Extra-pulmonary effects of inhaled nitric oxide in swine with and without phenylephrine


Summary
We have compared the effects of inhaled nitric oxide (iNO) and i.v. nitroglycerin (ivGTN) on the haemodynamic response to phenylephrine-induced hypertension (PEHT) in anaesthetized pigs. PEHT did not change either pulmonary vascular resistance or gas exchange throughout all experiments. Both treatments lowered pulmonary arterial pressure to the same extent (−12.4% iNO; −13.7% ivGTN) and passively via an effect on left atrial pressure (−26.3% iNO; −31.4% ivGTN). Both treatments failed to reverse the decrease in renal blood flow (RBFc) induced by PEHT, but both treatments lowered pulmonary oedema, and exerts a platelet anti-aggregating effect. A swine model has been used in the past to study the treatment of experimental pulmonary hypertension induced by hypoxia, a thromboxane analogue, oleic acid-induced ARDS or sepsis. Phenylephrine is a sympathomimetic amine commonly used in in vitro studies of the L-arginine:NO pathway or to counteract clinical hypotension. We have developed a phenylephrine-induced hypertension (PEHT) model in anaesthetized pigs. We wished to test the hypothesis that iNO does not affect the systemic circulation. Accordingly, we compared the effects of iNO and ivGTN on pulmonary and systemic haemodynamics and gas exchange, in addition to plasma and urinary concentrations of nitrite and nitrate during and beyond the period of infusion (iNO: 6.4% and 4.9%, respectively). In four control pigs (no PEHT), iNO markedly increased RBFc (+109%), glomerular filtration rate (+72.5%) and UF (+68.7%). We conclude that iNO may have direct cardiac and renal effects, probably via intervention of NO carrier forms such as S-nitroso compounds. (Br. J. Anaesth. 1997; 79: 631–640).

Key words

I.v. endothelium-independent nitrovasodilators such as sodium nitroprusside and nitroglycerin (ivGTN) produce their vasodilator effects by providing nitric oxide. In contrast with i.v. medication, inhaled nitric oxide (iNO) is used commonly as a selective pulmonary vasodilator in experimental and clinical conditions. Inhaled NO is “microselective” in that it dilates only vessels directly adjacent to the alveolar units being ventilated. Therefore, in a hypoxaemic patient, iNO may improve oxygenation by improving ventilation/perfusion matching with redistribution of blood flow from unventilated shunted areas to ventilated but underperfused areas. Some authors also explain the improved oxygenation with iNO by the fact that it produces local bronchodilation, decreases vascular permeability and the appearance of pressure-driven pulmonary oedema, and exerts a platelet anti-aggregating effect.

Materials and methods
This study was approved by the Institutional Research and Animal Welfare Committee. The animals were treated according to the Canadian Council on Animal Care guidelines. We studied 28 female pigs, mean weight 22.84 (SEM 0.27) kg. The pigs were premedicated with azaperone 2 mg kg−1 i.m., ketamine 15 mg kg−1 i.m. and atropine 4 μg kg−1 i.m. After induction of anaesthesia with fentanyl 5 μg kg−1 i.v. and pentobarbitone 6.5 mg kg−1 i.v., the trachea was intubated and the lungs ventilated using a volume-controlled ventilator (Ventilator 7200 AE, Puritan Bennett, Carlsbad, California). The lungs were ventilated with 100% oxygen to achieve arterial oxygen tension well above 400 mmHg.

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CA, USA). Tidal volume was set at 15 ml kg⁻¹, F₁O₂ at 0.30 (Oxygen Monitor 5590, Hudson RCI, Temecula, CA, USA) and ventilatory frequency adjusted to maintain the partial pressure of arterial carbon dioxide (P₁CO₂) at 4.6–6.0 kPa (Blood gases analyser IL 1620, Coulter Electronics Ltd, Ville Saint-Laurent, QC, Canada). Anaesthesia was maintained with continuous infusion of fentanyl 3.8 µg kg⁻¹ h⁻¹ and pentobarbitone 9.8 mg kg⁻¹ h⁻¹. Neuromuscular block was produced with a mixture of tubocurarine 0.2 mg kg⁻¹ h⁻¹ and pancuronium 0.15 mg kg⁻¹ h⁻¹. Lactated Ringer’s solution was infused at a rate of 15 ml kg⁻¹ h⁻¹ to meet maintenance needs during open-chest surgery.

