Inhibition of phosphodiesterase 1 augments the pulmonary vasodilator response to inhaled nitric oxide in awake lambs with acute pulmonary hypertension

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Evgenov, Oleg V., Cornelius J. Busch, Natalia V. Evgenov, Rong Liu, Bodil Petersen, George E. Falkowski, Beata Pethő, Ádám Vas, Kenneth D. Bloch, Warren M. Zapol, and Fumito Ichinose. Inhibition of phosphodiesterase 1 augments the pulmonary vasodilator response to inhaled nitric oxide in awake lambs with acute pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 290: L723–L729, 2006. First published November 18, 2005; doi:10.1152/ajplung.00485.2004.—Phosphodiesterase 1 (PDE1) modulates vascular tone and the development of tolerance to nitric oxide (NO)-releasing drugs in the systemic circulation. Any role of PDE1 in the pulmonary circulation remains largely uncertain. We measured the expression of genes encoding PDE1 isoforms in the pulmonary vasculature and examined whether or not selective inhibition of PDE1 by vinpocetine attenuates pulmonary hypertension and augments the pulmonary vasodilator response to inhaled NO in lambs. Using RT-PCR, we detected PDE1A, PDE1B, and PDE1C mRNAs in pulmonary arteries and veins isolated from healthy lambs. In 13 lambs, the thromboxane A2 analog U-46619 was infused intravenously to increase mean pulmonary arterial pressure to 35 mmHg. Four animals received an intravenous infusion of vinpocetine at incremental doses of 0.3, 1, and 3 mg·kg−1·h−1. In nine lambs, NO was administered in a random order at 2, 5, 10, and 20 ppm before and after an intravenous infusion of 1 mg·kg−1·h−1 vinpocetine. Administration of vinpocetine did not alter pulmonary and systemic hemodynamics or transpulmonary cGMP or cAMP release. Inhaled NO selectively reduced mean pulmonary arterial pressure, pulmonary capillary pressure, and pulmonary vascular resistance index, while increasing transpulmonary cGMP release. The addition of vinpocetine enhanced pulmonary vasodilation and transpulmonary cGMP release induced by NO breathing without causing systemic vasodilation but did not prolong the duration of pulmonary vasodilation after NO inhalation was discontinued. Our findings demonstrate that selective inhibition of PDE1 augments the therapeutic efficacy of inhaled NO in an ovine model of acute chemically induced pulmonary hypertension.

Guanosine 3′,5′-cyclic monophosphate; vinpocetine; sheep

PULMONARY HYPERTENSION (PH) is a life-threatening condition that is characterized by an increased pulmonary arterial pressure due to an elevated pulmonary vascular resistance. Impaired production of endogenous vasodilators, such as nitric oxide (NO) and prostacyclin, and excessive release of vasoconstrictors, such as thromboxane A2 and endothelin, play an important role in the pathophysiology of PH (15, 24). Treatment of PH with oral or intravenous vasodilating agents including NO-releasing drugs and analogs of prostacyclin can be associated with potentially catastrophic systemic arterial hypotension. In contrast to systemically administered vasodilators, inhaling low concentrations of NO produces pulmonary vasodilation in well-ventilated lung regions without causing systemic arterial hypotension because NO is rapidly bound by hemoglobin upon entry into the intravascular space (19, 31).

Because the vasodilator effects of NO are largely mediated via cGMP-dependent mechanisms (19, 34), it has been hypothesized that inhibition of the cGMP-metabolizing phosphodiesterases (PDEs) would augment the ability of inhaled NO (iNO) to dilate the pulmonary vasculature by further increasing cGMP levels in pulmonary vascular smooth muscle cells (15). In the lungs, at least six cGMP-metabolizing PDE families (PDE 1, 2, 3, 5, 9, and 10) have been identified (8, 28, 29, 35, 37). We have previously reported that zaprinast, an inhibitor of several cGMP-metabolizing PDEs, potentiates and markedly prolongs pulmonary vasodilation induced by iNO when administered in lambs with chemically induced PH (16, 17). Subsequently, oral administration of the more potent, clinically approved PDE5 inhibitor sildenafil has been shown to produce selective pulmonary vasodilation in experimental models, as well as in patients with PH (21, 25, 26, 39). In addition, sildenafil augmented the pulmonary vasodilator response to iNO, when administered in an aerosolized form in lambs with PH (18), suggesting an important modulatory role of PDE5 on pulmonary vascular tone. However, the specific roles of other cGMP-metabolizing PDEs in modulating the pulmonary vasodilator response to iNO remain largely unexplored.

