Synergistic Effects of ANP and Sildenafil on cGMP Levels and Amelioration of Acute Hypoxic Pulmonary Hypertension

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We hypothesized that the phosphodiesterase 5 inhibitor, sildenafil, and the guanosine cyclase stimulator, atrial natriuretic peptide (ANP), would act synergistically to increase cGMP levels and blunt hypoxic pulmonary hypertension in rats, because these compounds act via different mechanisms to increase the intracellular second messenger. Acute hypoxia: Adult Sprague-Dawley rats were gavaged with sildenafil (1 mg/kg) or vehicle and exposed to acute hypoxia with and without ANP (10^{-8}–10^{-5} *M*). Sildenafil decreased systemic blood pressure (103 ± 10 vs. 87 ± 6 mm Hg, $P < 0.001$) and blunted the hypoxia-induced increase in right ventricular systolic pressure (RVSP; percent increase 73.7% ± 9.4% in sildenafil-treated rats vs. 117.2% ± 21.1% in vehicle-treated rats, $P = 0.03$). Also, ANP and sildenafil had synergistic effects on blunting the hypoxia-induced increase in RVSP ($P < 0.001$) and on rising plasma cGMP levels ($P < 0.05$). Chronic hypoxia: Other rats were exposed to prolonged hypoxia (3 weeks, 0.5 atm) after subcutaneous implantation of a sustained-release pellet containing lower (2.5 mg), or higher (25 mg) doses of sildenafil, or placebo. Higher-dose, but not lower-dose sildenafil blunted the chronic hypoxia-induced increase in RVSP ($P = 0.006$). RVSP and plasma sildenafil levels were inversely correlated in hypoxic rats ($r^2 = 0.68$, $P = 0.044$). Lung cGMP levels were increased by both chronic hypoxia and sildenafil, with the greatest increase achieved by the combination. Plasma and right ventricular (RV) cGMP levels were increased by hypoxia, but sildenafil had no effect. RV hypertrophy and pulmonary artery muscularization were also unaffected by sildenafil. In conclusion, sildenafil and ANP have synergistic effects on the blunting of hypoxia-induced pulmonary vasoconstriction. During chronic hypoxia, sildenafil normalizes RVSP, but in the doses used, sildenafil has no effect on RV hypertrophy or pulmonary vascular remodeling. Exp Biol Med 229:920–925, 2004

Key words: atrial natriuretic peptide; cyclic GMP; pulmonary hypertension; rats; sildenafil

Introduction

Hypoxic pulmonary hypertension occurs in animals and humans exposed to acute or sustained hypoxia (1). The initial event is acute hypoxic pulmonary vasoconstriction, followed later by remodeling of small and medium-sized pulmonary arteries (2) and right ventricular (RV) hypertrophy.

Intracellular cGMP is a second messenger that mediates pulmonary vascular relaxation via activation of cGMP-related protein kinases and K-channel activation (3, 4). The natriuretic peptides (atrial natriuretic peptide [ANP] and brain natriuretic peptide) and nitric oxide (NO) increase intracellular cGMP production by activating particulate and soluble guanylate cyclase, respectively. Both NO and the natriuretic peptides have vasodilator effects on the pulmonary vascular bed and have been shown to blunt acute and chronic hypoxic pulmonary hypertension (5, 6). Previous studies suggest that NO and the natriuretic peptides play important physiologic roles in modulating pulmonary vascular and right ventricular hypertrophic responses during both acute and chronic hypoxic exposure (6, 7).

Phosphodiesterases (PDEs) comprise a superfamily of enzymes that inactivate cAMP and cGMP (8). Among the 11 families of PDEs described, PDE5 is one of the isoenzymes that selectively degrade cGMP into the inactive form of guanosine 5′-monophosphate. Normoxic pulmo-

This research project was funded by grant AHA0240190N from James R. Klinger.

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Received December 1, 2003.
Accepted June 14, 2004.

1535-3702/04/2299-0920$15.00

nary arteries have a large amount of PDE5 activity that further increases with hypoxic pulmonary hypertension (9). By accelerating the degradation of cGMP, this enhancement of PDE5 activity could limit the vasodilatory effects of cGMP during chronic hypoxia.