After placing the electrocardiographic leads, we inserted an arterial pressure catheter (carotid artery), two venous infusion cannulae (external jugular vein, auricular vein), a pulmonary artery thermodilution catheter (for cardiac output), a core temperature probe and central venous catheter. Median sternotomy was performed, the pericardium opened and two catheters (12-gauge) inserted, one into the main pulmonary artery and the other into the left atrial appendage for pressure monitoring in addition to arterial and venous blood sampling for blood-gas analysis. The pericardium and chest wall were closed with sutures and metallic wires. Via a laparotomy, the left renal vein (renal venous blood sampling site) and both ureters were catheterized, and the left ovarian vein was ligated because of drainage into the renal vein. After instrumentation, 45 min were allowed to elapse to obtain a stable physiological state.

Each experiment was divided into four stages (I–IV) of 20 min (table 1). Animals were enrolled in the experiments in random order.

Control arm. In control studies we evaluated the effect of time (experiment A), PEHT alone (experiment B) and iNO alone (experiment C).

Experimental arm. The four stages in the experiments were: (I) baseline measurements, (II) induction of PEHT by continuous infusion of phenylephrine diluted in saline (mean 14.68 (SEM 0.85) µg kg⁻¹ min⁻¹), (III) intervention to treat PEHT with an infusion of ivGTN (experiment D) or 40 ppm of iNO (experiment E), and (IV) a recovery period where both the hypertensive stimulus and treatment were discontinued.

ADMINISTRATION OF IVGTN

The dose of ivGTN (Nitroject, Omega Laboratories Ltd, Montréal, QC, Canada) was started at 10 µg kg⁻¹ min⁻¹ and increased progressively (3-min interval) to reduce mean pulmonary arterial pressure to the level observed after iNO. The period of measurement (stage III) was then started. The mean final dose of ivGTN used in experiment D was 92.43 (SEM 13.45) µg kg⁻¹ min⁻¹.

ADMINISTRATION OF iNO

A mixture of NO–N₂, 972 ppm (Cylinder NO 972 ppm, <5 ppm of NO₂, Vitalaire Canada, Montréal, QC, Canada) was injected cyclically into the inspiratory limb of the ventilator system using a method developed in our institution. Concentrations of gaseous NO and NOₓ were monitored continuously using a chemiluminescence analyser (CLD700AL NO/NOₓ analyser, Ecophysics Tecan AG, Dürten, Switzerland). Based on our previous work, we chose to study the effects of iNO 40 ppm. This dose was shown in several studies to have a significant pulmonary vasodilator effect while ensuring an inspired fraction of NO₂ below 1 ppm with our system.

RENA1 MONITORING

Thirty minutes before the beginning of baseline measurements (stage I), a bolus of insulin 2 g and para-amino-hippuric acid (PAH) 2 g dissolved in 50 ml of glucose were administered, immediately followed by an infusion of insulin 2 g and PAH 4 g dissolved in 1000 ml of glucose at a constant rate of 1 ml min⁻¹. Blood samples (4 ml) were obtained simultaneously from the left renal vein and carotid artery in order to measure packed cell volume (PCV), PAH and inulin concentrations, in addition to arterial and venous pNOₓ concentrations. Blood samples were collected at the beginning of stage I and at the end of each 20-min period. Urine was collected separately in graduated cylinders from both ureters at 5-min interval during the whole procedure to measure urinary flow (UF), PAH and inulin concentrations, and uNOₓ. The concentration of PAH in both urine and blood was measured using the technique described by Bratton and Marshall. Renal plasma flow (RPF) was taken as PAH clearance (ClRH–Cᵣ × (UF/Cᵤ), where Cᵤ PAH concentration in urine and Cᵣ PAH concentration in arterial blood plasma). Renal plasma flow was corrected (RPFc) for PAH extraction coefficient.

