thus allowing constrictors such as endothelin (ET)-1 to act unopposed (14, 39). Vasoconstriction is further enhanced by increased ET-1 synthesis in hypoxia (24). However, available CGRP receptors remain in the hypoxic lung vasculature (as indicated by increased CGRP binding) (25), allowing for protection by exogenous CGRP (18).

CGRP is a 37-amino acid polypeptide hormone produced by tissue-specific, alternative RNA splicing of the calcitonin gene (3) and is expressed in sensory neurons. CGRP-like immunoreactivity is localized in nerve fibers of the airway mucosa and around vascular smooth muscle (4, 24, 37). Moreover, CGRP and its mRNA have been localized in the perikarya of intrapulmonary ganglia and in neuroendocrine cells of the airway epithelium (4, 19, 26), indicating CGRP synthesis and storage in these cells. These neuroendocrine epithelial cells, both solitary and clustered, have been shown to function as airway O2 sensors that respond to altered airway O2 content (23, 37, 44) by modulating local pulmonary vascular tone (37, 38). CGRP is therefore strategically localized, interconnecting neuroendocrine cells, airway epithelium, and local vasculature in a local microcircuit (37).

Because of a strong clinical need for further understanding of peptidergic mechanisms associated with neonatal PH, we established a neonata hypoxia model of PPH in rats based on limited prior information (5, 12, 20, 32). We characterize here the chronic adult PH effects of hypoxic exposure at birth and examine their relationships with levels of immunoreactive circulating CGRP.

**MATERIALS AND METHODS**

**Experimental protocol.** Pregnant Sasco Sprague-Dawley rats (primipara) were kept in a hypobaric hypoxia chamber (Biotron, Univ. of Wisconsin) from gestational day 16 (term = 21 days). In experiment I they gave birth at a barometric pressure (Pb) of 380 mmHg (equivalent to inspired O2 fraction (FiO2) 10%). However, because of high incidence of neonate deaths in the first week after birth in hypoxia (85%) with the use of 10% O2, subsequent pups were placed with their mothers in normobaric normoxia 1–2 days postpartum. In experiment II pups were born at a Pb of 520 mmHg (equivalent to Fio2 15%) and remained in hypoxia for 21 days. Survival rate was similar to that of pups born in normoxia (96.5%). Pups from both experiments were then reared in normoxia for 3 mo. At that time (12 and 15 wk of age, respectively), one group from each experiment was reexposed to hypobaric hypoxia equivalent to Fio2 at 10% for 7 days (H–H group) while another group remained in normoxia (H–N group). Moreover, in both experiments, age-matched rats of normoxic mothers were born and raised in normoxia (ambient air, ~21% O2) under similar conditions regarding cage size, lighting, food, and water. These control rats were exposed to hypobaric hypoxia for the first time during the last 7 days (N–H group) or were normoxic throughout (N–N group).

Numbers of rat pups were adjusted by culling to achieve similar numbers per group and number within experiments. In experiment I, 12 normoxic pups and 12 surviving hypoxic pups were separated from their mothers on day 1 or 2 after birth and raised by 4 normoxic and 4 hypoxic adoptive mothers, respectively. To achieve identical sample sizes (3 pups per mother), hypoxic pups were moved from larger litters to mothers with only one or two young, and normoxic pups were culled. Because of additional deaths among rats born in hypoxia, these numbers were further reduced from six pups per group to four and five per group. In experiment II, to avoid bias by maternal factors, 16 normoxic and 16 hypoxic pups were switched on day 2 after birth between 4 normoxic and 4 hypoxic adoptive mothers, respectively, resulting in 8 pups per treatment group (4 per mother). A total of 21 and 32 pups were used for experiments I and II, respectively. All rats were treated and housed humanely according to an animal care protocol approved by the University of Wisconsin-Madison Research Animal Resources Center and the Animal Welfare Act (assurance no. A-3368-01).

