Sildenafil Acutely Reverses the Hypoxic Pulmonary Vasoconstriction Response of the Newborn Pig

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ABSTRACT: Sildenafil is a pulmonary vasodilator shown to be effective in neonates, but conflicting data exist regarding its effect on arterial oxygenation. To address this issue, we tested the sildenafil effect on the piglet's hypoxic pulmonary vasoconstriction (HPV) response. A segmental lung atelectasis was created by obstructing the corresponding bronchus. Total pulmonary and specific flows to the atelectatic and contra-lateral lobes were measured by magnetic resonance (MR) before and 30-min post sildenafil (0.2 and 1 mg/kg i.v.) or saline administration. Flow was reduced ($p < 0.01$) in the atelectatic and increased in the contra-lateral lobe indicating an effective HPV response. Sildenafil at both doses significantly ($p < 0.01$) increased flow solely to the atelectatic lobe. At a dose of 1 mg/kg, sildenafil induced a decrease in Pao_2 from 285 ± 37 to 161 ± 22 mm Hg ($p < 0.01$). We conclude that the HPV response in the newborn is capable of almost completely reducing blood flow to nonventilated lung units and is reversed following sildenafil i.v. administration in a dose-dependent manner. In the presence of lung parenchymal disease, the use of i.v. sildenafil as a pulmonary vasodilator may worsen arterial oxygenation by reversing the HPV response in nonventilated lung units. (*Pediatr Res* **64: 251–255, 2008**)

Inhaled nitric oxide (NO) is a selective pulmonary vasodilator that significantly reduces the neonatal morbidity and mortality associated with pulmonary hypertension (1,2). Given the cost and need for a dedicated apparatus to administered inhaled NO, there has been a recent interest in alternative pharmacological approaches to achieve pulmonary vasodilation in infants.

Among the clinically approved and commercially available compounds, sildenafil, a phosphodiesterase 5 (PDE-5) inhibitor, is one of the most comprehensively studied drugs and found beneficial in the pulmonary hypertension treatment of adult and pediatric subjects (3). Except for the penile vasculature, PDE-5 expression is highest in the lung and even when parenterally administered, sildenafil has a pulmonary preferential vasodilatory effect (4).

The use of sildenafil as a pulmonary vasodilator in infants with lung disease is controversial. On one hand, innumerous clinical studies documented a significant improvement in arterial oxygenation (2,3,5–8). Yet, lung parenchymal disease (*e.g.* meconium aspiration syndrome) is commonly associated with pulmonary hypertension (9) and worsening of arterial oxygenation has been reported in animal models of this disease (10–12). As such, it has been suggested that sildenafil inhibits the hypoxic pulmonary vasoconstrictor (HPV) response leading to increased blood flow to nonventilated lung units (10). This speculative mechanism has never been properly evaluated.

Little is known about the HPV response in the newborn. When compared with the adult animal data, the HPV response is reduced early in life in sheep and rabbits (13–15), but after repeated stimulation, its magnitude is increased in the newborn pig (16).

Thus, the issue at stake is whether parenteral sildenafil administration at doses low enough to treat pulmonary hypertension reverses the HPV response in the newborn. A single recent study attempted to evaluate the intravenous sildenafil effect on the regional pulmonary blood flow of the meconium-induced pulmonary hypertension piglet model (17). In that study, sildenafil, at a dose of 0.2 mg/kg i.v., reduced pulmonary arterial pressure without significantly altering arterial oxygenation. Yet, the interpretation of the sildenafil effect on regional blood flow to well and poorly ventilated areas was made technically impossible to evaluate in that study, given the widespread parenchymal disease associated with meconium aspiration.

The purpose of this study was to comparatively evaluate blood flow changes to ventilated and nonventilated lung units in a newborn animal model of acute lobar atelectasis by flow-sensitive magnetic resonance (MR) imaging methods. We hypothesized that in the newborn, i.v. sildenafil, even in low doses, reverses the HPV response of nonventilated (atelectic) lung units.

