Intensive Care Med (1999) 25: 1147–1154

EXPERIMENTAL

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Effect of aerosolized prostacyclin and inhaled nitric oxide on experimental hypoxic pulmonary hypertension

Received: 8 March 1999
Final revision received: 2 July 1999
Accepted: 13 July 1999

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Abstract *Objective*: To compare the effect of different concentrations of inhaled nitric oxide and doses of nebulized prostacyclin on hypoxia-induced pulmonary hypertension in pigs.
Design: Prospective, controlled animal study.
Setting: Animal research facilities of an university hospital.
Interventions: After reducing the fraction of inspired oxygen (FIO_2) from 1.0 to 0.1, two groups of five pigs each were submitted to inhalation of three concentrations of nitric oxide (5, 10 and 20 ppm) or three doses of prostacyclin (2.5, 5, 10 ng × kg^{-1} × min^{-1}).
Results: All doses of prostacyclin and concentrations of nitric oxide resulted in a decrease in mean pulmonary arterial pressure and pulmonary vascular resistance when compared to hypoxic ventilation ($p < 0.001$) which was independent of the dose or concentration of either drug used. While inhalation of nitric oxide caused a reduction in mean pulmonary arterial pressure back to values obtained during ventilation with FIO_2 1.0, values achieved with prostacyclin were still significantly higher when compared to measurements prior to the initiation of hypoxic ventilation. However, direct comparison of the effect of 20 ppm nitric oxide and 10 ng × kg^{-1} × min^{-1} prostacyclin on mean pulmonary arterial pressure revealed no differences between the drugs. All other hemodynamic and gas exchange parameters remained stable throughout the study.
Conclusions: Inhalation of clinically used concentrations of nitric oxide and doses of prostacyclin can decrease elevated pulmonary arterial pressure in an animal model of hypoxic pulmonary vasoconstriction without impairing systemic hemodynamics or gas exchange.

Key words Prostacyclin · Nitric oxide · Hypoxic pulmonary vasoconstriction

Introduction

Hypoxic pulmonary vasoconstriction is an important physiologic adaptation of the pulmonary vascular bed to improve gas exchange in acute and chronic cases of regional or ubiquitous alveolar hypoxia and a decreased arterial and mixed venous oxygen tension [1], such as the acute respiratory distress syndrome (ARDS) [2], asthma, chronic obstructive pulmonary disease (COPD) [3] and one-lung ventilation during thoracic surgery [4]. However, the reduction of blood flow to hypoxic lung segments can contribute to sometimes severe acute pulmonary hypertension, aggravating pulmonary edema [5] and restricting right ventricular performance.

Intravenous use of vasodilators can decrease the elevated pulmonary arterial pressure, however, the concomitant dilation of the systemic vasculature and the increase of pulmonary shunt due to the non-selective re-

laxation of pulmonary vessels of both ventilated and non-ventilated lung segments limit the use of the intravenous route of administering these drugs [6]. Therefore, gaseous or nebulized vasodilators such as nitric oxide and prostacyclin have been proposed to dilate selectively the pulmonary vessels of ventilated lung regions by administering them with the inspiratory gas.

Inhaled nitric oxide (NO) can cause relaxation of the pulmonary vascular smooth muscle cells by stimulating the formation of intracellular cyclic guanosine monophosphate. Its vasodilatory effect is restricted to the lung, because systemically absorbed, intravascular NO is rapidly inactivated by binding to hemoglobin. However, it has been shown that NO is taken up by the lung [7] and dose-dependent extrapulmonary effects of NO, such as platelet inhibition [8] and the formation of toxic metabolites like nitrite (NO_2) and methemoglobin have to be considered when using the drug. Concentrations of 20 to 100 ppm NO have been shown to decrease elevated pulmonary arterial pressures and to improve right ventricular function in experimental settings of hypoxic pulmonary hypertension [9–12].

Prostacyclin (PGI_2), an arachidonic acid metabolite with a short half-life of 2–3 min, is predominantly derived from the endothelium and binds to membrane receptors of the vascular smooth muscle cells where it activates adenylate cyclase which causes vasodilation. Inhalation of PGI_2 aerosol induces selective dilation of the pulmonary vasculature of ventilated lung segments unless overdosing results in a spillover and a subsequent decrease of arterial pressure and an increase in pulmonary shunt. In experimental hypoxic pulmonary hypertension, doses between 1 and 20 $ng \times kg^{-1} \times min^{-1}$ PGI_2 were effective in decreasing elevated pulmonary vascular resistance and in improving right ventricular performance [9, 11, 12]. Unlike NO, no toxic side effects of prostacyclin have been reported, even when given over extended time periods [13]. Therefore, PGI_2 aerosol has been proposed as an alternative to inhaled NO in situations of pulmonary hypertension of various etiologies [14, 15]. However, when doses of 1–20 $ng \times kg^{-1} \times min^{-1}$ of PGI_2 were compared to 20–100 ppm of inhaled NO in settings of hypoxia-induced pulmonary vasoconstriction in healthy animals, NO was found to be more effective in dilating the pulmonary circulation than PGI_2 [9, 11]. Such an experimental comparison has not been performed for doses of NO below 20 ppm, which are usually used in clinical practice. Therefore, we compared the effect of 2.5 to 10 $ng \times kg^{-1} \times min^{-1}$ PGI_2 aerosol to 5 to 20 ppm of inhaled NO on pulmonary arterial pressure and pulmonary vascular resistance in an animal model of acute hypoxic pulmonary vasoconstriction.

