Nitric Oxide Modulation of Pulmonary Blood Flow Distribution in Lobar Hypoxia

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Background: Nitric oxide, endogenously produced or inhaled, has been shown to play an important role in the regulation of pulmonary blood flow. The inhalation of nitric oxide reduces pulmonary arterial pressure in humans, and the blockade of endogenous nitric oxide production increases the pulmonary vascular response to hypoxia. This study was performed to investigate the hypothesis that intravenous administration of an nitric oxide synthase inhibitor and regional inhalation of nitric oxide can markedly alter the distribution of pulmonary blood flow during regional hypoxia.

Methods: Hypoxia (5% O_2) was induced in the left lower lobe of the pig, and the blood flow to this lobe was measured with transit-time ultrasound. Nitric oxide was administered in the gas ventilating the hypoxic lobe and the hyperoxic lung regions with and without blockade of endogenous nitric oxide production by means of N^ω-nitro-L-arginine methyl ester (L-NAME).

Results: Hypoxia in the left lower lobe reduced blood flow to that lobe to $27 \pm 3.9\%$ (mean $\pm$ SEM) of baseline values ($P < 0.01$). L-NAME caused a further reduction in lobar blood flow in all six animals to $12 \pm 3.5\%$ and increased arterial oxygen tension (Pa_{O_2}) ($P < 0.01$). Without L-NAME, the inhalation of nitric oxide (40 ppm) to the hypoxic lobe increased lobar blood flow to $66 \pm 5.6\%$ of baseline ($P < 0.01$) and, with L-NAME, nitric oxide delivered to the hypoxic lobe resulted in a lobar blood flow that was $88 \pm 9.3\%$ of baseline (difference not significant). When nitric oxide was administered to the hyperoxic lung regions, after L-NAME infusion, the blood flow to the hypoxic lobe decreased to $2.5 \pm 1.6\%$ of baseline and Pa_{O_2} was further increased ($P < 0.01$).

Conclusions: By various combinations of nitric oxide inhalation and intravenous administration of an nitric oxide synthase inhibitor, lobar blood flow and arterial oxygenation could be markedly altered during lobar hypoxia. In particular, the combination of intravenous L-NAME and nitric oxide inhalation to the hyperoxic regions almost abolished perfusion of the hypoxic lobe and resulted in a Pa_{O_2} that equalled the prehypoxic values. This possibility of adjusting regional blood flow and thereby of improving Pa_{O_2} may be of value in the treatment of patients undergoing one-lung ventilation and of patients with acute respiratory failure. (Key words: Anesthetics: nitric oxide. Hypoxia. Lung(s): hypoxic vasoconstriction. Pharmacology: N^ω-nitro-L-arginine methyl ester.)

IN 1980 Furchgott and Zawadski[1] reported that vasodilation caused by acetylcholine is endothelium dependent and that endothelial cells, when stimulated, can release substances that cause relaxation of vascular smooth muscle. One such substance has been identified as nitric oxide.[2,3] Endogenous production of nitric oxide has been demonstrated by Gustafsson *et al.*,[4] who measured the nitric oxide present in the exhaled breath of rabbits and humans. When the rabbit was hypoxic, the exhaled nitric oxide concentration decreased. Nitric oxide synthase, constitutive and inducible, has been found in many cell types in the human lung.[5] Inhaled nitric oxide at concentrations of 5–80 ppm has been shown to diminish or eradicate both the hypoxic vasoconstrictor response and the pulmonary hypertension caused by hypoxia and thromboxane analogues in conscious lambs[6,7] and in a porcine model of adult respiratory distress syndrome (ARDS).[8] Nitric oxide inhalation can reduce pulmonary hypertension in healthy volunteers breathing hypoxic gas[9] and in patients with ARDS.[10]

In anesthetized guinea pigs, when endogenous nitric oxide production was blocked by administration of L-arginine analogues, Persson *et al.*[11] found that hypoxemia

developed and that respiratory frequency increased. They also found an increase in pulmonary vascular resistance (PVR), which was more marked during hypoxic conditions than during normoxia. More recently, Sprague and coworkers[12] found that the blockade of endogenous nitric oxide production in the anesthetized rabbit with induced unilateral hypoxia caused a shift of blood from the hypoxic lung and improved arterial oxygenation. These findings indicate that administration of nitric oxide inhibits hypoxic pulmonary vasoconstriction and that blockade of endogenous nitric oxide production enhances it. In heterogenous lung injury the combination of nitric oxide and nitric oxide synthase inhibitors or another vasoconstrictor may improve gas exchange, although results are conflicting.[13–15]

We hypothesized that regional inhalation of nitric oxide and blockade of endogenous nitric oxide production would have an effect on the distribution of pulmonary blood flow during regional hypoxia. This hypothesis was tested in an animal model of unilobar hypoxia.

Materials and Methods

Animals and Anesthesia

The study was approved by the Animal Research Ethics Committee of Uppsala University.

Twenty-one pigs of Swedish country breed, weighing 22–35 kg, were used in the study. The animals were premedicated with pentobarbital ($20 \ mg \cdot kg^{-1}$) and 0.5 mg atropine intraperitoneally. Anesthesia was induced 30 min later with 180–360 mg intravenous pentobarbital and was maintained with a continuous infusion of chlormetiazole[16] ($800–1,600 \ mg \cdot h^{-1}$) and pancuronium bromide ($4–8 \ mg \cdot h^{-1}$). After induction the animals were placed in the supine position for the remainder of the study.

An intravenous injection of 0.5 mg fentanyl was given before surgery (see below), and additional doses of 0.2–0.3 mg were given as necessary. The level of anesthesia was considered appropriate when the surgical procedure did not cause any changes in heart rate or blood pressure. Isotonic saline, $10 \ ml \cdot kg^{-1} \cdot h^{-1}$, was given for hydration.