METHODS AND MATERIALS

Twenty newborn pigs (4–6 d old) were studied. General anesthesia was induced with ketamine (20 mg/Kg, i.m.) and akemizine (0.5 mg/Kg, i.m.) and initially maintained with 2% isoflurane and subsequent to the placement of a femoral vein catheter with intravenous pentobarbital (30 mg/kg induction followed by continuous infusion of 0.2 mg/kg/min).

The animals were maintained warm with the use of a heating blanket and the body temperature together with heart rate, respiratory rate, oxygen saturation (pulse oximetry, Novametrix Cosmo, Pittsburgh, PA, USA), and end-tidal CO_2 (Puritan Bennett, Pleasanton, CA, USA) continuously measured. At the end of the study, the animals were killed with an overdose of pentobarbital and the lung tissue frozen for cGMP content measurement. All procedures

Abbreviations: HPV, hypoxic pulmonary vasoconstriction response; **PDE-5,** phosphodiesterase-5; **PC,** phase contrast; **ROI,** region of interest

were conducted according to criteria established by the Canadian Council on Animal Care and approved by The Hospital for Sick Children Research Institute Animal Care Review Committee.

Mechanical ventilation and hemodynamic measurements. An incision was made in the neck, and both internal jugular vein and carotid artery were dissected. A 5-F Argyle umbilical catheter was introduced in the carotid artery and advanced into the aorta and a 3.5 F Swan-Ganz catheter into the jugular vein and guided into the pulmonary artery by fluoroscopy (OmniDiagnost Eleva Philips, USA). Both catheters were connected to Hewlett-Packard 1280 transducers for continuous measurement of systemic and pulmonary arterial pressures (Hewlett-Packard, Waltham, MA, USA).

A tracheostomy was performed and a cuffed tracheal tube inserted for mechanical ventilation (Servo 300-Siemens Medical Systems, Erlangen, Germany). The animals were ventilated on pure oxygen at a tidal volume of 5 mL/kg, positive end expiratory pressure = 5 cm H_2O. The ventilatory rates were adjusted to maintain an end-tidal Pco_2 = 40 mm Hg.

Right lobar atelectasis. To induce right lower lobe atelectasis, a 3.5-F vascular balloon catheter (Stouffville, Ontario, Canada) was advanced through the endotracheal tube and fluoroscopy guided to place its tip immediately above the entrance to the right lower lobe bronchus. MR measurements were obtained before and after bronchus occlusion (inflation of the vascular balloon catheter). In preliminary experiments, we determined that right lower lobe atelectasis was consistently obtained in less than 5-min postbronchial occlusion (Fig. 1).

Sildenafil administration. The sildenafil powder (generously supplied by Pfizer, New York, New York, USA) was reconstituted in normal saline (2 mg/mL) and administered intravenously over 10 min at a dose of 0.2 ($n = 7$) or 1 mg/kg ($n = 7$). Control animals ($n = 6$) received a similar volume of saline solution. Measurements were obtained pre and 30-min postsildenafil or saline administration.

cGMP tissue content. Cyclic guanine monophosphate (cGMP) lung tissue content was measured as a surrogate marker for PDE-5 activity in the atelectatic and contralateral lobes of control animals ($n = 3$) to comparatively evaluate the effect of alveolar hypoxia (30 min). The Perkin-Elmer kit (AlphaScreen® cGMP assay, Montreal Quebec, Canada) was used for this purpose, following manufacturer instructions.

Magnetic resonance imaging. Studies were performed on a 1.5 Tesla MR scanner (GE Signa EXCITE III; General Electric Healthcare, Milwaukee, WI, USA) equipped with high-performance gradients (slew rate of 150 mT/m/ms; amplitude of 40 mT/m) and an eight-channel birdcage receiver coil.

