

# Organic mononitrites of 1,2-propanediol act as an effective NO-releasing vasodilator in pulmonary hypertension and exhibit no cross-tolerance with nitroglycerin in anesthetized pigs

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**Purpose:** Clinically available intravenous (IV) nitric oxide (NO) donor drugs such as nitroglycerin (GTN) cause systemic hypotension and/or tolerance development. In a porcine model, novel NO donor compounds – the organic mononitrites of 1,2-propanediol (PDNO) – were compared to GTN with regard to pulmonary selectivity and tolerance development. The vasodilatory effects of inorganic nitrite were investigated.

**Materials and methods:** In anesthetized piglets, central hemodynamics were monitored. At normal pulmonary vascular resistance (PVR), IV infusions of PDNO (15–60 nmol kg<sup>-1</sup> min<sup>-1</sup>), GTN (13–132 nmol kg<sup>-1</sup> min<sup>-1</sup>), and inorganic nitrite (dosed as PDNO) were administered. At increased PVR (by U46619 IV), IV infusions of PDNO (60–240 nmol kg<sup>-1</sup> min<sup>-1</sup>) and GTN (75–300 nmol kg<sup>-1</sup> min<sup>-1</sup>) before and after a 5 h infusion of GTN (45 nmol kg<sup>-1</sup> min<sup>-1</sup>) were given.

**Results:** At normal PVR, PDNO (n=12) and GTN (n=7) caused significant dose-dependent decreases in mean systemic and pulmonary arterial pressures, whereas inorganic nitrite (n=13) had no significant effect. At increased PVR, PDNO (n=6) and GTN (n=6) significantly decreased mean systemic and pulmonary pressures and resistances, but only PDNO reduced the ratio between pulmonary and systemic vascular resistances significantly. After the 5 h GTN infusion, the hemodynamic response to GTN infusions (n=6) was significantly suppressed, whereas PDNO (n=6) produced similar hemodynamic effects to those observed before the GTN infusion.

**Conclusion:** PDNO is a vasodilator with selectivity for pulmonary circulation exhibiting no cross-tolerance to GTN, but GTN causes non selective vasodilatation with substantial tolerance development in the pulmonary and systemic circulations. Inorganic nitrite has no vasodilatory properties at relevant doses.

**Keywords:** nitrites, nitrates, nitric oxide donors, tachyphylaxis, PDNO

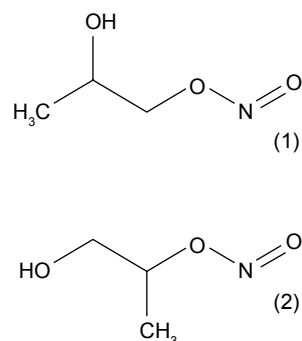
## Introduction

Pulmonary hypertension is a common finding in medical intensive care unit patients and is associated with increased mortality.<sup>1</sup> One-third of the patients with refractory acute respiratory distress syndrome have echocardiographic findings showing isolated pulmonary hypertension, and one-third exhibit findings of pulmonary hypertension and right ventricle dilatation; the latter was associated with poor outcomes.<sup>2</sup> Acute pulmonary hypertension arises from mechanical obstruction of the pulmonary vessels and from pulmonary vasoconstriction.<sup>3</sup> Indeed, pulmonary hypertension, regardless of etiology, threatens the integrity of the circulatory system since right ventricle insufficiency and failure occasionally occur.<sup>4</sup> The vicious cycle of pulmonary hypertension

and right ventricle failure involves systemic hypotension, low cardiac output (CO), diminished right coronary flow due to decreased pressure gradient, and right ventricle ischemia.<sup>5</sup> In addition, dilatation of the right ventricle compromises left ventricular function.<sup>6,7</sup> Consequently, relieving the afterload of the right ventricle is essential to restore circulation in severe cases of acute pulmonary hypertension.<sup>4</sup>

A vasodilator to be used in severe pulmonary hypertension must be effective in pulmonary circulation while having limited vasodilatory effects in systemic circulation, as systemic hypotension may be harmful.<sup>4</sup> Inhaled agents such as nitric oxide (NO) restrict their vasodilatory effects to the pulmonary circulation and are relatively effective in treating certain conditions.<sup>8,9</sup> However, an intravenous (IV) vasodilator is needed in conditions in which pulmonary vasodilation would be beneficial in larger pulmonary arteries, where inhaled agents cannot reach.<sup>10,11</sup> In contrast to inhaled vasodilators, IV vasodilators such as sodium nitroprusside and nitroglycerin (GTN) often also reach systemic arteries, resulting in systemic hypotension.<sup>12,13</sup> Thus, it is of clinical interest to search for and develop an optimal pharmacological approach to acute pulmonary vasoconstriction in various conditions where inhaled agents are insufficient. Previously, Adrie et al<sup>14</sup> found that by utilizing an ultrafast-releasing NO donor given intravenously, vasodilatation could be limited to the pulmonary circulation. Although there are a considerable number of experimental NO donor substances, only a few are clinically available, such as a few organic nitrates and sodium nitroprusside. Novel NO donor compounds for clinical use are lacking.<sup>15</sup> A significant drawback with organic nitrates such as GTN is the development of tolerance, most likely linked to successively decreased NO generation.<sup>16</sup> Despite tolerance development, organic nitrates – particularly GTN – are effective and recommended antianginal medications.<sup>17</sup> Certain NO donors, notably organic nitrites, seem to provoke less development of tolerance possibly due to a better maintained NO generation during infusions in vivo.<sup>18–20</sup> Recently, we synthesized novel NO donors for IV infusion, the organic mononitrites of 1,2-propanediol (PDNO, Figure 1), which were potent vasodilators, especially in the pulmonary circulation.<sup>21</sup>

