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Pulmonary Vasoconstriction during Regional Nitric Oxide Inhalation

Evidence of a Blood-borne Regulator of Nitric Oxide Synthase Activity

Kristina Hambraeus-Jonzon, M.D., Ph.D., Luni Chen, M.D.,† Filip Fredén, M.D., Ph.D.,‡ Peter Wiklund, M.D., Ph.D.,§ Göran Hedenstierna, M.D., Ph.D.‖*

Background: **Inhaled nitric oxide (INO) is thought to cause selective pulmonary vasodilation of ventilated areas. The authors previously showed that INO to a hyperoxic lung increases the perfusion to this lung by redistribution of blood flow, but only if the opposite lung is hypoxic, indicating a more complex mechanism of action for NO. The authors hypothesized that regional hypoxia increases NO production and that INO to hyperoxic lung regions (HL) can inhibit this production by distant effect.**

Methods: **Nitric oxide concentration was measured in exhaled air (NO_E), NO synthase (NOS) activity in lung tissue, and regional pulmonary blood flow in anesthetized pigs with regional left lower lobar (LLL) hypoxia (fraction of inspired oxygen [FIO_2] = 0.05), with and without INO to HL (FIO_2 = 0.8), and during cross-circulation of blood from pigs with and without INO.**

Results: **Left lower lobar hypoxia increased exhaled NO from the LLL (NO_ELLL) from a mean (SD) of 1.3 (0.6) to 2.2 (0.9) parts per billion (ppb) ($P < 0.001$), and Ca^{2+}-dependent NOS activity was higher in hypoxic than in hyperoxic lung tissue (197 [86] *vs.* 162 [96] pmol · g^{-1} · min^{-1}, $P < 0.05$). INO to HL decreased the Ca^{2+}-dependent NOS activity in hypoxic tissue to 49 [56] pmol · g^{-1} · min^{-1} ($P < 0.01$), and NO_ELLL to 2.0 [0.8] ppb ($P < 0.05$). When open-chest pigs with LLL hypoxia received blood from closed-chest pigs with INO, NO_ELLL decreased from 2.0 (0.6) to 1.5 (0.4) ppb ($P < 0.001$), and the Ca^{2+}-dependent NOS activity in hypoxic tissue decreased from 152 (55) to 98 (34) pmol · g^{-1} · min^{-1} ($P = 0.07$). Pulmonary vascular resistance increased by 32 (21)% ($P < 0.05$), but more so in hypoxic ($P < 0.01$) than in hyperoxic ($P < 0.05$) lung regions, resulting in a further redistribution ($P < 0.05$) of pulmonary blood flow away from hypoxic to hyperoxic lung regions.**

Conclusions: **Inhaled nitric oxide downregulates endogenous NO production in other, predominantly hypoxic, lung regions. This distant effect is blood-mediated and causes vasoconstriction in lung regions that do not receive INO.**

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HYPOXIA causes pulmonary vasoconstriction (HPV), resulting in redistribution of the pulmonary blood flow to better-oxygenated parts of the lungs.[1] HPV is intrinsic to the pulmonary vascular smooth muscle, as demonstrated by hypoxic contraction of isolated pulmonary vascular smooth muscle cells.[2] Hypoxia in small pulmonary arteries inhibits an outward potassium current, causing membrane depolarization and calcium entry through the voltage-dependent calcium channels.[3] This results in increased cytosolic free calcium and vasoconstriction.[4–6] HPV can be modulated by several factors, including the unstable free radical nitric oxide (NO),[7] which is produced in the lungs and elsewhere, by a variety of cells.[8–10] Inhibition of NO synthase (NOS) increases pulmonary vascular resistance (PVR). The increase in PVR by NOS blockade is more pronounced during hypoxia than normoxia, indicating that endogenous NO is an important modulator of HPV but plays a minor role in pulmonary blood flow regulation during normoxic conditions.[11–14] When anesthetized rabbits are exposed to unilateral hypoxia, the redistribution of pulmonary blood flow away from the hypoxic lung is enhanced in a dose-dependent manner by intravenous administration of the NOS inhibitor *N*-nitro-L-arginine methylester.[13] We found a similar enhancement of the blood flow redistribution away from a hypoxic lung to the other lung, which was ventilated with hyperoxic gas containing NO, in anesthetized human patients.[15] Because no such redistribution of blood flow by regional inhalation of NO (INO) occurred during bilateral normoxia or hyperoxia, the results suggest an interaction effect between hypoxic lung regions and INO. As a possible explanation for this observation, we hypothesized that regional hypoxia increases NO production in the hypoxic region, opposing HPV, and that INO can inhibit this production by distant effect, thereby potentiating HPV. To test the hypothesis, we studied the regional NO production as expressed by regional NO concentration in exhaled air (NO_E) and the regional amount of NOS as expressed by regional Ca^{2+}-dependent NOS activity, as well as pulmonary gas exchange and central hemodynamics in anesthetized pigs. We

* Senior Consultant, Department of Anesthesiology and Intensive Care, § Professor, Department of Urology, Karolinska Hospital, Stockholm, Sweden. † Research Fellow, ‖ Professor, Department of Clinical Physiology, ‡ Senior Consultant, Department of Anesthesiology and Intensive Care, University Hospital, Uppsala.

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Address reprint requests to Dr. Hambraeus-Jonzon: Karolinska Hospital, Department of Anesthesiology and Intensive Care, S-171 76 Stockholm, Sweden. Address electronic mail to: hambraeus.jonzon@telia.com.

compared the NO production with pulmonary vascular tone and distribution of blood flow during global and regional hypoxia and during regional INO, and tested whether a distant effect of INO was realized by a blood-borne mediator in a two-pig cross-circulation model.

Our results showed that INO downregulates endogenous NO production in lung regions that do not receive INO, and that this distant effect is blood-mediated and causes a substantial vasoconstriction. This distant effect of INO may therefore be of importance in regulating pulmonary artery pressure and gas exchange and may be one explanation why improvement in pulmonary artery pressure may not equal improvement in oxygenation by INO.

Materials and Methods

Material and Anesthesia

The study was approved by the Animal Research Ethics Committee of Uppsala University. A total of 45 pigs (Swedish country breed; weight, 25–35 kg) were premedicated with 20 mg/kg pentobarbital and 0.5 mg atropine intraperitoneally 30 min before the induction of anesthesia. An ear vein was cannulated for induction and maintenance of anesthesia, and a pulse oximeter was attached to the pig. Anesthesia was induced with 8–10 mg/kg intravenous pentobarbital and maintained with 0.8–1.6 g/h chlormetiazole and 0.2–0.4 mg/h fentanyl. A 4–8-mg/h dose of pancuronium bromide was infused for muscle relaxation. Oxygen saturation and inspiratory and end-tidal concentrations (C_{ET}) of oxygen and carbon dioxide were continuously monitored (Datex AS/3 Anaesthesia Monitor; Datex Ohmeda, Helsinki, Finland) during the entire experiment. Before surgery, 0.5 mg fentanyl was given intravenously and repeated if systemic blood pressure increased more than 20% above the initial control value. A large-bore catheter was inserted into the left external jugular vein for infusion of 10–20 ml $\cdot$ kg^{-1} $\cdot$ h^{-1} warm buffered Ringer solution, and a suprapubic catheter was inserted for measurement of urinary output. A heating mattress and warm blankets were used to maintain a normal and stable body temperature. The animals were studied while they lay supine.