Ventilation

A tracheotomy was performed and a cuffed endotracheal tube with an inner diameter of 6.0 mm was inserted. During the preparation, adequate ventilation was supplied by a servomechanism-controlled ventilator (Servo 900 C, Siemens Elema, Lund, Sweden). The respiratory frequency was maintained at 20 breaths/min, and the inspired fraction of oxygen was 0.4. Another cuffed endotracheal tube (4.5 mm internal diameter) was introduced through the tracheal stoma to the left of the main tube. An introducer inside this smaller tube enabled positioning in the left lower lobe (LLL) bronchus. By inflating the cuff, the LLL was isolated and could be ventilated independently. The position of the tube could be checked and adjusted at the site of the thoracotomy so that the LLL was inflated with the smaller tube but not when ventilation was provided by the main tube. This arrangement was tested by submerging the tube leading to the LLL under water and checking for air leakage when the remaining lung regions were ventilated. It also was possible to ensure that the left middle and upper lobes and the right lung were ventilated by the main tube. The LLL was ventilated with an additional volume-controlled ventilator (Servo 900 C, Siemens Elema) synchronized with the main ventilator. Thirty percent of the total minute ventilation was administered to the LLL, which corresponded to the LLL's weight as a percentage of the total weight of both lungs.‖ After thoracotomy, both ventilators were adjusted to give a positive end-expiratory pressure of 5 cmH$_2$O. The inspired fraction of oxygen was increased to 0.8, and respiratory frequency was maintained at 20 breaths/min with tidal volumes adjusted to obtain an arterial carbon dioxide tension (Pa$_{CO_2}$) of 37–47 mmHg. At the beginning of each measurement period (see Experimental Protocol, below) the lungs were ventilated three times with two times normal tidal volume to prevent atelectasis.

A mixture of 200 ppm nitric oxide in pure nitrogen was administered with volumetrically calibrated flowmeters (AGA, Lidingö, Sweden) and was connected to the low-flow inlet of the ventilator with a Y-piece. The oxygen cell of the ventilator was used to measure the inspired fraction of oxygen. The inspired gas passed through a canister containing soda lime to absorb any nitrogen dioxide. The concentrations of inspired nitric oxide and nitrogen dioxide were measured close to the endotracheal tubes by means of chemiluminescence (9841 NOx, Measurement Controls, Englewood, CO). Inspired nitrogen dioxide was less than 0.5 ppm.

‖ Fredén F, Hayberg Å, Hedenstierna G: Unpublished data. 1993.

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Preparation

An ear vein was cannulated for inducing and maintaining anesthesia. A 7-French triple-lumen balloon-tipped catheter (Swan-Ganz) was introduced *via* the right external jugular vein to the pulmonary artery for blood sampling, pressure recording, and cardiac output ($\dot{Q}_T$) measurements. A large-bore catheter was inserted into the contralateral jugular vein for infusion, with its tip in the superior caval vein. The right carotid artery was cannulated to measure arterial blood pressure and to sample blood gases. The catheters were connected to appropriate pressure transducers (Sorenson Transpac, Abbott Critical Care Systems, North Chicago, IL).

By means of a left-sided lateral thoracotomy, the artery to the LLL was identified and freed from surrounding tissues. The artery was enclosed in an ultrasonic flow probe, which was connected to a flowmeter (probe 6 SB and flowmeter T208, Transonic, Ithaca, NY) for continuous measurement of blood flow to the LLL ($\dot{Q}_{LLL}$).

Measurements

Mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), and pulmonary capillary wedge pressure (PCWP) were recorded (7010 monitor, Marquette Electronics, Milwaukee, WI). Pressures were averaged over the entire respiratory cycle, and the midthorax was used as a zero reference level.

$\dot{Q}_T$ was measured by means of thermodilution: 10 ml isotonic saline at room temperature was injected as a bolus, and the $\dot{Q}_T$ was computed (7010, Marquette). Three injections were given for each measurement, and the mean was calculated. The injections were evenly distributed over the respiratory cycle.

Mixed venous and arterial blood samples were collected for blood gas analysis (ABL 3, Radiometer, Copenhagen, Denmark) and measurement of oxygen saturation and hemoglobin concentration (OSM 3, Radiometer). Mean airway pressure and expired minute volume were recorded from both ventilators on each measurement occasion.

Experimental Protocol

The protocol is shown in figure 1. After preparation, 30 min was allowed before a set of baseline measurements was made. Each set of measurements consisted of MAP, MPAP, heart rate, PCWP, $\dot{Q}_T$, $\dot{Q}_{LLL}$, arterial and mixed venous blood gas tensions, arterial and mixed venous blood oxygen saturations, and airway pressures. After baseline measurements had been recorded, the
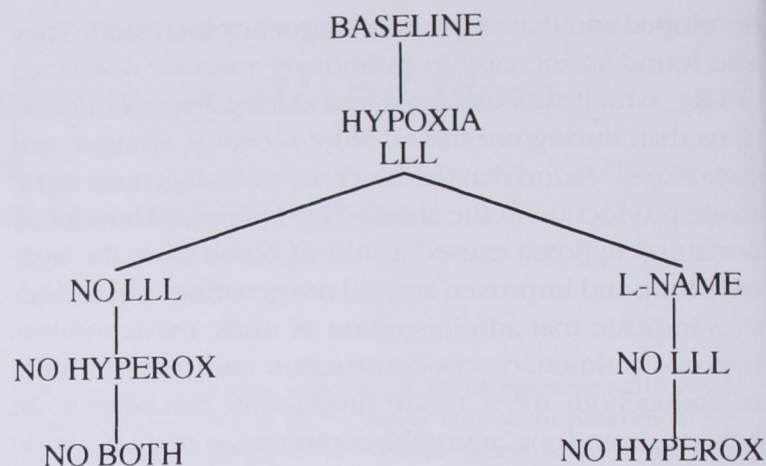

Fig. 1. Experimental protocol. Nitric oxide (NO) was delivered to the hypoxic left lower lobe (NO LLL), to the hyperoxic regions of the lungs (NO HYPEROX), and, in the left arm of the protocol, to both lungs at the same time (NO BOTH). In the right arm of the protocol, nitric oxide was administered after the infusion of N$^\omega$-nitro-L-arginine methyl ester (L-NAME).

