

Sildenafil Is a Pulmonary Vasodilator in Awake Lambs with Acute Pulmonary Hypertension

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Background: Phosphodiesterase type 5 (PDE5) hydrolyzes cyclic guanosine monophosphate in the lung, thereby modulating nitric oxide (NO)/cyclic guanosine monophosphate-mediated pulmonary vasodilation. Inhibitors of PDE5 have been proposed for the treatment of pulmonary hypertension. In this study, we examined the pulmonary and systemic vasodilator properties of sildenafil, a novel selective PDE5

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inhibitor, which has been approved for the treatment of erectile dysfunction.

Methods: In an awake lamb model of acute pulmonary hypertension induced by an intravenous infusion of the thromboxane analog U46619, we measured the effects of 12.5, 25, and 50 mg sildenafil administered via a nasogastric tube on pulmonary and systemic hemodynamics (n = 5). We also compared the effects of sildenafil (n = 7) and zaprinast (n = 5), a second PDE5 inhibitor, on the pulmonary vasodilator effects of 2.5, 10, and 40 parts per million inhaled NO. Finally, we examined the effect of infusing intravenous L-NAME (an inhibitor of endogenous NO production) on pulmonary vasodilation induced by 50 mg sildenafil (n = 6).

Results: Cumulative doses of sildenafil (12.5, 25, and 50 mg) decreased the pulmonary artery pressure 21%, 28%, and 42%, respectively, and the pulmonary vascular resistance 19%, 23%, and 45%, respectively. Systemic arterial pressure decreased 12% only after the maximum cumulative sildenafil dose. Neither sildenafil nor zaprinast augmented the ability of inhaled NO to dilate the pulmonary vasculature. Zaprinast, but not sildenafil, markedly prolonged the duration of pulmonary vasodilation after NO inhalation was discontinued. Infusion of L-NAME abolished sildenafil-induced pulmonary vasodilation.

Conclusions: Sildenafil is a selective pulmonary vasodilator in an ovine model of acute pulmonary hypertension. Sildenafil induces pulmonary vasodilation *via* a NO-dependent mechanism. In contrast to zaprinast, sildenafil did not prolong the pulmonary vasodilator action of inhaled NO. (Key words: PDE5; phosphodiesterase.)

NITRIC oxide (NO) is produced by NO synthase (NOS) through the conversion of L-arginine to L-citulline in the presence of oxygen.^{1,2} NO rapidly diffuses into subjacent vascular smooth muscle cells, stimulating soluble guanylate cyclase to produce guanosine-3',5'-cyclic monophosphate (cGMP).³ cGMP causes smooth muscle relaxation *via* several mechanisms such as activation of cGMP-dependent protein kinase and calcium-gated potassium channels.^{3,4} In the lung, five different phosphodiesterase (PDE) isoforms can inactivate cGMP: PDE isoforms 1, 2, 3, and 5,^{3,5} as well as the recently discovered PDE9.^{6,7}

Inhibitors of the cGMP-hydrolyzing PDE5, which is abundantly expressed in the lungs, especially in vascular smooth muscle cells,⁸ have been shown to be potent

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pulmonary vasodilators in experimental pulmonary hypertension.⁹⁻¹³ Moreover, PDE5 inhibition augments the pulmonary vascular response to administration of endothelium-dependent vasodilators or nitrovasodilators.^{9,10,12,14-17} Ichinose *et al.*¹⁸ demonstrated that intravenous zaprinast infusion markedly prolonged the pulmonary vasodilation induced by inhalation of gaseous NO in awake sheep. Fullerton *et al.*¹⁹ and Ziegler *et al.*²⁰ reported that the combination of a dipyridamole infusion and NO inhalation selectively dilated the pulmonary vasculature of patients with pulmonary hypertension. PDE5 inhibitors, alone or in combination with NO inhalation, have been proposed for the treatment of acute and chronic pulmonary hypertension.

Until recently, only a single PDE5 inhibitor, dipyridamole, was approved for clinical use. The clinical application of dipyridamole as a PDE5 inhibitor for the treatment of pulmonary hypertension is limited by nonselective effects on other PDEs and on adenosine metabolism.^{13,19-22}

In 1996, Boolell *et al.*^{23,24} described the use of the novel and highly selective PDE5 inhibitor sildenafil for the treatment of erectile dysfunction in men. Sildenafil was approved for clinical use by the United States Food and Drug Administration under the trade name Viagra.²⁵ In this study, we examined the ability of sildenafil administered by gavage to dilate the pulmonary vasculature. We report that, in awake lambs with acute pulmonary hypertension, low doses of sildenafil can decrease pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) without reducing the systemic vascular resistance (SVR). These pulmonary hemodynamic effects of sildenafil were prevented by concomitant infusion of the NOS inhibitor L-NAME. Furthermore, we demonstrate that, in contrast to zaprinast, sildenafil does not prolong the vasodilatory effect of inhaled NO.

Materials and Methods

The investigations were approved by the Subcommittee for Research Animal Care of the Massachusetts General Hospital.