Ventilation

A tracheotomy was performed, and a cuffed endotracheal tube (ID = 6.0 mm) was inserted. In 24 pigs, another cuffed endotracheal tube (ID = 4.5 mm) was inserted through the tracheostoma and positioned in the left lower lobar (LLL) bronchus, while temporally deflating the cuff of the tube positioned in the trachea. A medial sternotomy was performed in these pigs so that the tubes could be guided to a position that ensured separation of the LLL from the other lung regions. The separation was considered satisfactory if no air bubbles were detected when the tube leading to the LLL was submerged under water while the other lung regions were ventilated. The different and persistent fractions of expired oxygen during LLL hypoxia and hyperoxia to the other lung regions were regarded as additional proof of separation. The LLL was then ventilated with 30% of the total minute ventilation, corresponding to the relative weight of the LLL of that of both lungs. The sternotomy allowed inspection of the lungs to ensure that the left middle and upper lobes and the right lung (hyperoxic lung regions [HLs]) were ventilated through the main tube. The lungs of the pigs with closed thorax were mechanically ventilated by a Servo 900 C ventilator (Siemens Elema, Lund, Sweden) (n = 9) or an Engström Erica ventilator (Gambro Engström, Stockholm, Sweden) (n = 12). In the pigs with open thorax (n = 24), the LLL and the other lung regions were separately ventilated by two synchronized Servo 900 C ventilators. All ventilators were set at volume-controlled ventilation, 10 breaths/min, and an inspiration:expiration ratio of 1:1. A positive end-expiratory pressure of 5 cm H_2O was applied. The minute ventilation was adjusted to obtain an arterial carbon dioxide partial pressure of 33–45 mmHg (4.4–6.0 kPa) in the initial control situation, and was then kept constant throughout the experiment. The exhaled minute volumes (mean [SD]) were 2.2 (0.5) 1/min from the LLL and 5.0 (0.7) 1/min from the hyperoxic lung regions.

Hemodynamics

The right carotid artery was cannulated, and a triple-lumen thermistor-tipped balloon catheter (Criti Cath No 7 French; Ohmeda Pte Ltd., Singapore) was introduced *via* the right or left external jugular vein to the pulmonary artery. The catheters were connected to pressure transducers (Sorenson Transpac; Abbott Critical Care Systems, North Chicago, IL), and arterial and pulmonary blood pressures and temperature were recorded (7010 monitor; Marquette Electronics, Milwaukee, WI). Cardiac output ($\dot{Q}_T$) was measured by thermodilution in the pigs with closed thorax. The thermal indicator was 10 ml of 5% glucose at 0–2°C injected into the right atrium. The first measurement was ignored, and cardiac output was derived from the mean of the three consecutive measurements. Cardiac output and blood flow to the LLL ($\dot{Q}_{LLL}$) were measured continuously in the pigs with open thorax by enclosing the pulmonary artery and the artery to the LLL in ultrasonic flow probes (12 and 6 SB) connected to flowmeters (T208, Transonic volume flowmeter; Transonic Systems Inc., Ithaca, NY). Calculations were made of global PVR and of the PVR in the LLL (PVR_{LLL}), and the right lung and left upper and middle lobes (PVR_{HL}). We assumed that the whole lung was perfused under zone 3 conditions (mean pulmonary artery pressure [MPaP] and pulmonary capillary wedge pressure [PcwP] being higher than alveolar pressure), so that the perfusion pressure through the lung equaled

MPaP − PcwP. It must be borne in mind that the calculations of regional PVR (PVR_{LLL} and PVR_{HL}) merely reflect the blood flow distribution between the different lung regions, as the driving pressure (MPaP − PcwP) is assumed to be the same in all lung regions.

Cross-circulation

Twenty-four of the pigs were cross-circulated pairwise in 12 experiments. Each pair of pigs were siblings. A double-lumen dialysis catheter (Duo-Flow Catheter, 11.5 French; Medcomp Inc., Harleysville, PA) was introduced *via* the right external jugular vein, one in each pig, and placed with the distal opening in the right atrium as judged by pressure curves. Blood was drawn from the proximal lumen in each pig and pumped through polyvinyl-chloride tubings by two separate nonpulsatile roller pumps (PMO 10–220 Type; Gambro, Lund, Sweden) at a rate of 500 ml/min to the other pig, where the blood was delivered through the distal lumen of the double-lumen catheter. When trying to increase the flow to greater than 500 ml/min, the catheter sucked to the wall of the right atrium. The pigs were given 340 (16) IU/kg intravenous heparin before the cross-circulation was started.

Blood Gases

Mixed venous and arterial blood were collected for analysis of arterial and mixed venous oxygen partial pressures, arterial carbon dioxide partial pressure, and *p*H (ABL 3, Radiometer, Copenhagen, Denmark).

Nitric Oxide

Nitric oxide was given from a mixture of 200 or 1,000 parts per million (ppm) NO, balance nitrogen (AGA Specialgas; AGA Gas AB, Sundbyberg, Sweden) by a volumetrically calibrated flowmeter connected by a Y piece to the low-flow inlet of the Servo ventilator (n = 6) or by an I-NO vent (Ohmeda, Madison, WI) connected to the proximal part of the inspiratory limb of the Erica ventilator tubings (n = 6). The inspired gas was passed through a canister containing soda lime to absorb any NO_2. The concentrations of inspired NO and NO_2 were measured by chemiluminescence (analyzer M9841 NO_X; Lear Siegler Measurement Controls Corporation, Englewood, CO) in the inspiratory limb of the ventilator tubings, more than 50 cm from the Y piece to avoid contamination of expired gas.

The NO concentration in exhaled air was measured continuously by chemiluminescence (analyzer Model 42; Thermo Environmental Instruments Inc, Franklin, MA) from the hypoxic LLL and the hyperoxic lung regions alternately, in the expiratory limb of the ventilator tubings, more than 100 cm from the endotracheal tubes to ensure complete mixing and to avoid contamination of inspired gas. The average concentration over 10 breaths was used for statistical analysis.