Materials and methods

Animal preparation

The experimental protocol was approved by the appropriate governmental institution and the study was performed according to the Helsinki convention for the use and care of animals.

Ten female pigs (weight 30.4 ± 4.1 kg) were included in this study. Prior to the experiments, all animals were examined for the absence of infection. Following premedication with intramuscular azaperone (5–7 $mg \times kg^{-1}$) and atropine (0.01 $mg \times kg^{-1}$), anesthesia was induced with an intravenous injection of metomidate (2 $mg \times kg^{-1}$) via an auricular vein and maintained with a continuous infusion of thiopental (6–10 $mg \times kg^{-1} \times h^{-1}$), fentanyl (0.1 $\mu g \times kg^{-1} \times min^{-1}$) and pancuronium bromide (4–5 $\mu g \times kg^{-1} \times min^{-1}$).

All animals were positioned supine, intubated with an endotracheal tube, internal diameter. 8.0 to 9.0 mm and submitted to volume controlled, mechanical ventilation (Servo 300 A, Siemens Elema, Lund, Sweden). The respirator was set to deliver tidal volumes of 8–10 $ml \times kg^{-1}$, adjusted to maintain an arterial carbon dioxide tension ($PaCO_2$) of 30–40 mmHg at a constant respiratory rate of 20 breaths/min with an inspiration/expiration time ratio of 1:2 throughout the entire experiment. The end-expiratory pressure was adjusted to 5 cmH_2O.

Catheterization of a femoral vein and artery was performed percutaneously. A pulmonary artery catheter (model 93A-431–7.5 F, Baxter Healthcare, Irvine, Calif., USA) was advanced into a pulmonary artery under transduced pressure guidance through an 8.5-Fr sheath positioned in a femoral vein (Arrow Deutschland, Erding, Germany); an 18-G arterial line (Vygon, Ecouen, France) was introduced into a femoral artery.

The blood temperature, measured by means of the pulmonary artery catheter, was maintained at 37.1 ± 1.5 °C during the experiment using an infrared warming lamp and a warming pad. A continuous infusion of 3–5 $ml \times kg^{-1} \times h^{-1}$ of balanced electrolyte solution was administered for adequate hydration.

Data acquisition

Measurements were taken in the supine position according to the experimental protocol with zero reference level at the midchest. Mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) were transduced (PVP, Kirschseeon/Eglharting, Germany) and recorded (Datex, Model CS/3 Compact, Achim, Germany). Cardiac output was determined with standard thermodilution techniques (Baxter Deutschland, Unterschleissheim, Germany) and expressed as the mean of three measurements at end-expiration of different respiratory cycles. Heart rate was traced by the arterial blood pressure curve.

Arterial and mixed venous blood samples were collected anaerobically and immediately analyzed for partial pressure of oxygen and carbon dioxide and pH using standard blood gas electrodes (ABL 520, Radiometer Copenhagen, Denmark). The same device was used for measuring the fractional inspired oxygen (FIO_2). Species-specific spectrophotometry was performed to obtain arterial and mixed venous oxygen saturation, arterial methemoglobin fraction and total hemoglobin concentration (OSM 3 Hemoximeter, Radiometer Copenhagen, Denmark).

Pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) were calculated:

$$PVR = (MPAP - PAOP) \times 80 \times CO^{-1}$$
$$SVR = (MAP - CVP) \times 80 \times CO^{-1}$$

Administration of NO

NO was mixed into the inspiratory gas using the integrated NO application system of the Servo 300 A ventilator from a stock of 800 ppm NO in nitrogen (AGA, Bottrop, Germany). A CLD 700 AL chemiluminometer (ECO Physics, Duernten, Switzerland) and the NO/NO_2 monitoring system of the Servo 300 A ventilator were used for continuous determination of the NO and NO_2 concentrations in the inspiratory limb of the respiratory circuit.

FIO_2 was kept constant during NO inhalation by intermittent measurements of FIO_2 in the inspiratory limb and adaptation of the inspiratory oxygen concentration when necessary.

Administration of PGI_2

PGI_2 was supplied as the sodium salt of epoprostenol (Flolan, Glaxo Wellcome, Hamburg, Germany) dissolved in a glycine buffer of pH 10.5 distributed by the manufacturer at a concentration of 10 $\mu g \times ml^{-1}$. The stock solution was prepared on the day of the experiment and stored on ice until use. Different concentrations of PGI_2 were prepared by further diluting the stock solution with saline immediately before inhalation.