Selective PDE inhibitors have been used to better characterize the distribution and role of cAMP and cGMP in vasodilation. In an awake lamb model of acute pulmonary hypertension, sildenafil, a potent and selective PDE5 inhibitor, selectively dilated pulmonary vessels (10). This effect was blocked by the NO synthase (NOS) inhibitor L-NAME, suggesting that sildenafil acts via an NO-dependent mechanism. In mice exposed to chronic hypoxia, oral sildenafil attenuated hypoxia-induced pulmonary hypertension (11).

In the present study, we hypothesized that ANP and sildenafil act synergistically to inhibit acute hypoxic vasoconstriction in rats via an additive effect on increasing cGMP levels. We further examined the effect of relatively low-dose but continuously released sildenafil on pulmonary arterial tone and structure, hypothesizing that it would blunt chronic hypoxic pulmonary hypertension in association with increased lung cGMP levels.

Materials and Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the Rhode Island Hospital, Providence, Rhode Island. Sprague-Dawley rats weighing 150–250 g at the beginning of the experiment were used. Female rats were used because peak plasma sildenafil levels occur more rapidly after oral administration (20 min from ingestion) and plasma half-life is longer (approximately 2 hrs) in female than in male rats (12).

Drugs. ANP was purchased from Sigma Chemical (St. Louis, MO) and sildenafil was kindly provided by Dr. Lakshminarayana Sudershan (Biotech Pvt. Ltd., Hyderabad, India)

Acute Hypoxia. Rats were anesthetized with pentobarbital (20 mg/kg ip) and ketamine (60 mg/kg im), and were ventilated via a tracheal cannula with room air using a Harvard Apparatus Rodent Ventilator (Millis, MA) set at a rate of 90 breaths/min and pressure of 6 cm H_2O inspiratory and 2 cm H_2O expiratory. A catheter was advanced into the right ventricle via the right jugular vein for continuous RV pressure recording. A thermodilution catheter was introduced into the left carotid artery for measurement of cardiac output (Cardiotherm 500; Columbus Instruments, Columbus, OH). Systemic blood pressure was recorded via a left femoral artery catheter that was also used for arterial blood gas and cGMP sampling, and ANP administration.

After instrumentation, rats were gavaged with either sildenafil 1 mg/kg in 0.2 ml of sterile water, or 0.2 ml of water alone via a rigid oral gastric tube. Thirty minutes were allowed for gastric absorption, after which rats underwent two 15-min exposures to acute hypoxia, separated by 10 min of normoxic recovery. Hypoxia was achieved by exposing rats to a mixture of 11% O_2 (balance N_2), during which blood gases were obtained. After 15 mins during the second hypoxic exposure, rats were given 200-μl iv bolus injections of ANP in four sequential concentrations (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} *M*) every 2 mins. Cardiac output (CO), arterial blood gases, and serum cGMP levels were measured at baseline and at the end of the first and second hypoxic exposures. Cardiac index (CI) was calculated by the ratio of CO and body weight (BW). Systemic blood pressure (SBP) and RV pressure were monitored continuously throughout the entire experiment. Total pulmonary resistance index was calculated as the RV systolic pressure/cardiac index. All hemodynamic measurements were made with the investigators blinded to treatment group.

Chronic Hypoxia. To establish the effects of sildenafil on chronic hypoxic pulmonary hypertension, matrix-driven 21-day sustained release pellets containing lower dose of sildenafil (2.5 mg per pellet, or 0.6 mg/kg/day), a higher dose (25 mg per pellet, or 6 mg/kg/day), or placebo (Innovative Research of America, Sarasota, FL) were implanted subcutaneously in the posterior neck of anesthetized rats (medetomidine hydrochloride 0.07 mg/kg im) and reversed with atipamezole hydrochloride (0.35 mg/kg im). These doses are lower than those used successfully in some previous studies (11), but we proposed that the continuous release might achieve steadier levels than oral administration via drinking water (13). In addition, we intended to assess efficacy at lower doses than those reported to produce systemic effects, such as those described in the rat retina (14). After 1 day of recovery, animals were randomized to 3 weeks of normoxia or hypobaric hypoxia (0.5 atm). Hypobaric chambers were opened three times weekly to add food and water, and for cleaning. Twelve-hour light exposure cycles, standard rat chow, and water ad libitum were provided to all rats. Normoxic rats were kept in the same room adjacent to the hypobaric chamber. At the end of the 3-week exposure period, rats were anesthetized with pentobarbital (20 mg/kg ip) and ketamine (60 mg/kg im), and hemodynamics were measured as described for acute hypoxia. After hemodynamic measurements were completed, blood (1–2 ml) was collected via the RV catheter for measurement of plasma sildenafil levels.