Recent evidence suggests that PDE1 contributes to the regulation of systemic vascular tone (32, 38). Vinpocetine [14-ethoxycarbonyl-(3α,16α-ethyl)-14,15-eburnamenine], a synthetic alkaloid derivative, is one of the most selective PDE1 inhibitors currently available (1, 12, 38). Vinpocetine has been recently shown to limit nitroglycerin-induced tolerance in isolated rat aortas, as well as to attenuate hypoxic pulmonary vasoconstriction in an in situ perfused rat lung preparation (20, 28). On the basis of these observations, we hypothesized that PDE1 is expressed in the ovine pulmonary vasculature and that systemic administration of vinpocetine would produce pulmo-

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nary vasodilation and also enhance vasodilator responsiveness to iNO in lambs with acute chemically induced PH. We found that three PDE1 isozymes (PDE1A, PDE1B, and PDE1C) are expressed in pulmonary arteries and veins isolated from healthy lambs. We also demonstrated that administration of vinpocetine alone did not attenuate PH but augmented the pulmonary vasodilation induced by iNO.

**Materials and Methods**

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

**Isolation of pulmonary vessels and analysis of PDE1 isozyme expression.** Four healthy lambs were killed with an intravenous injection of 100 mg/kg pentobarbital (Abbott Laboratories, North Chicago, IL). The chest cavity was immediately exposed through a midline sternotomy, and the lungs and heart were carefully removed en bloc and placed in ice-cold phosphate-buffered saline solution (MP Biomedicals, Aurora, OH). The main and fourth-generation pulmonary arteries and the main and fourth-generation pulmonary veins were dissected free, cleaned of the surrounding parenchyma, and rapidly frozen in liquid N2 before being homogenized. Total RNA was extracted from the main pulmonary artery with the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). cDNA was generated with reverse transcriptase and random primers (Promega, Madison, WI) and was amplified by PCR and primers designed to detect PDE1A (5'-CGGGATCACTTTATGATHTAYGA-3', 5'-CGAATCTGCTTCACTTCTG-3'), PDE1B (5'-CGGGATCATCTTGACTGTTATCT-3'), and PDE1C (5'-CGGGATCACTTTATGATHTAYGA-3', 5'-CGAATCTGCTTCACTTCTG-3').

**RNA expression.** Four healthy lambs were killed with an intravenous injection of 100 mg/kg pentobarbital (Abbott Laboratories, North Chicago, IL). The chest cavity was immediately exposed through a midline sternotomy, and the lungs and heart were carefully removed en bloc and placed in ice-cold phosphate-buffered saline solution (MP Biomedicals, Aurora, OH). The main and fourth-generation pulmonary arteries and the main and fourth-generation pulmonary veins were dissected free, cleaned of the surrounding parenchyma, and rapidly frozen in liquid N2 before being homogenized. Total RNA was extracted from the main pulmonary artery with the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). cDNA was generated with reverse transcriptase and random primers (Promega, Madison, WI) and was amplified by PCR and primers designed to detect PDE1A (5'-CGGGATCACTTTATGATHTAYGA-3', 5'-CGAATCTGCTTCACTTCTG-3'), PDE1B (5'-CGGGATCATCTTGACTGTTATCT-3'), and PDE1C (5'-CGGGATCACTTTATGATHTAYGA-3', 5'-CGAATCTGCTTCACTTCTG-3').

**Biochemical analysis.** Four healthy lambs were killed with an intravenous injection of 100 mg/kg pentobarbital (Abbott Laboratories, North Chicago, IL). The chest cavity was immediately exposed through a midline sternotomy, and the lungs and heart were carefully removed en bloc and placed in ice-cold phosphate-buffered saline solution (MP Biomedicals, Aurora, OH). The main and fourth-generation pulmonary arteries and the main and fourth-generation pulmonary veins were dissected free, cleaned of the surrounding parenchyma, and rapidly frozen in liquid N2 before being homogenized. Total RNA was extracted from the main pulmonary artery with the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). cDNA was generated with reverse transcriptase and random primers (Promega, Madison, WI) and was amplified by PCR and primers designed to detect PDE1A (5'-CGGGATCACTTTATGATHTAYGA-3', 5'-CGAATCTGCTTCACTTCTG-3'), PDE1B (5'-CGGGATCATCTTGACTGTTATCT-3'), and PDE1C (5'-CGGGATCACTTTATGATHTAYGA-3', 5'-CGAATCTGCTTCACTTCTG-3').