Aerosolization of PGI_2 was performed using the ultrasonic drug nebulizer (Model 6302 595 E 400 E, Siemens Elema, Lund, Sweden) of the respirator. The nebulizer was connected to the Y-piece in the inspiratory limb of the ventilatory circuit and the chamber was filled with 30 ml of each PGI_2 dilution. To assess the amount of PGI_2 delivered with the inspiratory gas, the total volume of the PGI_2 solution in the nebulizer chamber was measured prior to and after nebulization and the fraction of aerosolized PGI_2 was calculated as: actual amount of PGI_2 nebulized = volume nebulized × concentration of the PGI_2 solution/duration of PGI_2 nebulization.

Experimental protocol

After preparing the animals for the experiment, control measurements were performed at FIO_2 of 1.0. Thereafter, hypoxia was induced by decreasing the FIO_2 under intermittent monitoring to 0.09–0.11 by increasing the inspiratory nitrogen concentration. Baseline recordings of all parameters during hypoxia were obtained after stable conditions were achieved. Regarding all animals ($n = 10$), ventilation with the hypoxic gas mixture resulted in a decrease of arterial oxygen tension (PaO_2) and mixed venous oxygen tension (PvO_2) from 521 ± 43 to 42 ± 8 mmHg and from 52 ± 7 to 26 ± 5 mmHg, respectively, with a concomitant increase in MPAP from 16 ± 2 to 30 ± 6 mmHg and of PVR from 173 ± 39 to 368 ± 108 dyne × s × cm^{-5}. Subsequently, the animals were divided randomly into two groups.

In the NO group ($n = 5$), three different concentrations of NO (5, 10 and 20 ppm) were administered for 10 min in increasing order followed by a 10-min time period without NO inhalation to avoid residual effects of the previous concentration. In the PGI_2 group ($n = 5$), three different doses of prostacyclin (2.5, 5 and 10 ng × kg^{-1} × min^{-1}) were nebulized for 10 min in increasing order followed by a 10-min time period without PGI_2 aerosolzation to avoid residual effects of the previous dose. Measurements were obtained prior to and at the end of each NO or PGI_2 inhalation period. At the end of the study, all animals were sacrificed by intravenous administration of potassium chloride.

Statistical analysis

All data are expressed as mean ± standard deviation. Statistical analyses were performed using the NCSS 6.0.7. (Jerry Hintze, 1995) software package. The data within each group were analyzed by analysis of variance (ANOVA) for repeated measurements. For significant ANOVA results, post hoc Bonferroni's multiple comparison test was performed for comparison of study points within one group of animals (NO group, PGI_2 group).

After positively testing for normal distribution, a two-sample *t*-test was used to analyze the hypothesis that there was no difference in MPAP and PVR between the NO group and the PGI_2 group at corresponding control measurements without inhalation of NO or PGI_2.

Since we did not study the complete dose-response curves of each drug, we could not determine and compare the effect of equipotent doses of PGI_2 or concentrations of NO on MPAP and PVR. Therefore, comparison of the effect of both drugs on MPAP and PVR between the two groups was performed only for the highest dose of PGI_2 (10 ng × kg^{-1} × min^{-1}) and the highest concentration of NO (20 ppm), assuming that a maximum effect on the pulmonary vasculature was achieved during this setting. To test the zero hypothesis that there was no difference between the maximum effect of NO and PGI_2 on MPAP and PVR, we performed a two-sample *t*-test after normal distribution of the MPAP and PVR data for each group at the highest dose of PGI_2 and concentration of NO had been confirmed. $p < 0.05$ was considered as significantly different.

Results

All animals survived the entire study period. Prior to the study, no differences were observed between the two groups of animals for body weight (NO group 31.4 ± 3.4 kg, PGI_2 group 29.4 ± 4.8 kg) and total hemoglobin concentration (NO group 8.0 ± 1.0 g × 100 ml^{-1}, PGI_2 group 8.6 ± 1.3 g × 100 ml^{-1}). Hemoglobin remained constant in both groups throughout the study period.

Gas exchange

Blood gas parameters are summarized in Tables 1 and 2. Regarding all animals ($n = 10$), the initial drop in PaO_2 due to the induction of hypoxia (FIO_2 0.09–0.11) resulted in a decrease in mean PvO_2 from 52 ± 7 to 26 ± 5 mmHg. Thereafter, PaO_2 and PvO_2 remained unchanged during continued hypoxic ventilation throughout the entire investigation in both groups.

$PaCO_2$ demonstrated a slight decrease from 38 ± 5 to 35 ± 3 mmHg in all animals ($n = 10$) after FIO_2 was reduced, reaching statistical significance in the NO group when these animals were analyzed alone ($p < 0.001$, Table 2), but with no further changes during hypoxic ventilation with or without inhalation of NO or PGI_2 within either group. PaO_2, PvO_2, $PaCO_2$ and pH revealed no difference between the NO group and the PGI_2-treated animals.

