Beneficial Effects of Phosphodiesterase 5 Inhibition in Pulmonary Hypertension Are Influenced by Natriuretic Peptide Activity

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Background—Phosphodiesterase type 5 (PDE5) inhibitors (eg, sildenafil) are a novel, orally active approach to the treatment of pulmonary arterial hypertension. The role of natriuretic peptides in the response to sildenafil was examined in mice lacking NPR-A, a guanylyl cyclase–linked natriuretic peptide receptor, in which pulmonary hypertension was induced by hypoxia.

Methods and Results—Mice homozygous for NPR-A (NPR-A+/+) and null mutants (NPR-A−−) were studied. Sildenafil inhibited the pressor response to acute hypoxia in the isolated perfused lungs of both genotypes. This effect was greater in the presence of atrial natriuretic peptide in the perfusate in NPR-A+/+ mice but not NPR-A−− animals. In vivo, NPR-A mutants had higher basal right ventricular (RV) systolic pressures (RVSPs) than did NPR-A+/+ mice, and this was not affected by 3 weeks of treatment with sildenafil (25 mg·kg−1·d−1). Both genotypes exhibited a rise in RVSP and RV weight with chronic hypoxia (10% O2 for 21 days); RVSP and RV weight were reduced by continuous sildenafil administration in NPR-A+/+ mice, but only RVSP showed evidence of a response to the drug in NPR-A−− mice. The effect of sildenafil on hypoxia-induced pulmonary vascular muscularization and cyclic GMP levels was also blunted in NPR-A−− mice.

Conclusions—The natriuretic peptide pathway influences the response to PDE5 inhibition in hypoxia-induced pulmonary hypertension, particularly its effects on RV hypertrophy and vascular remodeling. (Circulation. 2003;107:234-237.)

Key Words: hypertension, pulmonary ■ natriuretic peptides ■ remodeling

Pulmonary arterial hypertension is a life-threatening condition for which therapeutic options are limited. Phosphodiesterase type 5 (PDE5) inhibitors (eg, sildenafil) are under investigation as a novel, orally active therapy for this condition. PDE5 is abundant in the lung and hydrolyses cyclic GMP, a mediator of vasorelaxation and antifibrotic effects in vascular tissue.1–3 Chronic PDE5 inhibition has been shown to elevate pulmonary cyclic GMP levels and abrogate hypoxia-induced pulmonary hypertension and vascular remodeling in animal models, and to reduce pulmonary artery pressure in primary pulmonary hypertension.4–8

The major factors stimulating cyclic GMP synthesis in pulmonary vascular tissue are nitric oxide (NO) and the natriuretic peptides (atrial natriuretic peptide [ANP], brain natriuretic peptide [BNP], and c-type natriuretic peptide [CNP]).9 Natriuretic peptide levels are elevated in all forms of pulmonary hypertension and may influence the response to PDE5 inhibitors in this condition. The cardiovascular response to the natriuretic peptides is transduced by NPR-A, a guanylyl cyclase–linked receptor.10,11 We have examined the effect of sildenafil in mice lacking functional NPR-A exposed to hypoxia, a commonly used model of experimental pulmonary hypertension.

Methods

Animals

NPR-A receptor–deficient mice (NPR-A−−) were bred in-house from stock and produced as described previously.10,11 Studies were conducted on homozygous NPR-A+/+ and NPR-A−− from brother-sister mating aged 10 to 12 weeks fed standard chow and water ad libitum. Genotype was confirmed by polymerase chain reaction of genomic DNA with standard techniques.

Isolated Perfused Mouse Lung

The effects of sildenafil±ANP on hypoxia-induced pulmonary vasoconstriction (HPV) were examined in isolated perfused lungs.7,11 Each preparation was challenged twice at 15-minute intervals with hypoxia (2% O2/5% CO2/93% N2 for 10 minutes) and the pressor response recorded: HPV1 and HPV2. Sildenafil (final reservoir concentration 100 nmol/L) was added where indicated between the two hypoxic challenges. ANP (300-ng bolus) was given to some animals during HPV1 to confirm phenotype and examine the effect on the response to sildenafil in HPV2.
Chronic Dosing Study
Mice were exposed to normal air or normobaric hypoxia (FiO$_2$ 10%) for 3 weeks. Sildenafil (25 mg · kg$^{-1}$ · d$^{-1}$) or vehicle was administered in the drinking water. Intake was assessed by daily weighing of the drinking water, and the sildenafil concentration in the water was adjusted to maintain the dose. Right ventricular systolic pressure (RVSP) was measured via direct cardiac puncture in the anesthetized animal. Cardiac chamber weights were recorded and the lungs snap-frozen for histology and cyclic GMP measurements.