LLL was ventilated with a hypoxic gas mixture for the remainder of the study (5% O_2, 5% CO_2, and 90% N_2; AGA). This gas mixture was used to obtain a zero gas exchange in the LLL, by establishing approximately the same oxygen and carbon dioxide tensions in the alveoli as in the mixed venous blood. After 30 min of LLL hypoxia, a new set of measurements was performed, and subsequently the animals were divided into two groups.

In the first group (n = 9) 40 ppm nitric oxide was administered according to one of the following procedures: (1) to the hypoxic LLL, (2) to the hyperoxic right lung and upper and middle lobes of the left lung, or (3) to both lungs. Procedure 1 was performed first in four pigs and procedure 2 first in the other five; when comparing these two subgroups no differences were found. Procedure 3 always took place at the end of the experiment. Each phase lasted 30 min and was terminated with measurements.

In the second group (n = 6) an intravenous dose of N$^\omega$-nitro-L-arginine methyl ester (L-NAME) was infused (30 mg/kg). The L-NAME was dissolved in 20 ml isotonic saline immediately before the infusion, which was given over a period of 10 min. New measurements were obtained 5 and 20 min after the infusion was terminated. Thereafter 40 ppm nitric oxide was administered to (1) the hypoxic LLL or (2) the hyperoxic lung regions. Again, each phase lasted 30 min and was terminated with measurements.

In six additional pigs, the same experimental protocol as described above was performed. After measurements at baseline and during lobar hypoxia, nitric oxide was

Table 1. Group 1, with Intact Endogenous NO Production

	Baseline	Hypoxia LLL	NO to LLL	NO to Hyperoxic Regions	NO to Both Lungs
HR (beats/min)	153 ± 7	148 ± 10	159 ± 6	155 ± 7	157 ± 8
MAP (mmHg)	110 ± 5	114 ± 5	116 ± 4	114 ± 5	113 ± 6
MPAP (mmHg)	18 ± 1	$23 \pm 1^*$	22 ± 2	$19 \pm 2\ddagger$	$19 \pm 2\ddagger$
PVR (dyne $\cdot$ s $\cdot$ cm^{-5})	277 ± 33	360 ± 34	356 ± 61	290 ± 38	269 ± 46
LLLVR (dyne $\cdot$ s $\cdot$ cm^{-5})	$1,067 \pm 143$	$8,705 \pm 3,382\dagger$	$2,004 \pm 447\S$	$5,114 \pm 1,229$	$1,952 \pm 410\S$
HYPEROX VR (dyne $\cdot$ s $\cdot$ cm^{-5})	383 ± 46	388 ± 35	447 ± 84	311 ± 43	312 ± 56
$\dot{Q}_T$ (L/min)	3.5 ± 0.3	3.7 ± 0.4	3.6 ± 0.4	3.5 ± 0.4	3.2 ± 0.4
$\dot{Q}_{LLL}/\dot{Q}_T$ (%)	27.3 ± 2.4	$7.2 \pm 1.4\dagger$	$18.2 \pm 2.7\dagger\S$	$7.5 \pm 1.4\dagger^{.**}$	$16.3 \pm 2.6\dagger\S\dagger\dagger$
PCWP (mmHg)	6 ± 1	7 ± 1	8 ± 1	7 ± 1	$9 \pm 1^*$
Pv$_{CO_2}$ (mmHg)	53 ± 4	54 ± 2	50 ± 2	53 ± 2	49 ± 1
Pa$_{CO_2}$ (mmHg)	43 ± 2	46 ± 2	42 ± 2	44 ± 2	$41 \pm 1\ddagger$
Pv$_{O_2}$ (mmHg)	56 ± 2	52 ± 2	$44 \pm 2\dagger\S$	$50 \pm 2^*$	$45 \pm 3\dagger\ddagger$
Pa$_{O_2}$ (mmHg)	417 ± 47	$300 \pm 48^*$	$141 \pm 28\dagger\S$	$266 \pm 38\dagger\P$	$187 \pm 33\dagger\ddagger$
Paw HYPEROX (cmH$_2$O)	9.1 ± 0.1	9.3 ± 0.1	9.4 ± 0.2	9.6 ± 0.2	9.6 ± 0.2
Paw LLL (cmH$_2$O)	9.0 ± 0.2	8.9 ± 0.2	9.0 ± 0.2	8.9 ± 0.2	9.0 ± 0.2

HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance; LLLVR = left lower lobe vascular resistance; HYPEROX VR = hyperoxic regions vascular resistance; $\dot{Q}_T$ = cardiac output; $\dot{Q}_{LLL}/\dot{Q}_T$ = fractional blood flow of the left lower lobe; PCWP = pulmonary capillary wedge pressure; Pv$_{CO_2}$ = mixed venous blood carbon dioxide tension; Pa$_{CO_2}$ = arterial blood carbon dioxide tension; Pv$_{O_2}$ = mixed venous blood oxygen tension; Pa$_{O_2}$ = arterial blood oxygen tension; Paw HYPEROX = mean airway pressure of the hyperoxic regions; Paw LLL = mean airway pressure of the hypoxic lobe.