**Surgical preparation.** Thirteen lambs weighing 21.4 (2.8) kg [means (SD)] were anesthetized with an intramuscular injection of 15 mg/kg ketamine hydrochloride (Fort Dodge Animal Health, Fort Dodge, IA) and 0.5 mg/kg xylazine (Lloyd Laboratories, Shenandoah, IA). A tracheostomy was performed, and an 8.0-mm cuffed tracheostomy tube (SIMS Portex, Keene, NH) was inserted. The lungs were ventilated at 15 breaths/min, 15 ml/kg tidal volume, and an inspired fraction of oxygen (FiO2) of 0.5 by a volume-controlled ventilator (model 613; Harvard Apparatus, South Natick, MA). Anesthesia was maintained with inhalation of 1.2–1.5% isoflurane (Baxter Healthcare, Deerfield, IL) and intravenous injections of 1 mg/kg morphine (Elkins-Sinn, Cherry Hill, NJ). Through a left thoracotomy in the fourth intercostal space, a polyvinyl chloride catheter (1.5-mm inner diameter (ID)) was implanted into the left atrium. An 8-Fr introducer (Cordis, Miami, FL) was placed in the left external jugular vein, and a 7-Fr flow-directed thermal dilution catheter (Edwards Lifesciences, Irvine, CA) was introduced into the pulmonary artery. In addition, a polyvinyl chloride catheter (1.5-mm ID) was inserted into the left common carotid artery (6). The catheters were continuously flushed with a saline solution containing 2 IU/ml heparin (Abbott Laboratories, North Chicago, IL).

After emergence from general anesthesia, the animals were housed in a large-animal mobile restraint unit (Lomir, Malone, NY) and allowed at least 3 h of recovery. The study commenced if the following baseline exclusion criteria did not occur: a peripheral white blood cell count <4,000 or >12,000 mm⁻³, mean pulmonary arterial pressure >20 mmHg, or a body temperature measured by the pulmonary artery catheter >40°C (16, 17).

**Hemodynamic measurements.** Mean arterial pressure (MAP), mean pulmonary arterial pressure (PAP), and mean left atrial pressure (LAP) were measured continuously, and pulmonary arterial occlusion pressure (PAOP) was measured intermittently with calibrated pressure transducers (Maxxim Medical, Athens, TX) zero-referenced at the midchest. The pressure signals were amplified (model 7700; Hewlett Packard, Palo Alto, CA) and recorded using a DT-220 converter and a WinDat 2.25 data acquisition program (DATAQ Instruments, Akron, OH). Heart rate was obtained from the phasic arterial pressure trace. Effective pulmonary capillary pressure (Pc) was derived from the PAOP tracing according to a technique described by Holloway et al. (14). In brief, when the balloon of the pulmonary artery thermal dilution catheter was rapidly inflated, the pressure at the distal port initially declined rapidly and then more slowly to approach PAOP. Pc was estimated visually as the inflection point between the rapid and the slow exponential components of the PAOP tracing. Cardiac output was determined by a cardiac output computer (SAT-2, Edwards Lifesciences) as the average of four measurements after injection of 10 ml of ice-cold isotonic saline. Body surface area was calculated as body wt in kg⁰.⁰⁶⁷ × 0.084. Cardiac index (CI) was calculated as cardiac output divided by body surface area, systemic vascular resistance index (SVRI) as MAP/CI × 80, and pulmonary vascular resistance index (PVRI) as (PAP – LAP)/CI × 80 (6).

**Delivery of NO.** The tracheostomy tube 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. Using volumetrically calibrated flowmeters, we mixed oxygen and air to produce FiO2 at 0.6, which was continuously monitored (Oxygen Monitor 5590; Hudson, Temecula, CA). NO gas (800 ppm in nitrogen; INO Therapeutics, Clinton, NJ) was introduced into the inspiratory limb of the breathing circuit immediately before the reservoir bag (5, 17). The concentration of NO was continuously measured with a calibrated chemiluminescence analyzer (Sievers 280; Ionics Instruments, Boulder, CO) on the inspiratory limb of the two-way valve. Exhaled gases were scavenged via a Venturi exhalation trap maintained at negative atmospheric pressure by the central vacuum system. The NO concentration in ambient air was ≤0.006 ppb.