Nitric Oxide Synthase Activity

Nitric oxide synthase activity was measured in hypoxic and hyperoxic lung tissue from the open-chest pigs in the single-pig and cross-circulation studies, by the conversion of L-[U^{14}C] arginine to L-[U^{14}C] citrulline. For the citrulline formation assay, tissues were homogenized in ice-cold homogenization buffer containing 320 mM sucrose, 10 mM HEPES, 0.1 mM EGTA, 1 mM DL-dithiothreitol, 10 μg/ml trypsin inhibitor, 10 μg/ml leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride, and 2 μg/ml aprotinin (adjusted to pH 7.2 at 20°C with 1 M HCl). The homogenate was centrifuged at 10,000*g* for 30 min at 4°C, and the soluble fraction was used for the measurement of NOS activity. The tissue extract was added to tubes prewarmed to 37°C, containing 100 μl of a buffer consisting of 50 mM potassiumphosphate, pH 7.2, 50 mM L-valine, 100 μM NADPH, 1 mM L-citrulline, 20 μM L-arginine and L-[U^{14}C] arginine (Amersham, United Kingdom; 150,000 disintegration per minute [dpm]), and 1.2 mM $MgCl_2$. Duplicate incubations for 10 min at 37°C were performed for each sample in the presence or absence of either EGTA (2 mM) or EGTA plus Nω-monomethyl-L-arginine (2 mM each), to determine the level of the Ca^{2+}-dependent (neuronal [nNOS] type I NOS and endothelial [eNOS] type III NOS) and the Ca^{2+}-independent (inducible [iNOS] type II NOS) NOS activity, respectively. The method does not distinguish between the two isoforms of Ca^{2+}-dependent NOS. The reaction was terminated by removal of substrate and dilution by the addition of 1.5 ml of 1:1 (vol/vol) H_2O/Dowex AF 50W-X8, pH 7.5 (supplied by Sigma-Aldrich Sweden AB, Stockholm, Sweden). Five milliliters of H_2O was added to the incubation mix, and 2 ml of the supernatant was removed and examined for the presence of L-[U^{14}C] citrulline by liquid scintillation counting. The level of citrulline is expressed as picomoles per gram of tissue (wet weight) per minute.

Experimental Protocol

Three sets of experiments were made. Inhaled gas was either hyperoxic (fraction of inspired oxygen [F_{IO_2}] = 0.8 or 1.0, balance nitrogen), hypoxic (F_{IO_2} = 0.05, balance nitrogen), or NO in hyperoxic gas (F_{IO_2} = 0.8, balance nitrogen). Hemodynamic and ventilatory parameters were recorded, inhaled and exhaled NO was measured, and mixed venous and arterial blood was sampled. Finally, the pigs were killed with an intracardiac injection of potassium chloride.

Study of the Effects of Global Hypoxia. The effects of global hypoxia were studied in six pigs with closed thorax (global-hypoxia group). The lungs were ventilated with F_{IO_2} = 0.8, balance nitrogen, for 30 min, followed by hypoxic ventilation during 5–10 min. To test the reproducibility over time, this procedure (30 min of hyperoxia, 5–10 min of hypoxia) was then repeated twice in each pig. The duration of hypoxic gas

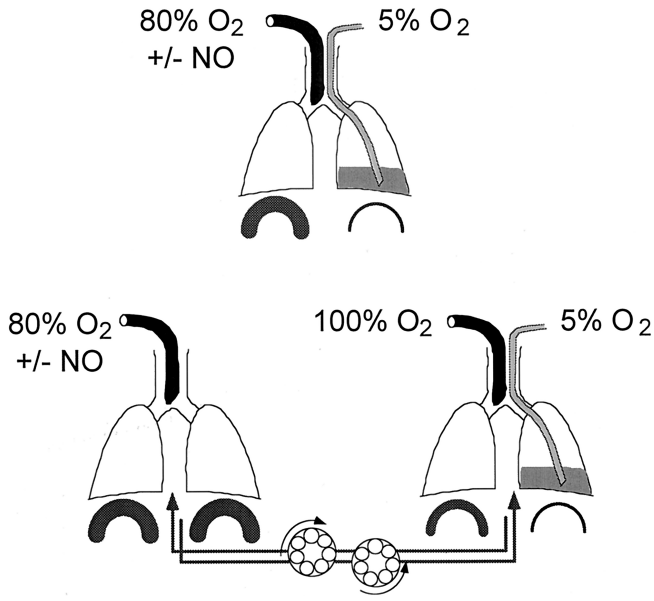

Fig. 1. Schematic drawing of the experimental set-ups. (*Top*) Single-pig study. Open-chest pigs, with or without inhaled nitric oxide (NO) to hyperoxic lung regions, during regional left lower lobe hypoxia. (*Bottom*) Cross-circulation study. Closed-chest pigs, with or without inhaled NO, cross-circulated with open-chest pigs with regional left lower lobe hypoxia.

ventilation was determined by the severe compromise of the circulation and the need to resume hyperoxic ventilation to keep the pigs alive. Data were collected at the end of each ventilator setting, resulting in data collection after 30, 40, 70, 80, 110, and 120 min.

Three other closed-chest pigs served as time controls (time-control group). The lungs were continuously ventilated with F_{IO_2} = 0.8, balance nitrogen. Data were collected at the same time intervals as in the global-hypoxia group.

Study of the Effects of Regional Hypoxia and Selective Inhaled Nitric Oxide to Hyperoxic Lung Regions during Regional Hypoxia. In the control group (n = 6), both lungs of pigs with open thorax were ventilated with F_{IO_2} = 0.8, balance nitrogen, for 90 min. The LLL was then continuously ventilated with hypoxic gas for 180 min during hyperoxic ventilation of the other lung regions (fig. 1, top). Data were collected at 90-min intervals. At the end of each experiment, pieces from both the hypoxic and hyperoxic lung regions were excised and immediately frozen in liquid nitrogen for analysis of NOS activity.

In the NO group (n = 6), the same protocol was followed as in the control group, but during the last 90 min of LLL hypoxia, NO 39 (SD, 1) ppm was added to the hyperoxic gas ventilating the right lung and the left upper and middle lobes (fig. 1, top).

Study of the Effects of Blood Cross-circulated from Pigs Exposed to Inhaled Nitric Oxide to Open-chest Pigs with Left Lower Lobar Hypoxia. Twenty-four pigs were cross-circulated pairwise in 12 experiments, divided into two groups (fig. 1, bottom). One of the pigs in each pair had closed thorax and the other open thorax. Both lungs of the pigs with closed thorax were ventilated with F_{IO_2} = 0.8, balance nitrogen, during the entire experiment. In six of these pigs, NO 72 (SD, 3) ppm was added to the inhaled hyperoxic gas, starting after 90 min and continuing until the experiment was completed 110 min later. We gave 72 ppm of NO, instead of 39 ppm as in the single-pig model, because the cross-circulated blood from the pigs exposed to INO was diluted in the open-chest pigs' blood volume (not exposed to INO). Furthermore, 94% of $\dot{Q}_T$ perfused the hyperoxic lung regions exposed to INO in the single-pig study, whereas we could cross-circulate no more than 500 ml of blood per minute, corresponding to approximately 17 % of $\dot{Q}_T$. In the pigs with open thorax, the LLL was continuously ventilated with hypoxic gas during hyperoxic (F_{IO_2} = 1.0) ventilation of the other lung regions. Data collected after 90 and 200 min were used for statistical calculations. At the end of each experiment, pieces from both the hypoxic and hyperoxic lung regions were excised and immediately frozen in liquid nitrogen for analysis of NOS activity.