Comparisons were performed with ANOVA for repeated measurements and Fisher's test of least significance.

* $P < 0.05$ *versus* baseline.

$\dagger$ $P < 0.01$ *versus* baseline.

$\ddagger$ $P < 0.05$ *versus* hypoxia LLL.

$\S$ $P < 0.01$ *versus* hypoxia LLL.

$\P$ $P < 0.05$ *versus* NO to LLL.

** $P < 0.01$ *versus* NO to LLL.

$\dagger\dagger$ $P < 0.01$ *versus* NO to hyperoxic regions.

administered to the hypoxic lobe at doses of 5, 10, 20, 40, 80, and 160 ppm, and measurements were performed after 30 min at each dose.

At the end of the experiment, the animals were killed with an intravenous injection of KCl, and the lungs were removed for inspection.

Calculations

The fractional $\dot{Q}_{LLL}$ was calculated as $\dot{Q}_{LLL}/\dot{Q}_T$. PVR was calculated according to the formula[17] (MPAP − PCWP/$\dot{Q}_T$) $\times$ 80 (where pressures are expressed in millimeters mercury, $\dot{Q}_T$ in liters per minute, and PVR in dynes $\cdot$ seconds $\cdot$ centimeters^{-5}). The vascular resistance of the LLL was calculated as (MPAP − PCWP/$\dot{Q}_{LLL}$) $\times$ 80 and that of the hyperoxic lung regions was calculated as (MPAP − PCWP/$\dot{Q}_T$ − $\dot{Q}_{LLL}$) $\times$ 80 and expressed in the same units as PVR.

Rubertsson *et al.*[18] have shown a difference between the $\dot{Q}_T$ of the pig in the main pulmonary artery when measured by thermodilution ($\dot{Q}_{T\,thermo}$) or by ultrasonic flow probes ($\dot{Q}_{T\,probe}$), thermodilution giving higher values. The equation $\dot{Q}_{T\,thermo} = \dot{Q}_{T\,probe} \times 1.26 + 0.38$ (r = 0.98, $P < 0.01$) provided good correlation. The $\dot{Q}_T$ measured by thermodilution was therefore recalculated according to the formula of Rubertsson *et al.*[18] The absence of a correction would not affect the relation between $\dot{Q}_T$ and $\dot{Q}_{LLL}$.

Statistics

All data in the tables are given as means ± SEM. Comparisons were made by analysis of variance for repeated measurements. In all analysis of variance tests, Fisher's least significance method was used. Differences were considered significant at $P < 0.05$.

Results

Only $\dot{Q}_{LLL}$ is given as mean ± SEM in the text; for most other data, see tables 1 and 2 and figures 2–4. All dif-

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Table 2. Group 2, Where NO Was Given after Infusion of L-NAME

	Baseline	Hypoxia LLL	20 min after L-NAME	NO to LLL	NO to Hyperoxic Regions
HR (beats/min)	131 ± 9	144 ± 11	149 ± 9	157 ± 15	129 ± 4
MAP (mmHg)	93 ± 5	99 ± 4	138 ± 2†·§	134 ± 6†·§	136 ± 4†·§
MPAP (mmHg)	15 ± 1	19 ± 1	36 ± 1†·§	22 ± 2†·**	17 ± 2**·‡‡
PVR (dyne·s·cm^{-5})	254 ± 41	256 ± 18	778 ± 102†·§	312 ± 23**	278 ± 66**
LLLVR (dyne·s·cm^{-5})	2,534 ± 738	7,566 ± 1,878	95,938 ± 44,556†·§	2,694 ± 400**	63,666 ± 21,181
HYPEROX VR (dyne·s·cm^{-5})	306 ± 46	267 ± 19	792 ± 99†·§	386 ± 14**	280 ± 66**
$\dot{Q}_T$ (L/min)	3.7 ± 0.2	4.8 ± 0.4†	3.0 ± 0.3*·§	2.8 ± 0.2*·§	2.8 ± 0.2*·§
$\dot{Q}_{LLL}/\dot{Q}_T$ (%)	17.8 ± 2.6	4.2 ± 0.7†	2.2 ± 0.8†	15.7 ± 2.9§·**	0.6 ± 0.4†·††
PCWP (mmHg)	4 ± 1	4 ± 1	8 ± 2*·‡	9 ± 2*·‡	8 ± 1
Pv_{CO_2} (mmHg)	48 ± 2	53 ± 1†	57 ± 1†·‡	54 ± 1†·¶	58 ± 1†·‡·‡‡
Pa_{CO_2} (mmHg)	41 ± 2	46 ± 1†	47 ± 1†	43 ± 1	46 ± 2†
Pv_{O_2} (mmHg)	53 ± 2	54 ± 2	47 ± 1†·§	36 ± 2†·§·**	50 ± 2††
Pa_{O_2} (mmHg)	397 ± 18	244 ± 17†	320 ± 19†·§	96 ± 17†·§·**	435 ± 17§·**·††
Paw HYPEROX (cmH$_2$O)	9.3 ± 0.2	9.4 ± 0.1	9.4 ± 0.2	9.6 ± 0.2	9.7 ± 0.2
Paw LLL (cmH$_2$O)	8.6 ± 0.2	8.6 ± 0.2	8.7 ± 0.2	8.8 ± 0.3	8.9 ± 0.2

HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance; LLLVR = left lower lobe vascular resistance; HYPEROX VR = hyperoxic regions vascular resistance; $\dot{Q}_T$ = cardiac output; $\dot{Q}_{LLL}/\dot{Q}_T$ = fractional blood flow of the left lower lobe; PCWP = pulmonary capillary wedge pressure; Pv_{CO_2} = mixed venous blood carbon dioxide tension; Pa_{CO_2} = arterial blood carbon dioxide tension; Pv_{O_2} = mixed venous blood oxygen tension; Pa_{O_2} = arterial blood oxygen tension; Paw HYPEROX = mean airway pressure of the hyperoxic regions; Paw LLL = mean airway pressure of the hypoxic lobe.