Table 1: Study design. *No intervention, †PE = Phenylephrine infusion at mean 15.75 (SEM 0.85) µg kg⁻¹ min⁻¹; ‡PE = phenylephrine infusion at 14.68 (1.36) µg kg⁻¹ min⁻¹; iNO = inhaled nitric oxide 40 ppm; ivGTN = nitroglycerine infusion at 92.43 (13.45) µg kg⁻¹ min⁻¹.

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**Extra-pulmonary effects of inhaled NO**

\[(\text{F}_{\text{PAH}} = (C_v - C_c)/C_a \text{ where } C_v = \text{PAH concentration in renal vein blood plasma}), \text{ and renal blood flow corrected for PAH extraction (RBFC)} \text{ was taken as } \text{RPFc/(1-PCV). Because PAH acetylation was observed with some pigs, RBFC was calculated for each 5-min interval using PAH and inulin clearances and extractions. Inulin concentration was measured by an antrone colorometric technique, and whole kidney glomerular filtration rate (GFR) was calculated for each 5-min interval by the standard clearance formula.**

**Measurements of pNOX and uNOX**

Plasma samples obtained by centrifugation were diluted 10-fold with distilled water and deproteinized by adding 5% volume of zinc sulphate to a final concentration of 15 g litre\(^{-1}\). After centrifugation at 1000 \(\times g\) for 15 min at room temperature, 100 \(\mu\)l of supernatant were applied to a microreaction purge vessel chamber (270B NO analyser, Sievers Research Inc, Boulder, CO, USA) equipped with a temperature regulator and a condenser, which allowed direct introduction of prepared plasma and urine samples into the reducing solution. pNOX and uNOX concentrations were measured by conversion to NO using hot acidic vanadium (III) chloride. NO was eluted in a stream of nitrogen and detected by an ozone-induced chemiluminescence reaction. The NO/NOX analyser was connected directly to a data acquisition system (Mac Lab, Lamont Scientific Ltd., Downsview, Ont, Canada). Each sample was analysed in duplicate and measured once at the end of each 20-min period, as described previously.

**Cardiorespiratory variables**

Systolic (SAP), mean (MAP) and diastolic (DAP) systemic arterial pressures, mean pulmonary arterial pressure (PAP), left atrial pressure (LAP), central venous pressure (CVP), heart rate (HR) and cardiac output (CO; 5 ml of cold normal saline were injected in triplicate for each measurement) were recorded every 5 min in each period. Arterial and mixed venous blood-gas tensions were measured once at the end of each 20-min period.

**Data analysis**

In the control arm, the sample size was \(n = 4\) for each experiment (table 1). In the experimental arm, the sample size was \(n = 8\) for each experiment. Half of the pigs in experiments D and E were used to evaluate renal effects and the other half to measure pNOX\(^-\) and uNOX\(^-\) concentrations.

Data distribution of each variable was Gaussian. For each stage, mean values of four measurements (obtained every 5 min) or a single measurement (obtained only once during each stage) were chosen as the values for the period. Pulmonary (PVR) and systemic (SVR) vascular resistances were calculated using standard formulae. All values are reported as mean (SEM) for each variable in each stage (I–IV) of the experiments.


For each experiment, the effect of stage was evaluated with the one-factor repeated measures analysis of variance (F-ANOVA) and, when necessary, multiple comparisons were made using Fisher’s protected least significant difference (LSD\(_p\)) test.

Between the five experiments, for the difference between two stages (e.g. stages-difference

**Figure 1** Variation in renal blood flow corrected for PAH extraction (RBFC). A. Control arm: mean (SEM) values for RBFC for each sampling time in each stage (I–IV) for experiment A (no intervention during the four stages), experiment B (infusion of phenylephrine 15.75 (0.85) \(\mu\)g kg\(^{-1}\) min\(^{-1}\) during stages II and III), and experiment C (iNO 40 ppm during stages II and III). RBFC decreased with time in experiment A. Infusion of phenylephrine significantly reduced RBFC while iNO considerably increased RBFC. *Significant difference for F-ANOVA (\(P < 0.05\)) and for the LSD\(_p\) test of the stage compared with stage I (\(P < 0.008\)). B. Experimental arm: mean (SEM) values for RBFC for each sampling time in each stage (I–IV) for experiment D (PEHT + ivGTN treatment) and experiment E (PEHT + iNO treatment). Infusion of phenylephrine significantly reduced RBFC, and nitrergic treatments failed to reverse the decrease. *Significant difference for F-ANOVA (\(P < 0.05\)) and for the LSD\(_p\) test of the stage compared with stage I (\(P < 0.008\)). (See text for further details.)
between stages III and I), one-way analysis of variance (W-ANOVA) was used and multiple comparisons were made using Fisher’s protected least significant difference (LSDW) test.