The anesthetized animals were imaged supine and mechanically ventilated using an MR-compatible ventilator (LTV model 1200, Viasys Health Care, Minneapolis, MN, USA). Electrocardiographic leads were attached to the chest to provide a gating signal for the MR data acquisition.

For localization, three sets of images covering the chest in the coronal, axial, and sagittal planes were acquired at the beginning of the examination using a two-dimensional nongated steady state free precession imaging sequence. Visualization of the previously inserted balloon was possible by momentarily inflating the balloon with saline during the acquisition of these images.

Regional pulmonary blood flow was then measured using a velocity-sensitive MR technique known as phase-contrast (PC) MRI (18) and proven to have an accuracy of within 3% of the actual measurement (19). The vessel feeding the lower right segment was targeted first by prescribing an imaging plane perpendicular to the vessel at the level of the balloon. The contralateral vessel was then targeted and the scan was repeated. These cardiac-gated scans resulted in 20 cross-sectional images of each peripheral artery throughout the cardiac cycle, with pixel intensities equal to the blood-flow velocity through the imaging plane (Fig. 2). Velocity scans of both the vessels were repeated after induction of segmental atelectasis, and again after administration of sildenafil, the resulting change in volume flow was calculated.

Specific imaging parameters for this sequence were field-of-view = 12 × 12 cm, in-plane spatial resolution ≈ 0.5 × 0.5 mm², slice thickness = 5 mm, aliasing velocity = ±100 cm/s, effective temporal resolution = 17 to 34 ms, and number of signal averages = 3.

Flow analysis. Quantitative analysis of the PC MR data was performed using commercial software with customized routines (MATLAB–Mathworks, Natick, MA, USA). First, conventional background-phase correction was applied to the data to remove background velocity errors from the images (20). A region of interest (ROI) was then drawn to identify the lumen of each targeted pulmonary artery in the PC MR data. The average velocity over the ROI was multiplied by the area of the ROI to calculate the flow volume in each image. Summation of these volumes over all 20 images produced the volume flow per cardiac cycle. From these values, percentage changes in flow relative to baseline (*i.e.* before atelectasis) were calculated at each stage of the experiment.

Data analysis. Data were evaluated by one-way analysis of variance with multiple comparisons obtained by the Tukey-Krammer test when appropriate. Statistical significance was accepted at $p < 0.05$. All statistical analyses were performed with the Number Cruncher Statistical System (NCSS, Kaysville, Utah, USA). Results are presented as mean ± SEM.

RESULTS

Hemodynamic and oxygenation changes induced by right lobar atelectasis. Right lower lobe atelectasis resulted in a significant increase in mean pulmonary arterial pressure ($p < 0.01$) and decrease in PaO_2 ($p < 0.01$) without altering cardiac output or systemic blood pressure (Table 1). Regional blood flow to the atelectatic lung lobe was significantly ($p < 0.01$) reduced by 64%, whereas the flow to the contralateral lung region tended to increase, but the change was not statistically significant ($p = 0.25$; Table 1).