Comparisons were performed with ANOVA for repeated measurements and Fisher's test of least significance.

* $P < 0.05$ *versus* baseline.

† $P < 0.01$ *versus* baseline.

‡ $P < 0.05$ *versus* hypoxia LLL.

§ $P < 0.01$ *versus* hypoxia LLL.

¶ $P < 0.05$ *versus* 20 min after L-NAME.

** $P < 0.01$ *versus* 20 min after L-NAME.

†† $P < 0.01$ *versus* NO to LLL.

‡‡ $P < 0.05$ *versus* NO to LLL.

ferences described are statistically significant ($P < 0.05$), unless otherwise stated. The data obtained for baseline and LLL hypoxia in Results and in figure 2 apply to both groups together; data for the separate groups are given in tables 1 and 2.

Baseline

At baseline, the $\dot{Q}_T$, systemic pressure, MPAP, and PCWP demonstrated similar values to ones previously reported for mechanically ventilated pigs with healthy lungs.[19] The $\dot{Q}_{LLL}$ was 23 ± 2.1% of the total $\dot{Q}_T$, a value that is in accordance with a previous report.[19] However, $\dot{Q}_{LLL}/\dot{Q}_T$ was on average lower in the pigs that later received L-NAME (group 2; mean 18 ± 2.6%) than in those who did not (group 1; mean 27 ± 2.4%). Arterial oxygenation was good: mean arterial oxygen tension (Pa_{O_2}) was greater than 400 mmHg. The animals were normocapnic with a Pa_{CO_2} averaging 42 mmHg.

Left Lower Lobe Hypoxia

LLL hypoxia caused an increase in MPAP of 4 mmHg from a mean baseline value of 17 mmHg. The $\dot{Q}_T$ increased slightly by an average of 0.5 l·min^{-1}. The increase was only significant in the pigs that later received L-NAME (group 2). The $\dot{Q}_{LLL}/\dot{Q}_T$ was markedly reduced (to a mean 6 ± 1% for both groups). No changes in PVR were seen. The vascular resistance in the hypoxic LLL was markedly increased, while that in the hyperoxic regions remained essentially unaltered. The Pa_{O_2} decreased to an average of 275 mmHg and the Pa_{CO_2} increased by 5 mmHg in the second group.

L-NAME during Left Lower Lobe Hypoxia

The infusion of L-NAME caused a prompt and persistent effect on the systemic and pulmonary circulation. Recordings obtained 5 and 20 min after infusion did not differ. The data presented are from the 20-min recordings. These showed significant increases in MAP
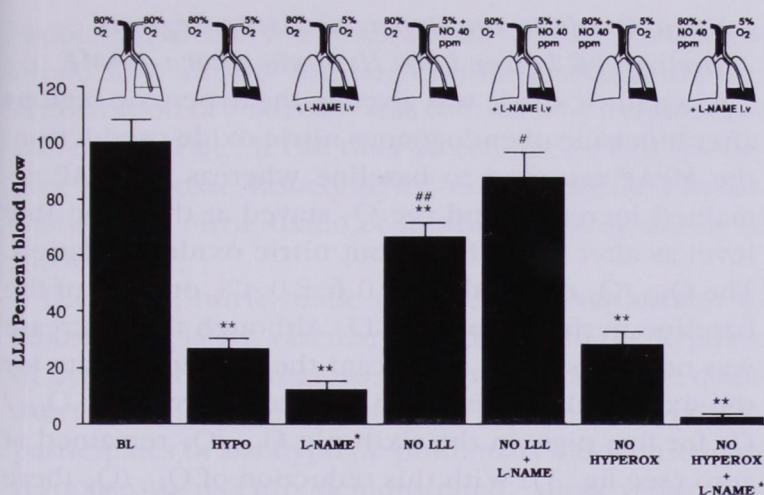
Fig. 2. Perfusion of the hypoxic lobe as a percentage of baseline (BL). This figure combines the findings of the two groups; data for separate groups are given in tables 1 and 2. Hypoxia (HYPO) reduced lobar blood flow. When Nᵚ-nitro-L-arginine methyl ester (L-NAME) was given there was a reduction of blood flow in all six animals, and nitric oxide (NO) given to the hyperoxic regions after L-NAME (NO HYPEROX + L-NAME) caused a further reduction of lobar blood flow in five of six pigs; the sixth remained at zero blood flow. Nitric oxide to the hypoxic lobe (NO LLL) resulted in a blood flow that was 66% of baseline values, whereas nitric oxide to the hypoxic lobe after L-NAME (NO LLL + L-NAME) resulted in a blood flow of 88% of baseline. When nitric oxide was given to the hyperoxic regions (NO HYPEROX), blood flow did not differ from HYPO. Comparisons with baseline (**$P < 0.01$) and with hypoxia (#$P < 0.05$ and ##$P < 0.01$) were performed with analysis of variance for repeated measurements and Fisher's test of least significant difference. Star = individual data given in figure 3.

and MPAP, and also in the PCWP. PVR increased by a factor of 3. The $\dot{Q}_T$ was decreased when compared with baseline values and when compared with LLL hypoxia results. The $\dot{Q}_{LLL}/\dot{Q}_T$ decreased in all six pigs from a mean of $4.2 \pm 0.7\%$ to $2.2 \pm 0.8\%$, but this reduction was not clearly significant by analysis of variance that also included the large change in $\dot{Q}_{LLL}/\dot{Q}_T$ by hypoxia *per se* (see fig. 3). At the same time there was a significant improvement of arterial oxygenation.