Effluent blood from the lungs was drawn from the left ventricle (LV) into 10-ml syringes pretreated with EDTA (10 μl of 10% EDTA/ml blood; no. 2670, Ricca Chemical) and Trasyrryl (200 kallikrein-inactivating unit/ml blood; no. A-1153, Sigma), transferred to glass tubes, and placed briefly on ice until centrifugation (804 × 1000g, 10 min). The plasma was collected, lyophilized, and stored at ~70°C for subsequent analysis of peptide levels; plasma and blood volume measurements were also used to determine hematocrit.

Lungs were then perfused with heparinized saline, removed, and weighed. One 3-mm-thick transverse slice was sampled from the midregions of the left lobe and the right lower (diaphragmatic) lobe for histochemistry and morphometry, and the remainder was weighed, snap frozen, and stored at ~70°C for subsequent peptide assays. The lung pellet remaining after tissue peptide extraction for radioimmunooassay was lyophilized and used for dry weight. Hearts were isolated and fixed in 10% buffered Formalin; they were later blotted, dissected, and weighed while moist. The weight ratio of RV to LV plus septum (S) [RV/LV + S] was used to evaluate RV hypertrophy.
Radioimmunoassay. Lungs were partially thawed, diced with a razor blade into 3 × 3-mm cubes, and homogenized (TissueMizer Mark II, Tekmar). Lung tissue peptides were extracted by boiling tissue homogenates in saline for 15 min, followed by centrifugation (2,300 g at +4°C for 30 min) and collection of supernatants. The lung pellets were boiled again for 15 min, using 0.5 M acetic acid, followed by centrifugation. For each lung, the combined supernatants were stored lyophilized until assay. Radioimmunoassays for CGRP were performed in duplicates on lung extracts and LV plasma according to previously published methods (11, 37, 38). As lung water and dry tissue mass are elevated in hypoxic rats, thereby significantly increasing lung weight, peptide levels are expressed as picomoles per whole lung instead of wet weight to avoid falsely low values. This was done under the assumption that the amount of CGRP containing lung parenchyma (not including edema and increased connective tissue) was similar between groups as body weights are similar (Table 1). Moreover, because hematocrit was increased in hypoxic rats, peptide levels are expressed as picomoles per liter of blood to avoid differences due to reduced plasma volume (38).

Morphometric evaluation of pulmonary vascular medial thickness index. We used histological sections of noninflated, noninjected routinely fixed lung samples (7) stained with Miller elastin stain (28) to determine medial thickness index on naturally expanded, cross-sectioned circular pulmonary vessels ranging from 50 to 100 μm in outer diameter. The selected vessels were presumed to be arteries because of their round shapes and distinct internal and external elastic laminae. The surface area of the cross-sectioned media was measured in each randomly selected vessel as outlined by the internal and external laminae. Average vessel diameter (2 × radius; 2r) was calculated from the total pixel area inside the circumference of the external lamina of the vessel as follows using the correction factor: 3.12 pixels = 1 μm, pixel area = 3.12² = 9.73, pixel area/9.73 = μm area. Total pixel area is πr², r² = pixel area/π, and r (in μm) = the square root of pixel area/9.73 π. Average diameter per vessel is 2r. Medial area was then normalized for each vessel by dividing by average diameter of that vessel. This method is more precise than direct measurement and averaging of the medial thickness and diameter, respectively, in two perpendicular sites, as previously employed (17, 36, 38). For the above measurements, we applied an Image Pro Plus morphometry software program to vessel images imported into a Gateway 486 computer using a Nikon Labophot microscope and a high-resolution color camera. A minimum of 10 vessels per rat was measured, and the individual means were used to calculate the group means.