In the present study, it was hypothesized that PDNO is more selective for pulmonary versus systemic circulation than GTN and that there is no cross-tolerance between PDNO and GTN, while GTN strongly develops tolerance to itself in both pulmonary and systemic circulations. In a porcine model of pharmacologically increased pulmonary vascular resistance (PVR), the systemic and pulmonary hemodynamic



**Figure 1** Chemical structures of the organic mononitrites of 1,2-propanediol.

**Note:** (1) 1-(Nitrosooxy)-propan-2-ol (CAS number 950478-72-5) and (2) 2-(nitrosooxy)-propan-1-ol (CAS number 950478-73-6).

effects of IV-infused PDNO and GTN were compared to investigate the potency of the respective NO donor compounds in these circulations. The infusions of PDNO and GTN in the same model after a 5 h infusion of GTN were repeated to investigate the tolerance and cross-tolerance profiles between PDNO and GTN. The circulatory effects of PDNO, inorganic nitrite, and GTN at normal PVR were also compared, since inorganic nitrite has been proposed to exhibit vasodilatory properties.

## Materials and methods

The study was approved by the local animal ethics committee (II Local Ethical Committee for Experiments on Animals, Wrocław University of Environmental and Life Sciences, Wrocław, Poland) and conducted in accordance with the European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.<sup>22</sup> The experiments were carried out at the Department of Reproduction and Clinic of Farm Animals, Wrocław University of Environmental and Life Sciences.

## Anesthesia and surgical preparation

Domestic piglets ( $n=38$ ; 3–4 months old; body weight range: 15–27 kg) were fasted for the night before the experiments but had free access to water. The procedures for anesthesia and catheter instrumentation (intubation, carotid arterial line, central venous catheter, pulmonary artery catheter, peripheral IV catheter in an ear vein, and urinary catheter through a minilaparotomy) and basic care were recently described.<sup>23,24</sup> In brief, anesthesia was induced with zolazepam ( $4 \text{ mg kg}^{-1}$  intramuscular [IM]; Zoletil forte®, Virbac, Carros, France), tiletamine ( $4 \text{ mg kg}^{-1}$  IM; Zoletil forte), and medetomidine ( $0.08 \text{ mg kg}^{-1}$  IM; Domitor vet®, Orion Pharma, Espoo, Finland). Anesthesia was maintained

with propofol ( $3\text{--}6\text{ mg kg}^{-1}\text{ h}^{-1}$  IV; Fresenius Kabi Poland, Warsaw, Poland) and fentanyl ( $0.8\text{--}1.3\text{ }\mu\text{g kg}^{-1}\text{ h}^{-1}$  IV; Polfa, Warsaw, Poland). The higher rate was infused during surgery for the toleration of instrumentation and then lowered during the postoperative study period. Anesthetic depth was monitored, and additional IV bolus doses of fentanyl ( $25\text{ }\mu\text{g}$ ) and propofol ( $10\text{ mg}$ ) were administered when needed. The animals were ventilated in the pressure-controlled mode (Servo 300 ventilator; Siemens-Elma, Solna, Sweden) at  $5\text{ cm H}_2\text{O}$  positive end-expiratory pressure. The inspiratory pressure ( $13\text{--}30\text{ cm H}_2\text{O}$ ) and frequency ( $15\text{--}30\text{ min}^{-1}$ ) were adjusted to maintain normoventilation. The inspired fraction of oxygen ( $\text{FiO}_2$ ) was adjusted ( $0.21\text{--}0.40$ ) to maintain adequate oxygenation of arterial blood. All animals received cefuroxime ( $500\text{ mg IV}$ ; Zinacef®, GlaxoSmithKline plc, London, UK) before instrumentation. After instrumentation, the animals received heparin ( $2,000\text{ IU IV}$ ) and were allowed a  $1\text{ h}$  intervention-free period to reach stable baseline values. After the experiments, the animals were killed using sodium pentobarbital ( $80\text{ mg kg}^{-1}\text{ IV}$ ; Morbital®, Biovet, Pulawy, Poland).

## Hemodynamic and respiratory monitoring

The animals were monitored as previously described.<sup>23,24</sup> Invasive mean systemic arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), heart rate (HR), peripheral oxygen saturation,  $\text{FiO}_2$ , and end-tidal carbon dioxide concentration were continuously monitored. Pulmonary capillary wedge pressure (PCWP) was measured intermittently by occlusion of the pulmonary artery catheter. CO was obtained intermittently by using the thermodilution technique (AS/3, Datex, Helsinki, Finland). Body temperature was monitored via the pulmonary artery catheter thermistor, and the animals were kept normothermic ( $37^\circ\text{C}\text{--}38^\circ\text{C}$ ) using heated blankets.

## Laboratory analyses

Immediate analyses of blood gases, hemoglobin, and methemoglobin (ABL520, Radiometer A/S, Copenhagen, Denmark) were conducted.

## Calculations

Cardiac index was calculated by dividing CO by body surface area.<sup>25</sup> Oxygen saturation was calculated as  $\text{PO}_2^{2.94}/(\text{PO}_2^{2.94} + \text{P}_{50}^{2.94})$ . A value of  $4.76\text{ kPa}$  was used as the porcine

partial pressure of oxygen ( $\text{PO}_2$ ), where hemoglobin was half saturated ( $\text{P}_{50}$ ) and adjusted with the fixed acid Bohr coefficient.<sup>26</sup> Right ventricle rate pressure product (RV RPP) was calculated as MPAP times HR, and systemic vascular resistance index (SVRI) and pulmonary vascular resistance index (PVRI) were calculated with the standard formula.<sup>27</sup>

## Protocol dose–response experiments at normal PVR

Cumulative IV infusions ( $5\text{ min}$  at each dose, using syringe pumps into a carrier flow of glucose/saline of  $15\text{ mL kg}^{-1}\text{ h}^{-1}$ ) of the respective substances (PDNO at  $15\text{--}60\text{ nmol kg}^{-1}\text{ min}^{-1}$ , 1,2-propanediol+ $20\text{ mM}$  inorganic nitrite at doses corresponding to PDNO at  $45\text{--}60\text{ nmol kg}^{-1}\text{ min}^{-1}$ , and GTN at  $13\text{--}132\text{ nmol kg}^{-1}\text{ min}^{-1}$ ) were conducted. Hemodynamic parameters were investigated in each group.