Table 1 Gas exchange and metabolic data for all animals treated with PGI_2 ($n = 5$). Values are mean ± SD (PaO_2 arterial oxygen tension, PvO_2 mixed venous oxygen tension, $PaCO_2$ arterial carbon dioxide tension, *Met-Hb* arterial methemoglobin fraction)

	Baseline FIO_2 1.0	Hypoxia FIO_2 0.1	2.5 ng · $kg^{-1} \cdot min^{-1}$ PGI_2	Hypoxia FIO_2 0.1	5 ng · $kg^{-1} \cdot min^{-1}$ PGI_2	Hypoxia FIO_2 0.1	10 ng · $kg^{-1} \cdot min^{-1}$ PGI_2	Hypoxia FIO_2 0.1
PaO_2 (mm Hg)	542 ± 30	41 ± 9	45 ± 8	39 ± 10	39 ± 7	37 ± 11	37 ± 6	44 ± 4
PvO_2 (mm Hg)	50 ± 10	24 ± 6*	25 ± 4*	23 ± 6*	23 ± 4*	21 ± 6*	22 ± 5*	24 ± 6*
$PaCO_2$ (mm Hg)	36 ± 4	34 ± 3	32 ± 5	32 ± 3	33 ± 3	34 ± 4	34 ± 3	33 ± 3
pH	7.49 ± 0.06	7.53 ± 0.05	7.54 ± 0.04	7.54 ± 0.05	7.54 ± 0.06	7.53 ± 0.06	7.53 ± 0.04	7.53 ± 0.03
Met-Hb (%)	0.86 ± 0.36	0.80 ± 0.44	0.94 ± 0.50	0.76 ± 0.39	0.72 ± 0.36	0.84 ± 0.27	0.72 ± 0.33	0.62 ± 0.24

* $p < 0.001$ when compared to initial ventilation with FIO_2 1.0

Table 2 Gas exchange and metabolic data for all animals treated with NO ($n = 5$). Values are mean ± SD (PaO_2 arterial oxygen tension, PvO_2 mixed venous oxygen tension, $PaCO_2$ arterial carbon dioxide tension, *Met-Hb* arterial methemoglobin fraction)

	Baseline FIO_2 1.0	Hypoxia FIO_2 0.1	5 ppm NO	Hypoxia FIO_2 0.1	10 ppm NO	Hypoxia FIO_2 0.1	20 ppm NO	Hypoxia FIO_2 0.1
PaO_2 (mm Hg)	499 ± 46	43 ± 8	42 ± 8	42 ± 9	39 ± 8	40 ± 13	38 ± 9	41 ± 9
PvO_2 (mm Hg)	54 ± 3	28 ± 5*	27 ± 6*	29 ± 14*	24 ± 8*	24 ± 8*	23 ± 6*	23 ± 5*
$PaCO_2$ (mm Hg)	41 ± 4	35 ± 2*	34 ± 3*	33 ± 3*	34 ± 1*	35 ± 2*	34 ± 0.4*	34 ± 2*
pH	7.49 ± 0.05	7.55 ± 0.04	7.54 ± 0.05	7.56 ± 0.03	7.56 ± 0.03	7.54 ± 0.02	7.55 ± 0.02	7.54 ± 0.05
Met-Hb (%)	0.96 ± 0.59	1.06 ± 0.76	0.86 ± 0.73	1.02 ± 0.81	1.06 ± 0.58	0.82 ± 0.62	1.02 ± 1.00	1.00 ± 0.71

* $p < 0.001$ when compared to initial ventilation with FIO_2 1.0

Table 3 Hemodynamic data for all animals treated with PGI_2 ($n = 5$). Values are mean ± SD (*HF* heart rate, *MAP* mean arterial pressure, *CVP* central venous pressure, *PAOP* pulmonary artery occlusion pressure, *CO* cardiac output, *SVR* systemic vascular resistance)

	Baseline FIO_2 1.0	Hypoxia FIO_2 0.1	2.5 ng · $kg^{-1} \cdot min^{-1}$ PGI_2	Hypoxia FIO_2 0.1	5 ng · $kg^{-1} \cdot min^{-1}$ PGI_2	Hypoxia FIO_2 0.1	10 ng · $kg^{-1} \cdot min^{-1}$ PGI_2	Hypoxia FIO_2 0.1
HF ($l \cdot min^{-1}$)	104 ± 14	107 ± 20	120 ± 26	115 ± 20	118 ± 31	119 ± 27	116 ± 30	125 ± 22
MAP (mm Hg)	107 ± 21	99 ± 20	104 ± 19	104 ± 17	103 ± 18	105 ± 17	105 ± 18	104 ± 18
CVP (mm Hg)	6 ± 2	6 ± 2	6 ± 2	6 ± 2	6 ± 2	6 ± 2	6 ± 2	7 ± 2
PAOP (mm Hg)	6 ± 2	6 ± 3	7 ± 2	6 ± 2	6 ± 3	6 ± 3	7 ± 3	6 ± 3
CO ($l \cdot min^{-1}$)	4.8 ± 0.4	4.4 ± 0.4	4.4 ± 0.3	4.5 ± 0.8	4.6 ± 0.7	4.4 ± 0.6	4.6 ± 0.7	4.7 ± 0.6
SVR ($dyne \cdot s \cdot cm^{-5}$)	1853 ± 338	1783 ± 396	1873 ± 552	1825 ± 607	1733 ± 540	1838 ± 473	1659 ± 432	1650 ± 402