Sildenafil effect. Sildenafil infusion resulted in a significant ($p < 0.01$) decrease in pulmonary and systemic arterial pressures (Fig. 3). At a dose of 0.2 mg/kg, sildenafil reduced the
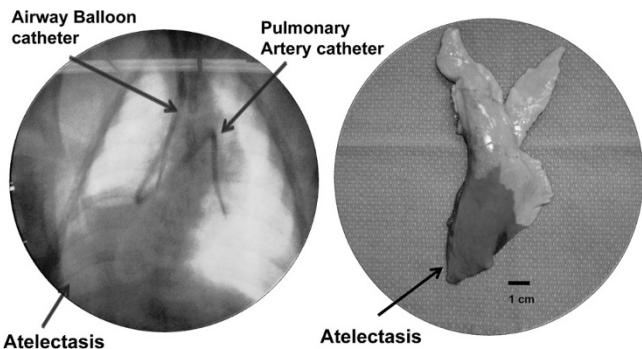
Figure 1. Newborn pig chest fluoroscopy illustrating the placement of the bronchus occluder catheter in the right lung (*left panel*) and the resulting right lower lobe atelectasis on postmortem assessment (*right panel*).
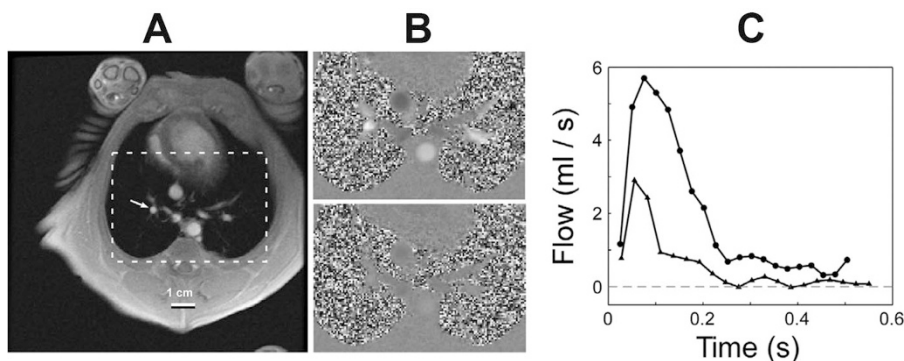
Figure 2. Representative MR blood-flow measurement in one animal. (*A*) Anatomical slice depicting the artery feeding the right lower segment of the lung (*arrow*). (*B*) Two velocity maps corresponding to the boxed area in (*A*) but taken from different points in the cardiac cycle: systolic flow (*top*) and diastolic flow (*bottom*). Bright pixels indicate superior flow and dark pixels inferior flow. (*C*) Blood flow measured in the feeding artery before (●) and after (▲) atelectasis of the right lower segment of the lung. Flow integrated over the cardiac cycle was approximately 1.0 mL and 0.3 mL before and after collapse, respectively.

Table 1. *Hemodynamic, pH and arterial oxygenation changes following right lower lobe atelectasis*

Lobar atelectasis	P_{sa}	P_{pa}	CO	$Q_{Atelectatic}$	$Q_{Contralateral}$	PVR	SVR	pH	PaO_2
Pre	77 ± 2	19 ± 1	163 ± 16	58 ± 9	52 ± 12	0.15 ± 0.02	0.47 ± 0.04	7.40 ± 0.01	368 ± 20
Post	76 ± 1	24 ± 1**	147 ± 11	21 ± 4**	77 ± 9	0.24 ± 0.002**	0.58 ± 0.05	7.39 ± 0.01	273 ± 18**

Data are Mean ± SE; N = 6.

** $p < 0.01$ as compared with pre levels by Student t-test.

P_{SA}, mean systemic arterial pressure (mmHg); P_{pa}, mean pulmonary arterial pressure (mmHg); CO, Cardiac Output (ml · kg/min); $Q_{atelectatic}$, regional blood flow to the right lower atelectatic lobe region of interest (ml · kg/min); $Q_{contralateral}$, regional blood flow to the contralateral lobe region of interest (ml · kg/min); PVR, pulmonary vascular resistance (mmHg · ml^{-1}·min · kg); SVR, systemic vascular resistance (mmHg · ml^{-1}·min · kg); PaO_2, partial pressure of oxygen in arterial blood (mmHg); pH, arterial blood pH.

pulmonary arterial pressure to a level comparable to the preatelectasis measurement. A further nonstatistically significant reduction in pulmonary arterial pressure was observed in the animals treated with 1 mg/kg.