Nitric Oxide to the Left Lower Lobe during Left Lower Lobe Hypoxia

The delivery of 40 ppm nitric oxide to LLL did not affect systemic pressure or MPAP nor $\dot{Q}_T$. The vascular resistance in the hypoxic LLL decreased by approximately 75%. $\dot{Q}_{LLL}/\dot{Q}_T$ increased during hypoxia from $7 \pm 1.4\%$ to $18 \pm 2.7\%$, but the latter value was still significantly reduced, to 66% of baseline. The increase in $\dot{Q}_{LLL}/\dot{Q}_T$ was accompanied by a reduction in Pa_{O_2}.

When 40 ppm nitric oxide was administered to both lungs at the same time during LLL hypoxia, $\dot{Q}_{LLL}$ and Pa_{O_2} did not differ from values obtained when nitric oxide was delivered to the LLL alone, nor did the vascular resistance in the LLL alter, but there were reductions in PVR and in the vascular resistance of the hyperoxic regions.

The effect of inhaling nitric oxide for 30 min at step-wise increased concentrations (0, 5, 10, 20, 40, 80, and 160 ppm) to the hypoxic LLL was tested in six pigs. Figure 4 shows the results for $\dot{Q}_{LLL}/\dot{Q}_T$. As small a concentration as 5 ppm nitric oxide produced a $\dot{Q}_{LLL}/\dot{Q}_T$ ratio of 60% of the baseline value. With 40 ppm nitric oxide, the $\dot{Q}_{LLL}/\dot{Q}_T$ increased to 83% of baseline, but neither 80 nor 160 ppm nitric oxide led to any further significant increase in the $\dot{Q}_{LLL}/\dot{Q}_T$, which leveled at 87% of the baseline value. Thus, the results show a dose-response relation with almost complete response at 40 ppm nitric oxide.

Nitric Oxide to the Hypoxic Left Lower Lobe after Blockade of Endogenous Nitric Oxide Production with L-NAME

When 40 ppm nitric oxide was administered to the hypoxic LLL after the infusion of L-NAME the MPAP di-
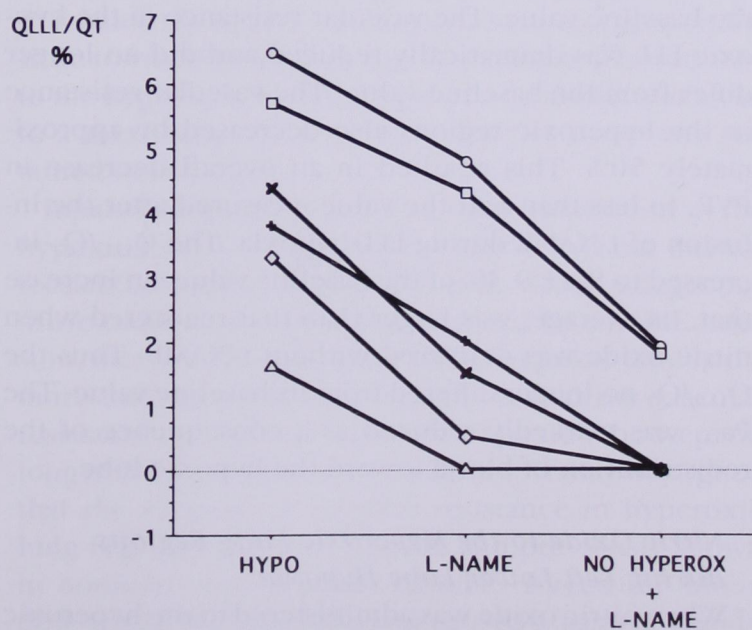

Fig. 3. Individual data for the six pigs in the group given Nᵚ-nitro-L-arginine methyl ester (L-NAME). Perfusion of the hypoxic lobe as a percentage of cardiac output at hypoxia (HYPO), after L-NAME infusion (L-NAME), and after L-NAME with nitric oxide (NO) to the hyperoxic regions (NO HYPEROX + L-NAME). L-NAME caused a reduction of blood flow in all six pigs. Addition of nitric oxide to the hyperoxic regions caused a further reduction in the five pigs that still had perfusion of the LLL after the infusion of L-NAME.
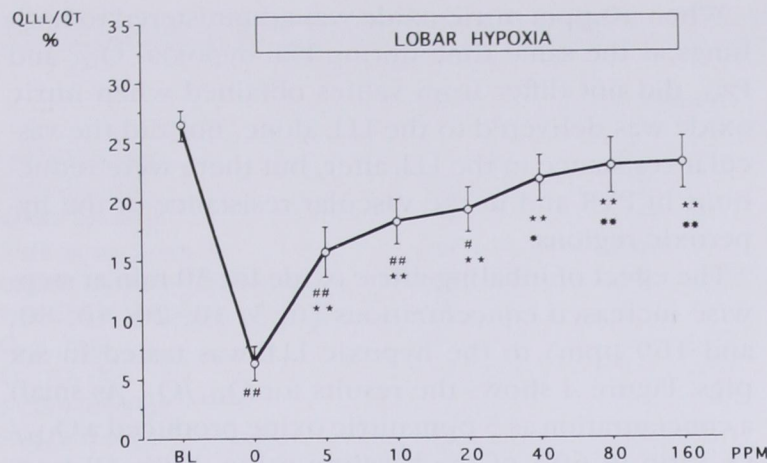
Fig. 4. Dose-response experiment. Perfusion of the hypoxic lobe as a percentage of cardiac output. At baseline (BL), inspired fraction of oxygen is 0.8 to both lungs, at nitric oxide (NO) concentrations of 0 ppm and greater it is 0.05 to LLL and 0.8 to the other lung regions. Nitric oxide was delivered to the hypoxic lobe in increasing doses, and there was an increase in blood flow with concentrations to 40 ppm. With greater concentrations there was only a minor increase in the perfusion of the hypoxic lobe. Comparisons were performed with analysis of variance for repeated measurements and Fisher's test of least significant difference. $\#P < 0.05$ and $\#\#P < 0.01$ compared with baseline; $^*P < 0.05$ and $^{**}P < 0.01$ compared with 0 ppm; and multipointed star $P < 0.05$ and double multipointed star $P < 0.01$ compared with 5 ppm.