**P**/0.05 was considered significant, except for multiple comparisons where the global significance level of 0.05 was adjusted for the number of null hypotheses tested (P<0.008 for multiple comparisons within group LSDF and P<0.005 for multiple comparisons between the five groups LSDW).

**Results**

For each experiment, the cardiovascular, renal and metabolic (pNOX, uNOX) effects observed during each stage are presented.

(III–I) = between stages III and I), one-way analysis of variance (W-ANOVA) was used and multiple comparisons were made using Fisher’s protected least significant difference (LSDW) test.

P<0.05 was considered significant, except for multiple comparisons where the global significance level of 0.05 was adjusted for the number of null hypotheses tested (P<0.008 for multiple comparisons within group LSDF and P<0.005 for multiple comparisons between the five groups LSDW).

**Figure 2** Variation in glomerular filtration rate (GFR). A: Control arm: mean (SEM) values for GFR for each sampling time in each stage (I–IV) for experiment A (no intervention during the four stages), experiment B (infusion of phenylephrine 15.75 (0.85) µg kg⁻¹ min⁻¹ during stages II and III), and experiment C (iNO 40 ppm during stages II and III). GFR decreased with time in experiment A. Infusion of phenylephrine had no effect on GFR while iNO considerably increased GFR. *Significant difference for F-ANOVA (P<0.05) and for the LSDF test of the stage compared with stage I (P<0.008). †Significant difference between groups for W-ANOVA (P<0.05) and for the LSDW test (P<0.005) on the difference (III–II) and (IV–III). (See text for further details.)

**Figure 3** Variation in urinary flow (UF). A: Control arm: mean (SEM) values for UF for each sampling time in each stage (I–IV) for experiment A (no intervention during the four stages), experiment B (infusion of phenylephrine 15.75 (0.85) µg kg⁻¹ min⁻¹ during stages II and III), and experiment C (iNO 40 ppm during stages II and III). UF decreased with time in experiment A. Infusion of phenylephrine had no effect on UF while iNO considerably increased UF. *Significant difference for F-ANOVA (P<0.05) and for the LSDF test of the stage compared with stage I (P<0.008). †Significant difference between groups for W-ANOVA (P<0.05) and for the LSDW test (P<0.005) on the difference (III–II) and (IV–III). (See text for further details.)

**CONTROL ARM**

In experiment A on the effect of time, there was no change in renal haemodynamics and diuresis: stage IV was different from the three preceding periods for RBFc (−22.9%; fig. 1A), GFR (−20.8%; fig. 2A) and UF (−19.5%, fig. 3A).
During experiment B, phenylephrine significantly increased PAP by 40% (LSDF comparison of stage II with stage I: $P = 0.0001$), but PVR remained unchanged (F-ANOVA: $P = 0.42$). PEHT significantly increased MAP and SVR: +65.9% and +64.5%, respectively. LAP and CVP exhibited similar changes: +67.9% and +49%, respectively. Phenylephrine had no effect on HR or CO (F-ANOVA, $P = 0.17$ and $P = 0.33$, respectively). The haemodynamic changes were stable during stage III. Compared with stage I, phenylephrine induced a similar decrease in RBF$\text{c}$ for stage II (−34%) and III (−37%) but other renal variables remained unchanged (figs 2A and 3A). The vasoconstrictor did not significantly modify arterial (92.6 (4) to 96 (5.1) μmol litre$^{-1}$) or venous (89.8 (5.5) to 94.1 (8.3) μmol litre$^{-1}$) $\text{pNOX}$ or $\text{uNOX}$−$\text{c}$ (988.2 (73.4) to 1132.4 (102.6) μmol litre$^{-1}$) concentrations.