Hemodynamics

Hemodynamic parameters are summarized in Tables 3 and 4. No changes between or within the NO group and the PGI_2 group were observed for heart rate, MAP, CVP, PAOP or SVR throughout the study. Cardiac output was consistently higher in the NO-treated animals, however, with no changes within either group during the study.

Effect of NO and PGI_2 inhalation

Inhalation of all concentrations of NO resulted in a significant but not dose-dependent decrease in MPAP ($p < 0.001$) and PVR ($p < 0.001$) back to levels obtained during ventilation with FIO_2 1.0. There was no effect of inhaled NO on mean methemoglobin fraction nor on the other hemodynamic and gas exchange parameters. After interruption of NO administration, MPAP and PVR returned to values measured prior to NO inhalation (Fig. 1, 2).

Nebulization of PGI_2 aerosol during hypoxic ventilation resulted in a significant, but not dose-dependent, decrease in PVR back to baseline values obtained at FIO_2 1.0 for all doses tested ($p < 0.001$). Similarly, MPAP equally decreased during inhalation of all doses of prostacyclin when compared to phases during hypoxia without PGI_2 application ($p < 0.001$). However, in contrast to the results with inhaled NO, MPAP values were still significantly higher than those measured during ventilation with FIO_2 1.0. No effect of PGI_2 aerosol inhalation on other hemodynamic and gas exchange parameters was observed. After interruption of PGI_2 ad-

Table 4 Hemodynamic data for all animals treated with NO ($n = 5$). Values are mean ± SD (*HF* heart rate, *MAP* mean arterial pressure, *CVP* central venous pressure, *PAOP* pulmonary artery occlusion pressure, *CO* cardiac output, *SVR* systemic vascular resistance)

	Baseline FIO_2 1.0	Hypoxia FIO_2 0.1	5 ppm NO	Hypoxia FIO_2 0.1	10 ppm NO	Hypoxia FIO_2 0.1	20 ppm NO	Hypoxia FIO_2 0.1
HF ($l \cdot min^{-1}$)	114 ± 30	139 ± 39	133 ± 39	135 ± 38	128 ± 33	124 ± 33	130 ± 34	127 ± 47
MAP (mm Hg)	116 ± 28	114 ± 27	92 ± 53	109 ± 32	113 ± 27	109 ± 28	112 ± 29	106 ± 21
CVP (mm Hg)	5 ± 2	6 ± 3	6 ± 4	6 ± 2	6 ± 3	6 ± 3	7 ± 2	6 ± 3
PAOP (mm Hg)	5 ± 3	6 ± 3	5 ± 3	5 ± 3	5 ± 3	5 ± 3	5 ± 4	6 ± 2
CO ($l \cdot min^{-1}$)	5.7 ± 0.7	6.6 ± 1.3	6.8 ± 1.8	6.3 ± 1.8	7.0 ± 1.8	6.9 ± 1.3	6.6 ± 2.2	6.8 ± 2.5
SVR ($dyne \cdot s \cdot cm^{-5}$)	1552 ± 200	1389 ± 608	1140 ± 739	1381 ± 563	1276 ± 466	1227 ± 458	1157 ± 366	1316 ± 600

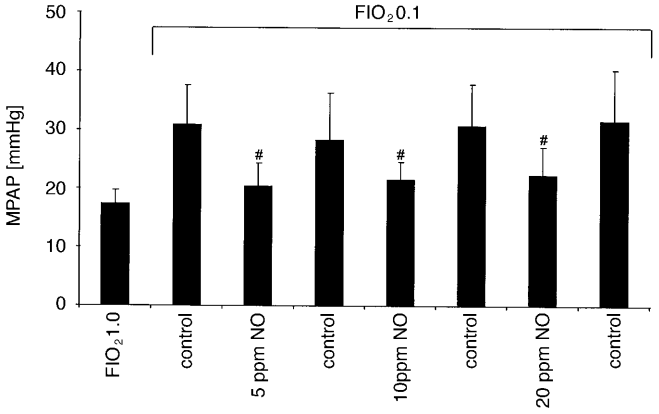

Fig. 1 Regarding the animals of the NO group ($n = 5$), the increase in mean pulmonary arterial pressure *MPAP* due to ventilation with a hypoxic gas mixture was reversed by all concentrations of NO back to values observed during ventilation with FIO_2 1.0. Comparison of the effect of 20 ppm NO and 10 ng × kg^{-1} × min^{-1} PGI_2 revealed no statistically significant difference when using a two-sample *t*-test. All data are presented as mean ± standard deviation. #$p < 0.001$ when compared to hypoxic ventilation with FIO_2 0.1 before and after inhalation of NO