The pigs with open thorax receiving blood from closed-chest pigs without INO constituted the control group (n = 6). The pigs with open thorax receiving blood from closed-chest pigs with INO constituted the NO group (n = 6).

Statistics

Data in the text and tables are presented as mean (SD). A two-way analysis of variance for repeated measures on one factor was applied to disclose any differences within groups (pA; tables 1 and 2) and interaction effects between groups (pAB; tables 2 and 3). Data collection periods (fixed) and pigs (n = 6, random) made up the two block factors for comparisons within groups, and the Scheffé *post hoc* test was used (global-hypoxia group and control group in single-pig study). A one-way analysis of variance was applied to disclose any differences in NOS activity between the control and NO groups (pA). A P value < 0.05 was considered significant. All analyses were performed with the SOLO Statistical System (version 4.0; BMDP Statistical Software Inc., Los Angeles, CA).

Results

Effects of Global Hypoxia

Mean systemic artery pressure decreased, while MPaP and NO_E (fig. 2B) increased reproducibly during the hypoxic periods ($P < 0.001$; table 1). Arterial and mixed venous oxygen tension decreased ($P < 0.001$) during 5–10 min of global hypoxia, causing a severe arterial oxygen desaturation down to 25–29%, as calculated by standard equations.[16] Systemic and pulmonary artery

Table 1. Effects of Repeated Brief Periods (5–10 min) of Global Hypoxia

	pA	Global Hyperoxia	Global Hypoxia	Global Hyperoxia	Global Hypoxia	Global Hyperoxia	Global Hypoxia
F_{IO_2}		0.8	0.05	0.8	0.05	0.8	0.05
NO_E (ppb)	< 0.001	1.4 (0.5)	1.9†‡ (0.8)	1.3 (0.5)	2.1*†‡ (0.8)	1.3 (0.5)	2.1*†‡ (0.8)
Q_T (l/min)	< 0.001	3.4 (1.7)		3.9 (1.6)		3.2 (1.5)	2.5*†‡ (1.7)
MaP (mm Hg)	< 0.001	75 (11)	56† (12)	81 (4)	51*†‡ (8)	73 (9)	43*†‡ (5)
MPaP (mm Hg)	< 0.001	16 (3)	31* (3)	18 (4)	32*†‡ (6)	18 (4)	29* (11)
Pa_{O_2} (mm Hg)	< 0.001	394 (64)	19* (1)		20* (2)		21* (1)
Pv_{O_2} (mm Hg)	< 0.001	44 (5)	10* (2)		10* (2)		11* (4)
Pa_{CO_2} (mm Hg)	> 0.07	36 (6)	37 (6)		35 (5)		35 (5)

Significant difference from the * initial, † second, and ‡ third periods of hyperoxic ventilation. Cardiac output (Q_T) was not measured during the first and second periods of hypoxic ventilation, and blood gases were not measured during the second and third periods of hyperoxic ventilation.

pA = overall *P* value for effect of global hypoxia within the group; F_{IO_2} = fraction of inspired oxygen; NO_E = nitric oxide concentration in exhaled gas; ppb = parts per billion; MaP = mean systemic arterial pressure; MPaP = mean pulmonary arterial pressure; Pa_{O_2} = arterial oxygen tension; Pv_{O_2} = partial pressure of oxygen in mixed venous blood; Pa_{CO_2} = arterial carbon dioxide tension.

pressures, cardiac output, and NO_E all returned to baseline values ($P > 0.07$) during hyperoxic ventilation between the periods of hypoxic ventilation (table 1). In the time-control pigs that were ventilated constantly with an F_{IO_2} of 0.8, no measurable changes were observed in hemodynamic or ventilatory parameters, NO_E (fig. 2A), or blood gases during a 2-h period. However, cardiac output increased over time from 2.3 (0.3) to 3.0 (0.7) 1/min.

Effects of Regional Hypoxia

Although arterial and mixed venous oxygen tensions decreased ($P < 0.01$) during LLL hypoxia, arterial oxygen partial pressure was within or above normal limits. MPaP increased ($P < 0.001$) and both $\dot{Q}_T$ ($P < 0.05$) and the relative blood flow to the LLL ($\dot{Q}_{LLL}/\dot{Q}_T$) ($P < 0.001$) decreased (table 2). After 90 min of LLL hypoxia, $\dot{Q}_{LLL}/\dot{Q}_T$ had decreased from 23 (7) to 7 (3) % of $\dot{Q}_T$.

Table 2. Effects of Regional Hypoxia (Single Pig Study, Control Group), and Effects of Selective INO to the Hyperoxic Lung Regions during LLL Hypoxia (Single Pig Study, NO Group)

	Group	Global Hyperoxia	90 min of LLL Hypoxia	180 min of LLL Hypoxia	pA	pAB
F_{IO_2LLL}		0.8	0.05	0.05		
F_{IO_2HL}		0.8	0.8	0.8		
INO_{HL} (ppm)	NO			39 (1)		
NO_{ELLL} (ppb)	Control	1.3 (0.6)	1.8* (0.5)	2.2* (0.9)	< 0.001	
	NO		2.2 (0.6)	2.0† (0.8)		0.014
NO_{EHL} (ppb)	Control	1.3 (0.5)	1.5 (0.5)	1.9* (0.5)	0.034	
Q_T (l/min)	Control	3.11 (0.35)	2.91 (0.44)	2.75* (0.50)	0.030	
	NO		2.65 (0.70)	2.70 (0.84)		> 0.07
Q_{LLL}/Q_T (%)	Control	23 (7)	7* (3)	7* (3)	< 0.001	
	NO		6 (2)	3† (2)		0.023
MaP (mm Hg)	Control	70 (7)	70 (7)	68 (12)	> 0.07	
	NO		79 (10)	74 (10)		> 0.07
MPaP (mm Hg)	Control	22 (3)	28* (6)	28* (4)	< 0.001	
	NO		25 (1)	20† (2)		0.045
PcwP (mm Hg)	Control	10 (3)	10 (3)	10 (3)	> 0.07	
	NO		10 (1)	10 (1)		> 0.07
PVR (mm Hg · l^{-1} · min^{-1})	Control	3.81 (1.39)	6.40* (0.94)	6.55* (0.82)	< 0.001	
	NO		6.00 (2.33)	4.04† (1.57)		0.009
*p*H	Control	7.43 (0.04)	7.40 (0.08)	7.39 (0.07)	> 0.07	
	NO		7.44 (0.03)	7.43 (0.04)		> 0.07
Pa_{O_2} (mm Hg)	Control	320 (10)	160* (107)	138* (78)	0.001	
	NO		221 (68)	261 (102)		> 0.07
Pv_{O_2} (mm Hg)	Control	42 (3)	35* (7)	34* (7)	0.004	
	NO		37 (4)	39 (4)		> 0.07
Pa_{CO_2} (mm Hg)	Control	44 (5)	48 (7)	49 (6)	> 0.07	
	NO		42 (2)	43 (2)		> 0.07
MetHb (%)	Control	1.0 (0.1)	0.9 (0.1)	0.8 (0.2)	> 0.07	
	NO		1.0 (0.2)	1.4† (0.2)		0.010

* Significant effect of regional hypoxia **within the control group** (pA). † Significant interaction effect of inhalation of nitric oxide (INO; pAB) to the hyperoxic lung regions during left lower lobe (LLL) hypoxia.