Animal Preparation

Seventeen Suffolk lambs weighing 16–25 kg were anesthetized by inhalation of halothane in 50% oxygen and 50% nitrous oxide. Their tracheas were intubated and their lungs mechanically ventilated at 15 breaths/min and 15 ml/kg tidal volume with a large animal

ventilator (Harvard Apparatus, Natick, MA). An 8-French introducer (Cordis, Miami, FL) was placed in the right external jugular vein for introduction of a 7-French thermomodulation pulmonary artery catheter (Edwards Laboratories, Santa Anna, CA) on the following day. The left carotid artery was cannulated with a polyvinyl chloride catheter (2-mm ID) advanced 30 cm into the aorta for continuous arterial pressure monitoring and sampling arterial blood. A tracheotomy was performed. Lambs were allowed to recover from anesthesia and cannulation overnight. On the next day, a cuffed tracheotomy tube (8-mm ID; Portex, Keene, NH) was inserted to allow spontaneous ventilation. Catheters were aspirated before the study and continuously flushed (2 ml/h) with lactated Ringer's solution without heparin during the experiments. Lambs were housed in a Babraham cage with free access to food and water. When experiments involved administration of sildenafil, a nasogastric tube was briefly introduced, and proper location of the tube was confirmed by suctioning of gastric contents. Experiments were performed if the animal met the following criteria: body temperature (measured with the thermomodulation catheter) less than 40°C and mean PAP less than 20 mmHg.

Hemodynamic Measurements

Systemic arterial pressure (SAP), PAP, and central venous pressure were measured continuously, and pulmonary artery occlusion pressure was measured intermittently using calibrated saline-filled membrane pressure transducers (Argon, Athens, TX) zeroed at midchest level. Pressure transducers were connected to a biomedical amplifier (Hewlett Packard 7754B, Andover, MA), and data were continuously recorded at 150 Hz on a personal computer using an analog-to-digital interface with a data acquisition system (DI-220; Dataq Instruments, Akron, OH). Thermomodulation cardiac output (CO) was measured as the average of three determinations after injection of 5 ml of 4°C Ringer's lactate solution. PVR and SVR were calculated using standard formulas. After baseline measurements, an intravenous infusion of the thromboxane A₂ analog U46619 (Cayman Chemicals, Ann Arbor, MI) was administered at a rate of 1.0 to 2.0 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and was titrated to achieve a mean PAP of 25–30 mmHg. The pulmonary vasodilator response (ΔPAP) to gavage administration of a PDE5 inhibitor or to NO inhalation was measured as the vasodilator induced reduction in PAP as a percent of the PAP during steady state pulmonary hypertension induced by infusion of U46619. The ratio of PVR to SVR (PVR/SVR) was

calculated. A decrease in PVR/SVR ratio in response to a vasodilator was interpreted as selective pulmonary vasodilation.^{26,27}

Delivery of NO

During the study, the tracheotomy was connected to a circuit consisting of a 5-l reservoir bag and a two-way nonrebreathing valve (Hans Rudolph, Kansas City, MO) to separate inspired from expired gas. Oxygen and nitrogen were blended (Oxygen Blender; Bird Corporation, Palm Springs, CA) to produce an inspired oxygen fraction of 0.5 delivered at a fresh gas flow rate of 10 l/min. NO gas (800 or 80 parts per million by volume [ppm] NO in N₂; Airco, Murray Hill, NJ) was introduced into the inspiratory limb of the breathing circuit immediately before the reservoir bag. Inspired oxygen fraction (Oxygen Meter #5590; Hudson, Temecula, CA) and the concentration of NO (CLD 700 AL, Eco Physics, Dürnten, Switzerland) were monitored continuously.

Measurement of Plasma cGMP Levels and Plasma Sildenafil Concentrations

For each sample, 3-isobutyl-1-methylxanthine (IBMX; Sigma Chemicals, St. Louis, MO; final concentration 0.5 mM) was added to 1 ml of citrated blood, and the mixture was centrifuged at 3,000g at 4°C for 10 min. Plasma cGMP concentrations were measured using a commercial radioimmunoassay (Biomedical Technologies Inc., Stoughton, MA). Plasma cGMP concentrations are expressed as picomoles cGMP per milliliter plasma.

The concentration of sildenafil in heparinized sheep plasma was measured using the ASTED (automated sequential trace enrichment of dialysates) system followed by high-performance liquid chromatography as described by Cooper *et al.*²⁸

Experimental Protocols

Protocol 1: Dose-Response Study of Sildenafil Administration during U46619-induced Pulmonary Hypertension. Five lambs were studied while spontaneously breathing at and inspired oxygen fraction of 0.5. After baseline measurements, an intravenous infusion of U46619 (1.0–2.0 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was titrated to achieve a stable elevation of PAP (25–30 mmHg), and hemodynamic measurements were repeated after 10 min. A cumulative dose-response curve was obtained by administering 12.5, 12.5, and 25 mg sildenafil *via* the nasogastric tube at 15-min intervals. This time interval was chosen based on pilot experiments showing that the maximal sildenafil-induced pulmonary vasodilation oc-

curs within 10–15 min after gastric administration, and that this effect lasted for at least 1.5 h (data not shown). At the end of each 15-min period, hemodynamic measurements were obtained. In four lambs, arterial blood was sampled for measurements of plasma cGMP and sildenafil concentrations before sildenafil administration, and 15 min after administration of 12.5, 25, and 50 mg sildenafil.

Protocol 2: Effects of Sildenafil and Zaprinast Administration on Pulmonary Vasodilation Produced by Inhaled NO During U46619-induced Pulmonary Hypertension. In 12 lambs, steady state pulmonary hypertension was induced by the intravenous infusion of U46619, and hemodynamic measurements were obtained as described previously. The lambs then breathed incremental concentrations of NO (2.5, 10, and 40 ppm each administered for 5 min). After the inhalation at each concentration of NO was terminated, PAP was allowed to return to baseline (< 10 min). Hemodynamic measurements were obtained immediately before and at the end of each NO inhalation period. The duration of the pulmonary vasodilator response to NO inhalation was measured as the time required for PAP to return to the pre-NO inhalation baseline value after the discontinuation NO inhalation.