## Protocol dose–response and GTN tolerance experiments at increased PVR

Pulmonary hypertension was induced by a continuous IV infusion of the thromboxane  $\text{A}_2$  mimetic U46619 (dissolved to  $30\text{ }\mu\text{g mL}^{-1}$  in saline). After reaching a stable pulmonary hypertension (MPAP of  $\sim 35\text{ mmHg}$  at a required U46619 dose of  $75\text{--}150\text{ ng kg}^{-1}\text{ min}^{-1}$ ), either PDNO ( $60, 120,$  and  $240\text{ nmol kg}^{-1}\text{ min}^{-1}$ ) or GTN ( $75, 150,$  and  $300\text{ nmol kg}^{-1}\text{ min}^{-1}$ ) was infused using IV syringe pumps at increasing doses into a carrier flow of  $10\text{ mL}^{-1}\text{ kg}^{-1}$ . Each dose was infused over  $2\text{ min}$  and administered at  $12\text{ min}$  intervals, during which the hemodynamic parameters recovered to similar values to those noted prior to the NO donor infusion. After completing three doses of either PDNO or GTN, the animals recovered over  $15\text{--}30\text{ min}$ , and then the other NO donor (PDNO or GTN) was infused IV as described earlier. The order of the NO donors was randomized. This was followed by a  $5\text{ h}$  infusion of GTN at  $45\text{ nmol kg}^{-1}\text{ min}^{-1}$ . At the end of this infusion, the described procedure was repeated (ie, induction of pulmonary hypertension by U46619 [ $75\text{--}150\text{ ng kg}^{-1}\text{ min}^{-1}$ ]), and the responses to  $2\text{ min IV}$  infusions of the three doses of PDNO and GTN were investigated at  $12\text{ min}$  intervals with a  $15\text{--}30\text{ min}$  period in between the NO donors. Ventilation was adjusted to keep end-tidal carbon dioxide at  $5.0\%$ . The animals received a continuous saline IV infusion ( $10\text{ mL kg}^{-1}\text{ h}^{-1}$ ), which was also the carrier flow for the drugs throughout the experiment.

Full hemodynamic data including cardiac index and blood gases were collected at baseline, during U46619 prior to NO donor infusion, and at the end of the largest dose of the NO donors in the presence of U46619 infusion. In addition,

MPAP, MAP, and HR were recorded at the end of each dose of the NO donors.

## Drugs

All chemicals were of analytical grade and obtained from Sigma-Aldrich (St Louis, MO, USA). U46619 was from Cayman Chemicals (Larodan Fine Chemicals AB, Malmö, Sweden). GTN was from Schwarz Pharma AG (Monheim, Germany). PDNO was prepared according to Nilsson et al.<sup>21</sup> Other drugs, anesthetics, and infusion fluids were obtained as indicated in the “Materials and methods” section.

## Statistical analysis

Data are expressed as mean  $\pm$  standard error of the mean. Normality was tested with the Shapiro–Wilk test, which indicated that the data followed approximate normal distribution. One-way repeated analysis of variance (ANOVA) was used in the dose–response experiments for comparisons in the respective groups, where dose was the repeated factor (Table 1, Figure 2 for GTN). Two-way repeated ANOVA was used for intragroup and intergroup comparisons in the PDNO and PD+inorganic nitrite groups in Figure 2, where the drug (PDNO or PD+inorganic nitrite) was one factor, and the dose was the repeated factor. Two-way repeated ANOVAs were used in the GTN tolerance experiments where one factor was the different time points (Table 2) or the dose (Figure 3), and the other factor was before or after

the 5 h GTN infusion. One-way repeated ANOVA was used for comparisons of the changes in the PVRI/SVRI ratio by PDNO and GTN (Figure 4). Tukey’s testing was used post hoc for multiple comparisons;  $p < 0.05$  was considered statistically significant. Computer software was used for statistical analyses (SigmaPlot and SigmaStat; Systat Software Inc., San Jose, CA, USA; IBM SPSS Statistics version 22.0 for Windows, IBM Corporation, Armonk, NY, USA).

## Results

### Dose–response experiments at normal PVR

IV infusions of PDNO (15–60 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=12) and GTN (13–132 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=7) caused dose-dependent significant reductions in MPAP and MAP in contrast to PD+inorganic nitrite IV (n=13) in the corresponding doses (Figure 2 and Table 1).

### Dose–response experiments at increased PVR

The pulmonary vasoconstrictor U46619 (75–150 ng kg<sup>-1</sup> min<sup>-1</sup> IV, n=6) induced severe pulmonary hypertension, increased RV RPP, and minor systemic hypertension (Table 2). Short IV infusions (2 min) of PDNO (60, 120, and 240 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=6) and GTN (75, 150, and 300 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=6) showed dose-dependent decreases in MPAP, PVRI, RV RPP, MAP, and SVRI (Figure 3 and