Tissue Collection and Preparation. After animals were euthanized by pentobarbital injection (120 mg/kg ip), the thorax was opened immediately, and the heart and lungs were removed. The left lung was frozen in liquid nitrogen and stored at –80°C pending cGMP measurements. Double fixation of the right lung was achieved in the distended state by infusion of 4% aqueous buffered formalin into the trachea at 25 cm H_2O pressure and into the pulmonary trunk at 5 cm H_2O pressure. The hearts were dissected into RV free wall and left ventricle plus septum, weighed, frozen in liquid nitrogen, and stored at –80°C until needed for cGMP

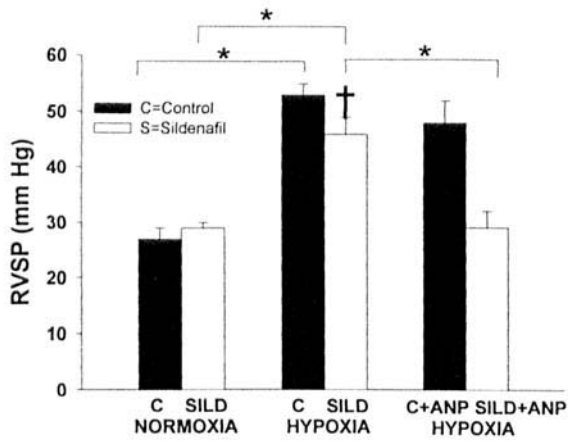

Figure 1. Mean right ventricular systolic pressure (RVSP) measurements in rats exposed to two episodes of acute hypoxia after gavage with sildenafil (SILD) or vehicle. Atrial natriuretic peptide (ANP) was infused into rats from both groups during the second hypoxic exposure; ANP and sildenafil had synergistic vasodilatory effects; *$P < 0.001$ when compared with prior point in time within the group; †$P = 0.03$, between the two groups as a percent change from the prior point in time; $n = 13$ for sildenafil group and $n = 8$ for control group. Values are mean ± SEM.

measurements. The ratio of RV free wall weight to body weight (RV:BW) was used as an index of RV hypertrophy.

The right lung was processed for paraffin embedding. Sections (5 μm) were cut for light microscopy and stained with hematoxylin-eosin. In each rat, 30 intraacinar vessels accompanying an alveolar duct or alveolus were analyzed. Their type was identified as muscular, partially muscular, or nonmuscular. Vessels with a layer of smooth muscle cells comprising ≥3/4 of the vessel perimeter were categorized as fully muscularized. Vessels with a smooth muscle layer comprising <3/4 of the vessel perimeter were categorized as partially muscularized, and vessels that had no identifiable smooth muscle cells were categorized as nonmuscularized vessels.

Biochemical Assays. Cyclic GMP concentrations were measured using a commercial radioimmunoassay kit (Biomedical Technologies Inc., Stoughton, MA) and were expressed as picomoles per milliliter. The detection assay was 0.05 pmol/ml. Sildenafil levels were kindly measured by Pfizer Laboratories (Sandwich, UK) in a blinded fashion using high-performance liquid chromatography and expressed as nanograms per milliliter of plasma (15). The assay is linear between 1 to 250 ng/ml.

Data Analysis. Results were expressed as mean ± SE. The significance of differences between control and experimental rats was determined by unpaired *t* test. The significance of differences between two treatments within the same group was determined by paired *t* test. When more than two means were compared, as with serial values within the same group at multiple time points, a one-way repeated measures analysis of variance (ANOVA) was used, with Tukey posthoc analysis. Means between multiple parallel experimental groups in the chronic hypoxia studies were compared using two-way ANOVA. The correlation between sildenafil levels and RV pressures was determined by linear regression analysis to determine Pearson Correlation coefficients. Differences were considered to be statistically significant at $P < 0.05$.

