NPR-A–Deficient Mice Show Increased Susceptibility to Hypoxia-Induced Pulmonary Hypertension

Lan Zhao, PhD; Lu Long, MB; Nicholas W. Morrell, MD; Martin R. Wilkins, MD

Background—Mice in which the gene encoding NPR-A, a guanylyl cyclase–linked natriuretic peptide receptor, has been disrupted were used to examine the contribution of natriuretic peptides to maintaining pulmonary vascular homeostasis in normal- and low-oxygen environments.

Methods and Results—Wild-type (+/+), heterozygous (+/−), and homozygous null mutants (−/−) were studied. The response of the pulmonary vasculature to atrial, B-type, and C-type natriuretic peptides (ANP, BNP, and CNP) during acute hypoxia was studied in isolated perfused lungs. Right ventricular systolic pressure (RVSP), RV weight, and pulmonary vascular remodeling were measured in each genotype exposed to normal air and after 7 and 21 days in a hypoxic atmosphere (10% O2). ANP and BNP (300 ng) reduced pulmonary artery pressure during acute hypoxia-induced pulmonary vasoconstriction in +/+ mice, but this effect was attenuated in +/− and absent in −/− mice. CNP (600 ng) had little effect in all 3 genotypes. RVSP and RV weight were similar in the 3 genotypes housed in a normal-O2 environment. Seven and 21 days of hypoxia produced a pronounced and significantly greater increase in RVSP and RV weight in −/− mice compared with +/+ or +/− mice and more rapid muscularization of distal pulmonary arterioles.

Conclusions—ANP and BNP do not contribute to maintaining normal pulmonary artery pressure but play an important role in attenuating the pulmonary vascular response to hypoxia. NPR-A mediates the vasorelaxant effect of ANP in pulmonary vasculature. (Circulation. 1999;99:605-607.)

Key Words: genes ■ hypertension ■ lung ■ peptides

The normal adult pulmonary circulation is a low-pressure, low-resistance vascular bed. Chronic hypoxia leads to pulmonary hypertension, characterized by pulmonary vasoconstriction, vascular remodeling, and right ventricular (RV) hypertrophy.

The natriuretic peptides, specifically atrial and B-type (ANP and BNP), have been shown to reduce elevated pulmonary vascular tone and attenuate hypoxia-induced pulmonary hypertension in the rat. Of the 3 natriuretic peptide receptors (NPRs) described, NPR-A and NPR-B are guanylyl cyclase–linked and mediate the actions of ANP, BNP, and C-type natriuretic peptide (CNP) through cGMP. NPR-C is not linked to guanylyl cyclase; it is thought to have a clearance role but may mediate antitrophic effects in some tissues through cAMP or phosphoinositol. We used mice in which the gene encoding NPR-A has been disrupted to define the receptor subtype responsible for the vascular effects of the natriuretic peptides in the pulmonary circulation and the contribution of these peptides to the regulation of pulmonary vascular tone and remodeling.

Methods

Animals
Mice deficient in the NPR-A receptor were produced as described previously and provided by Dr David Garbers (Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas). The wild-type and mutant homozygous null mice used in these studies were siblings (10 to 12 weeks of age) from the breeding of heterozygous mutants.

Isolated Perfused Lung Preparation
Mice were anesthetized (pentobarbitone 20 mg/kg IP) and the lungs ventilated and perfused in situ according to a protocol modified from the rat. The lungs were ventilated with air at constant end-expiratory pressure (12 to 15 cm H2O) at 60 breaths per minute and perfused with DMEM and 4% Ficoll at a flow rate of 0.06 mL/g body wt/min with a nonpulsatile pump (Masterflex model 7519). Pulmonary artery pressure was measured with a Gould pressure transducer (Gould RE 550). Hypoxic pulmonary vasoconstriction (HPV) was produced by ventilating with 2% O2, 5% CO2, and 93% N2 for 15 minutes. Animals received either ANP, BNP, or CNP (Sigma Chemical Co) during the stable phase of the first hypoxic challenge, HPV1, and any change in pressure was recorded. The lungs were then ventilated with air for 10 minutes before a second hypoxic challenge, HPV2.

Chronic Hypoxia Study
Mice were exposed to normal air or placed in a specially constructed environmental chamber and exposed to hypoxia (FiO2 10%) for 7 or 21 days, as described previously. RV systolic pressure (RVSP) was measured in the anesthetized animal (pentobarbitone 20 mg/kg IP). The heart was excised, and the right and left ventricles (plus septum) were separated and weighed. The trachea was cannulated, and lungs
were fixed in inflation with 10% formalin in PBS before paraffin embedding and sectioning.

**Pulmonary Artery Morphometry**

Coronal lung sections were stained with elastic van Gieson. The proportion of arteries at the level of the alveolar duct containing smooth muscle was used to measure the extent of distal vessel muscularization. Vessels were viewed under 400 magnification by an observer unaware of the experimental conditions pertaining to each section, and the presence of a double-elastic lamina was taken as evidence of muscularization.1

**Statistical Analysis**

Data are expressed as mean±SEM. Statistical analysis was done by ANOVA using Fisher’s post hoc test for multiple comparisons. Differences were considered statistically significant at \( P<0.05 \).