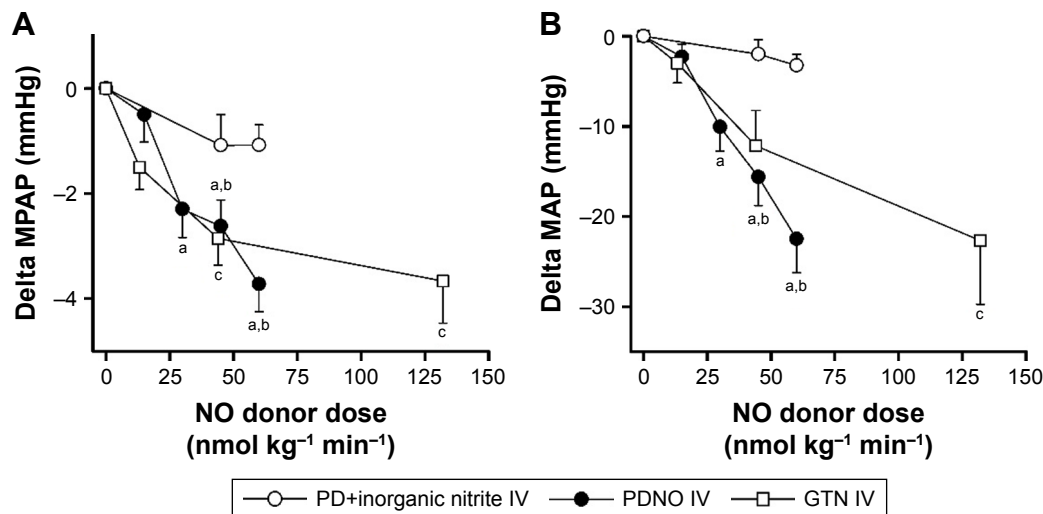
**Table 1** Effects of IV inorganic nitrite, organic nitrite, and nitroglycerin on central hemodynamics

	MAP (mmHg)	CVP (mmHg)	MPAP (mmHg)	PCWP (mmHg)	Cardiac index (L min <sup>-1</sup> m <sup>-2</sup> )	SVRI (mmHg L <sup>-1</sup> min m <sup>2</sup> )	PVRI (mmHg L <sup>-1</sup> min m <sup>2</sup> )	HR (beats min <sup>-1</sup> )
PDNO								
Baseline	91 $\pm$ 3	5 $\pm$ 1	17 $\pm$ 1	6 $\pm$ 1	3.6 $\pm$ 0.3	25 $\pm$ 3	3.1 $\pm$ 0.4	94 $\pm$ 5
15 nmol kg <sup>-1</sup> min <sup>-1</sup>	88 $\pm$ 4	5 $\pm$ 1	17 $\pm$ 1	6 $\pm$ 1	3.5 $\pm$ 0.3	25 $\pm$ 4	2.9 $\pm$ 0.5	87 $\pm$ 7
30 nmol kg <sup>-1</sup> min <sup>-1</sup>	82 $\pm$ 5 <sup>a</sup>	6 $\pm$ 1	15 $\pm$ 1 <sup>a</sup>	6 $\pm$ 1	3.2 $\pm$ 0.2	25 $\pm$ 3	2.8 $\pm$ 0.4	92 $\pm$ 5
45 nmol kg <sup>-1</sup> min <sup>-1</sup>	78 $\pm$ 4 <sup>a</sup>	5 $\pm$ 1	14 $\pm$ 1 <sup>a</sup>	6 $\pm$ 1	3.7 $\pm$ 0.3	22 $\pm$ 3	2.4 $\pm$ 0.3	98 $\pm$ 9
60 nmol kg <sup>-1</sup> min <sup>-1</sup>	68 $\pm$ 4 <sup>a</sup>	6 $\pm$ 1	13 $\pm$ 1 <sup>a</sup>	6 $\pm$ 1	3.5 $\pm$ 0.3	21 $\pm$ 3	2.1 $\pm$ 0.1	92 $\pm$ 5
PD+inorganic nitrite								
Baseline	89 $\pm$ 3	5 $\pm$ 1	17 $\pm$ 1	5 $\pm$ 1	3.9 $\pm$ 0.3	23 $\pm$ 2	3.0 $\pm$ 0.3	93 $\pm$ 5
45 nmol kg <sup>-1</sup> min <sup>-1</sup>	87 $\pm$ 3	4 $\pm$ 1	15 $\pm$ 1	4 $\pm$ 1	4.0 $\pm$ 0.3	23 $\pm$ 2	3.0 $\pm$ 0.4	90 $\pm$ 5
60 nmol kg <sup>-1</sup> min <sup>-1</sup>	86 $\pm$ 3	4 $\pm$ 1	16 $\pm$ 1	4 $\pm$ 1 <sup>b</sup>	3.9 $\pm$ 0.3	22 $\pm$ 2	3.0 $\pm$ 0.3	93 $\pm$ 5
GTN								
Baseline	94 $\pm$ 2	5 $\pm$ 1	17 $\pm$ 1	5 $\pm$ 1	3.2 $\pm$ 0.2	28 $\pm$ 2	3.8 $\pm$ 0.4	85 $\pm$ 9
13 nmol kg <sup>-1</sup> min <sup>-1</sup>	91 $\pm$ 2	5 $\pm$ 1	16 $\pm$ 1	5 $\pm$ 1	3.3 $\pm$ 0.3	27 $\pm$ 3	3.6 $\pm$ 0.4	79 $\pm$ 10
44 nmol kg <sup>-1</sup> min <sup>-1</sup>	82 $\pm$ 4	4 $\pm$ 1	14 $\pm$ 1 <sup>c</sup>	4 $\pm$ 1	3.2 $\pm$ 0.2	25 $\pm$ 3	3.3 $\pm$ 0.4	79 $\pm$ 8
132 nmol kg <sup>-1</sup> min <sup>-1</sup>	71 $\pm$ 8 <sup>c</sup>	4 $\pm$ 1	14 $\pm$ 1 <sup>c</sup>	5 $\pm$ 1	2.9 $\pm$ 0.3	25 $\pm$ 5	3.1 $\pm$ 0.3	82 $\pm$ 10

**Notes:** Hemodynamic parameters at baseline and effects of 5 min IV infusions of the organic mononitrites of 1,2-propanediol (PDNO, n=12), 1,2-propanediol PDNO with 20 mM inorganic nitrite (PD+inorganic nitrite, doses corresponding to PDNO 45 and 60 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=13), and nitroglycerin (GTN, n=7) in ventilated and anesthetized piglets. Superscripted a, b, and c denote statistical differences compared to baseline of PDNO, PD+inorganic nitrite, and GTN infusions, respectively. Data are expressed as mean  $\pm$  standard error of the mean.