Table 1. Body weights of rats at the end of experiments

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<tr>
<td>10% (21)</td>
<td>316 ± 64 (5)</td>
<td>399 ± 50 (6)</td>
<td>298 ± 30 (4)</td>
<td>418 ± 54 (6)</td>
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<tr>
<td>15% (32)</td>
<td>332 ± 33 (8)</td>
<td>358 ± 30 (8)</td>
<td>427 ± 37 (8)</td>
<td>419 ± 38 (8)</td>
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Values are means ± SE; nos. in parentheses are sample sizes. See MATERIALS AND METHODS for detailed descriptions of treatments. H, hypoxia; N, normoxia; 10%, rats born in a hypobaric O₂ environment equivalent to 10% inhaled O₂ and maintained in this environment for only 2–3 days; 15%, rats born in a hypobaric O₂ environment equivalent to 15% inhaled O₂ and maintained in this environment for 21 days by ANOVA, no significant differences between any groups in either experiment. *Significantly lower than 15% group by use of Student’s t-test for unpaired data at P ≤ 0.05.

Table 2. Blood gases in anesthetized, spontaneously breathing rats from the 15% study at the end of experiments

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<tr>
<td>PaO₂</td>
<td>44.0 ± 3.7*</td>
<td>28.7 ± 1.9*</td>
<td>87.4 ± 4.7*</td>
<td>72.0 ± 7.2*</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>15.5 ± 1.2</td>
<td>18.7 ± 1.1</td>
<td>21.1 ± 2.0</td>
<td>29.1 ± 2.8*</td>
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Values are means ± SE; n = 8 rats/group. PaO₂, arterial Po₂; PaCO₂, arterial PCO₂. *All groups are significantly different from one another by use of Student-Newman-Keuls test at P ≤ 0.05. †Significantly higher than all other groups by use of Student-Newman-Keuls test at P < 0.05. No other differences found between groups.

Statistical analysis. Results are expressed throughout as group means ± SE. Data were evaluated by use of ANOVA followed by Student-Newman-Keuls test for multiple comparisons. Where so indicated, Student’s t-test for unpaired data was used to compare a specific test group with its matched control. The latter test was used mostly to detect trends toward differences that might have proven significant with larger sample sizes in the Student-Newman-Keuls test.

RESULTS

Body weights did not differ significantly between any two treatment groups among either 10% or 15% rats at the end of each experiment (Table 1); overall mean body weight among rats was 382 g. However, males were, on average, 70% heavier than females (492 ± 12 vs. 290 ± 9 g). Sex composition was 52% females and 48% males and was similar within individual treatment groups.

Blood gas analysis in the 15% O₂ experiment confirmed that rats exposed for the first time to hypoxia at the end of the experiment (N–H) had lower PaO₂ compared with N–N controls (Table 2). However, in H–N rats, PaO₂ was above N–N controls, suggesting hyperventilation in the former. Similarly, PaCO₂ was lower in all hypoxia groups compared with normoxic controls, supporting expected hyperventilation in these groups as well as the H–N rats.

Mean Pₚa among rats born in a hypobaric O₂ environment (equivalent to 10% inhaled O₂) and maintained in this environment for only 1–3 days (Fig. 1A), hereafter referred to as 10% rats (H–N group; n = 4), was at normoxic control levels (N–N; n = 6) at the end of the experiment over 3 mo later. However, when reexposed to 10% hypoxia after 3 mo (H–H group; n = 5), these rats developed elevated Pₚa (19% increase) compared with age-matched rats born and maintained in normoxia and exposed to hypobaric hypoxia for the first time at age 15 wk (N–H group; n = 6). Furthermore, mean Pₚa among rats born in a hypobaric O₂ environment equivalent to 15% inhaled O₂ and maintained in this environment for 21 days (Fig. 1B), hereafter referred to as 15% rats, remained elevated after over 3 mo of normoxia (H–N; n = 8) compared with controls (N–N; n = 8), indicating PPH. Additionally, Pₚa levels in rats reexposed to hypoxia (H–H; n = 7) rose to 40% above those of age-matched rats exposed to hypoxia for the first time (N–H; n = 8). Mean RV
pressures followed closely the same pattern as $P_{PA}$ (Fig. 1, A and B).