Figure 4 illustrates the total and regional changes in pulmonary blood flow in the control and sildenafil-treated animals. Administration of 1 mg/kg sildenafil significantly increased the regional blood flow to the right lower atelectatic lobe ($p < 0.01$) without statistically altering the flow in the contralateral lower lobe (Fig. 4). The regional flows shown in Fig. 4 represent only that fraction of the cardiac output reaching the lower segments of the left and right lungs, and as such are not equal to the total flow.

Sildenafil did not significantly alter either the total pulmonary blood flow (cardiac output) or pulmonary or systemic vascular resistance (Fig. 5). When compared with the control group, arterial oxygenation progressively decreased after sildenafil administration and was significantly lower ($p < 0.01$) at the 1 mg/kg dose (Fig. 6).

The cGMP content of the atelectatic right lobe was 115 ± 12 pmol/mg tissue protein and not significantly different when compared with the left contralateral lobe (127 ± 15 pmol/mg tissue

protein). These tissue cGMP content data suggest that atelectasis does not acutely increase PDE-5 activity in the piglet lung.

DISCUSSION

Ventilating anesthetized adult cats with a hypoxic gas mixture, Liljestrand was the first to report the association between alveolar hypoxia and increased pulmonary vascular resistance (21). This physiologic response minimizes ventilation-perfusion mismatch, by reducing blood flow to poorly ventilated areas, thus optimizing blood oxygenation. In the newborn, the HPV response has been documented to exist, but it remains unknown how this response varies with age in humans. In sheep (14,22) and rabbit (15), the magnitude of response was reported to be lowest in the newborn and increased with age.

Lung parenchymal disease is a common finding in infants with pulmonary hypertension, and meconium aspiration is the most common associated pathology and present in as many as 41% of the patients in one study (9). If intact, the HPV response would limit blood flow to lung units filled with meconium, thus maximizing ventilation-perfusion mismatch. The goal of this study was to evaluate whether intravenous administration of sildenafil reverses the HPV response in the newborn.

We developed a newborn animal model of unilateral lobar atelectasis and used PC MR imaging to simultaneously measure regional blood flow to the atelectatic and contralateral lung units. We purposefully did not attempt to use pulmonary vasoconstrictor agonists (*i.e.* thromboxane A_2 analogue) to further increase the pulmonary arterial pressure. This was done to study the sildenafil effect on the atelectasis-induced physiologically mediated HPV response in the absence of other confounding pharmacological agonist effects on the pulmonary circulation.
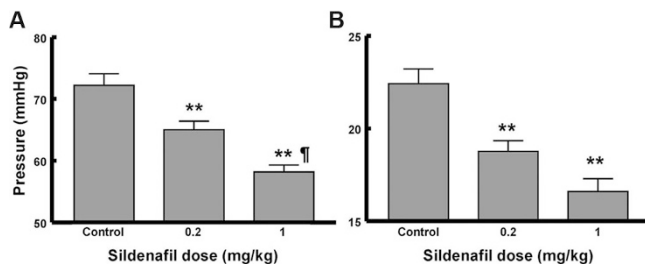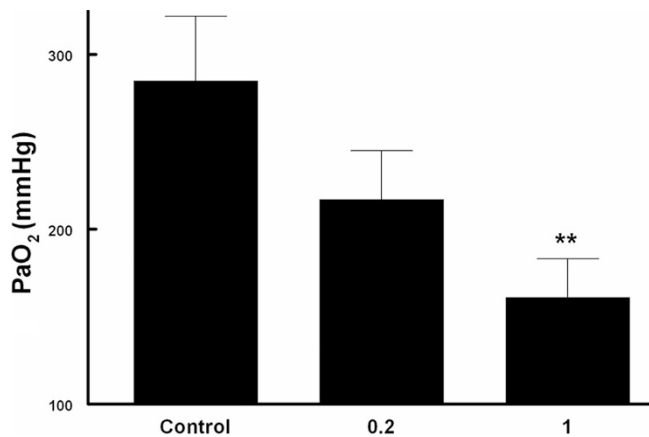