**Experimental protocol.** During the experiments, the lambs were awake and breathed spontaneously while receiving an intravenous infusion of lactated Ringer solution (10 ml/kg·h⁻¹). All measurements and samples were obtained at baseline and before and at the end of each treatment.

Following baseline measurements, we intravenously administered the stable endoperoxide analog of thromboxane A₂ U-46619 (Cayman Chemical, Ann Arbor, MI), dissolved in lactated Ringer solution, at a rate of 1.6 (0.4) µg·kg⁻¹·min⁻¹ to increase PAP to ~35 mmHg (5). After a 30-min stabilization period, four lambs received an intravenous infusion of vinpocetine (Gedeon Richter, Budapest, Hungary) in sequential incremental doses of 0.3, 1, and 3 mg·kg⁻¹·h⁻¹, which were administered for 1 h each. Of note, the maximal dose of vinpocetine was selected based on our pilot experiments, where bolus intravenous injection of the drug at a higher dose (5 mg/kg over 5
min) caused signs of acute toxicity, including convulsions, profound tachycardia, and hypertension.

In nine additional lambs with U-46619-induced acute PH, NO gas was administered via inhalation in a random sequence at 2, 5, 10, or 20 ppm. At each dose level, NO was inhaled for 10 min followed by a 15-min NO-free period. All hemodynamic parameters returned to pretreatment values during the latter period. A loading intravenous dose of 3 mg/kg vinpocetine was then administered over 3 min, followed by a continuous intravenous infusion of 1 mg·kg⁻¹·h⁻¹. After a 30-min stabilization period, NO was inhaled again at the same concentrations and in the same order as was given before vinpocetine administration. After the final measurements, the animals were killed with an intravenous injection of 100 mg/kg pentobarbital.

**Blood samples and analysis of blood gases.** Blood samples were simultaneously obtained from the carotid and pulmonary arteries and analyzed for pH, PO₂, and PCO₂ with a blood gas analyzer (Radiometer, Copenhagen, Denmark). Barometric pressure was noted daily. Using the alveolar gas equation, we calculated alveolar oxygen tension, assuming a respiratory quotient of 0.8. Assuming a hemoglobin oxygen binding capacity of 1.31 ml/g and pulmonary end-capillary oxygen saturation of 100%, we calculated oxygen content of arterial blood, pulmonary end-capillary blood, and mixed venous blood. Venous admixture (Qs/Qt) was calculated with a standard equation (23). In addition, arterial and mixed venous blood was collected in sample tubes containing a buffered sodium citrate solution and 3-isobutyl-1-methyl-xanthine (Sigma-Aldrich, St. Louis, MO) at final concentration of 0.5 mM. The mixture was centrifuged at 4°C (2,000 g for 10 min). The plasma supernatants were stored at −70°C.

**Transpulmonary cGMP and cAMP release.** Arterial and mixed venous plasma concentrations of cGMP or cAMP were determined by radioimmunoassay (BT-340 and BT-300; Biomedical Technologies, Stoughton, MA) as previously described (16). The quantity of cGMP or cAMP released by the lung per minute during each treatment was calculated as the product of cardiac output times the concentration and in the same order as was given before vinpocetine administration.