Medial thickness index among 10% rats born in hypoxia (Fig. 2A) was normal after 3 mo (H→N) but was markedly elevated (53% increase) after hypoxic reexposure (H→H) compared with age-matched rats exposed for the first time (N→H). On the other hand, among 15% rats (Fig. 2B), medial thickness index remained greater after 3 mo (H→N) compared with age-matched normoxic controls (N→N). These levels were similar to those of reexposed 10% and 15% rats (H→H). Hypoxia for the first time (7 days) at the end of the experiment (N→H) did not increase medial thickness index significantly in either experiment.

RV weight index among the 10% rats (Fig. 3A) was normal after 3 mo (H→N vs. N→N) and was equally elevated in the reexposed rats (H→H) and those exposed only after 3 mo (N→H). On the other hand, RV weight index of the 15% rats (Fig. 3B) remained high after 3 mo (H→N) at the index of age-matched rats exposed for the first time (N→H) and was further elevated by reexposure (H→H, 23%).

Hematocrit among both 10% and 15% rats was normal after 3 mo (H→N; 0.50 ± 0.06 and 0.69 ± 0.03, respectively) compared with controls (N→N; 0.59 ± 0.03 and 0.67 ± 0.00, respectively). However, hematocrit was equally elevated after hypoxic reexposure (H→H; 0.77 ± 0.04 and 0.81 ± 0.02, respectively) and in age-matched rats after the first hypoxic exposure as adults (N→H; 0.77 ± 0.04 and 0.81 ± 0.02, respectively).

Lung tissue levels of immunoreactive CGRP (Fig. 4) were not significantly different between the same treatment groups in the 10% and 15% experiments. Thus, in both experiments, the H→H and N→H rats had significantly higher lung CGRP levels after 3 mo of normoxia compared with H→N rats but did not differ from N→N controls. Although the means for H→N rats were 41 and 38%, respectively, below those of N→N controls, these differences may indicate trends but were not statistically significant.

LV blood levels of immunoreactive CGRP (Fig. 5) were significantly lower in all three hypoxic groups of both experiments compared with their respective normoxic control group (N→N). The 10% H→N group (Fig. 5A) had levels 85% below those of N→N rats, and, among 15% H→N rats (Fig. 5B), levels were 46% lower.
than normoxic controls. Among 15% rats, levels were further reduced to 69% below N$_{3}$ rats on reexposure to hypoxia (H$_{3}$H). Regression analysis of the 10% study data revealed no significant correlation between blood CGRP levels and PPA. Regression analysis of the entire 15% data set (Fig. 6) indicated a highly significant, strong negative correlation between PPA and blood CGRP levels ($r = -0.81$, $P = 0.000$), indicating that mean PPA increased exponentially with declining LV blood CGRP.

Mean systemic arterial pressure, only measured in experiment II, was reduced among N$\rightarrow$H rats (80 $\pm$ 6 mmHg) and unchanged in H$\rightarrow$H rats (115 $\pm$ 38 mmHg) and H$\rightarrow$N rats (92 $\pm$ 14 mmHg) compared with N$\rightarrow$N controls (116 $\pm$ 38 mmHg).