Thereafter, lambs received either 50 mg sildenafil *via* the nasogastric tube (n = 7), or a loading dose of zaprinast (2 mg/kg over 5 min) was administered followed by a zaprinast infusion (0.1 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; n = 5) as previously reported.¹⁸ Twenty minutes later, hemodynamic parameters were measured, and the U46619 infusion was increased to obtain a PAP of 25–30 mmHg. The doses of U46619 needed to maintain an elevated PAP were greater (2.2–4.0 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) after either sildenafil or zaprinast administration but did not differ between the two groups. Ten minutes after achieving a stable elevated PAP, lambs breathed incremental concentrations of NO gas. Hemodynamic measurements were repeated before and 5 min after inhaling each concentration of NO. NO inhalation was then discontinued, and the duration of the vasodilator response was recorded as described previously.

In three lambs of the sildenafil group, plasma sildenafil concentrations were measured before and 20 min after administration of 50 mg sildenafil as well as after the last NO inhalation period.

Protocol 3: Effect of L-NAME on Sildenafil-induced Pulmonary Vasodilation. After obtaining baseline hemodynamic measurements, five lambs received an intra-

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venous dose of the NOS inhibitor L-NAME (*N*^G-nitro-L-arginine methyl ester; Sigma Chemical Co.) of 25 mg/kg over 5 min, followed by a continuous infusion of 1 mg · kg⁻¹ · h⁻¹. Inhibition of endogenous NO production was confirmed by comparing the systemic vasodilation induced by an intravenous bolus of acetylcholine (0.2 μg/kg) injected immediately before and 10 min after the initial L-NAME bolus.²⁹ Acute pulmonary hypertension was then produced by infusion of U46619, as described previously. Doses of U46619 needed to achieve the same level of pulmonary hypertension were reduced by approximately 50% during L-NAME infusion as compared with protocols 1 and 2. Hemodynamic measurements were obtained before and 15 min after 50 mg sildenafil was administered *via* the nasogastric tube.

Chemicals

Sildenafil citrate (Viagra, Pfizer Inc., New York, NY) was obtained as tablets containing 50 mg sildenafil. For administration *via* the nasogastric tube, one tablet was dissolved in 20 ml water. Immediately before the study, 10 mg U46619 was dissolved in 50 ml lactated Ringer's solution. Zaprinast (2-*o*-propoxyphenyl-8-azapurin-6-one) was a generous gift from Rhône-Poulenc Rorer (Dagenham, Essex, United Kingdom). The stock solution of zaprinast was prepared in 0.1 N NaOH. This stock solution was diluted with lactated Ringer's solution to a final concentration of 8 mg/ml before use. L-NAME (40 mg) was dissolved in 40 ml lactated Ringer's solution.

Data Analysis

All data are expressed as mean ± SE. A one-way analysis of variance for repeated measurements followed by a *post hoc* Scheffé test was used to compare values obtained from animals of the same group at different times (protocols 1 and 3). For between-group comparisons, a two-way analysis of variance was performed. When significant differences were detected by analysis of variance, a *post hoc* Scheffé test was used (protocol 2). We used a linear regression model to test for a correlation between plasma sildenafil concentrations and the pulmonary vasodilator response (Δ PAP) and to examine the correlation between plasma sildenafil levels and plasma cGMP concentrations (Statistica for Windows; StatSoft, Inc., Tulsa, OK). Statistical significance was assumed at $P < 0.05$.

Results

Effects of Sildenafil during U46619-induced Pulmonary Hypertension

After administration of sildenafil *via* the nasogastric tube, the onset of pulmonary vasodilation occurred within 5 min and was maximal after 10 min. Pilot experiments revealed that 50 mg sildenafil produced pulmonary vasodilation for at least 1.5 h. Table 1 shows the effects of sildenafil on PAP, SAP, central venous pressure, pulmonary artery occlusion pressure, CO, PVR, SVR, and the PVR/SVR ratio. Sildenafil after serial doses of 12.5, 12.5, and 25 mg (cumulative dose 12.5, 25, and 50 mg) reduced PAP 21%, 28%, and 43% (fig. 1) and PVR by 18%, 23%, and 45%, respectively. There was a 12% decrease in SAP at a cumulative dose of 50 mg sildenafil (fig. 1). SVR and CO did not change after administration of any dose of sildenafil. The PVR/SVR ratio decreased by 10%, 22%, and 31% at 12.5, 25, and 50 mg sildenafil, respectively, suggesting that the vasodilator effect of sildenafil was relatively selective for the pulmonary vasculature (table 1).

Sildenafil administered at cumulative doses of 12.5, 25, and 50 mg increased plasma cGMP concentrations by 54%, 77%, and 154%, respectively (fig. 2A). Plasma sildenafil concentrations were less than the detectable level of 2.17 ng/ml before sildenafil administration and increased to 3.3 ± 2.6, 18.1 ± 4.9, and 28.8 ± 9.9 ng/ml 15 min after the cumulative administration of 12.5, 25, and 50 mg sildenafil, respectively (fig. 2B). Plasma sildenafil concentrations correlated with the sildenafil-induced decrease in PVR ($r = 0.73$; $P < 0.05$) and the increase of plasma cGMP concentrations ($r = 0.79$; $P < 0.05$) measured at the same time.

