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

Lan Zhao, PhD; Nicola A. Mason, PhD; Julian W. Strange, MRCP; Hamish Walker, MRCP; Martin R. Wilkins, MD

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 antitrophic 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 mmol/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 (FiO₂ 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). 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 HPV₁ response to ventilation with 2% O₂ was similar in both NPR-A⁻/⁺ and NPR-A⁻/⁻ mice (9.3±1.3 versus 9.7±0.6 mm Hg, n=4). HPV₂ was reduced (compared with HPV₁) by pretreatment with sildenafil in both genotypes (HPV₁/HPV₂ 45±2.4% in +/- mice; HPV₂/HPV₁ 58±5.3% in –/- mice; not significant). ANP reduced HPV₂ in NPR-A⁻/⁻ but not NPR-A⁻/⁺ mice (HPV₁/HPV₂ 65±6% in +/- mice; HPV₂/HPV₁ 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 (HPV₁/HPV₂ 29±2.1%), but not in NPR-A⁻/⁻ mice (HPV₁/HPV₂ 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% O₂) 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/left ventricular + septal 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.

Right Ventricular Hypertrophy
Three weeks hypoxia produced a significant increase in the ratios of right ventricular (RV) weight/left ventricular + septal 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.

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
limit the rise in pulmonary vascular resistance and RV
enous ANP and BNP levels, which act through cyclic GMP to
hypoxia-induced pulmonary hypertension.
peripheral pulmonary vessels than its effect on RVSP in
sildenafil on RV hypertrophy and the muscularization of
NPR-A pathway makes a greater contribution to the effect of
hypoxia and that NPR-A inhibits cardiac hypertrophy inde-
ing than in inhibiting the rise in RVSP during chronic
mice; a significant rise above basal
PDE5 inhibition with sildenafil attenuated the pressor re-
mice. The effect of sildenafil on RVSP during
functional NPR-A system is crucial to the effective reduction
NPR-A mice. This is at variance with our earlier work
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. Interest-
3 weeks of treatment with sildenafil had no effect on
the elevated RVSP or on altered cyclic GMP levels in
normoxic NPR-A mice.
vasculature of 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
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 hyper-
trophy 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.

Discussion
PDE5 inhibition with sildenafil attenuated the pressor re-
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 hypoxia sildenafil-treated
and hypoxic control mice reached statistical significance only
in the NPR-A group, the absolute and percentage reduc-
tions in RVSP were similar for both strains.
In contrast, the effect of sildenafil on RV mass and
muscularization 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
inhbiting RV hypertrophy and pulmonary vascular remodeling
than in inhibiting the rise in RVSP during chronic
hypoxia and that NPR-A inhibits cardiac hypertrophy inde-
pendent of its effect on blood pressure and ventricular
load.14 NPR-A-deficient mice are able to synthesize
ANP and BNP but do not benefit from the elevated lev-
eils.10–13 Previous studies suggest that ANP is the primary
source of cyclic GMP in hypoxia-adapted rats.9 Consistent
Figure 2. a. Proportion of muscularized distal vessels, and b,
change in lung cyclic GMP in NPR-A and NPR-A mice treated with sildenafil (closed bars) or vehicle (open bars) and
exposed to hypoxia or normal air for 3 weeks. Data are
mean±SEM. n=6 each group. *P<0.05, **P<0.01.
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.

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