**Table 1. Effects of incremental doses of intravenously infused vinpocetine in lambs with U-46619-induced acute pulmonary hypertension**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline PH</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP, mmHg</td>
<td>35.2 (9.2)</td>
<td>35.1 (1.3)</td>
<td>34.7 (3.4)</td>
<td>35.7 (1.1)</td>
</tr>
<tr>
<td>Pc, mmHg</td>
<td>19.9 (1.5)</td>
<td>19.2 (2.0)</td>
<td>19.6 (2.0)</td>
<td>20.4 (0.7)</td>
</tr>
<tr>
<td>PVRI</td>
<td>625 (29)</td>
<td>668 (49)</td>
<td>649 (90)</td>
<td>605 (72)</td>
</tr>
<tr>
<td>CI, 1·min⁻¹·m⁻²</td>
<td>3.2 (0.3)</td>
<td>3.2 (0.3)</td>
<td>3.1 (0.5)</td>
<td>3.4 (0.4)</td>
</tr>
<tr>
<td>SVRI</td>
<td>2794 (370)</td>
<td>2802 (90)</td>
<td>2867 (608)</td>
<td>2669 (545)</td>
</tr>
<tr>
<td>PaO₂/PcO₂</td>
<td>0.23 (0.02)</td>
<td>0.24 (0.01)</td>
<td>0.23 (0.03)</td>
<td>0.23 (0.03)</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>380 (88)</td>
<td>383 (32)</td>
<td>371 (59)</td>
<td>399 (31)</td>
</tr>
<tr>
<td>Qs/Qt</td>
<td>0.12 (0.03)</td>
<td>0.11 (0.01)</td>
<td>0.12 (0.02)</td>
<td>0.11 (0.01)</td>
</tr>
<tr>
<td>Transpulmonary cGMP release, μmol/min</td>
<td>5.7 (1.0)</td>
<td>6.0 (1.2)</td>
<td>5.8 (1.6)</td>
<td>6.1 (2.6)</td>
</tr>
<tr>
<td>Transpulmonary cAMP release, μmol/min</td>
<td>1.8 (4.7)</td>
<td>2.8 (7.5)</td>
<td>2.8 (6.4)</td>
<td>1.5 (4.8)</td>
</tr>
</tbody>
</table>

Values are means (SD), n = 4. PH, pulmonary hypertension; PAP, mean pulmonary arterial pressure; Pc, pulmonary capillary pressure; PVRI, pulmonary vascular resistance index; MAP, mean arterial pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PVRI/SVRI, PVRI-to-SVRI ratio; PaO₂/PcO₂, ratio of arterial oxygen tension-to-inspired oxygen fraction; Qs/Qt, venous admixture.
As shown in Fig. 2 and Table 2, the concomitant infusion of vinpocetine markedly enhanced the reductions in PAP and Pc induced by 2, 5, and 10 ppm of iNO (P < 0.05). In parallel, the combined administration of vinpocetine and iNO at all doses decreased PVRI below that obtained during iNO alone (P < 0.05). The combination of vinpocetine and iNO administered at 10 and 20 ppm also increased CI (Table 2, P < 0.05 vs. baseline PH), whereas other hemodynamic variables remained unchanged. Furthermore, transpulmonary cGMP release was greater during 5, 10, and 20 ppm of iNO administered in combination with vinpocetine than during iNO alone (Fig. 3, P < 0.05), whereas no significant changes in transpulmonary cAMP release occurred (Table 2). The vinpocetine infusion enabled iNO to reduce Qs/Qt starting from the dose of 2 ppm, as well as to improve Pao2/Fio2, starting from the dose of 5 ppm (Table 2, P < 0.05 vs. baseline PH). However, vinpocetine did not change T1/2 of the decay of pulmonary vasodilation after iNO was discontinued, as shown in Table 2. The mean plasma concentration of vinpocetine remained stable throughout this part of the study. The mean arterial concentration of methemoglobin did not exceed 1% during iNO administered alone or in combination with vinpocetine (data not shown).

**DISCUSSION**

Our study demonstrates that the PDE1A, PDE1B, and PDE1C isozymes are expressed in ovine pulmonary vasculature. Selective inhibition of the PDE1 family by intravenously administered vinpocetine does not attenuate acute chemically induced PH in awake lambs; however, it augments the pulmonary vasodilator effect of iNO. Similarly, vinpocetine alone does not modulate transpulmonary cGMP release, whereas the iNO-induced increase in transpulmonary cGMP release is augmented by concomitant infusion of this drug.

In mammalian tissues, PDE1 is expressed as three isozymes named PDE1A, PDE1B, and PDE1C (3). It is generally agreed that PDE1A and PDE1B selectively hydrolyze cGMP, while PDE1C hydrolyses cGMP and cAMP with equal affinity (32, 35). In the present investigation, we detected the expression of PDE1A, PDE1B, and PDE1C genes in the main pulmonary arteries, the fourth-generation pulmonary arteries, the main pulmonary veins, and the fourth-generation pulmonary veins isolated from healthy lambs. Our findings add to previous reports demonstrating the expression of the PDE1 isozymes in normal rabbit and bovine lungs, as well as in rat and human pulmonary arteries (28, 29, 35, 36).