Morphological Analysis
Transverse lung sections were stained with van Gieson’s Elastic method (EvG). Muscularization of distal pulmonary vessels was measured as previously described and calculated as the percentage of vessels <50 μm diameter with immunoreactivity for α-smooth muscle actin under light microscopy. Forty vessels per section and 3 sections per mouse were counted by an observer unaware of the experimental conditions pertaining to each section.

Cyclic GMP Measurement
Lung homogenates were lysed in ice-cold buffer with inhibitors and cyclic GMP assayed by radioimmunoassay (TRK500, Amersham) as described. The levels are expressed as picomoles per mg protein.

Statistics
Data are expressed as mean ± SEM. For each measured parameter, a one-way analysis of variation was performed across all groups of mice to detect significant variation between group means. For those parameters exhibiting variation, Bonferroni’s multiple-comparison tests were then performed between individual groups. P<0.05 was taken as evidence of significance.

Results
Isolated Perfused Mouse Lung
The HPV$_1$ response to ventilation with 2% O$_2$ was similar in both NPR-A$^{+/+}$ and NPR-A$^{-/-}$ mice (9.3±1.3 versus 9.7±0.6 mm Hg, n=4). HPV$_2$ was reduced (compared with HPV$_1$) by pretreatment with sildenafil in both genotypes (HPV$_2$/HPV$_1$ 45±2.4% in +/+ mice; HPV$_2$/HPV$_1$ 58±5.3% in −/− mice; not significant). ANP reduced HPV$_2$ in NPR-A$^{-/-}$ but not NPR-A$^{+/+}$ mice (HPV$_2$/HPV$_1$ 65±6% in +/+ mice; HPV$_2$/HPV$_1$ 98±5.6% in −/− mice, P<0.01). The combined effect of ANP and sildenafil was significantly greater (P<0.01) than sildenafil alone in NPR-A$^{-/-}$ mice (HPV$_2$/HPV$_1$ 29±2.1%), but not in NPR-A$^{+/+}$ mice (HPV$_2$/HPV$_1$ 60±7.3%).

Right Ventricular Systolic Pressure
In normal air, RVSP was higher in NPR-A$^{-/-}$ than NPR-A$^{+/+}$ animals and this was unaffected by sildenafil treatment (Figure 1a). RVSP increased in both genotypes treated with vehicle and exposed to hypoxia (10% O$_2$) for 3 weeks. Sildenafil treatment significantly reduced RVSP during chronic hypoxia in NPR-A$^{+/+}$ mice but not NPR-A$^{-/-}$ animals (P=0.058).

Right Ventricular Hypertrophy
Three weeks hypoxia produced a significant increase in the ratios of right ventricular (RV) weight/weight + septum weight and RV weight/body weight in both genotypes (Figure 1, b and c). This was attenuated by sildenafil in NPR-A$^{-/-}$ but not NPR-A$^{+/+}$ mice.

Figure 1. Response of NPR-A$^{+/+}$ and NPR-A$^{-/-}$ mice to chronic hypoxia and sildenafil. a, RVSP; b, ratio of RV to left ventricle (LV) plus septum (sep); and c, ratio of RV to body weight (BW) in mice kept in normal air or exposed to hypoxia (10% O$_2$) for 3 weeks and treated with sildenafil (25 mg · kg$^{-1}$ · d$^{-1}$, closed bars) or vehicle (open bars). Data are mean ± SEM. *P<0.05, **P<0.01. (Note #P<0.05 compared with similarly treated NPR-A$^{+/+}$ group.)