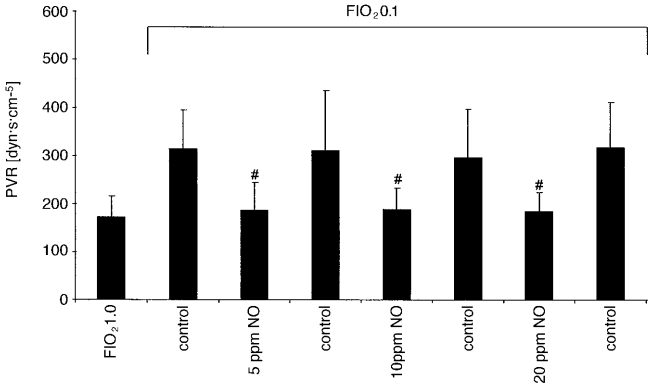

Fig. 2 Initiation of hypoxia in the animals of the NO group ($n = 5$) resulted in an increase of pulmonary vascular resistance *PVR* which was equally reversed to values achieved prior to hypoxic ventilation by all concentrations of NO. Interruption of NO restored hypoxic pulmonary vasoconstriction to values observed prior to inhalation of the drug. All data are presented as mean ± standard deviation. #$p < 0.001$ when compared to hypoxic ventilation with FIO_2 0.1 before and after inhalation of NO

ministration, hypoxic pulmonary vasoconstriction was restored to levels obtained after inducing hypoxia (Fig. 3, 4).

Direct comparison of the effect on MPAP and PVR during inhalation of the highest dose of PGI_2 and the highest concentration of NO revealed no differences in the efficacy of the two drugs in improving pulmonary hemodynamics with a decrease in MPAP from 29 ± 6 to 21 ± 5 mmHg following inhalation of 10 ng × kg^{-1} × min^{-1} PGI_2 and from 31 ± 7 to 22 ± 5 mmHg during administration of 20 ppm NO (Fig. 1, 4).

Discussion

The main finding of this study is that concentrations of inhaled NO and doses of PGI_2 used in clinical practice reduced the elevated pulmonary arterial pressure in a dose-independent fashion in an experimental model of hypoxic pulmonary hypertension. However, although direct comparison of the highest concentration of NO and the highest dose of PGI_2 used in this study revealed no difference in the efficacy of the two drugs, inhalation of NO resulted in a decrease in MPAP to values achieved prior to the onset of hypoxic ventilation, while values observed during nebulization of PGI_2 remained increased when compared to baseline values at FIO_2 1.0. The effect of both NO and PGI_2 on the pulmonary vasculature was reversed within 10 min after terminating inhalation. No effects on SVR, cardiac output or gas exchange were observed for either drug. Spontaneous breathing or mechanical ventilation with hypoxic gas mixtures is a common model for inducing pulmonary arterial hypertension and has been used in several investigations to evaluate the effect of inhaled vasodilators [9–12]. Hypoxic pulmonary vasoconstriction contributes to the elevated PVR observed in patients with ARDS and a number of clinical studies showed the efficacy of inhaled NO and nebulized PGI_2 in improving pulmonary hypertension in such patients [14, 16, 17]. However, there is a discrepancy in the concentrations of NO and doses of PGI_2 required to achieve an effect

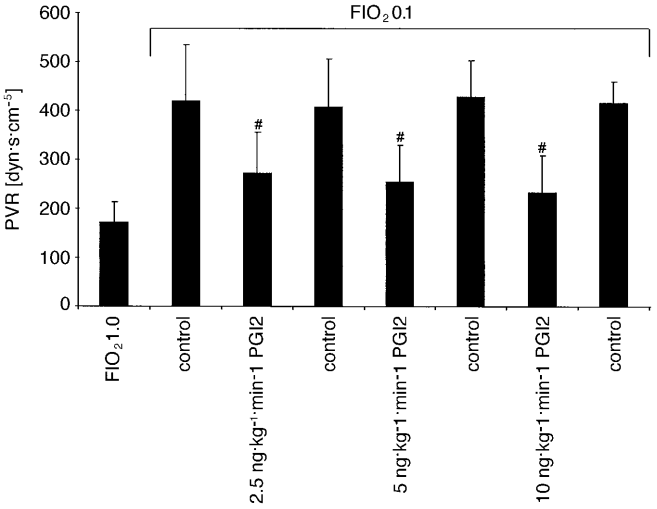

Fig. 3 Initiation of hypoxia in the animals in the PGI_2 group ($n = 5$) resulted in an increase in pulmonary vascular resistance *PVR* which was equally reversed to values achieved prior to hypoxic ventilation by all doses of PGI_2. Interruption of PGI_2 restored hypoxic pulmonary vasoconstriction to values observed prior to inhalation of the drug. All data are presented as mean ± standard deviation. #$p < 0.001$ when compared to hypoxic ventilation with FIO_2 0.1 before and after inhalation of PGI_2