Comparison of the Effects of Sildenafil and Zaprinast on Pulmonary Vasodilation Induced by Inhaled NO

At baseline and after U46619 infusion, lambs in the sildenafil and zaprinast groups did not differ in hemodynamic response to NO inhalation before PDE5 inhibitor administration (table 2). Twenty minutes after administration of 50 mg sildenafil *via* nasogastric tube, the PVR decreased 42 ± 3%, whereas infusion of zaprinast (2 mg/kg as a bolus over 5 min, followed by continuous infusion of 0.1 mg · kg⁻¹ · min⁻¹) reduced the PVR by 63 ± 3% measured at 20 min after starting the infusion. At this time, both sildenafil and zaprinast induced a small but significant decrease of SAP (18 ± 2% and 17 ± 3%, respectively) and SVR (23 ± 6% and 32 ± 6%, respec-

Table 1. Effects of Sildenafil on Hemodynamic Parameters

Parameter	Baseline	PHTN	Sildenafil		
			12.5 mg	25 mg	50 mg
PAP (mmHg)	13 ± 1	27 ± 2*	22 ± 2*†	19 ± 2*‡	15 ± 1‡
SAP (mmHg)	94 ± 6	111 ± 5§	106 ± 3	108 ± 3	97 ± 5#
CVP (mmHg)	2 ± 2	3 ± 2	3 ± 3	2 ± 3	0 ± 2
PAOP (mmHg)	5 ± 1	9 ± 2§	6 ± 1	6 ± 1	5 ± 2
CO (l/min)	3.3 ± 0.6	2.1 ± 0.6	2.1 ± 0.3	2.2 ± 0.4§	2.4 ± 0.4§
PVR (mmHg · l ⁻¹ · min ⁻¹)	3 ± 1	10 ± 2*	8 ± 1*	7 ± 1†	5 ± 1†
SVR (mmHg · l ⁻¹ · min ⁻¹)	31 ± 5	60 ± 10	52 ± 7	52 ± 8	46 ± 9
PVR/SVR	0.09 ± 0.01	0.17 ± 0.02*	0.15 ± 0.02	0.13 ± 0.01§#	0.12 ± 0.02†

Data are expressed as mean ± SE; n = 5.

* $P < 0.001$ versus baseline.

† $P < 0.01$.

‡ $P < 0.001$ versus PHTN.

§ $P < 0.05$.

|| $P < 0.01$.

$P < 0.05$.

PHTN = acute pulmonary hypertension induced by infusion of U46619 (all pressures are mean values); PAP = pulmonary artery pressure; SAP = systemic arterial pressure; CVP = central venous pressure; PAOP = pulmonary artery occlusion pressure; CO = cardiac output; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance.

tively) ($P < 0.05$; all values differ from predrug values). The PVR/SVR ratio was decreased at 20 min after initiation of sildenafil or zaprinast administration (0.21 ± 0.02 to 0.16 ± 0.01 and 0.23 ± 0.03 to 0.12 ± 0.02 , respectively; $P < 0.01$, both values differ from predrug values).

There was no difference in the quantity of U46619

infusion needed to reestablish pulmonary hypertension between the two drug groups. However, the increase of U46619 dose needed to reestablish the same magnitude of pulmonary hypertension as before PDE5 inhibition caused an additional decrease in CO and, thereby, an additional increase in SVR in both groups (table 2).

In three sheep that received sildenafil, plasma drug levels were measured before (below detection limit in all animals) and 20 min after administration of 50 mg sildenafil (37 ± 24 ng/ml), as well as at the end of the last NO inhalation period (12 ± 1 ng/ml) approximately 30 min later, confirming that sildenafil remained detectable in the blood at the end of the experiment.

The magnitude of the pulmonary vasodilator response to inhalation of 2.5, 10, and 40 ppm NO was unaffected by either sildenafil administration (Δ PAP $-11 \pm 1\%$, $-17 \pm 2\%$, and $-29 \pm 3\%$, respectively) or zaprinast infusion (Δ PAP $-13 \pm 3\%$, $-23 \pm 3\%$, and $-28 \pm 3\%$, respectively) and did not differ between the groups (fig. 3A). Systemic hemodynamic parameters during NO inhalation before and after sildenafil or zaprinast did not differ within or between drug groups (table 2).

The duration of the vasodilatory response to inhaled NO was markedly prolonged by zaprinast ($P < 0.001$ postdrug vs. control at each NO dose), but not by sildenafil (fig. 3B). The vasodilatory response to 2.5, 10, and 40 ppm inhaled NO lasted 18, 11, and 10 times longer, respectively, after zaprinast administration than after sil-