Figure 3. Mean systemic (*A*) and pulmonary (*B*) arterial pressures before (control) (*n* = 9), and 30 min after 0.2 (*n* = 10) and 1 mg/kg (*n* = 14) sildenafil iv administration. **$p < 0.01$ when compared with control values. ¶$p < 0.01$ when compared with 0.2 mg/kg dose.
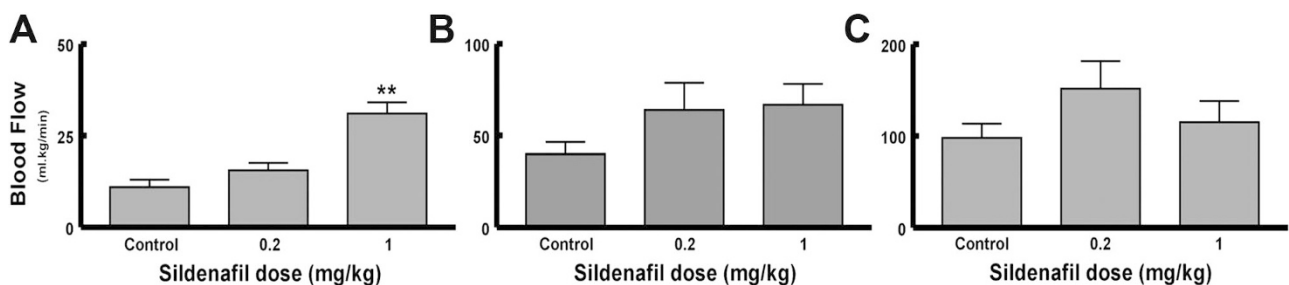
Figure 4. Atelectatic (*A*), contra-lateral (*B*) lobes and total pulmonary blood flow (*C*) before (control) (*n* = 3) and 30 min after 0.2 (*n* = 3) and 1 mg/kg (*n* = 3) sildenafil i.v. **$p < 0.01$ when compared with control values.

A

B
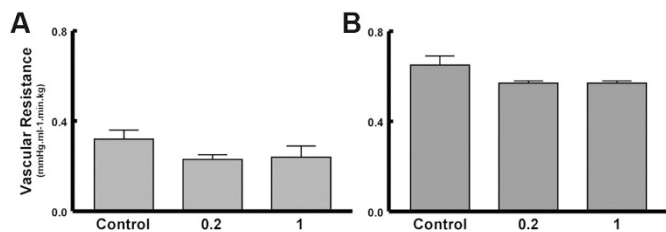
Figure 5. Pulmonary (*A*) and systemic (*B*) vascular resistance changes for control (*n* = 6), 0.2 (*n* = 5), and 1 mg/kg (*n* = 4) sildenafil administration.

Figure 6. Arterial oxygen tension (PaO_2) before (control) (*n* = 9), and 30 min after 0.2 (*n* = 10) and 1 mg/kg (*n* = 14) sildenafil i.v. administration. **p < 0.01 when compared with control values.

In this study, we documented the reversal of HPV response with sildenafil in a dose-dependent manner, resulting in increased ventilation-perfusion mismatch and reduced arterial oxygenation in piglets. These data are in-keeping with previous reports of intravenous sildenafil use in a piglet model of meconium-induced pulmonary hypertension, where worsening of oxygenation was also observed (10–12). In contrast with these previous studies, ours is the first to evaluate the effect of the drug on the HPV response of a noninjured lung.

In adult humans and animals, sildenafil administered either orally or intravenously suppresses the HPV response (23,24). In adult dogs, sildenafil partially reversed pulmonary vasoconstriction induced by inhaled hypoxic gas, but less so than inhaled NO (24). Yet a significant decrease in oxygenation and increase in V/Q mismatch was reported following intravenous administration of prostacyclin, but were not observed after oral administration of sildenafil to human subjects with chronic parenchymal lung disease (25).