F_{IO_2} = fraction of inspired oxygen; HL = hyperoxic parts of the lungs; ppm = parts per million; NO = nitric oxide; NO_E = nitric oxide concentration in exhaled gas; ppb = parts per billion; Q_T = cardiac output; Q_{LLL} = regional pulmonary blood flow to the left lower lobe; MaP = mean systemic arterial pressure; MPaP = mean pulmonary arterial pressure; PcwP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; Pa_{O_2} = arterial oxygen tension; Pv_{O_2} = partial pressure of oxygen in mixed venous blood; Pa_{CO_2} = arterial carbon dioxide tension; MetHb = methemoglobin.

Table 3. Effects in Open-chest Pigs with Regional LLL Hypoxia, Receiving Blood from Pigs with (NO Group) and without (Control Group) INO

	Group	90 min of LLL Hypoxia	200 min of LLL Hypoxia	pAB
$F_{IO_2}LLL$		0.05	0.05	
$F_{IO_2}HL$		1.0	1.0	
	NO		NO-blood	
$NO_E LLL$ (ppb)	Control	1.5 (0.8)	1.8 (0.8)	
	NO	2.0 (0.6)	1.5* (0.4)	0.011
$NO_E HL$ (ppb)	Control	1.3 (0.5)	1.6 (0.6)	
	NO	1.5 (0.5)	1.3* (0.4)	0.007
Q_T (l/min)	Control	2.89 (0.18)	2.80 (0.23)	
	NO	3.03 (0.38)	3.13 (0.39)	> 0.07
Q_{LLL}/Q_T (%)	Control	6 (1)	6 (1)	
	NO	7 (2)	5* (2)	0.012
MaP (mm Hg)	Control	82 (2)	81 (3)	
	NO	84 (6)	84 (6)	> 0.07
MPaP (mm Hg)	Control	31 (5)	32 (3)	
	NO	30 (6)	36* (6)	0.002
PcwP (mm Hg)	Control	14 (3)	15 (3)	
	NO	15 (3)	16 (4)	> 0.07
PVR (mm Hg · l^{-1} · min^{-1})	Control	5.71 (2.42)	5.95 (2.13)	
	NO	4.85 (1.26)	6.31* (1.40)	0.020
*p*H	Control	7.45 (0.05)	7.42 (0.05)	
	NO	7.46 (0.06)	7.41 (0.10)	> 0.07
Pa_{O_2} (mm Hg)	Control	229 (90)	181 (60)	
	NO	235 (133)	197 (128)	> 0.07
Pv_{O_2} (mm Hg)	Control	36 (3)	35 (5)	
	NO	38 (4)	35 (5)	> 0.07
Pa_{CO_2} (mm Hg)	Control	39 (4)	44 (4)	
	NO	39 (6)	46 (12)	> 0.07
MetHb (%)	Control	1.0 (0.1)	0.9 (0.2)	
	NO	1.0 (0.2)	1.7* (0.3)	< 0.001

* Significant interaction effect of blood cross-circulated from pigs exposed to inhalation of nitric oxide (INO; pAB).

F_{IO_2} = fraction of inspired oxygen; LLL = left lower lobe; HL = hyperoxic parts of the lungs; NO = nitric oxide; NO_E = nitric oxide concentration in exhaled gas; ppb = parts per billion; Q_T = cardiac output; Q_{LLL} = regional pulmonary blood flow to the left lower lobe; MaP = mean systemic arterial pressure; MPaP = mean pulmonary arterial pressure; PcwP = pulmonary capillary wedge pressure; Pa_{O_2} = arterial oxygen tension; PVR = pulmonary vascular resistance; Pv_{O_2} = partial pressure of oxygen in mixed venous blood; Pa_{CO_2} = arterial carbon dioxide tension; MetHb = methemoglobin.

Prolongation of the LLL hypoxia from 90 to 180 min did not cause any further redistribution of the blood flow ($P > 0.05$; fig. 3A). Exhaled NO from the LLL ($NO_E LLL$) increased by 51% ($P < 0.05$) during the first 90 min of LLL hypoxia and continued to increase during the following 90 min of hypoxic ventilation. After 180 min of LLL hypoxia, $NO_E LLL$ had increased by 81% and was significantly ($P < 0.01$) higher as compared with the initial control value (fig. 3B). Exhaled NO from the hyperoxic lung regions ($NO_E HL$) also increased (by 18% during the first 90 min) and was significantly ($P < 0.05$) higher after 180 min of LLL hypoxia than during the initial control period of bilateral hyperoxia. The redistribution of pulmonary blood flow and the increase in $NO_E LLL$ were rapid in onset, starting within seconds of the hypoxia start; however, whereas the redistribution of pulmonary blood flow

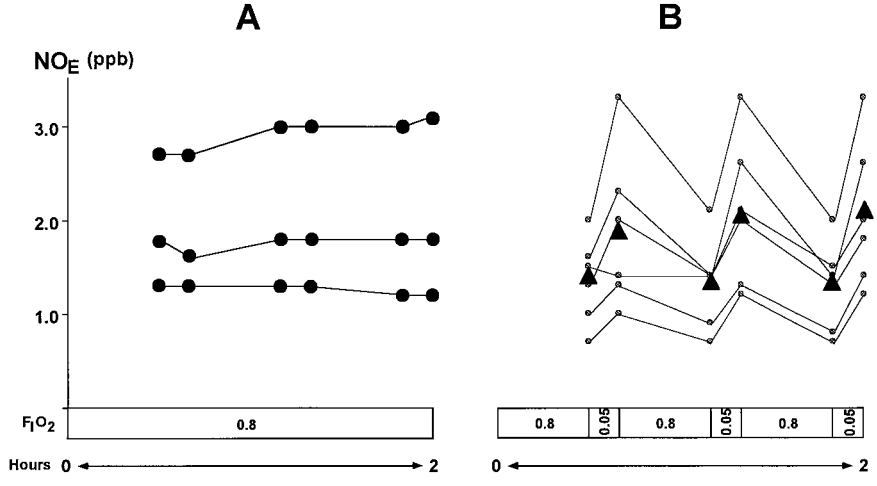

Fig. 2. Effects of global hypoxia on exhaled nitric oxide (NO_E). Triangles = mean. (*A*) NO_E (parts per billion [ppb]) remained stable during 2 h of global hyperoxia in three pigs. (*B*) NO_E increased ($P < 0.001$) during the brief periods of global hypoxia in six pigs. F_{IO_2} = fraction of inspired oxygen.