minished markedly, although it was still higher than the baseline value. The vascular resistance in the hypoxic LLL was dramatically reduced and did no longer differ from the baseline value. The vascular resistance in the hyperoxic regions also decreased by approximately 50%. This resulted in an overall decrease in PVR, to less than half the value measured after the infusion of L-NAME during LLL hypoxia. The $\dot{Q}_{LLL}/\dot{Q}_T$ increased to $88 \pm 9.3\%$ of the baseline value, an increase that, on average, was larger than that registered when nitric oxide was delivered without L-NAME. Thus the $\dot{Q}_{LLL}/\dot{Q}_T$ no longer differed from its baseline value. The Pa_{O_2} was markedly reduced as a consequence of the redistribution of blood toward the hypoxic lobe.

Nitric Oxide to the Hyperoxic Lung Regions during Left Lower Lobe Hypoxia

When nitric oxide was administered to the hyperoxic lung regions while the LLL was still hypoxic, no effects on pulmonary blood flow distribution were seen and there were no changes in MAP or $\dot{Q}_T$. The MPAP was slightly lower than during hypoxia without nitric oxide administration. The arterial blood gases remained unchanged, when compared with data obtained before nitric oxide administration.

Nitric Oxide to the Hyperoxic Lung Regions during Left Lower Lobe Hypoxia after L-NAME

When nitric oxide was given to the hyperoxic regions after blockade of endogenous nitric oxide production, the MPAP returned to baseline whereas the MAP remained increased and the $\dot{Q}_T$ stayed at the same low level as after L-NAME without nitric oxide inhalation. The $\dot{Q}_{LLL}/\dot{Q}_T$ diminished to $0.6 \pm 0.4\%$, or 2.5% of the baseline perfusion of the LLL. Although this decrease was not statistically significant the individual data for the six animals in this group showed a decrease in $\dot{Q}_{LLL}/\dot{Q}_T$ for five pigs; in the sixth pig $\dot{Q}_{LLL}/\dot{Q}_T$ remained at zero (see fig. 3). With this reduction of $\dot{Q}_{LLL}/\dot{Q}_T$ there was an increase in Pa_{O_2} from 320 mmHg after L-NAME to 435 mmHg. This increase was unexpectedly large and prompted an analysis of the oxygen content of pulmonary blood. The oxygen content of arterial and mixed venous blood and the venous admixture of the hyperoxic lung regions were calculated using standard formulas.[17,20] To make these calculations, the oxygen content of blood from the LLL was assumed to be the same as that of the mixed venous blood. These calculations showed that the oxygen content of arterial blood increased by 2.8 ml $O_2 \cdot l^{-1}$ blood when nitric oxide was added to the hyperoxic lung regions after L-NAME infusion. Blood was transferred at a rate of 50 ml/min from the hypoxic LLL to the hyperoxic regions and thereby increased the oxygen content of the arterial blood by 1 ml $O_2 \cdot l^{-1}$ blood. At the same time, the venous admixture in the hyperoxic lung regions was reduced from 9.4% to 3.4%, causing an increase in the systemic arterial oxygen content by a further 1.8 ml $O_2 \cdot l^{-1}$. These data suggest an improved ventilation-perfusion matching also in the hyperoxic lung regions when they receive nitric oxide after blockade of endogenous nitric oxide production.

Discussion

The major findings in the current study are that the degree of vasoconstriction in a hypoxic lobe could be decreased by inhaling nitric oxide or increased by impairing nitric oxide production with an nitric oxide synthase inhibitor. Moreover, by blocking the overall nitric oxide production by means of the nitric oxide synthase inhibitor and simultaneously administrating nitric oxide to the hypoxic lobe, $\dot{Q}_{LLL}$ was almost completely restored. The delivery of nitric oxide to the hyperoxic lung regions but not the hypoxic lobe during nitric oxide production blockade reduced $\dot{Q}_{LLL}$. This

reduction, seen in five of six animals (in the sixth, lobar blood flow already had been eliminated after the administration of L-NAME), was followed by a significant increase in Pa_{O_2}. It can thus be concluded that the degree of hypoxic vasoconstriction was modified by administering nitric oxide or inhibiting the synthesis of nitric oxide.

Therefore, nitric oxide was used as a vasodilator to adjust the relative vascular impedances in the hypoxic or hyperoxic lung regions. This finding raises the question as to what extent constitutive nitric oxide synthesis participates in the hypoxic pulmonary vasoconstrictor response. As recently demonstrated,[5] nitric oxide synthase is found in a variety of cell types in human airway and pulmonary endothelium. However, although endogenous nitric oxide very likely interferes with hypoxic pulmonary vasoconstriction, it should be remembered that hypoxic pulmonary vasoconstriction is thought to be a complex response mechanism in which several biochemical modulators are involved.[21]

Nitric oxide is synthesized from L-arginine with the help of nitric oxide synthase.[22] In the current study, a competitive inhibitor, L-NAME, was used to block nitric oxide production.[23] A dose of 10 mg/kg in rabbits eliminated any measurable nitric oxide in the exhaled air.[4] In pigs a dose of 10 mg/kg was shown to decrease pulmonary artery conductance and increase MPAP,[24] and in sheep a dose of 30 mg/kg was shown to increase PVR and PAP for at least 1 h.[13] To achieve a stable L-NAME effect in the current study, which lasted 90–120 min, 30 mg/kg was administered. This dose caused persistent changes in systemic arterial pressure and $\dot{Q}_T$, changes that were not seen in the group that did not receive L-NAME. The decrease in $\dot{Q}_T$ may in itself have affected the distribution of pulmonary blood flow, but because $\dot{Q}_T$ was constant once L-NAME had been given, the succeeding experiments should hardly have been biased by the L-NAME dose.