Sildenafil has been advocated for the treatment of infants with pulmonary hypertension based on favorable anecdotal and small-scale clinical studies (2). Oral (5,26) intratracheal or intravenous (27) therapeutic use of sildenafil has been reported to result in improvement in the arterial oxygenation of children with pulmonary hypertension. Sildenafil, however, is not a selective pulmonary vasodilator, and its intravenous administration has been shown to cause systemic hypotension in animals and humans (24,28,29).

A recently published Cochrane review of sildenafil use as a pulmonary vasodilator in infants reported a favorable clinical response, reduction of mortality, and absence of significant

side effects, but did not recommend it as a standard therapy (30). Yet, there are reports of worsening oxygenation after sildenafil administration to infants for the treatment of surgically induced pulmonary hypertension (31–33).

The possible explanation for the apparent discrepancy in the sildenafil effect on oxygenation among the different studies lies in the acute nature of the animal experimental models and the chronic characteristics of lung parenchymal disease. Exposure to hypoxia for at least 3 hours selectively suppresses the HPV response and animals living at high altitude exhibit a weak or absent response (34). The HPV response has also been shown to markedly decrease after 5 days in adult rabbits exposed to chronic hypoxia,(35) possibly secondary to an increase in NO production that abrogates the HPV response (36).

We speculate that in infants with pulmonary hypertension and associated lung disease the HPV response wanes over time contributing to the worsening gas exchange. Under these conditions of a time-suppressed HPV response, the sildenafil pulmonary vasodilatory effect will enhance blood perfusion to ventilated segments without altering it to the nonventilated lung units. As such, the end result will improve oxygenation. A similar phenomenon may be present in adults with chronic parenchymal lung disease accounting for the therapeutically beneficial effect of sildenafil (25). Furthermore, experimental testing of sildenafil in chronic animal models of lung parenchymal disease is necessary to test this hypothesis.

Last, hypoxia has been shown to upregulate PDE-5 activity in some (37), but not other chronic animal models (38). *In vitro* experiments on the magnitude of NO generation under hypoxic conditions support this speculation. Grimminger *et al.* showed that in rabbit lungs examined *ex vivo*, exhaled NO was significantly reduced after hypoxia (39), possibly as a result of an O_2-dependent reduction in airway epithelial NO production (40). The NO-mediated vasorelaxation was also found to be reduced in the perfused rabbit lungs (41), lending further support to the speculation that chronic hypoxia upregulates PDE-5 activity.

In this study, we evaluated the effect of acute hypoxia (reduced alveolar ventilation) on the PDE-5 activity. We used the lung cGMP level as a surrogate marker for PDE-5 activity. No significant differences in cGMP levels were observed in the atelectatic when compared with the contralateral lobe 30 min after balloon inflation. In rats, pentobarbital anesthesia and mechanical ventilation acutely increase the lung tissue cGMP content (42), possibly as a result of decreased PDE-5 activity. Thus, it is possible that a similar effect on the piglet preparation used in this study masked any alveolar hypoxia (atelectasis)-induced PDE-5 activity increase.

Anesthetics can also alter the HPV response, and isoflurane is known to significantly reduce its magnitude (43). A similar HPV response lowering effect was observed with pentobarbital in the *ex vivo* sheep lung (44). In this study, the atelectatic and nonatelectatic lung units were comparatively evaluated in each animal. As such, any anesthesia effect on the measurements would be similar for both lung regions.

In summary, we demonstrated that the newborn pig can mount a significant and effective acute HPV response that is acutely

reversed by intravenous sildenafil in a dose-dependent manner. The present results are in keeping with other data showing a reduction in arterial oxygenation after sildenafil treatment. Although not a model of pulmonary hypertension, the newborn piglet data obtained in this study raise concern regarding the therapeutic use of sildenafil as a pulmonary vasodilator when associated with acute lung parenchymal disease.

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