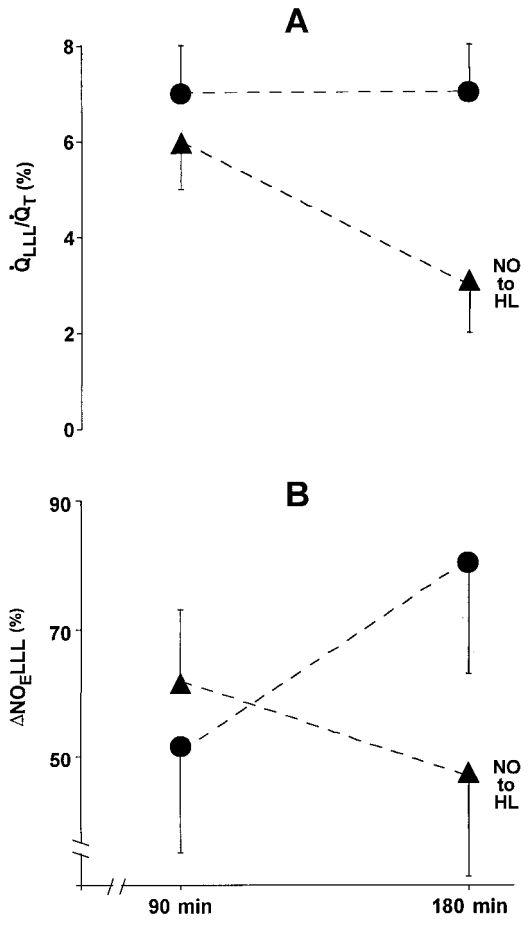

Fig. 3. Effects of selective inhaled nitric oxide (NO) to hyperoxic lung regions during left lower lobe (LLL) hypoxia (single-pig study). Circles = mean control group; triangles = mean NO group; whiskers = SEM; $\dot{Q}_{LLL}/\dot{Q}_T$ = relative blood flow to the hypoxic LLL expressed as percentage of cardiac output; $\Delta NO_E LLL$ = change (%) in exhaled NO from baseline measurements during bilateral hyperoxia. (*A*) Prolongation of regional LLL hypoxia from 90 to 180 min in the control group did not cause any further redistribution of the blood flow ($P > 0.05$), whereas INO to the hyperoxic lung regions (HLs) during the last 90 min of LLL hypoxia caused a further redistribution ($P < 0.05$) of pulmonary blood flow away from the hypoxic LLL. (*B*) $NO_E LLL$ continued to increase during prolonged LLL hypoxia in the control group, whereas it decreased ($P < 0.05$) in the NO group when NO was added to the gas ventilating the hyperoxic lung regions.

was completed within 15–20 min, $NO_E LLL$ increased slowly and constantly during the 180 min of LLL hypoxia. The Ca^{2+}-dependent NOS activity was higher (197 [86] pmol · g^{-1} · min^{-1}; $P < 0.05$) in the hypoxic as compared with hyperoxic lung tissue (162 [96] pmol · g^{-1} · min^{-1}) in the same animals (fig. 4, single-pig study, control group).

Effects of Selective Inhaled Nitric Oxide to Hyperoxic Lung Regions during Left Lower Lobar Hypoxia

Selective INO to the hyperoxic lung regions during ongoing LLL hypoxia (NO group) decreased MPaP ($P < 0.05$) and global PVR ($P < 0.01$; table 2). Regional PVR in the hyperoxic lung regions receiving INO decreased

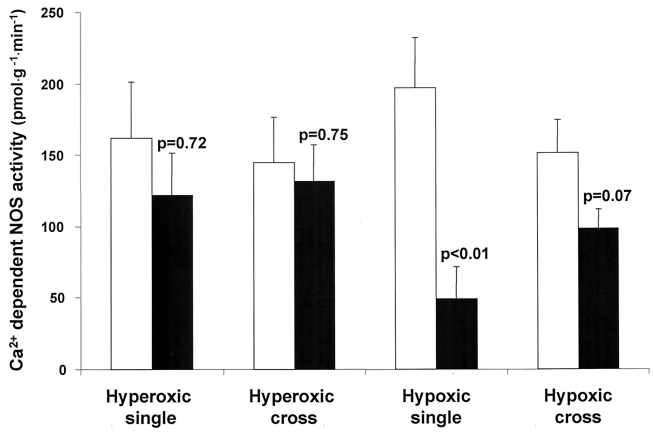

Fig. 4. Ca^{2+}-dependent nitric oxide synthase (NOS) activity in lung tissue. Single = single-pig study; cross = cross-circulation study; open bars = control groups; filled bars = NO groups; whiskers = SEM. The Ca^{2+}-dependent NOS activity was lower in lung tissue from the hypoxic left lower lobe, when NO was added to the hyperoxic gas ventilating the other lung regions (single-pig study, NO group) or when blood was cross-circulated from pigs with inhaled nitric oxide (cross-circulation study, NO group).

by 19 (38)%, whereas PVR_{LLL} increased by 61 (74)%, resulting in a further redistribution of the pulmonary blood flow away from the LLL, leaving only 3 (2)% of $\dot{Q}_T$ ($P < 0.05$) to perfuse the LLL (fig. 3A).

Whereas $NO_E LLL$ continued to increase during prolonged LLL hypoxia in the control group, $NO_E LLL$ decreased ($P < 0.05$) over the same time period when INO was added to the hyperoxic lung regions (fig. 3B, NO group). The decrease in $NO_E LLL$ was delayed in onset, emerging 35–60 min after starting INO. The Ca^{2+}-dependent NOS activity in hypoxic lung tissue was significantly lower ($P < 0.01$) in pigs with INO to hyperoxic lung regions (49 [56] pmol · g^{-1} · min^{-1}) as compared with pigs without INO (197 [86] pmol · g^{-1} · min^{-1}; fig. 4, single-pig study).

Effects in Open-chest Pigs with Regional Left Lower Lobar Hypoxia Receiving Blood from Closed-chest Pigs with and without Inhaled Nitric Oxide

Global PVR increased by 32 (21)% ($P < 0.05$) in the open-chest pigs when the cross-circulated blood came from pigs exposed to INO, but did not change during the same 90 min period when the cross-circulated blood came from pigs without INO (table 3). Moreover, both PVR_{LLL} and PVR_{HL} increased, the former by as much as 86% ($P < 0.01$; fig. 5A) and the latter by 29% ($P < 0.05$; fig. 5B). These changes in PVR were accompanied by a further redistribution of the pulmonary blood flow away from the hypoxic LLL ($P < 0.05$) to the hyperoxic lung regions. As in the single-pig experiments, $NO_E LLL$ increased constantly over time in the control group receiving blood from pigs without INO, whereas it decreased slowly over time in the NO group receiving blood from pigs with INO (table 3 and fig. 6A). MPaP increased