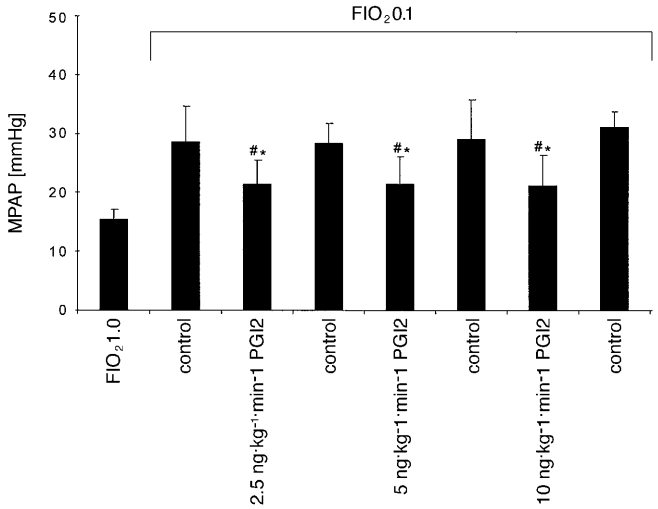

Fig. 4 Regarding the animals of the PGI_2 group ($n = 5$), the increase in mean pulmonary arterial pressure *MPAP* due to ventilation with a hypoxic gas mixture was reversed by all doses of PGI_2. However, values of MPAP during PGI_2 inhalation were still increased when compared to values achieved prior to hypoxia. Comparison of the effect of 20 ppm NO and 10 ng × kg^{-1} × min^{-1} PGI_2 revealed no statistically significant difference with a two-sample *t*-test. All data are presented as mean ± standard deviation. *$p < 0.001$ when compared to initial ventilation with FIO_2 1.0; #$p < 0.001$ when compared to hypoxic ventilation with FIO_2 0.1 before and after inhalation of PGI_2

on pulmonary arterial pressure in these investigations. It is suggested that these differences are due to associated diseases [18] and the inhomogeneous etiology of the syndrome in the patients studied, and it may be difficult to transfer these results to other situations of hypoxia-induced pulmonary hypertension such as COPD [19, 20]. Therefore, like in other studies [9, 11], a model of pulmonary hypertension solely due to ventilation with a hypoxic gas mixture was chosen in this study to avoid the possible influence of coexisting factors such as hypercapnia, lung edema and microembolism, which may occur and interfere with the effect of inhaled NO and nebulized PGI_2 on the elevated pulmonary arterial pressure when experimental models of acute lung injury due to surfactant depletion or oleic acid infusion are utilized. In this regard, the model of hypoxia-induced pulmonary hypertension can only provide limited information for situations of more complex acute lung injury but focus on the specific setting where the elevated pulmonary arterial pressure is exclusively or primarily due to hypoxia.

Several experimental studies have compared the effect of aerosolized prostacyclin and inhaled NO on hypoxic pulmonary vasoconstriction in otherwise healthy animals [9, 11, 12, 21]. However, the capability of both drugs to decrease the hypoxia-induced elevated pulmonary arterial pressure in a dose-dependent fashion has been analyzed only in one investigation [9], using concentrations of NO (20, 50 and 100 ppm) which were higher than the concentrations currently proposed and used in clinical practice. In contrast to this study by Booke and coworkers we did not see a dose dependency in the effect of lower concentrations of NO or lower doses of PGI_2. Differences in the experimental setting and the species used may account for this difference. Booke et al. investigated sheep who were ventilated in a side-separated mode, administering pure nitrogen to one lung and 21% oxygen to the other. This might have resulted in a more intense degree of hypoxic vasoconstriction requiring higher doses of vasodilators to achieve a reduction in pulmonary arterial pressure in the lung ventilated with nitrogen than in our model, where both lungs were ventilated with an FIO_2 of 0.09 to 0.11. A lack of dose dependency as observed in our study was also reported by other authors where an increase from approximately 1 to 2 ng × kg^{-1} × min^{-1} of nebulized PGI_2 did not result in an amplification of the effect on MPAP [11]. Therefore, the concentrations of NO and the doses of PGI_2 demonstrating a dose-dependent reduction of the increased pulmonary arterial pressure during hypoxia in our experimental setting may be still lower than the ones we used. Furthermore, we can not exclude the possibility that increasing the dose of NO to levels used by Booke et al. may have resulted in a further dose-dependent decrease in pulmonary arterial pressure, although values achieved with 5 ppm NO in our study did not differ significantly from those

achieved with an FIO_2 of 1.0, i.e., they were already reduced to baseline levels.