Vinpocetine has been shown to produce selective inhibition of the PDE1 isozymes in various tissues with IC50 ranging from ~10 to 50 μM (1, 12, 20, 22, 40). In preconstricted isolated rabbit aortic strips and in isolated perfused rat lungs exposed to hypoxia, vinpocetine produced vasorelaxation and increased cGMP but not cAMP levels (1, 12, 28). However, in the present in vivo study, intravenous infusion of vinpocetine at incremental doses up to 3 mg·kg−1·h−1 produced no effect on acute pulmonary vasoconstriction induced by U-46619. In addition, no changes were observed in transpulmonary cGMP or cAMP production and systemic hemodynamics. In contrast, zaprinast, which inhibits PDE1 with an IC50 similar to vinpocetine (1, 12, 22, 40), selectively decreased PAP and PVRI and increased transpulmonary cGMP release when administered intravenously as either a bolus (0.5–5 mg/kg over 5 min)
or a continuous infusion (6 mg·kg\(^{-1}·h^{-1}\)) or after inhalation in an aerosolized form (an effective dose of \(\approx 3–4\) mg·kg\(^{-1}·h^{-1}\)) in the same ovine model of PH (4, 16, 17). The difference in the pulmonary responses to vinpocetine and zaprinast may be attributed to the fact that zaprinast also inhibits PDE5 (IC\(_{50}\) = 1 \(\mu\)M) and PDE9 (IC\(_{50}\) = 35 \(\mu\)M) (1, 2, 7). Furthermore, sildenafil, a more potent and selective PDE5 inhibitor (IC\(_{50}\) = 3.5 nM for PDE5, IC\(_{50}\) = 280 nM for PDE1) (2), produced marked pulmonary vasodilation when administered in animal models (18, 39) and patients with PH (21, 25, 26). Thus the absence of a pulmonary vasodilator response to vinpocetine in lambs with U-46619-induced acute pulmonary hypertension is consistent with a selective PDE5 vasodilator action. The marked pulmonary vasodilation when administered in animal models (18, 39) and patients with PH (21, 25, 26). Thus the absence of a pulmonary vasodilator response to vinpocetine in lambs with U-46619-induced acute pulmonary hypertension is consistent with a selective PDE5 vasodilator action. However, when iNO was combined with intravenously infused vinpocetine, the reductions in PAP, PC, and PVRI were all markedly augmented, particularly at low doses of iNO (2–10 ppm). In addition to a further decrease of PAP, CI increased during coadministration of vinpocetine and iNO, indicating an additional dilatation of the pulmonary vasculature. This augmentation of the iNO-induced pulmonary vasodilation occurred in parallel with an additional rise in transpulmonary cGMP release. Interestingly, the cGMP-specific PDE activity in isolated ovine pulmonary veins has been demonstrated to be more sensitive to inhibition by zaprinast compared with pulmonary arteries (27). In the present study, we found no significant differences in the longitudinal expression of the PDE1 isozymes in the pulmonary vasculature. Therefore, the increase in transpulmonary cGMP release following combined administration of iNO and vinpocetine is unlikely to be attributed to a relatively larger accumulation of cGMP in pulmonary veins than in pulmonary arteries. Nevertheless, it is conceivable that vasodilation observed in our model of PH mainly occurred in pulmonary veins because thromboxane A\(_2\) or its analog U-46619 are potent constrictors of pulmonary veins in a number of species including dog, sheep, and human (9).