In experiment C, iNO alone did not significantly alter cardiorespiratory variables. However, renal function was affected: RBFe increased by +94% (LSDF, $P = 0.0046$) (fig. 1A), GFR by +63% (LSDF: $P = 0.00001$) (fig. 2A) and UF by +34% (LSDF: $P = 0.006$) (fig. 3A) during stage II; the changes in the three variables remained constant during stage III (−109%, +72.5% and +68.7%, respectively) (figs 1A–3A). UF remained high during stage IV LSD$\text{c}$.
P = 0.001 compared with stage I) (fig. 3A). The concentration of pNOX− was slightly modified by iNO in arterial (109.2 (3.1) to 119.8 (3.3) μmol litre−1) and venous (116.4 (4.7) to 124.3 (4.8) μmol litre−1) blood, and uNOX− increased from 691.2 (113.8) to 1011.3 (157.3) μmol litre−1 during stage II. ΔpNOX− increased continually during stage III compared with stage I (+22.1 and +21.4 μmol litre−1 in arterial and venous blood), and decreased on cessation of iNO. The value of uNOX− remained at the same high level during stages III and IV.

EXPERIMENTAL ARM

Infusion of phenylephrine in experiments D and E produced the same cardiovascular (table 2), renal (fig. 1B–3A) and metabolic (table 3) effects as in experiment B (W-ANOVA: P > 0.1 on difference (II–I) between experiments B, D and E).

Both nitrergic treatments decreased LAP (−31.4% ivGTN group = experiment D; −26.3% iNO group = experiment E) and CVP (−25.7% and −19.1%, respectively), achieving the same stages-difference (III–II) between experiments D and E (LSDW: P = 0.1 for LAP and P = 0.09 for CVP) (table 2). The effect on pulmonary hypertension was the same in both groups (table 2): PAP decreased by 13.7% and 12.4%, respectively. IvGTN decreased MAP (−39.4%) and SVR (−49.3%), and iNO induced a much smaller decrease in systemic haemodynamic variables (table 2). HR, CO and blood-gas values remained unchanged throughout the experiment.

The two nitrergic treatments did not reverse the decrease in RBCF induced by phenylephrine (fig. 1A), but they both increased UF (+148% for ivGTN group; +128% for iNO group) (P = 0.0005 for experiment D; +128% for experiment E) (fig. 1B). Comparison between experiments D and E on stages-difference (III–II) showed that ivGTN exerted more effect on UF than iNO (LSDW: P = 0.003). Infusion of ivGTN increased pNOX− concentrations compared with stage II (table 3): +22.8% for arterial blood and +26.2% for venous blood. The values in experiment E were +6.4% and +4.9% for arterial and venous blood, respectively (table 3). Both nitrergic treatments markedly increased uNOX− concentrations (+65.4% for experiment D and +46.3% for experiment E).

At the end of the experiment, cardiovascular, respiratory and renal (except for UF in experiments D and E (LSDW: P = 0.001 for comparison of stages IV to equivalent stages I; fig. 3B) variables essentially returned to their initial values. In experiment D, the increases in arterial and venous pNOX− (table 3) and uNOX− concentrations observed during stage III were still present during stage IV. Twenty minutes after cessation of iNO, pNOX− concentration significantly decreased (compared with stage III) in experiment E (table 3), whereas uNOX− remained high (1206.4 (108.9) μmol litre−1). Comparison between experiments D and E on stages-differences (IV–III) and (IV–I) confirmed that pNOX− returned to baseline values in the iNO group but not in the ivGTN group.

Discussion

To our knowledge, this is the first report of PEHT in swine treated with nitrergic therapies and the first study of the haemodynamic extra-pulmonary effects of iNO.

The model used in these studies is well established. There was no mortality during the surgical preparations and we demonstrated with experiment A that cardiorespiratory variables remained stable during the period of the study and there were only minimal changes in renal function.

EXPERIMENT B

Cardiovascular

Phenylephrine acts on both α1- and α2-adrenoceptors14 and induces hypertension (major α1-effect) by constricting resistance and, to a lesser degree, capacitance vessels.9 We observed no signs of tachyphylaxis with phenylephrine during the experiments. The observed passive post-capillary pulmonary hypertension with increased central filling pressures was secondary to the left ventricular haemodynamic effects of the acute increase in afterload induced by systemic hypertension. During the experiment, PVR did not change, in agreement with heterogeneous15 α-adrenergic innervation of large (5–7 mm in diameter) and small (2–3 mm in diameter) porcine pulmonary arteries and sparse or non-existent16 α-adrenergic innervation of swine pulmonary resistance vessels.