**DISCUSSION**

Our results indicate that hypoxia in the perinatal period has profound effects on the future lung health of an individual and may predispose one for PH later in life. It is particularly notable that 10% hypoxia perinatally and for just 1–2 days postpartum could cause elevated P$_{PA}$ on hypoxic reexposure as adults. This seems especially significant considering these data were collected mostly from survivors who would be expected to have a high level of resistance to hypoxia. Whereas a more moderate hypoxia of 15% O$_2$ allowed most pups to survive, birth and rearing in this environment for 21 days resulted in PPH, as measured more than 3 mo later, and more severe PPH on hypoxic reexposure than present among those rats exposed for the first time. Using similar protocols with Wistar albino rats and 1-wk neonate hypoxia (10%), Hampl and Herget (12) found P$_{PA}$ equally elevated in N$\rightarrow$H and H$\rightarrow$H rats. However, 2 wk after a repeat of hypoxia, isolated lungs showed heightened reactivity to acute hypoxia and increased perfusion pressure. Moreover, an exaggerated response to the alkaloid monocrotaline was demonstrated by Caslin et al. (5) and King et al. (20) in adult rats that were exposed to hypoxia neonatally. This response was indicated by more rapid and pronounced pulmonary vascular muscularization and higher RV hypertrophy index (RVH) (both associated with PH) compared with monocrotaline controls not exposed to neonatal hypoxia. These changes support our findings of neonatal hypoxia predisposing for exacerbated PH on an agonistic stimulus later in life. However, neonate hypoxia in rats has also been found to attenuate in vitro PA responses to hypoxia (12) or agonists such as norepinephrine and potassium chlo-

![Fig. 3. RV weight index (RV/(left ventricle + septum)) in 10% rats (A) and in 15% rats (B) and age-matched treatment groups (for more detailed descriptions of rat groups, see MATERIALS AND METHODS). *Significantly different from all other groups. †Not different from other groups marked the same. ††Not different from other groups marked the same. † and †† are significantly different from one another. (Student-Newman-Keuls test at $P \leq 0.05$.)](image)

![Fig. 4. Lung tissue calcitonin gene-related peptide (CGRP) levels in 10% rats (A) and in 15% rats (B) at the end of experiments. *Significantly lower than H$_{3}$H and N$_{3}$H groups but not different from N$_{3}$N by use of the Student-Newman-Keuls test at $P \leq 0.05$. †Not different from other groups marked the same.](image)
ride (32). This suggests that a whole animal approach is advantageous for summarizing the effects of neural, humoral, and other factors on hypoxic stimuli.

The exacerbated PH in H→H rats did not result indirectly from raised systemic or left atrial pressure, as there was no difference in systemic pressure between H→H and N→N groups (115.2 ± 4.83 and 116 ± 3.84 mmHg, respectively). Altered pulmonary pressures could potentially arise from increased cardiac output or a rise in left atrial pressure. However, cardiac output in rats is not increased by hypoxia (6), and an independent rise in left atrial pressure alone is not expected. We thus speculate that the hypoxic pressor response is primarily intrapulmonary, as suggested long ago by Daly and Hebb (8) and Laros (22).

Hypoxic exposure at the end of each experiment (N→H), when rats were adult, resulted in the typical elevation of P_P,A, P_R,V, hematocrit, medial thickness, and RV weight, as previously reported (15–17), although the medial thickness remodeling was not statistically significant after 1 wk of hypoxia. The PPH in 15% H→N rats was accompanied by increased medial thickness and RV weight ratio as expected, in contrast to normal values in 10% H→N rats, which had normal P_P,A. Accelerated smooth muscle changes in the pulmonary vasculature by monocrotaline (20), as previously mentioned, suggest increased vascular reactivity to several types of agents. The differences in vascular reactivity to hypoxia, reported by several laboratories, likely reflect differences in animal age and, perhaps more importantly, a difference in rat strains. We know, for example, that some strains, e.g., Hilltop rats, are highly sensitive to hypoxia (31) and that fawn-hooded rats develop spontaneous PH (34). The strain used here (Sasco Sprague-Dawley) is considered moderately reactive and appears to be less responsive to hypoxia than the Wistar rat (5, 12, 20, 32).

Normoxic rats with PPH (15% H→N) maintained high P_A,O2 and normal hematocrit through hyperventilating (P_CO2 reduced 28% below normal). Therefore, airway hypoxia is not likely the cause of their elevated P_P,A. This could potentially result from chronically reduced Cgrp levels noted in LV blood. Hyperventilation by adult (50-day-old) normoxic rats born in hypoxia was also noted by Okubo and Mortola (29).