Because of its ability to improve ventilation-perfusion matching, iNO is used to enhance systemic oxygenation in newborn and adult patients with PH or acute hypoxic respiratory failure (19). In contrast, systemic administration of vasodilators can lead to worsening of preexisting mismatching of ventilation and perfusion and subsequent deterioration of arterial oxygenation (30). Although the present lamb model is not designed to produce marked abnormalities of pulmonary gas exchange, it is characterized by a moderately increased Qs/Qt and decreased arterial oxygenation. We observed that iNO and vinpocetine are used to enhance systemic oxygenation in newborn and adult patients with PH or acute hypoxic respiratory failure (19). In contrast, systemic administration of vasodilators can lead to worsening of preexisting mismatching of ventilation and perfusion and subsequent deterioration of arterial oxygenation (30). Although the present lamb model is not designed to produce marked abnormalities of pulmonary gas exchange, it is characterized by a moderately increased Qs/Qt and decreased arterial oxygenation. We observed that iNO and vinpocetine, the reductions in PAP, PC, and PVRI were all markedly augmented, particularly at low doses of iNO (2–10 ppm). In addition to a further decrease of PAP, CI increased during coadministration of vinpocetine and iNO, indicating an additional dilatation of the pulmonary vasculature. This augmentation of the iNO-induced pulmonary vasodilation occurred in parallel with an additional rise in transpulmonary cGMP release. Interestingly, the cGMP-specific PDE activity in isolated ovine pulmonary veins has been demonstrated to be more sensitive to inhibition by zaprinast compared with pulmonary arteries (27). In the present study, we found no significant differences in the longitudinal expression of the PDE1 isozymes in the pulmonary vasculature. Therefore, the increase in transpulmonary cGMP release following combined administration of iNO and vinpocetine is unlikely to be attributed to a relatively larger accumulation of cGMP in pulmonary veins than in pulmonary arteries. Nevertheless, it is conceivable that vasodilation observed in our model of PH mainly occurred in pulmonary veins because thromboxane A\(_2\) or its analog U-46619 are potent constrictors of pulmonary veins in a number of species including dog, sheep, and human (9).

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**Table 2. Effects of inhaled nitric oxide administered alone or in combination with intravenously infused vinpocetine in lambs with U-46619-induced acute pulmonary hypertension**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline PH</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>iNO</td>
<td>110 (13)</td>
<td>111 (14)</td>
<td>109 (14)</td>
<td>107 (8)</td>
<td>109 (12)</td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>110 (12)</td>
<td>111 (15)</td>
<td>110 (13)</td>
<td>108 (13)</td>
<td>112 (14)</td>
</tr>
<tr>
<td>CI, l·min(^{-1})·m(^{-2})</td>
<td>iNO</td>
<td>3.3 (0.5)</td>
<td>3.3 (0.6)</td>
<td>3.3 (0.4)</td>
<td>3.3 (0.7)</td>
<td>3.1 (0.7)</td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>3.5 (0.4)</td>
<td>3.7 (0.8)</td>
<td>3.7 (0.6)</td>
<td>4.0 (0.7)†</td>
<td>3.9 (0.7)†</td>
</tr>
<tr>
<td>SVRI, dyne·s·cm(^{-2})·m(^{-2})</td>
<td>iNO</td>
<td>2679 (407)</td>
<td>2799 (553)</td>
<td>2701 (476)</td>
<td>2700 (487)</td>
<td>2837 (602)</td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>2647 (562)</td>
<td>2574 (779)</td>
<td>2501 (753)</td>
<td>2392 (679)</td>
<td>2401 (655)</td>
</tr>
<tr>
<td>PVRI/SVRI</td>
<td>iNO</td>
<td>0.26 (0.05)</td>
<td>0.24 (0.06)</td>
<td>0.22 (0.03)</td>
<td>0.21 (0.03)</td>
<td>0.19 (0.06)*</td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>0.26 (0.04)</td>
<td>0.21 (0.05)*</td>
<td>0.18 (0.04)*</td>
<td>0.16 (0.04)*†</td>
<td>0.16 (0.02)*†</td>
</tr>
<tr>
<td>PAO(_2)/FiO(_2), mmHg</td>
<td>iNO</td>
<td>371 (107)</td>
<td>381 (73)</td>
<td>416 (58)</td>
<td>479 (51)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>341 (89)</td>
<td>379 (86)</td>
<td>408 (80)*</td>
<td>489 (49)*</td>
<td>471 (71)*</td>
</tr>
<tr>
<td>Qs/Qt</td>
<td>V + iNO</td>
<td>0.10 (0.02)</td>
<td>0.09 (0.04)</td>
<td>0.08 (0.02)</td>
<td>0.08 (0.01)</td>
<td>0.05 (0.01)*</td>
</tr>
<tr>
<td>Transpulmonary cAMP release, μmol/min</td>
<td>iNO</td>
<td>2.8 (7.4)</td>
<td>2.9 (6.7)</td>
<td>2.2 (6.6)</td>
<td>2.2 (3.3)</td>
<td>2.6 (3.1)</td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>2.7 (7.2)</td>
<td>3.4 (3.3)</td>
<td>2.9 (7.1)</td>
<td>2.4 (5.3)</td>
<td>3.4 (5.5)</td>
</tr>
<tr>
<td>T(_{1/2}), s</td>
<td>iNO</td>
<td>341 (26)</td>
<td>26 (10)</td>
<td>26 (10)</td>
<td>31 (12)</td>
<td>26 (8)</td>
</tr>
<tr>
<td></td>
<td>V + iNO</td>
<td>27 (6)</td>
<td>29 (7.9)</td>
<td>35 (12)</td>
<td>34 (15)</td>
<td></td>
</tr>
<tr>
<td>Plasma vinpocetine concentration, ng/ml</td>
<td>iNO</td>
<td>221 (74)</td>
<td>245 (123)</td>
<td>214 (113)</td>
<td>216 (91)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (SD), \(n = 9\). iNO: inhaled nitric oxide; V, intravenously infused vinpocetine; T\(_{1/2}\), the half-time of the decay of pulmonary vasodilation. *\(P < 0.05\) vs. baseline PH; †\(P < 0.05\) vs. iNO group. For further information, see Table 1.
investigations in the same model (5, 18). Importantly, the intravenous infusion of vinpocetine enabled lower doses of iNO (2–10 ppm) to reduce venous admixture and thereby improve arterial oxygenation. These results suggest that by reducing pulmonary cGMP degradation, vinpocetine augments the iNO-induced increase of blood flow in better-ventilated lung areas.