Results

Effects of Sildenafil and ANP During Acute Hypoxia. Both sildenafil-treated and control groups had similar right ventricular systolic pressure (RVSP) and CI at baseline and 30 mins after gavage (Fig. 1). Blood gas analysis confirmed that the severity of hypoxia was similar in both groups (Table 1). Sildenafil blunted the hypoxia-induced increase in RVSP (percent increase was 73.7% ± 9.4% in the sildenafil group and 117.2% ± 21.1% in the control group, $P = 0.03$; Fig. 1), and decreased SBP 30 mins after gavage (Table 1), indicating that sildenafil had both pulmonary and systemic vasodilator effects. ANP and sildenafil had an additive blunting effect on the hypoxia-induced increase in RVSP (Fig. 1). In addition, the effect of adding sildenafil to ANP was pulmonary selective because total pulmonary resistance dropped significantly, whereas systemic vascular resistance remained unchanged (Table 1). All other parameters (CI, PaO_2, and $PaCO_2$) remained similar in the two groups at each point in time (Table 1).

Plasma cGMP levels were increased over normoxic baseline levels in acutely hypoxic rats treated with sildenafil, or the combination of sildenafil and ANP (Fig. 2). Although the combination of ANP and sildenafil tended to increase plasma cGMP levels more than sildenafil alone,

Table 1. Hemodynamic and Blood Gas Measurements during Acute Hypoxia[a]

	Control normoxia	Sildenafil normoxia	Control hypoxia	Sildenafil hypoxia	Control + ANP hypoxia	Sildenafil + ANP hypoxia
SBP	103 ± 6	87 ± 6*†	70 ± 3*	76 ± 5*	70 ± 3*	74 ± 4*
CI	285 ± 35	290 ± 25	275 ± 20	295 ± 50	233 ± 20	265 ± 35
TPRI	0.094 ± 0.06	0.1 ± 0.08	0.192 ± 0.03*	0.155 ± 0.06*†	0.206 ± 0.09	0.109 ± 0.08*†
PaO_2	90 ± 5	91 ± 4	49 ± 3*	47 ± 2*	43 ± 2*	46 ± 5*
$PaCO_2$	44 ± 3	45 ± 2	35 ± 2*	37 ± 1*	32 ± 2*	32 ± 2*

[a] Values are mean ± SEM. Pressures are measured in mm Hg, CI in mL/min/kg, resistance in mm Hg/mL/min/kg. ANP, atrial natriuretic peptide; SBP, systolic systemic blood pressure; CI, cardiac index; TPRI, = total pulmonary resistance index; PaO_2 = partial arterial pressure of oxygen.

* $P < 0.05$ within the group (normoxia and hypoxia, by one-way repeated measures); † $P < 0.05$ between control and sildenafil (unpaired *t* test); $n = 13$ for sildenafil group and $n = 8$ for control group.

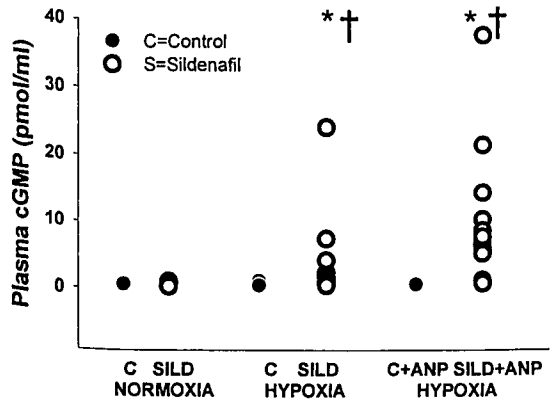

Figure 2. Plasma cGMP in rats during normoxic and acute hypoxic exposures. Levels were increased only in acutely hypoxic rats treated with sildenafil, or sildenafil and ANP. *$P < 0.005$ compared with normoxia; †$P < 0.009$ between sildenafil and control groups during hypoxia; $n = 13$ for sildenafil group and $n = 8$ for control group. Values are mean ± SEM.

the difference did not reach statistical significance (8.63 ± 3.76 vs. 4.48 ± 2.54 pg/ml). Sildenafil during normoxia, hypoxia alone, and ANP during hypoxia, did not alter plasma cGMP levels (Fig. 2).