In both experiments, lung tissue Cgrp was higher in the H→H and N→H rats but not in N→N rats compared with the H→N group. However, there was no significant measurable difference between H→H, N→H, and N→N groups. Previous reports by us and others (17, 36) suggest that lung Cgrp may increase during chronic hypoxia in adult rats, but levels vary between studies. The primary site for the increased lung Cgrp is believed to be airway neuroendocrine cells (36). Although the lung dynamics of Cgrp in hypoxia are unknown, reduced LV blood levels as seen by us in the H→H, H→N, and N→H rats could result
from impaired release to the blood stream from intrapulmonary sources.

Among our rats exposed to perinatal hypoxia, not only did PA and RV pressures remain persistently high but so did medial thickness and RV weight. These PH hallmarks did not disappear during more than 3 mo of normoxia, indicating PPH in the presence of normal \( P_{\text{O}_2} \) and hematocrit. Furthermore, in experiment II, the substantial reduction in LV blood CGRP among the H→N rats may indicate a causal relationship between reduced bioavailability of CGRP to its vascular receptors and the chronically increased \( P_{\text{PA}} \). In fact, blood CGRP levels among the rats from all groups in experiment II were negatively correlated with \( P_{\text{PA}} \). Specifically, the H→H group had the lowest levels of circulating CGRP and the highest \( P_{\text{PA}} \), and among H→N rats, \( P_{\text{PA}} \) was associated with significantly reduced CGRP levels after 3 mo of recovery in normoxia. These CGRP levels were similar to those of age-matched rats newly exposed to hypoxia (N→H).

The foregoing results supplement the many previous observations in our laboratory implicating reduced endogenous blood CGRP levels in HPH (17, 18, 37–39). The 10% H→N group is an exception in that blood CGRP levels remained extremely low 3 mo posthypoxia, whereas \( P_{\text{PA}} \) was normal. The low blood CGRP levels could have resulted from the relatively low lung levels in this group, suggesting reduced CGRP availability and/or impaired synthesis. The normal \( P_{\text{PA}} \) in the 10% H→N group might be explained by the fact that this was a small group (n = 4) composed of survivors of 10% neonate hypoxia, perhaps subjected to natural selection. A redundant vasodilator such as adrenomedullin, a member of the CGRP superfamil, could potentially be the alternative depressor agent among these surviving rats, as it may also bind to the CGRP-1 receptor (13); we believe this needs to be examined further. Small sample sizes in the 10% experiment may explain why we found few significant differences between groups overall and no significant correlation between blood CGRP and \( P_{\text{PA}} \) in that study.

We have previously shown prevention and reversal of HPH with exogenous \( \alpha \)-rat CGRP infused chronically into the pulmonary circulation of awake, unrestrained rats (18, 38). On the other hand, depletion of sensory CGRP with capsaicin resulted in exaggerated \( P_{\text{PA}} \) and RVH in hypoxia and augmented PA medial thickness in both normoxia and hypoxia (37). Moreover, we infused the selective CGRP-1 receptor antagonist CGRP-(8–37) and the nitric oxide synthase inhibitor \( N^\bullet \)-nitro-l-arginine methyl ester (\( \cdot \)NAME) into the closed circulation of in vitro lung preparations precontracted with potassium chloride (38). CGRP-(8–37) blocked vasodilation by exogenous CGRP, but \( \cdot \)NAME did not, suggesting that CGRP may act directly on the pulmonary vascular smooth muscle via CGRP-1 receptors in an endothelium-independent fashion, probably targeting resistance vessels. This previous information, taken together with results from the present research, supports a role of CGRP in HPH. We conclude that exposure to 15% perinatal hypoxia lowers blood CGRP levels in association with PPH and predisposes for PH later in life.

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