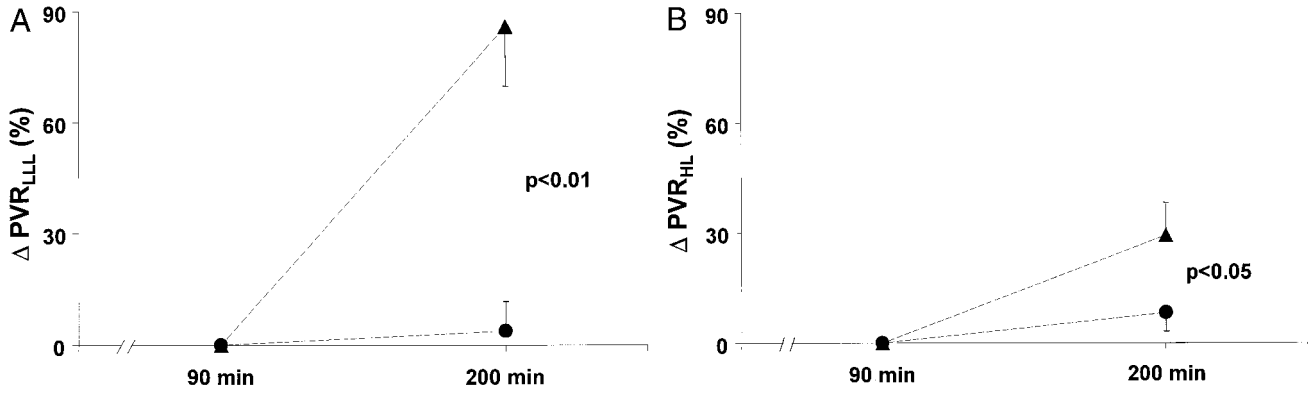

Fig. 5. Effects on pulmonary vascular resistance (PVR) in open-chest pigs with regional left lower lobe (LLL) hypoxia, receiving blood from pigs with and without inhaled nitric oxide. Circles = mean control group; triangles = mean NO group; whiskers = SEM; Δ = change; ΔPVR = change (%) in PVR from baseline measurements; HL = hyperoxic lung regions. The pigs in the nitric oxide group received blood from closed-chest pigs with inhaled nitric oxide during the last 110 min of the cross-circulation. When pigs with regional LLL hypoxia received blood from pigs with inhaled nitric oxide, PVR increased, but more so in hypoxic (*A*) than in hyperoxic (*B*) lung regions.

slowly over time in the NO group as compared with the control group, where no significant changes in MPaP were observed over time (fig. 6B). The Ca^{2+}-dependent NOS activity in the hypoxic LLL was lower when the cross-circulated blood came from pigs with INO (98 [34] pmol · g^{-1} · min^{-1}) as compared with cross-circulated blood from pigs without INO (152 [55] pmol · g^{-1} · min^{-1}; $P = 0.07$; fig. 4, cross-circulation study).

Discussion

The most important finding in our study is that INO has effects not only in lung regions directly receiving INO, but also in other lung regions. These distant effects are blood-mediated and mimic the effects found in other experiments where NOS inhibitors have been given intravenously to rabbits[13] and pigs[14] with regional hypoxia. We found that INO to hyperoxic lung regions decreased both NO_E and the Ca^{2+}-dependent NOS activity in tissue from hypoxic lung regions. When blood was cross-circulated from pigs exposed to INO, NO_E decreased from both hypoxic and hyperoxic lung regions, and PVR increased, more so in hypoxic than in hyperoxic lung regions. These changes were accompanied by a further redistribution of pulmonary blood flow away from hypoxic to hyperoxic lung regions. The redistribution is thus an effect of both vasodilation in ventilated areas that receive INO and vasoconstriction in areas that are not exposed to INO. Our results also show that NO_E increased both during moderate global hypoxia and during regional hypoxia in pigs, and the Ca^{2+}-dependent NOS activity was higher in hypoxic than in hyperoxic lung tissue from the same animal.

Nitric Oxide in Exhaled Air

Nitric oxide is present in exhaled air from both humans and animals,[17,18] with the largest contribution

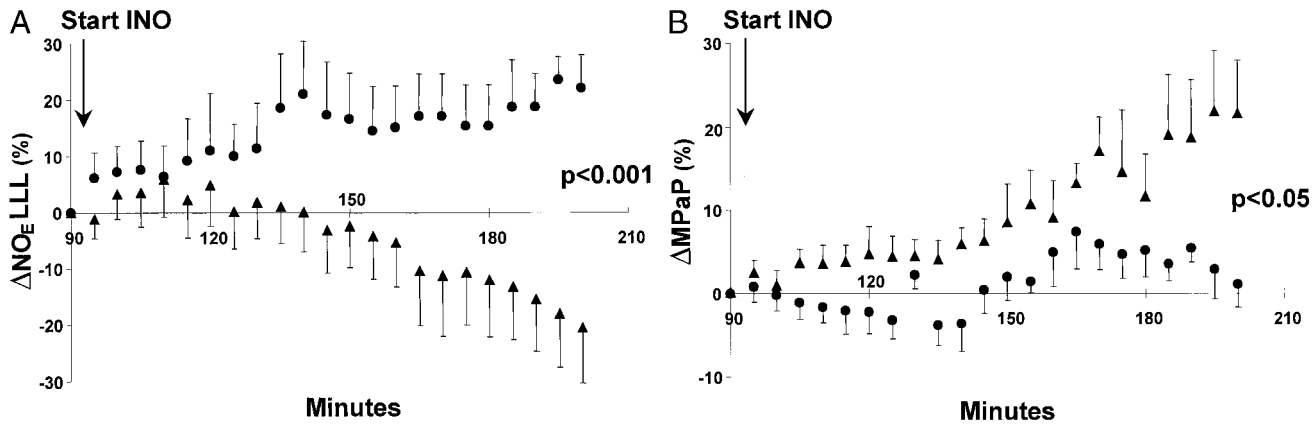

Fig. 6. Effects over time in open-chest pigs with regional left lower lobe (LLL) hypoxia, receiving blood from pigs with and without inhaled nitric oxide (INO). Blood was cross-circulated for 90 min between the closed- and open-chest pigs, before the time period showed above. Circles = control group (mean); triangles = NO group (mean); whiskers = SEM; Δ = change. (*A*) Exhaled NO from the LLL (NO_ELLL) decreased over time when blood was cross-circulated from closed-chest pigs with INO, but increased over the same time period when the cross-circulated blood came from closed-chest pigs without INO. (*B*) Mean pulmonary arterial pressure (MpaP) increased over time in the pigs receiving blood from closed-chest pigs with INO as compared with the pigs receiving blood from closed-chest pigs without INO.

from the upper airways.[19,20] NO_E measured from a tracheostoma, as in our study, reflects the NO production from the lower airways, provided that other factors influencing NO_E are kept constant. Several factors, for example, changes in ventilation, positive end-expiratory pressure, F_{IO_2}, *p*H, and total and regional blood flow, influence NO_E.[21] To eliminate any influence on our results by changes in ventilation or positive end-expiratory pressure, the minute ventilation, respiratory frequency, and inspiration:expiration ratio were kept constant, and a constant positive end-expiratory pressure of 5 cm H_2O was maintained throughout the study. Acute global hypoxia has been reported to decrease NO_E in isolated rabbit[21] and pig[22] lungs. We found that global hypoxia reproducibly increased NO_E in intact pigs, but it is hazardous to draw conclusions from such experiments. As the animal gets hypoxemic, numerous compensatory survival mechanisms are initiated,[23] and steady state conditions are difficult or impossible to maintain. However, NO_E also increased from the LLL exposed to regional hypoxia (table 2, control group). In these pigs, systemic hypoxemia was avoided, and cardiac output and vascular pressures remained normal, since the other lung regions were ventilated with hyperoxic gas.