Effects of Sildenafil During Chronic Hypoxia. After 3 weeks of hypoxia, hypoxic control and lower-dose sildenafil groups developed significantly increased RVSPs compared with normoxic controls (Table 2), hypoxic animals treated with higher-dose sildenafil had significantly reduced RVSPs and total pulmonary resistance indexes compared with chronic hypoxic controls (Table 2). At the completion of the 3-week exposures, plasma sildenafil levels were detected in all high-dose sildenafil-treated rats. However, levels were not detected in the placebo or lower-dose sildenafil groups, with one exception. The hypoxic high-dose sildenafil group also manifested a significant inverse correlation between plasma sildenafil levels and RVSP ($r = 0.82$, $r^2 = 0.68$, $P = 0.044$). Systemic pressure and CI were similar in all six groups (Table 2), whereas hematocrit was significantly elevated in the three hypoxic groups ($P < 0.05$, data not shown).

Despite the significant reduction in RVSP and total pulmonary resistance during chronic hypoxia in the higher-dose sildenafil group, RV hypertrophy as reflected by the RV:BW ratio was not affected by sildenafil treatment (Table 2). As expected, lower- and higher-dose sildenafil had no effect on RV:BW ratios during normoxia. Similarly, the degree of small pulmonary artery muscularization was not altered by treatment with sildenafil (41% fully muscularized and 28% partially muscularized in control-hypoxia vs. 43% fully muscularized and 27% partially muscularized in higher-dose sildenafil hypoxic rats, P = ns).

Higher-dose sildenafil in normoxic animals and chronic hypoxia alone increased lung cGMP levels similarly over levels in normoxic controls (Fig. 3), and the combination of the two had an additive effect (213 ± 28 pmol/ml in higher-dose hypoxic vs. 98 ± 30 pmol/ml in the higher-dose normoxic group, $P = 0.004$). In plasma and RV homogenates, cGMP levels were increased by chronic hypoxia, but sildenafil treatment had no additional effect (Figs. 4 and 5).

Discussion

Our study shows that a single dose of oral sildenafil has pulmonary vasodilator effects on acute hypoxia-induced pulmonary hypertension in rats and that this effect is potentiated by ANP. Compatible with our initial hypothesis, this potentiation is associated with a synergistic effect of sildenafil and ANP on the increase in plasma cGMP. Furthermore, we report that chronic administration of sildenafil by subcutaneously-implanted, sustained-release pellets decreases RVSP without affecting hypoxia-induced RV hypertrophy or pulmonary vascular remodeling, suggesting that, at the doses used, pulmonary vasodilation is the main effect of sildenafil. This reduction in RVSP is associated with a marked increase in cGMP levels in the lungs, but not in plasma or RVs of hypoxic rats receiving higher-dose sildenafil.

Previous studies have shown that sildenafil or other selective PDE5 inhibitors blunt acute pulmonary hypertension induced by pharmacologic agents such as U46619 (10). Sildenafil has also been shown to inhibit acute hypoxic pulmonary vasoconstriction in healthy volunteers (11, 16). Our results are consistent with these previous studies and extend these observations to rats given oral and subcutaneous sustained-release formulations of sildenafil. Our

Table 2. Hemodynamic Parameters During Chronic Hypoxia[a]

	Control/Normoxia	Control/Hypoxia	Lower dose sildenafil/normoxia	Lower dose sildenafil/hypoxia	Higher dose sildenafil/normoxia	Higher dose sildenafil/hypoxia
RVSP	30.4 ± 1.1	46.2 ± 2.5*	30.2 ± 2.0	44.4 ± 2.6*	31.0 ± 2.5	36.4 ± 1.5*†
CI	306 ± 40	335 ± 36	374 ± 78	348 ± 60	375 ± 28	333 ± 20
TPRI	0.099 ± 0.01	0.139 ± 0.01	0.078 ± 0.01	0.129 ± 0.01	0.081 ± 0.01	0.109 ± 0.012*†
RV:BW	6.9 ± 0.1	11.4 ± 0.9*	7.0 ± 0.4	12.0 ± 0.3	7.4 ± 0.3	11.3 ± 0.4*
Sild	0	0	0.9**	0	36.56 ± 4.45	44.57 ± 9.7

[a] Values are means ± SEM. RVSP, right ventricular systolic pressure; CI, cardiac index; TPRI, total pulmonary resistance index; RV:BW = right ventricle/body mass weight ratio; Sild, plasma sildenafil levels (ng/ml); $n = 6$–8 per group.
* $P < 0.05$ when compared with normoxia, † $P < 0.05$ between higher dose sildenafil and control hypoxic groups, ** only one animal had detectable levels.