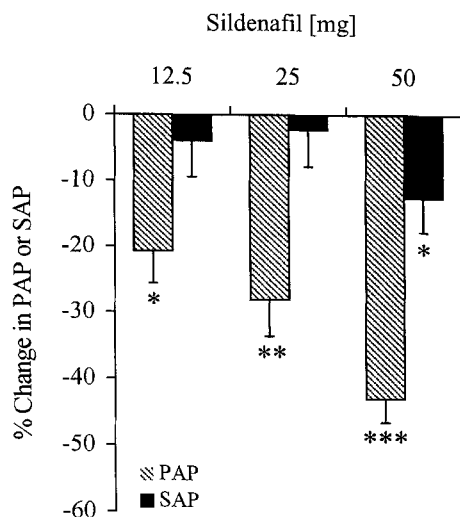
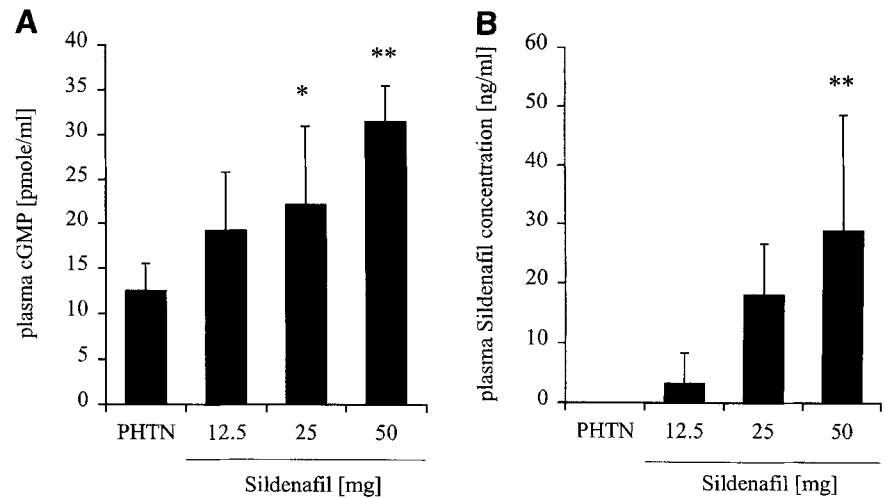


Fig. 1. Percent change of pulmonary arterial pressure (PAP) and systemic arterial pressure (SAP) after administration of increasing doses of sildenafil. Cumulative doses of 12.5, 25, and 50 mg sildenafil caused a significant reduction in PAP. Systemic hemodynamics were not altered, except for a 12% decrease of SAP at a dose of 50 mg sildenafil. * $P < 0.05$, ** $P < 0.01$, and * $P < 0.001$ versus baseline values during U46619-induced acute pulmonary hypertension (n = 5). Data are expressed as mean ± SE.**

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Fig. 2. Plasma cGMP concentrations (A) and plasma sildenafil concentrations (B) before (PHTN) and after the cumulative administration of 12.5, 25, and 50 mg sildenafil. Sildenafil caused a significant increase in plasma cGMP and sildenafil levels (* $P < 0.05$ and ** $P < 0.01$ vs. baseline PHTN). PHTN = acute pulmonary hypertension induced by U46619 infusion. $n = 4$. Data are expressed as mean \pm SE.



denafil administration ($P < 0.01$, groups differ at each NO dose; fig. 3B).

Effects of NO Synthase Inhibition on Sildenafil-induced Pulmonary Vasodilation

Infusion of L-NAME (25 mg/kg as a bolus, followed by continuous infusion of $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) reduced the transient acetylcholine-induced systemic hypotension by approximately 40% (data not shown).

Prior infusion of L-NAME completely prevented the pulmonary vasodilation induced by 50 mg sildenafil (PAP 27 ± 2 mmHg before and 28 ± 3 mmHg after sildenafil; $P = \text{NS}$). There was no change in PVR, SVR, CO, or PVR/SVR after sildenafil administration during L-NAME infusion. To evaluate the effects of L-NAME infusion on sildenafil-induced hemodynamic changes, these data were compared with data from protocol 2 (before and after administration of 50 mg sildenafil) without an L-NAME infusion. Sildenafil decreased PAP less in L-NAME-treated sheep than in those not receiving L-NAME ($4 \pm 5\%$ vs. $-40 \pm 1\%$; $P < 0.001$; fig. 4). Similarly, PVR decreased less in sheep that received L-NAME and sildenafil than in those that received sildenafil alone ($9 \pm 12\%$ vs. $-42 \pm 3\%$; $P < 0.05$). Sildenafil decreased SAP less in sheep that received L-NAME than in those that did not receive L-NAME ($10 \pm 3\%$ vs. $18 \pm 2\%$; $P < 0.05$).

Discussion

The main finding of our study is that enteral administration of sildenafil, a new PDE5 inhibitor, causes selective pulmonary vasodilation in awake sheep with

U46619-induced acute pulmonary hypertension (fig. 1). The pulmonary hemodynamic effects of sildenafil are likely to be mediated by augmentation of the endogenous NO/cGMP-dependent pathway of vasodilation. Surprisingly, and in contrast to other PDE5 inhibitors,^{13,16,18-20} enteral administration of sildenafil to sheep did not augment or prolong the pulmonary vasodilator effects of inhaled NO.

Sildenafil Induces Pulmonary Vasodilation

Nitric oxide, endogenously produced in pulmonary vascular endothelial cells or exogenously administered as an intravenous nitrovasodilator or as inhaled gaseous NO, reduces pulmonary vascular tone, at least in part, via stimulation of cGMP synthesis by soluble guanylate cyclase in pulmonary vascular smooth muscle cells.^{1,3,4} cGMP-mediated vasodilation is limited by the cGMP-metabolizing action of certain PDEs.⁵ Five different isoforms of PDEs that can metabolize cGMP (PDE 1, 2, 3, 5, and 9) are reported present in lung tissue.^{5,30} Several studies have demonstrated pulmonary vasodilation in response to PDE5 inhibitors such as zaprinast, dipyridamole, DMPPO, and E4021 both *in vitro*^{10-12,14,15} and *in vivo*.^{9,12,17}