**Abbreviations:** IV, intravenous; MAP, mean systemic arterial pressure; CVP, central venous pressure; MPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index; HR, heart rate.





**Figure 2** Effects of intravenous (IV) inorganic nitrite, organic nitrite, and nitroglycerin (GTN) on pulmonary and systemic arterial pressures.

**Notes:** Changes from baseline in mean pulmonary arterial pressure (delta MPAP, **A**) and mean systemic arterial pressure (delta MAP, **B**) by 5 min IV infusions of the organic mononitrites of 1,2-propanediol (PDNO,  $n=12$ ), PDNO with 20 mM inorganic nitrite (PD+inorganic nitrite, corresponding doses to PDNO 45 and 60  $\text{nmol kg}^{-1} \text{min}^{-1}$ ,  $n=13$ ), and nitroglycerin (GTN,  $n=7$ ) in ventilated and anesthetized piglets. a and c indicate statistical differences from baseline at indicated doses in PDNO and GTN groups, respectively. b denotes a statistical difference between PDNO and PD+inorganic nitrite groups at indicated doses. Data are expressed as mean  $\pm$  standard error of the mean.

Table 2). PDNO decreased the PVRI/SVRI ratio in contrast to GTN (Figure 4). Neither infusion affected arterial blood gases (data not shown).

### Dose–response experiments at increased PVR after a 5 h GTN infusion

After the 5 h infusion of GTN ( $45 \text{ nmol kg}^{-1} \text{min}^{-1}$ ), hemodynamic parameters (Table 2) and blood gases (data not shown) were similar compared to baseline values. U46619 ( $75\text{--}150 \text{ ng kg}^{-1} \text{min}^{-1} \text{IV}$ ,  $n=6$ ) induced severe pulmonary hypertension and increased RV RPP (Table 2). The short IV infusions of PDNO (60, 120, and  $240 \text{ nmol kg}^{-1} \text{min}^{-1}$ ) and GTN (75, 150, and  $300 \text{ nmol kg}^{-1} \text{min}^{-1}$ ) caused significant decreases in MPAP, PVRI, MAP, and SVRI (Figure 3 and Table 2). PDNO decreased MPAP and MAP to similar levels to those obtained before the GTN tolerance infusion, whereas the effects of GTN on MPAP and MAP were significantly less compared to the values obtained before the 5 h GTN infusion (Figure 3). Only PDNO decreased RV RPP (Table 2). Neither infusion affected arterial blood gases (data not shown).

### Discussion

In the present study, it was demonstrated that a novel NO donor, PDNO, is a potent vasodilator with enhanced selectivity toward pulmonary circulation but no major cross-tolerance to GTN. In comparison, GTN is a non-selective vasodilator exhibiting pronounced tolerance to itself in

both pulmonary and systemic circulations. It was also found that inorganic nitrite has no vasodilatory effects at doses corresponding to PDNO and GTN, showing the weak potency of this compound.

This report did not investigate the mechanism of the vasodilation produced by PDNO, but we recently found in rabbits that PDNO was an effective vasodilator at increased PVR (induced by U46619) by releasing NO, evidenced by a concomitant increase of NO in exhaled gas.<sup>21</sup> Furthermore, PDNO belongs chemically to the organic nitrites, which are known to be vasodilators by releasing NO.<sup>20</sup>