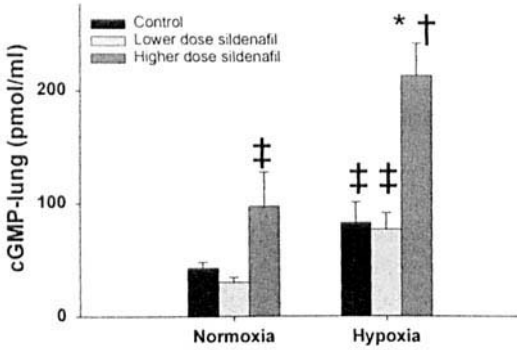

Figure 3. cGMP levels in the lung were significantly increased by hypoxia and higher-dose sildenafil; *P = 0.004 between the higher dose/normoxic group and higher dose/hypoxic group; †$P < 0.001$ between the higher dose/hypoxic group and control or lower-dose/hypoxic groups; ‡$P < 0.01$ between control/normoxic and control/hypoxic groups, between lower-dose/normoxic and lower-dose/hypoxic groups, and between higher-dose/normoxic and control or lower-dose/normoxic groups. n = 6–8 per group. Values are mean ± SEM.

findings also indicate that the acute vasodilator response to sildenafil is not species-specific.

We examined the combined effect of sildenafil and ANP because previous studies have suggested that endogenous ANP is at least partly responsible for the increase in lung cGMP levels that occurs during hypoxia (17). ANP has been shown to produce pulmonary vasodilation in numerous models of pulmonary hypertension (5, 6, 17), but its interaction with PDE5 inhibitors has only recently been investigated (18). We demonstrate that the combination of an agent that increases cGMP by inhibiting its degradation (sildenafil) and another that stimulates its production (ANP) produces synergistic pulmonary vasodilator effects. This is consistent with the results of Ichinose *et al.* (19), who demonstrated that nebulized sildenafil potentiates the pulmonary vasodilating effect of inhaled NO, most likely by potentiating the increase in intracellular cGMP levels. Although we were unable to detect any increase in plasma cGMP levels in response to ANP in vehicle-treated rats

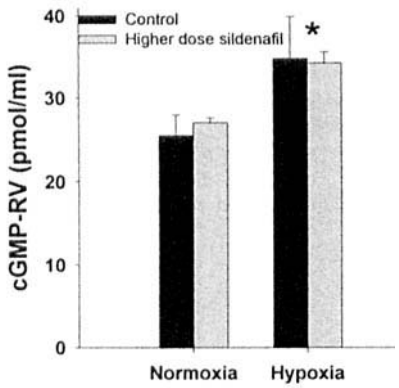

Figure 4. Right ventricular (RV) cGMP levels measured in normoxic and hypoxic animals receiving placebo or higher-dose sildenafil; *$P < 0.03$ between the normoxic and hypoxic groups. n = 6–8 per group. Values are mean ± SEM.

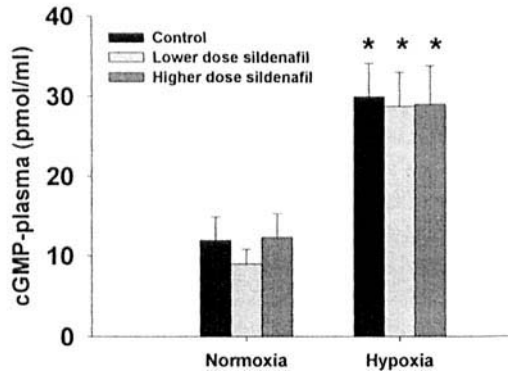

Figure 5. Hypoxia increased plasma cGMP levels compared with normoxic rats, but sildenafil treatment had no effect; *$P < 0.001$ between the normoxic and hypoxic groups. n = 6–8 per group. Values are mean ± SEM.