In this study, we have demonstrated that gavage administration of sildenafil alone, a new PDE5 inhibitor,^{23,24} causes pulmonary vasodilation in awake sheep with thromboxane analog-induced acute pulmonary hypertension (fig. 1). Plasma sildenafil levels increased with increasing sildenafil doses (fig. 2B) and correlated with the percent reduction in PVR, suggesting a dose-

Table 2. Effects of Sildenafil and Zaprinast on Hemodynamic Parameters during NO Inhalation

Parameter	Drug	Baseline	PHTN	Inhaled NO		
				2.5 ppm	10 ppm	40 ppm
PAP (mmHg)	before SIL	14 ± 1	28 ± 1*	24 ± 1†	22 ± 1‡	19 ± 1‡
	before ZAP	14 ± 1	27 ± 1*	24 ± 2#	21 ± 1‡	17 ± 1‡
	+ SIL	—	29 ± 1	26 ± 1†	23 ± 1‡	20 ± 1‡
	+ ZAP	—	27 ± 1	23 ± 1†	21 ± 1‡	19 ± 1‡
SAP (mmHg)	before SIL	86 ± 4	101 ± 3§	101 ± 3	99 ± 3	96 ± 2
	before ZAP	79 ± 4	90 ± 7	93 ± 6	93 ± 5	89 ± 5
	+ SIL	—	108 ± 6	105 ± 7	105 ± 7	105 ± 8
	+ ZAP	—	96 ± 12	94 ± 11	73 ± 18	95 ± 6
CVP (mmHg)	before SIL	3 ± 1	6 ± 2§	5 ± 2	6 ± 2	6 ± 2§
	before ZAP	4 ± 1	5 ± 1	4 ± 1	2 ± 1	3 ± 1
	+ SIL	—	8 ± 2	7 ± 2	7 ± 1	5 ± 1
	+ ZAP	—	6 ± 2	6 ± 1	3 ± 2	3 ± 1
PAOP (mmHg)	before SIL	4 ± 1	8 ± 2§	7 ± 2	8 ± 2	7 ± 2
	before ZAP	5 ± 1	8 ± 1§	7 ± 1	7 ± 1	7 ± 1
	+ SIL	—	9 ± 2	10 ± 2	9 ± 2	8 ± 1
	+ ZAP	—	10 ± 1	11 ± 1	9 ± 1	9 ± 1
CO (l/min)	before SIL	3.2 ± 0.3	2.5 ± 0.4	2.7 ± 0.8	2.5 ± 0.5	2.7 ± 0.6
	before ZAP	2.7 ± 0.5	2.0 ± 0.2	2.2 ± 0.3	2.3 ± 0.3	2.2 ± 0.3
	+ SIL	—	1.5 ± 0.2	1.6 ± 0.3	1.7 ± 0.4	2.0 ± 0.6
	+ ZAP	—	1.9 ± 0.4	1.9 ± 0.3	2.2 ± 0.5	2.4 ± 0.4
PVR (mmHg · l ⁻¹ · min ⁻¹)	before SIL	3 ± 1	9 ± 2‡	8 ± 2	7 ± 2**	6 ± 2†
	before ZAP	4 ± 1	10 ± 1*	8 ± 2**	6 ± 1†	5 ± 1‡
	+ SIL	—	14 ± 2	11 ± 2	9 ± 2**	7 ± 1†
	+ ZAP	—	10 ± 2	7 ± 2	7 ± 2**	5 ± 1†
SVR (mmHg · l ⁻¹ · min ⁻¹)	before SIL	28 ± 3	46 ± 8	49 ± 11	49 ± 10	47 ± 10
	before ZAP	32 ± 6	43 ± 5	43 ± 6	43 ± 6	41 ± 5
	+ SIL	—	72 ± 9	69 ± 10	68 ± 11	65 ± 13
	+ ZAP	—	59 ± 17	54 ± 14	34 ± 13	44 ± 10

Data are mean ± standard error. Sildenafil group, n = 7; zaprinast group, n = 5.

* $P < 0.001$ versus baseline.

† $P < 0.01$ versus PHTN.

‡ $P < 0.001$ versus PHTN.

§ $P < 0.05$.

|| $P < 0.05$ versus — drug.

$P < 0.01$.

** $P < 0.05$ versus PHTN.

PHTN = pulmonary hypertension induced by infusion of U46619; PAP = pulmonary artery pressure; SIL = sildenafil; ZAP = zaprinast; SAP = arterial pressure; CVP = central venous pressure; PAOP = pulmonary artery occlusion pressure; CO = cardiac output; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance.

response relationship between sildenafil and pulmonary vasodilation. Sildenafil doses used in this study (0.625, 1.25, and 2.5 mg/kg) are comparable to those given to humans (25–100 mg).^{23,24,31} However, the plasma sildenafil concentrations measured in our study were lower than the maximal sildenafil plasma concentrations reported after oral administration in humans.^{32,33} One reason for this may be a lower bioavailability in sheep than in humans since sheep are ruminators. Alternatively, at the time when hemodynamic measurements were obtained (15 min after sildenafil administration), maximal

sildenafil plasma concentrations may not have been attained, although the maximal pulmonary vasodilator effect was observed. Moreover, we observed a significant decrease in PAP that reached its maximum within 15 min after enteral administration of 50 mg sildenafil and remained stable for at least 1.5 h.