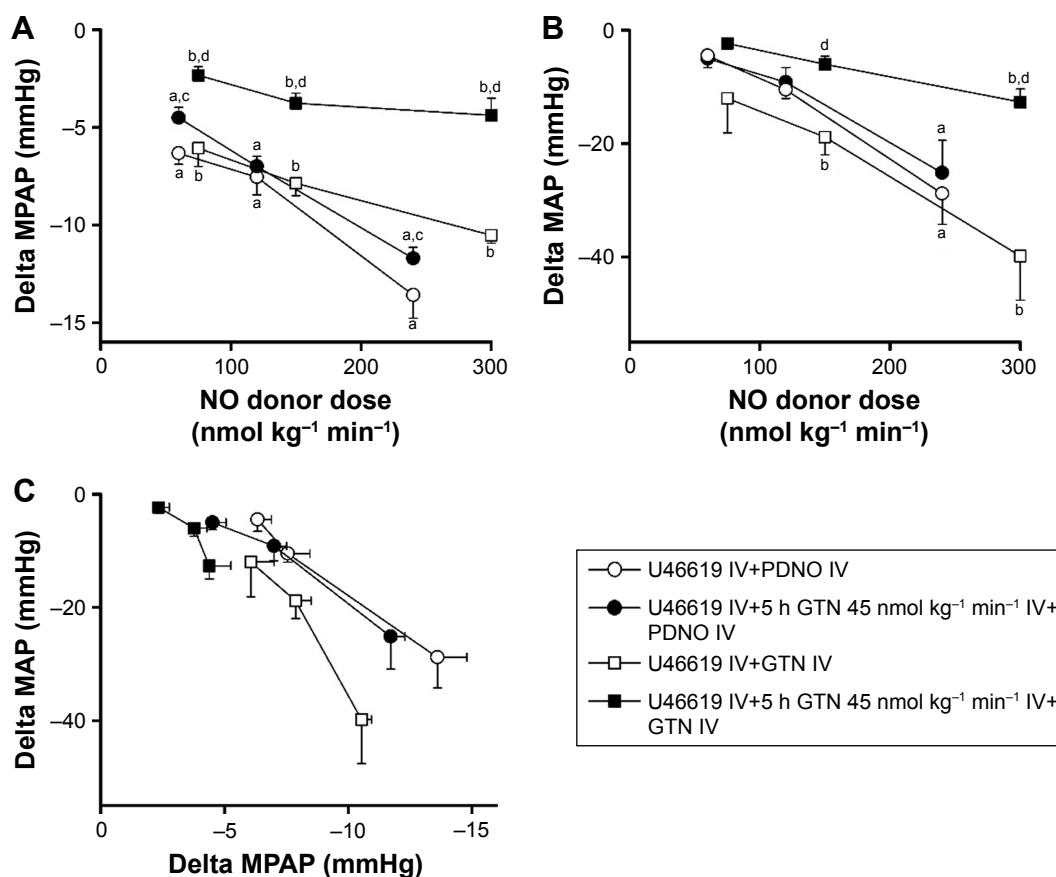
Previously, tolerance development by GTN has been shown in systemic circulation and in pulmonary NO formation from GTN<sup>16,28–30</sup> but not in pulmonary hemodynamics. In addition to confirming the development of systemic tolerance by GTN, the present study showed that the pulmonary vasodilatory response by GTN was also associated with some tolerance development. Tolerance development in pulmonary circulation was shown in MPAP and RV RPP, whereas the PVRI response was inhibited to a lesser degree after the 5 h GTN infusion (GTN decreased PVRI by 50% and 37% before and after the 5 h GTN infusion, respectively). The weaker inhibition of PVRI was mainly due to the opposing effects on PCWP, in that GTN decreased PCWP by 1 mmHg before the 5 h GTN infusion but increased PCWP by 3 mmHg after the 5 h GTN infusion (Table 2). Thus, PCWP affected the transpulmonary pressure gradient in opposing directions in the two experimental conditions,

Table 2 Effects of IV organic nitrite and nitroglycerine (GTN) in pulmonary hypertension, before and after GTN tolerance infusion

	MAP (mmHg)	CVP (mmHg)	MPAP (mmHg)	PCWP (mmHg)	Cardiac index (L min <sup>-1</sup> m <sup>-2</sup> )	SVRI (mmHg L <sup>-1</sup> min m <sup>2</sup> )	PVRI (mmHg L <sup>-1</sup> min m <sup>2</sup> )	HR (beats min <sup>-1</sup> )	RV RPP (mmHg beats min <sup>-1</sup> )
Before GTN tolerance infusion									
Baseline #1	86±5	7±1	18±1	8±1	3.7±0.4	24±3	2.9±0.3	83±6	1,500±100
U46619 before PDNO #1	103±6 <sup>a</sup>	9±1	37±3 <sup>a</sup>	8±1	2.9±0.9 <sup>a</sup>	36±5 <sup>a</sup>	1.1±1.7 <sup>a</sup>	92±3	3,400±200 <sup>a</sup>
U46619+PDNO 240 nmol kg <sup>-1</sup> min <sup>-1</sup> #1	74±7 <sup>c</sup>	6±1 <sup>c</sup>	24±2 <sup>c</sup>	7±1	3.2±0.4	24±4 <sup>c</sup>	5.7±1.1 <sup>c</sup>	89±3	2,100±200 <sup>c</sup>
U46619 before GTN #1	102±9 <sup>b</sup>	9±1	35±3 <sup>b</sup>	9±1	2.6±0.3 <sup>b</sup>	39±6 <sup>b</sup>	10.8±1.6 <sup>b</sup>	88±4	3,100±300 <sup>b</sup>
U46619+GTN 300 nmol kg <sup>-1</sup> min <sup>-1</sup> #1	62±4 <sup>d</sup>	6±1	24±2 <sup>d</sup>	8±1	3.1±0.4	20±3 <sup>d</sup>	5.9±1.1 <sup>d</sup>	89±4	2,200±200 <sup>d</sup>
After GTN 45 nmol kg <sup>-1</sup> min <sup>-1</sup> tolerance infusion									
Baseline #2	86±6	7±1	17±1	8±1	3.7±0.4	23±3	2.5±0.2	78±7	1,400±200
U46619 before PDNO #2	99±5	8±1	36±2 <sup>a</sup>	10±1	3.3±0.4	30±4	8.7±1.2 <sup>a</sup>	91±4	3,300±200 <sup>a</sup>
U46619+PDNO 240 nmol kg <sup>-1</sup> min <sup>-1</sup> #2	73±6 <sup>c</sup>	6±1	25±2 <sup>c</sup>	8±1	4.3±0.5 <sup>c,e</sup>	17±2 <sup>c</sup>	4.1±0.8 <sup>c</sup>	99±6	2,500±300 <sup>c</sup>
U46619 before GTN #2	92±6	9±1	34±2 <sup>b</sup>	6±1	3.2±0.7	31±6	9.5±1.6 <sup>b</sup>	86±6	2,900±100 <sup>b</sup>
U46619+GTN 300 nmol kg <sup>-1</sup> min <sup>-1</sup> #2	79±5 <sup>f</sup>	7±1	30±2 <sup>f</sup>	9±1	4.0±0.6 <sup>d,f</sup>	20±3 <sup>d</sup>	5.9±1.0 <sup>d</sup>	90±6	2,600±100

**Notes:** Hemodynamic parameters at baseline and effects of U46619 (75–150 ng kg<sup>-1</sup> min<sup>-1</sup> IV), of the organic mononitrites of 1,2-propanediol (PDNO, 240 nmol kg<sup>-1</sup> min<sup>-1</sup> IV), and of GTN (300 nmol kg<sup>-1</sup> min<sup>-1</sup> IV) before (#1) and after (#2) the GTN tolerance infusion (45 nmol kg<sup>-1</sup> min<sup>-1</sup> IV for 5 h) in ventilated and anesthetized piglets (n=6). Superscripted a and b denote statistical differences between baseline and U46619 prior to PDNO and GTN infusions, respectively (ie, describe the effects of the U46619 infusion). Superscripted c and d denote a statistical difference between U46619 prior to PDNO and GTN infusions, respectively (ie, describe the effects of the NO donors in the presence of U46619). Superscripted e and f denote a statistical difference before (#1) and after (#2) the GTN tolerance infusion at U46619+PDNO and GTN infusions, respectively (ie, describe the effects of the GTN tolerance infusion on the response to PDNO and GTN). Data are expressed as mean ± standard error of the mean.