exposed to acute hypoxia, we did observe an increase in plasma cGMP levels when ANP was given to acutely hypoxic rats pretreated with sildenafil. Thus, our findings indicate that sildenafil can significantly enhance hypoxia and ANP-induced increases in circulating cGMP levels. This effect is likely responsible for the synergistic effect of ANP on inhibition of hypoxia-induced pulmonary vasoconstriction by sildenafil. Furthermore, this synergistic effect of ANP was pulmonary-selective, inducing no further decline in systemic vascular resistance.

In chronically hypoxic rats, we used a unique method for administration of sildenafil; sustained-release sc pellets. This demonstrates the feasibility of this method in treating pulmonary hypertension, at least in rats when measurable plasma levels of sildenafil are achieved. The inverse correlation between RVSP and circulating sildenafil levels strengthens the inference that sildenafil was responsible for the hemodynamic responses observed. Chronic sildenafil treatment also did not affect systemic blood pressure, indicating that it has specific effects on the pulmonary circulation and is well tolerated by the systemic circulation, at least at these doses in rats.

As has been previously reported (20, 21), cGMP levels in the lung were increased by chronic hypoxia. Furthermore, hypoxia and sildenafil had additive effects on lung cGMP levels, consistent with the idea that even though cGMP levels are increased by chronic hypoxia, the increase is not maximal, and a further pulmonary hemodynamic benefit can be achieved by augmenting the increase in cGMP via inhibition of its metabolism. This is consistent with the idea that the increase in lung PDE5 during hypoxia may be countercompensatory, and thus, inhibition of PDE5 has salubrious effects on pulmonary hemodynamics.

In contrast to the changes in lung homogenate levels, plasma and RV cGMP levels were increased by chronic hypoxia, but were not influenced by sildenafil. These results, together with the fact that sildenafil did not influence the severity of RV hypertrophy or pulmonary vascular muscularization, indicates that sildenafil inhibits PDE5 and

increases cGMP levels in the lungs, achieving vasodilation, but at least in the doses used, not altering cardiac or pulmonary vascular structure. The latter observations are unexpected, because most studies have observed a reduction in pulmonary arterial muscularization accompanying sustained reductions in pulmonary artery (PA) pressure (6). However, previous studies in which sildenafil was administered to mice that were deficient in endothelial NOS or the natriuretic A receptor obtained similar findings, with gene-altered animals having significant reductions RV systolic pressure, but no differences in indexes of RV hypertrophy or vessel structure (11, 18). These findings suggest that inhibitory effects of cGMP on vascular tone and structure are disconnected, with vasodilation occurring at lower levels than effects on remodeling or via different signaling pathways. In support of the idea that effects on remodeling might have been observed at higher doses of sildenafil, Sebkhi *et al.* (13) observed reductions in both PA pressure and the degree of muscularization in rats ingesting 75 mg/kg/day of sildenafil in their drinking water (as opposed to 6 mg/kg/day in the current study). The lack of effect of sildenafil on RV hypertrophy might also be related to the failure of sildenafil to increase levels of RV cGMP levels above those achieved by hypoxia alone.

Several limitations need to be acknowledged in our study. In addition to using doses of sildenafil that may have been too low to bring about structural changes, we did not measure plasma levels of sildenafil with acute hypoxia. However, the increase in cGMP levels after sildenafil administration indicates that PDE5 inhibition was achieved. Further, we used plasma arterial cGMP levels as an indication of pulmonary PDE5 inhibition and we acknowledge that these could reflect activity in the systemic circulation, as well.

In conclusion, sildenafil has significant vasodilator effects on both acute and chronic hypoxia-induced pulmonary hypertension in rats. ANP has an additional acute vasodilatory effect in combination with sildenafil, consistent with the idea that greater vasodilatory effects can be achieved by simultaneously stimulating the synthesis and blocking the inactivation of cGMP. These results indicate that potentiation of cGMP elevations by using combinations of agents holds promise as an approach to treat pulmonary hypertension.

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