Renal effects

The kidney reacted to phenylephrine-induced increase in SVR by a decrease in RBCF,5 while GFR and UF remained unchanged. This suggests that phenylephrine constricted mainly the efferent arteriole and that GFR was maintained, despite a decrease in RBCF by an increase in glomerular filtration fraction.17

Metabolic effects

The baseline values of pNOX− were higher than those measured in a porcine model of ARDS (30 μmol litre−1).7 However, the measurement techniques were different (chemiluminescence vs high-pressure liquid chromatography based on the Griess reaction7) and the surgical stress of our procedure may have stimulated release of endogenous NO. Furthermore, reported values for basal concentrations of pNOX− (mainly NO3−) in humans vary greatly.18–20 Our basal values for uNOX− were in accordance with those published previously in humans.18–20

EXPERIMENT C

Inhaled NO alone induced no cardiorespiratory modifications, but considerably altered renal function.
Transport of EDRF–NO

Several authors have suggested that EDRF–NO interacts with circulating molecules to regulate its activity, allowing its transport (NO carriers) or promoting its degradation (NO scavengers). Metal nitrosylation with haem and non-haem proteins and the NO reaction with nucleophilic groups (e.g. sulphydryl, amine) of amino acids, peptides and proteins lead to the potential interaction and formation of many nitrosyl–haem adducts, S-nitrosothiols (S-nitroso-cysteine), S-nitrosylated proteins (S-nitrosyldicarboxylic acids, S-nitrosocarboxyhemoglobin), nitrosylated iron–sulphur clusters, dinitrosyl–iron complexes, nitrosoamines and others.

Renal effects

Renal haemodynamics and diuresis were altered by iNO (experiment C), strongly supporting the presence of extra-pulmonary effects. It is now widely accepted that EDRF–NO is an important modulator of RBF and that regional microcirculation may be mediated by endogenously produced EDRF–NO. Pharmacological block of EDRF–NO results in an increase in MAP and renal vascular resistance, and decrements in RBF, GFR, UF and sodium excretion. There is some controversy regarding a selective effect of EDRF–NO block on either afferent or efferent arteriolar tone. It would seem that EDRF–NO primarily alters afferent vascular tone, thereby modifying the ability of the pregglomerular vasculature to autoregulate glomerular capillary pressure. Several studies have emphasized an effect of EDRF–NO not only on glomerular but also on tubular function and the medullary circulation.

McKee, Scavone and Nathanson showed that EDRF–NO, through generation of cGMP and stimulation of cGMP-dependent protein kinase(s), mediates the actions of several intercellular messengers to regulate renal tubular Na–K-ATPase.

Inhibition of this sodium pump (e.g. by acetycholine, bradykinin) would decrease transcellular water and sodium reabsorption from the tubular lumen, resulting in diuresis and natriuresis. Moreover, low doses of NO synthase inhibitors, that have no effect on either RBF or GFR, induce antidiuretic and antinatriuretic effects. Here is the first report of exogenous iNO modulating renal function. Renal haemodynamic state may be influenced during iNO administration by circulating NO metabolites (e.g. NOX), renal NO delivery through release from carrier S-nitroso compounds (e.g. S-nitrosothiols) after nitrosylation in the pulmonary circulation, or a local renal effect owing to an unknown paracrine influence stimulated by iNO. In vitro studies have shown that NaNO2 did not influence renovascular tone in concentrations up to 0.1 mmol litre⁻¹. The diuretic effect of NaNO3 was observed with a plasma NO3⁻ concentration of 38 mmol litre⁻¹ in dogs. In view of the rapid renal response to application and discontinuation of iNO, we postulate that iNO may be accompanied by non-selective, extra-pulmonary effects caused by local delivery of NO on peripheral territories that include the renal bed.