An increase in *p*H increases NO_E.[21] We could not detect any changes in arterial *p*H in the pigs with open thorax, but the decrease in $\dot{Q}_{LLL}$ at the onset of HPV while the ventilation was unchanged might have caused regional respiratory alkalosis. NO_E is inversely related to the pulmonary blood flow. In isolated, blood-perfused rabbit lungs, changes in flow rate between 200 and 20 ml/min caused only minor changes in NO_E, whereas NO_E increased sharply at flow rates less than 20 ml/min.[21] This is because less of the NO that is produced in the lung is eliminated by the blood. The increase in NO_ELLL during the first 15–20 min of LLL hypoxia might, at least in part, be a result of the marked decrease in regional LLL blood flow at the onset of HPV and the ensuing regional respiratory alkalosis (table 2, control group). However, NO_ELLL continued to increase over time during prolonged regional hypoxia, when HPV was already fully developed and there was no further change in regional pulmonary blood flow or regional ventilation–perfusion relation. Furthermore, NO_ELLL increased over time in the pigs receiving blood from closed-chest pigs without INO, where no changes in Q_{LLL} were detected (table 3, control group).

Endogenous Nitric Oxide Production

Increased shear stress is a potent stimulus for endothelial NO synthesis in both systemic[24] and pulmonary[25] vessels. Vascular shear stress increases when blood flow or blood viscosity increases or when vessel diameter decreases.[26] Changes in shear stress might affect NO_E and NOS activity. However, changes in shear stress cannot explain the increase in PVR and the decrease in NO_E observed in our cross-circulation experiments.

Previous studies have shown severe hypoxia both to reduce[27,28] and to increase[29] NO production, whereas moderate hypoxia, compatible with life, has been shown to have no effect on[27,28] or increase[30–32] NO production in pulmonary endothelial cells. We found a higher Ca^{2+}-dependent NOS activity in tissue samples from hypoxic than from hyperoxic lung regions in the same animals (fig. 4, single-pig study, control group). Together with the increase in NO_ELLL measured between 90 and 180–200 min of regional LLL hypoxia (tables 2 and 3, control groups), this indicates an increased NO production in hypoxic lung regions, opposing HPV. This is consistent with the study by Hampl *et al.*,[30] which showed that hypoxia increases cytosolic free calcium in pulmonary artery endothelial cells and potentiates NO synthesis. Hampl and Archer[33] suggested that increased production of endogenous NO during hypoxia might be physiologically advantageous as it would prevent excessive increases in pressure in small pulmonary vessels.

Distant Effects of Inhaled Nitric Oxide

We found that INO has effects not only in lung regions directly receiving INO, but also in other lung regions. During INO to hyperoxic lung regions, both NO_ELLL, the Ca^{2+}-dependent NOS activity in tissue from hypoxic lung regions, and the regional blood flow to the hypoxic LLL decreased significantly. The separation between the lungs was complete since we could detect a decrease in NO_ELLL during ongoing inhalation of 40 ppm of NO (20,000 times higher concentration) to the hyperoxic lung regions. The effects on blood flow distribution during selective INO to the hyperoxic lung regions may reflect a preference for blood to pass through the vasodilated regions and need not reflect an active vasoconstriction. To distinguish between an active vasoconstriction in the hypoxic LLL and a passive shift of blood flow to the vasodilated hyperoxic lung regions, we had to conduct the cross-circulation experiments. They allow the calculation of global and regional PVR in lungs that are not exposed to INO and the measurement of NO_E from both hyperoxic and hypoxic lung regions. When pigs were cross-circulated with NO– blood but not directly exposed to INO, NO_E decreased from both hypoxic and hyperoxic lung regions, and global PVR increased. These changes were much more pronounced in hypoxic than in hyperoxic lung regions. These results suggest that INO downregulates endogenous NO production, even in regions that do not receive INO, and more so in hypoxic than hyperoxic regions. There are previous reports that support our findings. Thus, NO has been shown to function as a negative feedback modulator of its own synthesis.[34–39] NO binds to iron-containing proteins including NOS, thereby inhibiting the activity of constitutive NOS from rat and bovine

cerebellum,[34,35] from bovine aortic endothelial cells,[36] and from cultured pulmonary artery endothelial cells.[37] This has been suggested as a possible explanation for the rebound pulmonary vasoconstriction sometimes observed after withdrawal of INO.[37–39] The hypothesis was further supported when INO for 48 h was shown to reversibly reduce the pulmonary endogenous NO production in isolated perfused rat lungs,[38,40] and INO for 3 weeks was shown to attenuate hypoxic pulmonary vascular remodeling.[41] However, the same group later showed that 3 weeks of INO attenuated the upregulation of cyclic guanosine monophosphate production caused by prolonged hypoxia, but did not alter the eNOS protein levels, NOS activity, or endothelium-dependent vasodilatation during normoxic and hypoxic conditions.[39] The negative feedback mechanism for NO may be model-dependent and dependent on the level of endogenous NO. In humans with endothelial pulmonary dysfunction, there may be very little endogenous NO to downregulate. Our findings form the basis of a paradigm shift in view of the mechanisms by which INO acts. The generally held explanation has been that INO dilates vessels in ventilated lung areas, decreasing MPaP and redistributing blood flow to the ventilated areas, away from the nonventilated regions. Our findings show that the effect of INO is dual, dilating vessels in ventilated regions and actively constricting vessels in regions that do not receive INO. This dual effect on the pulmonary vasculature will cause different degrees of pressure reduction and redistribution of blood flow, depending on whether the nonventilated region is large or small. The more tissue that is not receiving INO, because of collapse or consolidation as in acute respiratory failure or ventilation with NO-free gas, as in the present study, the more of the lung will constrict by INO and the less visible is the overall vasorelaxant effect by INO. This might be a cause of "nonresponse," in addition to other possible mechanisms, as, for example, attenuation of the effects of NO by scavenging, competition by other vasoactive substances, and morphologic derangement that may not allow a response to INO. Our findings suggest a possible explanation of the observations of a significant improvement of arterial oxygenation, without any significant change of MPaP in patients with acute respiratory distress syndrome.[42–44]

In conclusion, inhalation of NO downregulates endogenous NO production in lung regions that do not receive INO, and more so in hypoxic than in hyperoxic regions. This distant effect is mediated by a blood-borne factor. INO thus causes a dual effect: vasodilation in regions that receive INO and vasoconstriction in other, predominantly hypoxic regions that do not receive INO. The vasoconstrictive effect seems to be substantial in the pig, but the possible importance in other species remains to be shown, and the identity of the transmitter of the distant effect has to be disclosed.

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