**Abbreviations:** IV, intravenous; MAP, mean systemic arterial pressure; CVP, central venous pressure; MPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index; HR, heart rate; RV RPP, right ventricle rate pressure product.



**Figure 3** Organic nitrite and nitroglycerine (GTN) in pulmonary hypertension, before and after GTN tolerance infusion.

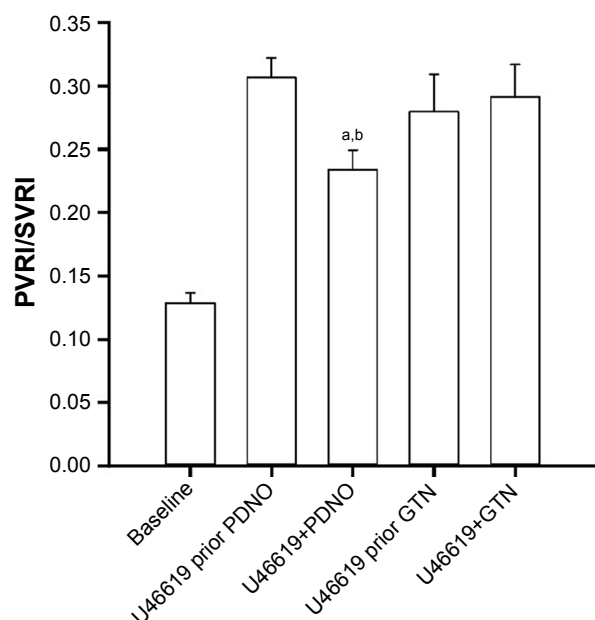
**Notes:** Changes in mean pulmonary arterial pressure (MPAP, **A**) and mean systemic arterial blood pressure (MAP, **B**) by intravenous (IV) infusions (2 min of each dose with 12 min interval) of the organic mononitrites of 1,2-propanediol (PDNO) at 60, 120, and 240 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=6 and GTN (at 75, 150, and 300 nmol kg<sup>-1</sup> min<sup>-1</sup>, n=6) in pharmacologically induced pulmonary hypertension (induced by the thromboxane A<sub>2</sub> mimetic, U46619, at 75–150 ng kg<sup>-1</sup> min<sup>-1</sup> IV) in anesthetized and ventilated piglets before and after a 5 h GTN infusion at 45 nmol kg<sup>-1</sup> min<sup>-1</sup>. Panel (**C**) showing delta MPAP versus delta MAP at the three doses of the NO donors merges (**A**) and (**B**). a and b denote statistical change from 0 at the respective doses in PDNO and GTN groups. c and d denote statistical differences at the respective doses before and after the 5 h GTN infusion in PDNO and GTN groups (open symbols versus filled symbols). Data are expressed as mean ± standard error of the mean.

leading to an exaggerated PVRI response to GTN after the 5 h GTN infusion.

Previous studies and our preliminary data suggest that organic nitrites, in contrast to organic nitrates, are devoid of tolerance development.<sup>18,19</sup> The present study showed limited cross-tolerance between GTN and PDNO. Previously, ample cross-tolerance between several organic nitrates has been shown,<sup>16,31</sup> whereas the cross-tolerance between organic nitrites and organic nitrates was limited.<sup>19,32</sup> Several mechanisms, which may coexist, have been proposed to explain different aspects of GTN tolerance, including inactivation of the bioactivation pathway, neurohormonal activation, desensitization of soluble guanylyl cyclase, and supersensitization to vasoconstrictors.<sup>33</sup> A tentative explanation of the differences in tolerance development and the lack of substantial cross-tolerance between organic nitrites and organic nitrates involves the distinct bioactivation mechanisms of organic nitrites and organic nitrates. GTN is thought to be

bioactivated by mitochondrial aldehyde dehydrogenase,<sup>34,35</sup> although other enzymes can also convert GTN to NO.<sup>33</sup> It has been suggested that organic nitrite bioactivation occurs via glutathione transferases.<sup>36–38</sup> GTN is a recommended symptomatic therapy for angina pectoris,<sup>17</sup> but tolerance development may be an obstacle to long-term infusions of GTN. In clinical use, it is advantageous to use an NO donor like an organic nitrite lacking tolerance development that also fulfills the summary beneficial effects of GTN in the respective disease condition. It has not yet been investigated whether chronic treatment with PDNO causes tolerance.