It can be argued that the calculation of PVR, and in particular that of a single lobe, suffers from several uncertainties. Thus, if the perfusion in a part of a lung or a lobe has been altered from zone III to zone II or I conditions, the calculation of vascular resistance will be erroneous. Furthermore, the small flow values registered in a hypoxic lobe will produce a large degree of scatter, as can be seen from the standard deviations under prevailing conditions. The variation in vascular resistance must therefore be interpreted with caution.

There is increasing evidence that the bronchial circulation has an influence on the hypoxic pulmonary

vasoconstriction and that the bronchial vessels regulate the tone of the larger pulmonary arteries.[25] However, it seems less likely that inhaled nitric oxide exerts any effect *via* the bronchial vessels because of the inactivation of nitric oxide in the blood by its binding to hemoglobin. In addition, inhaled nitric oxide to one lung was found to have no measurable effect on the vascular tone of the other lung in anesthetized patients during individual lung ventilation.[26] The extent to which blockade of endogenous nitric oxide production by the infusion of L-NAME affects bronchial circulation and influences pulmonary vascular tone remains to be evaluated.

Inhaled nitric oxide has been shown to cause a dose-dependent decrease in MPAP in various animals and humans,[6,7,9] and a decrease in airway resistance.[27,28] Inhaled concentrations as low as 1–5 ppm have been reported to lower pulmonary artery pressure substantially whereas concentrations as great as 300 ppm or more are required to counter bronchoconstriction. The pig is known to develop a very strong vascular response to hypoxia,[29] which may raise the question as to whether the response to nitric oxide in pigs differs from that in humans. A dose-response study in six pigs was therefore undertaken and showed that 40 ppm nitric oxide exerted an almost full effect and that an increase in inhaled nitric oxide concentration to 160 ppm added little to vasodilatation and redistribution of blood flow to the hypoxic lobe. It can thus be concluded that close to a maximum effect of inhaled nitric oxide was obtained in this study.

Inhaled nitric oxide reduced vasoconstriction in the hypoxic lobe and increased the blood flow but did not reestablish control values. However, when endogenous nitric oxide production was blocked by L-NAME infusion, nitric oxide inhalation to the hypoxic lobe had a more marked vasodilatory effect, so that the vascular resistance and blood flow in the hypoxic lobe were no longer different from control values. It may be argued that the increase in vascular resistance in hyperoxic lung regions caused by L-NAME and previously shown in normoxic and hypoxic rabbits[11] forced the blood flow to the hypoxic lobe and caused a passive vasodilatation by distention and recruitment of the vascular bed. Of interest, the vascular resistance in the hyperoxic lung regions (left upper and middle lobes and right lung), which increased threefold after L-NAME infusion, was reduced to almost half when nitric oxide was administered to the hypoxic lobe. Whether this indicates that a reflex mechanism exists between hyp-

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oxic and hyperoxic lung regions or is an effect of nitric oxide that has diffused or circulated in the blood is unclear.

When nitric oxide was inhaled to the hyperoxic lung regions after L-NAME infusion blood flow to the hypoxic lobe was reduced in five pigs and remained at zero in the sixth (see fig. 3). At the same time Pa_{O_2} increased significantly. This method of minimizing shunting by constricting vessels in a shunted area has also been tried in animal models of ARDS. In an ovine model of ARDS, Rovira *et al.*[13] did not find any improvement in gas exchange by adding intravenous L-NAME to inhaled nitric oxide. In a canine model of ARDS Putensen *et al.*[14] used another nitric oxide synthase inhibitor, N^G-mono-methyl-L-arginine (L-NMMA), and its administration with nitric oxide caused a more pronounced reduction of shunt than did nitric oxide alone. There is evidence that constitutive types of nitric oxide synthase are more sensitive to L-NAME and inducible nitric oxide synthase more sensitive to L-NMMA.[30] Our model used regional hypoxia in a healthy lung, in which constitutive nitric oxide synthase probably dominates, and hence L-NAME had a strong effect. In ARDS there is an increase in inducible nitric oxide synthase, which may explain why L-NMMA had an effect whereas L-NAME did not. In patients with ARDS[15] a peripheral chemoreceptor stimulant, almitrine, was combined with inhaled nitric oxide, and the combination improved Pa_{O_2} without increasing pulmonary artery pressure. The use of a vasoconstrictor may in the future be a way of potentiating the effect of inhaled nitric oxide.

The Pa_{O_2} increase observed when nitric oxide was delivered to the hyperoxic regions after L-NAME infusion was remarkable and cannot be fully explained in terms of blood redistribution away from the hypoxic lobe, although blood flow in this lobe was less than 1% of $\dot{Q}_T$. In fact, it was found that the venous admixture in the hyperoxic regions decreased when nitric oxide was inhaled to these parts of the lungs. This result suggests an improved ventilation-perfusion matching when nitric oxide is inhaled after endogenous nitric oxide production has been blocked.

In conclusion, lobar blood flow during lobar hypoxia could be markedly varied. The combination of an intravenous nitric oxide synthase inhibitor and nitric oxide to the hyperoxic regions reduced lobar blood flow and caused a marked increase in Pa_{O_2}. This method of reducing the shunt could be valuable in the treatment of patients undergoing one-lung ventilation and of patients with acute respiratory failure.

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