A difference in the efficacy of inhaled NO and PGI_2 aerosol on hypoxia-induced pulmonary vasoconstriction has been reported by other investigators. Although PGI_2 decreased elevated PVR in a study by Welte et al. [11], the effect of approximately $1\ ng \times kg^{-1} \times min^{-1}$ PGI_2 aerosol on pulmonary hypertension was always exceeded by the inhalation of 50 ppm NO. Booke et al. found that the maximum vasodilatory effect of PGI_2, observed with a dose of $10\ \mu g \times min^{-1}$ was comparable to the effect of 20 ppm inhaled NO [9]. While an increase in the NO concentration resulted in a further decrease in MPAP, doubling of the dose of PGI_2 had no further effect on pulmonary arterial pressure. In accordance with these data we found that in contrast to inhaled NO, the effect of PGI_2 aerosol on MPAP did not reach statistical significance when compared to baseline values (FIO_2 1.0) in each group. However, in contrast to the results of Booke et al., direct comparison of the highest concentration of NO (20 ppm) and the highest dose of PGI_2 ($10\ ng \times kg^{-1} \times min^{-1}$) used in our investigation revealed no such difference in the potency between the drugs. Besides the differences mentioned before in the experimental setup and the animal species used, this may be explained by the small diameter of the double lumen endotracheal tube used by Booke et al., which may have restricted the transfer of the aerosolized PGI_2 particles into the lung, thereby reducing the efficacy of PGI_2 inhalation. Furthermore, an insufficient fit of the NO concentrations and PGI_2 doses used in the other investigations may have influenced the results. While NO was applied in rather high concentrations, PGI_2 aerosol was administered in doses which do not exceed intravenous or inhaled doses used in clinical practice. Therefore, reduction of the NO concentrations in our investigation when compared to Booke and coworkers could be the reason for the equal action of the two drugs in our experiment when a direct comparison was performed. However, since we did not know the complete dose-response curves of both compounds we cannot be certain that the concentration of NO and the dose of PGI_2 compared in our investigation were actually equipotent. In addition, when interpreting these results, one has to take into account that the number of animals investigated in our study and the other investigations was rather small, and testing of small sample sizes can increase the likelihood of a rise in β error and a decrease in power, i.e., the probability of missing an effect which is actually present, which further increases the difficulty in obtaining statistically reliable results.

Toxic side effects of NO with regard to the methemoglobin fraction were not observed in our study. Generally, the application of up to 50 ppm NO to the inspiratory gas is considered safe for the clinical use of NO [22, 23]. However, NO has a potential for cytotoxicity by initiating lipid peroxidation of cell membranes [24] and inactivation of pulmonary surfactant [25], and it is not known whether even low concentrations of NO may thereby result in additional lung damage. Therefore, it seems best to use the lowest concentration of NO possible. Rebound effects following the interruption of NO administration as reported by other authors [26] were not observed in our study, probably due to the short inhalation time. Although the effect of exogenous NO on the regulation of endogenous NO production as the possible reason for this rebound phenomenon is not entirely clear, it is common practice to withdraw NO progressively after long-term inhalation to avoid this potential side effect.

It has been suggested that the nebulization of PGI_2 has certain advantages over the inhalation of NO regarding the mode of application (e.g., administration to non-intubated patients) and the necessity of monitoring possible toxic side effects [9]. Although this is in part correct, some reservations concerning the use of PGI_2 aerosol should be noted. With the currently available nebulizers it is almost impossible to assess the actual amount of prostacyclin being administered into the alveoli. Using radiolabeled aerosol, a recent study found that only 0.37–3.68 % of the nebulized compound was actually deposited in the lung during mechanical ventilation [27]. Furthermore, long-term treatment over several days can be very expensive, taking into account the relative high price of PGI_2 compared to NO.

Our results indicate that inhalation of clinical doses of PGI_2 ($10\ ng \times kg^{-1} \times min^{-1}$) and concentrations of NO (20 ppm) in hypoxia-induced pulmonary hypertension in pigs can improve the elevated pulmonary artery pressure to a similar degree, although it is notable that PGI_2 did not achieve a decrease in MPAP to baseline values. The rather small sample size of our study and of other investigations using the same animal model may be a drawback and larger numbers may be necessary for definite results. Based on our data, PGI_2 may actually be an alternative in the treatment of hypoxic pulmonary vasoconstriction.

Reservations must be made concerning the lack of reliable nebulizers which allow a more precise dosing of the drug and avoid accidental overdosing with the risk to induce systemic side-effects. In this regard, the availability of ventilators with integrated application and monitoring systems for NO makes the administration of nitric oxide more suitable than the nebulization of prostacyclin. Furthermore, to avoid possible toxicity, much smaller doses of NO than indicated by other experimental work may be sufficient to improve hypoxic pulmonary vasoconstriction.

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