Enhancement of iNO-induced pulmonary vasodilation and transpulmonary cGMP release has previously been reported following coadministration of zaprinast or sildenafil in the lamb model and patients with PH (16–18, 25). In addition, zaprinast markedly prolonged the duration of the pulmonary vasodilation after iNO was discontinued (16, 17). In contrast, vinpocetine had no effect on the duration of the iNO-induced pulmonary vasodilation in the present study. The latter observation suggests that other PDEs modulate the duration of iNO action. Overall, our findings indicate that the effectiveness of vinpocetine in influencing pulmonary hemodynamics, gas exchange, and hydrolysis of cGMP only becomes evident during NO inhalation, i.e., only when large amounts of cGMP accumulate in the pulmonary vasculature.

Recent evidence suggests that upregulation of PDE1A expression may be one of the key mechanisms responsible for impairing responsiveness to NO-releasing drugs, at least, in the systemic vasculature (20, 32). For example, in a nitrate-tolerant rat model induced by chronic nitroglycerin treatment, PDE1A enzyme activity, protein levels, and mRNA expression were found to be all increased. Correspondingly, vinpocetine increased the sensitivity of tolerant aortic rings to subsequent nitroglycerin exposure (20). It is well described that a significant number of patients with respiratory failure and/or PH do not respond to iNO with pulmonary vasodilation (19). Although increased expression of PDE1 isozymes has not been demonstrated in the pulmonary vasculature of patients with PH, it is conceivable that upregulation of PDE1 in the pulmonary circulation might contribute to the reduced responsiveness to iNO in such patients. If that were the case, inhibition of PDE1 might improve pulmonary vascular responsiveness to iNO. This hypothesis merits further investigation in the experimental and clinical settings.

To conclude, three PDE1 isozymes (PDE1A, PDE1B, and PDE1C) are expressed in ovine pulmonary vasculature. Selective inhibition of PDE1 by systemically administered vinpocetine does not attenuate acute PH in awake lambs. However, vinpocetine infusion augments the pulmonary vasodilatory response and transpulmonary cGMP release following NO inhalation without altering its pulmonary selectivity. Our study suggests that selective inhibition of PDE1 may represent a useful adjuvant to enhance the efficacy of iNO therapy in subjects with PH.

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DISCLOSURES

Massachusetts General Hospital has licensed patents covering the inhalation of nitric oxide to INO Therapeutics, a division of Linde Gas Therapeutics, and Dr. Zapol receives a portion of the royalties. Dr. Zapol and Dr. Bloch are members of the Scientific Advisory Board of INO Therapeutics Inc. Dr. Pethe and Dr. Vas are full-time employees of Gedeon Richter Ltd.

REFERENCES