In contrast to organic nitrites and organic nitrates, inorganic nitrites (NO<sub>2</sub><sup>-</sup>) at the low doses (90–120 nmol kg<sup>-1</sup> min<sup>-1</sup> IV) used in this study were insufficient for vasodilatation both in pulmonary and systemic circulations. This finding is supported by previous work, which has convincingly shown that larger doses of inorganic nitrites are needed for vasodilatation of the pulmonary and systemic vascular beds in various



**Figure 4** Organic nitrite and nitroglycerine (GTN) at increased pulmonary vascular resistance.

**Notes:** Pulmonary vascular resistance index (PVRI) divided by systemic vascular resistance index (SVRI) in pharmacologically induced pulmonary hypertension (induced by the thromboxane  $A_2$  mimetic, U46619, at  $75\text{--}150\text{ ng kg}^{-1}\text{ min}^{-1}$  intravenous [IV]) and effects of IV infusions (2 min) of the organic mononitrites of 1,2-propanediol (PDNO at  $240\text{ nmol kg}^{-1}\text{ min}^{-1}$ ,  $n=6$ ) and GTN ( $300\text{ nmol kg}^{-1}\text{ min}^{-1}$ ,  $n=6$ ) in anesthetized and ventilated piglets. a denotes a statistical difference between U46619 prior to PDNO and U46619+PDNO. b denotes a statistical difference between U46619+PDNO and U46619+GTN. Statistical testing was done on the change in PVRI/SVRI from U46619 infusion only and U46619 infusion in combination with PDNO and GTN infusions, respectively. Data are expressed as mean  $\pm$  standard error of the mean.

species,<sup>39–42</sup> including humans.<sup>43–45</sup> Larger doses of inorganic nitrites, which are necessary for vasodilatation, also cause increased levels of methemoglobin.<sup>39,40,43–45</sup> Since methemoglobinemia may be an adverse effect of some organic nitrites,<sup>46–49</sup> methemoglobin was monitored in the present study, yielding normal values (data not shown). Since the infusions of PDNO in the present study had a short duration, it cannot be excluded that a long-term infusion of PDNO causes methemoglobinemia. However, 6 h infusions of PDNO in a sheep model of renal ischemia reperfusion injury caused only a minor increase (0.3%) in methemoglobin levels.<sup>50</sup>

The present experiments were done both at normal PVR and at increased PVR. Acute pulmonary hypertension during intensive care can lead rapidly to circulatory failure, and reducing the afterload of the right ventricle (ie, pulmonary vasodilation) remains an important treatment strategy.<sup>4</sup> Treatment with only inhaled agents may be insufficient since larger pulmonary arteries are not reached by inhalation, in contrast to IV agents.<sup>10</sup> When using IV NO donors, a delicate balance exists between satisfactory pulmonary NO delivery and harmful systemic hypotension. The PVRI/SVRI ratio is a means

of examining pulmonary versus systemic vasodilatation that has been used when investigating selective pulmonary vasodilatation by inhaled NO.<sup>51</sup> PDNO decreased the PVRI/SVRI ratio (ie, increased selectivity for the pulmonary circulation), but GTN did not affect the PVRI/SVRI ratio (ie, no selectivity). We acknowledge that PDNO also had systemic effects, especially at higher doses. Several mechanisms may lie behind the selectivity of PDNO for pulmonary circulation. Previously, it has been shown that the vasodilatation of an NO donor given intravenously could be confined to the pulmonary circulation by using an NO donor with an extremely short half-life,<sup>14,52</sup> which also may be a viable mechanism for PDNO. Organic nitrites hydrolyze rapidly in neutral aqueous solutions, promoting a short half-life in blood.<sup>53</sup> Preliminary unpublished data in rabbits indicate a significant difference in pulmonary NO generation from PDNO, measured as exhaled NO, when administering PDNO intravenously (ie, large increase in NO concentration in exhaled gas) compared to in the left ventricle (minor increase in the NO concentration in exhaled gas). These data indicate a rapid disappearance of PDNO from the circulation. Furthermore, we recently discovered that organic nitrites differ in pharmacodynamics.<sup>21</sup> We suggested that the relative efficacy of different organic nitrite molecules in pulmonary versus systemic circulations was determined by their molecular properties (ie, the less polar the molecule, the more effective in the pulmonary circulation).<sup>21</sup> However, we also found that the most non-polar of the organic nitrites caused significant amounts of methemoglobin.<sup>21</sup> The polarity of the organic nitrite molecules probably determines cell membrane permeation characteristics and breakdown rates, thus affecting the speed of disappearance from the circulation.<sup>21</sup> In addition, lung tissue has a high capacity for organic nitrite bioactivation.<sup>36</sup>

The study has several limitations. The investigation was only performed in one type of model of pulmonary hypertension, and we used a model in which the PVR was increased by a pharmacological substance (U46619). This model has been extensively used when studying pulmonary hypertension (eg, in the initial experiments with inhaled NO<sup>54</sup>) but may not mimic the full scenario of a clinical disease condition. Furthermore, the present study only uses one animal species, but we have previously shown that PDNO was a vasodilator in another experimental animal (rabbits).<sup>21</sup> Although we suggest these results may be extrapolated to other types of pulmonary vascular constriction and other animal species including humans, such work has yet to be conducted. PDNO was synthesized in our laboratory by a method recently described.<sup>21</sup> The concentration of the active



ingredient was measured,<sup>21</sup> and short-term stability (weeks) is certain (unpublished data).

## Conclusion

PDNO was shown in this study to act as a vasodilator with selectivity for pulmonary circulation, exhibiting no cross-tolerance to GTN. In contrast, GTN caused non-selective vasodilation with substantial tolerance development in both pulmonary and systemic circulations. Inorganic nitrite had no vasodilatory properties at relevant doses. PDNO has the potential to be a novel IV NO donor for use in intensive care patients suffering from acute pulmonary hypertension. Before human exposures can be contemplated, a full preclinical safety program needs to be performed with PDNO.

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## Disclosure

Authors Lars E Gustafsson and Kristofer F Nilsson wish to declare potential financial competing interests due to their roles as co-applicants in two international patents (US 8,552,068, US 8,030,511 and EP 2004576) and co-ownership of Attgeno AB pertaining to the current subject matter. The author Claes Frostell wishes to declare financial interest in the clinical use of inhaled NO. The authors report no other conflicts of interest in this work.

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