Our data support the concept of using inhibitors of cGMP-metabolizing PDEs in the treatment of patients with pulmonary hypertension. Further studies examining the clinical effects and toxicity of inhibitors of this PDE class are warranted. As a clinically available agent,

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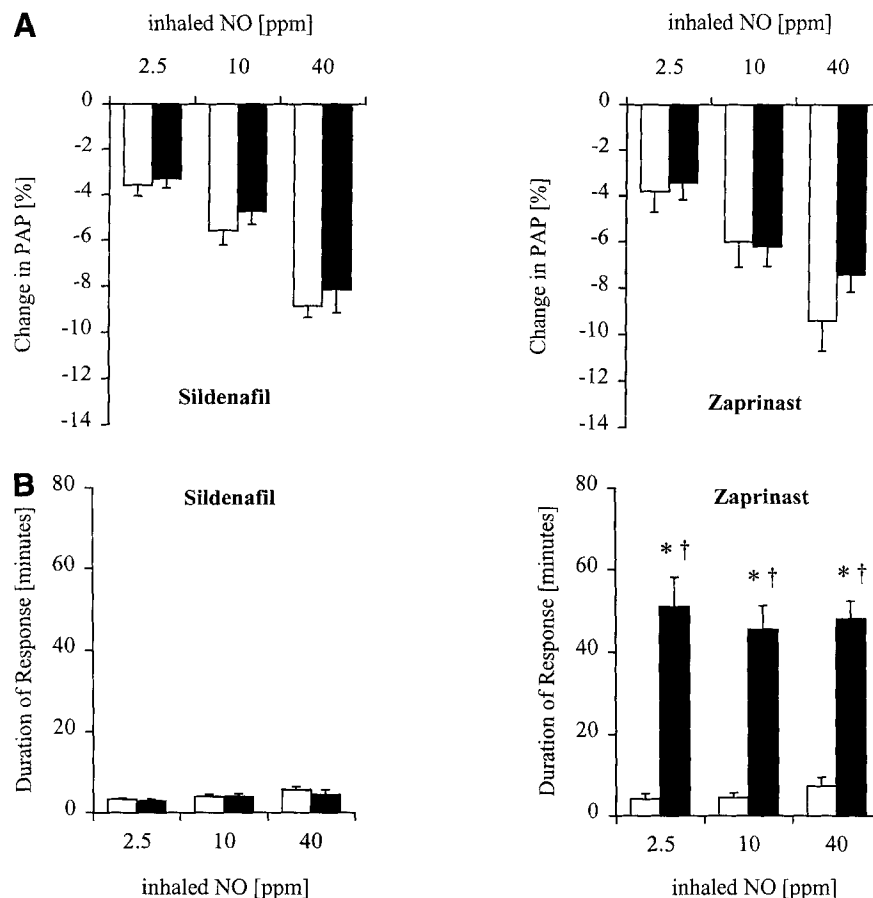


Fig. 3. Effect of sildenafil (50 mg *via* nasogastric tube) and zaprinast (2 mg/kg over 5 min loading dose followed by infusion of $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ intravenously) on the magnitude (A) and the duration (B) of the change in PAP induced by inhalation of 2.5, 10, and 40 ppm NO. Clear bars = control values before administration of sildenafil or zaprinast. Black bars = values after administration of sildenafil or zaprinast. * $P < 0.001$ versus control; † $P < 0.001$ versus the sildenafil group. $n = 7$ in the sildenafil group; $n = 5$ in the zaprinast group. Data are expressed as mean \pm SE.

sildenafil may be a useful agent for the treatment of pulmonary hypertension.

Hemodynamic Effects of Sildenafil Require Endogenous NO-stimulated cGMP Production

In the present study, prior blockade of endogenous NO production by infusion of the NOS inhibitor L-NAME abolished subsequent pulmonary vasodilation induced by sildenafil (fig. 4). Braner *et al.*⁹ reported that pulmonary vasodilation in response to zaprinast was blocked by infusion of the NOS inhibitor N^G -nitro-L-arginine in newborn lambs,⁹ and Ziegler *et al.*¹³ noted, in fetal lambs, a diminished pulmonary vasodilator response to dipyridamole after administration of nitro-L-arginine, another NOS inhibitor. These data suggest that pulmonary vasodilation induced by sildenafil was caused by augmentation of endogenous NO signaling. It is likely that sildenafil augments the vasodilation mediated by endogenous NO by impairing the PDE5-mediated metabolism of cGMP. Similarly, sildenafil-mediated vasorelaxation of

the corpus cavernosum requires stimulation of cGMP production by NO released from nonadrenergic, noncholinergic nerves.³⁴⁻³⁶ Activation of the NO-cGMP pathway is supported by the observation that the magnitude of the sildenafil-mediated decrease in PVR correlated with the increase in plasma cGMP levels.