Metabolic effects

Nitric oxide reaching the circulation can be metabolized via three pathways: (i) interaction with dissolved oxygen in blood to form NO2⁻, (ii) reaction with oxyhaemoglobin to form methaemoglobin which in turn is reduced back to haemoglobin and NO3⁻ mainly by the NADPH–methaemoglobin reductase and peroxynitrite pathways, and (iii) combination with deoxyhaemoglobin to form the stable nitrosohaemoglobin (HbNO) or with carrier molecules to form S-nitrosothiols among others (see above). However, to date, the mechanisms by which NO is inactivated and eliminated are not fully known.

The concentration of pNOX⁻ increased almost linearly throughout inhalation, and decreased on cessation of iNO. This result is in agreement with those of previous reports which have shown a linear temporal increase in pNOX⁻. Approximately 40 µmol of uNOX⁻ were excreted in urine during the 40-min inhalation period, a result similar to that of Wennmalm and colleagues, and the rate of elimination was maintained over the following 20 min. This implies that 23.6% of the retained NO was excreted in urine as uNOX⁻ within 1 h after the start of inhalation. This early and fast elimination of iNO corroborates a previous study that showed 69% of inhaled NO was excreted in urine as NO2⁻ within 24 h and another 4% from 24 to 48 h after the start of inhalation. Moreover, the decrease in pNOX⁻ and maintenance of diuresis and high uNOX⁻ concentrations after cessation of iNO suggest that NOX is stored in packaged forms before its release without vasoactive but with diuretic effects, transformation and elimination as NOX⁻.

EXPERIMENTAL ARM

Cardiovascular effects

Inhaled and i.v. nitricergic treatments partially reversed the passive pulmonary hypertension induced by phenylephrine. With the PEHT swine model, iNO 40 ppm was less effective in reducing PAP (50% reversal of the increase) than during hypoxia (108.7% at 40 ppm), thromboxane-mimetic infusion (61.9% at 40 ppm), experimental ARDS (57.1% at 40 ppm) or sepsis (84% at 10 ppm; 64.8% at 40 ppm). PVR remained unchanged throughout the experiment suggesting that the pulmonary resistance vasculature was not constricted and that both treatments did not act at this level in the PEHT swine model. Because the pulmonary vascular tone was unchanged by nitricergic treatments superimposed on PEHT, baseline gas exchange variables also remained unchanged.

The increase in PAP induced by phenylephrine was adjusted to comparable levels in both groups. Both treatments induced similar decreases in LAP, and consequently PAP and CVP. The decrease in LAP may be explained by a decrease in left atrial pressure.
filling, increase in left compliance, decrease in left ventricular afterload or a combination of these effects. The first hypothesis is unlikely because CO did not change and there was no clinical sign of an increase in lung water as gas exchange variables remained constant. The second assumption implies, from the formed cGMP, a block of α1 effects on cardiac contraction and relaxation, and a positive lusitropic effect increasing relaxation and diastolic distensibility of the heart. A direct cardiac effect of endothelium-independent nitrovasodilators, such as depressed myocardial contractility, improved ventricular relaxation and diastolic distensibility is accepted. The decrease in systemic afterload (third postulate) was manifest with ivGTN infusion which induced a substantial decrease in SVR. An endogenous EDRF–NO signalling pathway has been found to regulate cardiac myocyte function. The paracrine actions of EDRF–NO stimulated by cardiac contraction and relaxation, are also well established. The second and third hypotheses imply extra-pulmonary effects with iNO. In a previous study, we suggested that iNO had a direct cardiac effect. There was a slight modification of left ventricular afterload with iNO, as the observed decreases in SAP and MAP were statistically significant (LSD0.05; P=0.0001 and P=0.004, respectively) (table 2). We propose that iNO had a direct cardiac effect to explain the similar action of ivGTN and iNO on PAP, LAP and CVP. Our data indicate a larger decrease in SAP and MAP than that for DAP, suggesting that factors other than aortic compliance or SVR are modified by iNO. These other factors are usually related to changes in the mechanical performance of the heart as a pump. A trend for iNO to decrease SVR or MAP was observed in several publications.