Pulmonary Vascular Morphology
NPR-A$^{-/-}$ mice showed greater muscularization of pulmonary arterioles than did NPR-A$^{+/+}$ mice, but this did not reach statistical significance (Figure 2a). The proportion of muscularized vessels increased markedly with chronic hypoxia in both NPR-A$^{+/+}$ and NPR-A$^{-/-}$ mice. Sildenafil treatment appeared to attenuate muscularization only in hypoxic NPR-A$^{-/-}$ mice, but this did not reach statistical significance.

Cyclic GMP Levels
Hypoxia increased cyclic GMP levels in the NPR-A$^{+/+}$ lung, and there was a further increase with sildenafil treatment (Figure 2b). Lung cyclic GMP levels were similar in NPR-A$^{-/-}$ and NPR-A$^{+/+}$ mice in normal air. Hypoxia alone and sildenafil alone produced no significant change in lung cyclic
GMP levels in mutant mice; a significant rise above basal levels was recorded with hypoxia plus sildenafil, but this was not significantly greater than hypoxia alone.

**Discussion**

PDE5 inhibition with sildenafil attenuated the pressor response to acute hypoxia in the isolated lung in both NPR-A+/+ and NPR-A−/− mice. The effect of sildenafil on RVSP during chronic hypoxia was also similar in both genotypes. Although the difference in RVSP between hypoxic sildenafil-treated and hypoxic control mice reached statistical significance only in the NPR-A−/− group, the absolute and percentage reductions in RVSP were similar for both strains.

In contrast, the effect of sildenafil on RV mass and muscle of pulmonary vessels was notably different between NPR-A+/+ and NPR-A−/− mice. Recent studies in NPR-A−/− mice suggest that NPR-A plays a greater role in inhibiting RV hypertrophy and pulmonary vascular remodeling than in inhibiting the rise in RVSP during chronic hypoxia and that NPR-A inhibits cardiac hypertrophy independently of its effect on blood pressure and ventricular afterload. Our data indicate that the natriuretic peptide–NPR-A pathway makes a greater contribution to the effect of sildenafil on RV hypertrophy and the muscle of peripheral pulmonary vessels than its effect on RVSP in hypoxia-induced pulmonary hypertension.

Exposure of the whole animal to hypoxia elevates endogenous ANP and BNP levels, which act through cyclic GMP to limit the rise in pulmonary vascular resistance and RV pressure load. NPR-A−/− deficient mice are able to synthesize ANP and BNP but do not benefit from the elevated levels. Previous studies suggest that ANP is the primary source of cyclic GMP in hypoxic-adapted rats. Consistent with this, NPR-A−/− mice showed a small, nonsignificant rise in lung cyclic GMP levels during exposure to hypoxia (compared with NPR-A+/+ mice). A significant rise in lung cyclic GMP levels was recorded in sildenafil-treated hypoxic NPR-A−/− mice, which may be expected to contribute to the fall in RVSP in these animals.

Basal RVSP was greater in NPR-A−/− mice than in NPR-A+/+ mice. This is at variance with our earlier work but in keeping with other studies. The development of phenotype with continued breeding of the colony is well recognized. Our findings support the view that the natriuretic peptides contribute to the low pulmonary vascular tone associated with the normal adult in a normal oxygen environment. Interestingly, 3 weeks of treatment with sildenafil had no effect on the elevated RVSP or on altered cyclic GMP levels in normoxic NPR-A−/− mice.

The isolated lung data and the change in cyclic GMP levels during hypoxia-sildenafil treatment show that the pulmonary vasculature of NPR-A−/− animals is not completely unresponsive to PDE5 inhibition during hypoxia. The biochemical source of cyclic GMP that permits a response to PDE5 inhibition in the absence of natriuretic peptide activity is NO, but the contribution of NO to the response in NPR-A−/− mice has not been examined.

The lack of data on drug levels in individual mice is one limitation of the study. Because dosing was assessed by measuring water intake, the dose may have varied between animals in a cage, but there were no outliers in any of the groups.

In conclusion, the pulmonary vasculature of NPR-A−/− mice responds to the PDE5 inhibitor, sildenafil, but its effects, particularly on chronic hypoxia-induced RV hypertrophy and pulmonary vascular remodeling, are blunted. A functional NPR-A system is crucial to the effective reduction of hypoxia-induced morphological changes of the pulmonary vasculature by sildenafil.

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