There are several limitations to the correlation of circulating plasma cGMP levels with plasma sildenafil levels and with the sildenafil-induced reduction of PVR. First, Hamet *et al.*³⁷ noted that extracellular cGMP concentrations incompletely correlated with intracellular cGMP concentrations after stimulation of rat mesenteric smooth muscle cells by atrial natriuretic peptide. They showed that cGMP egression into the extracellular space involves an adenosine triphosphate-dependent active transporter system that may be modulated by temperature and intracellular cyclic adenosine monophosphate levels. Second, the cGMP released into the circulation after sildenafil administration is likely to be derived from the systemic as well as the pulmonary circulation. Fi-

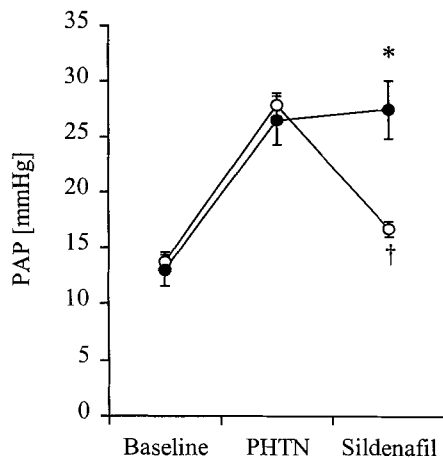


Fig. 4. The pulmonary vasodilation produced by sildenafil (50 mg) is abolished by concomitant infusion of L-NAME. PHTN = pulmonary hypertension induced by U46619 infusion. Open circles = the group of animals not receiving L-NAME infusion ($n = 7$, data from protocol 2); closed circles = the group of animals receiving L-NAME infusion ($n = 6$). * $P < 0.01$ versus group without L-NAME infusion; † $P < 0.001$ versus PHTN. Data are expressed as mean \pm SE.

nally, in our study, the increase of plasma cGMP levels in response to cumulative doses of sildenafil may reflect cGMP accumulation, as well as decreased metabolism.

Pulmonary Selectivity of Sildenafil-induced Vasodilation

In our ovine model of acute pulmonary hypertension, PDE5 inhibition by sildenafil predominantly caused vasodilation of the pulmonary vasculature (fig. 1 and table 1). These results are consistent with other reports demonstrating selective pulmonary vasodilation by PDE5 inhibitors in experimental models of acute^{9,13,18} and chronic pulmonary hypertension.^{11,12}

Acute pulmonary vasoconstriction seems to be accompanied by activation of the NO-cGMP vasodilator pathway as a key physiologic compensatory mechanism.³⁸⁻⁴⁰ Because, in the present study, as in previous studies from our laboratory,^{18,41,42} infusion of U46619 caused greater vasoconstriction of the pulmonary than the systemic vasculature (table 1), it is possible that compensatory activation of NO and cGMP production was more pronounced in the pulmonary vasculature, favoring pulmonary over systemic vasodilation in response to PDE5 inhibition. Alternatively, it is possible that PDE5 enzyme activity may be greater in pulmonary rather than systemic vascular smooth muscle, contributing to a greater impact of sildenafil on the former.

Nonetheless, we noted a significant reduction of SAP at

the 50-mg sildenafil level (tables 1 and 2). Thus, our observations warn that administration of PDE5 inhibitors may cause marked systemic hypotension under conditions associated with increased cGMP production, including treatment with nitrovasodilators²⁵ or in pathologic states associated with abundant NO production, such as sepsis.⁴³

Effects of Sildenafil on Inhaled NO-induced Pulmonary Vasodilation

Inhaled NO decreases the PVR and improves arterial oxygenation in patients with pulmonary hypertension of various causes.⁴⁴⁻⁴⁶ Fullerton *et al.*¹⁹ reported 10 patients with pulmonary hypertension after cardiac surgery who failed to respond with a decrease in PAP and PVR to 40 ppm inhaled NO or to 0.2 mg/kg intravenous dipyridamole but who had a marked pulmonary vasodilator response to their combination. Similarly, dipyridamole¹³ or zaprinast^{16,18} augmented the pulmonary vasodilation induced by NO inhalation in fetal lambs. In contrast, in this study of older lambs, neither zaprinast nor sildenafil increased the magnitude of the pulmonary vasodilator response to inhaled NO (table 2). Our data are supported by a study by McMahon *et al.*,¹⁰ who reported that zaprinast did not enhance the ability of acetylcholine, substance P, or nitrovasodilators to cause pulmonary vasodilation in an *in situ* perfused lung lobe model in adult cats.

In the current study, as well as in a previous study,¹⁸ we observed that zaprinast markedly prolonged the pulmonary vasodilator action of inhaled NO after NO inhalation was discontinued (fig. 3B). These observations are in agreement with those of McMahon *et al.*,¹⁰ who also reported that zaprinast prolonged the duration of NO-induced pulmonary vasodilation. In contrast, sildenafil did not augment the duration of pulmonary vasodilation after discontinuing NO inhalation (fig. 3B). The reason for this difference in the ability of zaprinast and sildenafil, both selective inhibitors of PDE5, to modulate NO-dependent pulmonary vasodilation is unknown and merits further investigation. One possible explanation is that zaprinast inhibits cGMP-metabolizing phosphodiesterases that are not inhibited by sildenafil. For example, zaprinast has been reported to inhibit human PDE1 isoforms Hcam1 and Hcam3A, both of which are expressed in the lung,⁴⁷ whereas only very high concentrations of sildenafil inhibit PDE1.³⁴ Moreover, high concentrations of zaprinast, but not sildenafil, have been reported to inhibit the recently described PDE9, a phosphodiesterase isoform that is detected in pulmonary tissues.⁷

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Conclusion

At low doses, the new PDE5 inhibitor sildenafil is a selective pulmonary vasodilator in awake lambs with U46619-induced acute pulmonary hypertension. Sildenafil-induced vasodilation seems to be mediated by augmentation of the endogenous NO-cGMP signal transduction system. Sildenafil did not improve the efficacy of inhaled NO-induced pulmonary vasodilation and, in contrast to zaprinast, did not prolong the duration of action of inhaled NO.

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