### Renal effects

In experiments D and E, nitricergic treatments superimposed on phenylephrine infusion resulted in increases in UF without reversing the phenylephrine-induced decrease in RBFc. The primary phenylephrine site of action in the kidney seems to be the efferent arteriole in contrast with the afferent arteriole for EDRF–NO. This could explain the lack of reversibility for the decrease in RBFc induced by phenylephrine for both nitricergic treatments. Our findings also support a direct diuretic (and natriuretic?) effect of exogenous NO. Another hypothesis is the involvement of regulatory systems, either intrarenal such as the kallikrein–kinin system, or prostaglandins (PGE2), or extrarenal such as atrial natriuretic factor (ANF) or the ouabain-like endogenous factor that increase diuresis. However, EDRF–NO released from the endocardium or from the endothelial cells of the coronary vasculature, directly or through a decrease in LAP, or both, inhibits the release of ANF. The results of experiment C and those from another study gave greater credence to intervention of exogenous NO in packaged forms.

Inhaled NO had a major effect on the kidney and a slight (on SAP and MAP) or no statistically apparent (on DAP and SVR) systemic effect. The kidney could be more sensitive to exogenous NO than the systemic circulation as demonstrated by the high sensitivity of RBF to NO synthase inhibition. Indeed, the renovasculature showed significant modifications with low quantities of NO. Another hypothesis is that the kidney could more easily concentrate NO carriers or recuperate NO from its transport forms (local catabolism of S-nitroso compounds such as S-nitrosothiol, etc.). The persistent diuresis after discontinuing PEHT and nitricergic treatments (stage IV) was in agreement with previous data. It correlated with the persistent high pNOX− and uNOX− concentrations in experiment D or previous hypotheses such as intervention of other regulatory systems or a particular renal sensitivity to NO carriers for experiments C and E. It would imply that NO transported via GTN and that transported when NO is inhaled have similar (on the heart and kidney) and different (on the systemic circulation) effects. These could be explained by the nature of the implied packaged forms and their respective metabolism, particularly biotransformation of GTN to NO.

### Metabolic effects

Because we deproteinized the plasma samples, NO (from iNO or released by GTN)-derived metabolites (NO2− and NO3−) and GTN-derived metabolites (1,3- and 1,2-glyceryl dinitrate, and glyceryl mononitrate) were potentially measured as pNOX−. At the end of stage III, infusion of ivGTN significantly increased arterial and venous pNOX− concentrations (table 3), and we observed only a statistical trend for iNO to increase pNOX− concentrations. This difference could be explained by a greater NOX intake for ivGTN than for iNO and the larger number of metabolites of GTN. Other studies found significant differences in pNOX− concentrations between baseline and iNO treatment with a longer period of inhalation and larger sample size.

During stage IV, pNOX− concentrations remained high in experiment D (ivGTN), but decreased in experiment E (iNO), whereas uNOX− concentrations remained high in all experiments. NO might be released during stage IV from its carrier proteins with diuretic and without vasoactive effect, and metabolized in NOX−. Moreover, GTN-derived metabolites could be present at the end of stage IV and measured as NO3−. The absence of haemodynamic effects of these compounds during stage IV could be explained by their low potency or the nitrate tolerance phenomenon. The explanation for the pNOX− decrease in experiment E is disruption of iNO administration.

In summary, previous studies have shown clearly a marked effect of iNO on pulmonary hypertension in pulmonary vasconstriction models. Furthermore, in the passive PEHT model without pulmonary vasoconstriction, iNO maintained its pulmonary antihypertensive property. These results suggest that iNO may have direct cardiac effects. This study also demonstrated a marked effect of iNO on renal function, similar to that of ivGTN. We conclude that
iNO had extra-pulmonary effects in an in vivo swine model and consequently that iNO cannot be considered as a pure selective agent on the pulmonary vasculature.

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References


2. Weitberg E, Rudelli A, Lundberg JM. Nitric oxide in hypertension and consequently that iNO cannot had extra-pulmonary effects in an swine model and consequently that iNO cannot had extra-pulmonary effects in an


34. Shah AM, Evans HG, Lewis MJ. Phenylephrine-induced cardiac phosphatidylinositol (PI) hydrolysis is inhibited by cyclic GMP. Circulation 1990; 82 (Suppl. III): III-270 (1073).