**Results**

**Isolated Perfused Lung**

Baseline pulmonary artery pressure after 20 minutes of equilibration was similar for all 3 genotypes (mean±SEM, mm Hg: +/+, 20.3±0.9, \( n=8 \); +/-, 21.4±0.5, \( n=8 \); −/−, 20.6±0.3, \( n=5 \)). Likewise, the pressor response to acute hypoxia (HPV) did not differ significantly between the 3 genotypes (+/+, 7.3±2.2; +/-, 8.2±1.8; −/−, 7.8±0.8 mm Hg). ANP 300 ng given as a bolus during HPV1 produced a significantly greater fall in pulmonary artery pressure in the wild-type mice than in the −/− mutants (mm Hg: 3.0±0.3, \( n=5 \) versus 0.2±0.2, \( n=3 \), \( P<0.05 \)); indeed, the response to ANP in the −/− mice was negligible. The +/- mice exhibited an intermediate response (1.9±0.9 mm Hg). The vasorelaxant effect of ANP in the wild-type mice extended to the pressor response to a second exposure to hypoxia 5 minutes later (HPV2), which was significantly \( (P<0.05) \) attenuated compared with HPV1, (HPV1/HPV1, 57.4±6.1%). HPV1 and HPV2 did not differ in the −/− mutants (HPV1/HPV1, 106.1±6.1%). NPR-A–deficient mice showed no response to ANP (data not shown). The response to CNP 600 ng was very small (mm Hg: +/+, 0.4±0.2, \( n=3 \); +/-, 1.0±0.3, \( n=4 \); −/−, 0.5±0.1, \( n=2 \)) and did not differ significantly between the 3 groups. A reduction in HPV comparable to that produced by ANP 300 ng in wild-type mice was achieved with CNP 30 \( \mu \)g in all 3 groups (data not shown).

**Chronic Hypoxia Study**

The response of mice with each genotype to chronic hypoxia is shown in the Table. The 3 genotypes did not differ significantly in basal RVSP, RV mass, or the degree of distal muscularization of the pulmonary vasculature. After 7 days of exposure to hypoxia (10% \( \text{O}_2 \)), the wild-type and heterozygous mice showed no significant change in RVSP or RV weight (except when the latter was corrected for left ventricular or body weight). In contrast, chronically hypoxic −/− mutants showed a marked 53% increase in RVSP and a similar increase in RV weight. After 21 days of hypoxia, RVSP and RV weight were increased in all 3 genotypes, but to a strikingly greater degree in −/− mice (the increase in left ventricular weight is largely an artifact of including the septal wall with the left ventricle). Morphometric data show that exposure to hypoxia was accompanied by more rapid extension of smooth muscle into alveolar duct arterioles in −/− mice. Analysis of the data according to sex shows that males and females responded similarly.

**Discussion**

In this study, mice deficient in NPR-A with no pulmonary vasodilator response to ANP or BNP had normal pulmonary artery pressures and RV weights in a normal oxygen environment but demonstrated increased susceptibility to pulmonary hypertension when chronically exposed to hypoxia. Previous studies have reported that circulating ANP and BNP levels are elevated by hypoxia.7,8 Furthermore, raised pulmonary artery pressure can be decreased by administration of exogenous peptide,9 and chronic administration2 or overexpression3 of ANP reduces hypoxia-induced pulmonary hypertension, vascular remodeling, and RV hypertrophy in the rat. However, such studies do not address the contribution of endogenous natriuretic peptides to the regulation of the pulmonary circulation. Our data provide the first compelling evidence that endogenous ANP and BNP have an important role in attenuating hypoxia-induced pulmonary hypertension.

The observation that mice lacking NPR-A have normal pulmonary artery pressures in a normal atmosphere indicate that neither ANP nor BNP is responsible for the low pulmonary vascular tone of the normal adult mouse. Studies in vitro10 and in mice deficient in endothelial nitric oxide synthase11 suggest that this is an important role for nitric oxide.
Messenger RNA for all 3 natriuretic peptide receptor subtypes has been demonstrated in lung tissue. Significantly, ANP and BNP have no effect on the pressor response to hypoxia in the isolated perfused lungs of NPR-A–deficient mice, whereas heterozygotes exhibit a partial response. Thus, the vasorelaxant effect of ANP and BNP in the mouse pulmonary circulation is mediated solely by NPR-A.

Our data suggest a very limited role for CNP and its putative receptor, NPR-B, in regulating pulmonary vascular tone. CNP in a dose twice the EC50 for relaxing aortic rings had little effect on the pressor response to hypoxia in either wild-type or mutant mice, although very high doses (30 µg) did reduce HPV in all 3 genotypes. NPR-B mRNA levels are increased in hypoxic rat lung; therefore, CNP may assume a more significant role in chronic hypoxia, but not sufficient to compensate for the lack of effect of ANP and BNP. NPR-C, conversely, appears to be downregulated in the hypoxic rat lung. This receptor has no direct role in mediating the vasorelaxant effects of ANP or BNP in the pulmonary circulation, but if it functions as a clearance receptor, downregulation would serve to increase the levels of circulating ANP and BNP available for NPR-A–mediated vasorelaxation.

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References

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