Nature and site of action of endogenous nitric oxide in vasculature of isolated pig lungs

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Cremona, George, Tim Higenbottam, Motoshi Takao, Edward A. Bower, and Leslie W. Hall. Nature and site of action of endogenous nitric oxide in vasculature of isolated pig lungs. *J. Appl. Physiol.* 82(1): 23–31, 1997.—The site of action of endogenous and exogenous nitric oxide (NO) in isolated pig lungs was investigated by using arterial, double, and venous occlusion, which allowed precapillary, postcapillary, and venous segments to be partitioned into arterial, precapillary, postcapillary, and venous segments. N^G-nitro-L-arginine (L-NNA; 10^{-5} M) increased resistance in the arterial ($35 \pm 6.6\%$, $P = 0.003$), precapillary ($39.3 \pm 5.1\%$, $P = 0.001$), and venous ($18.3 \pm 4.8\%$, $P = 0.01$) segments, respectively. Sodium nitroprusside (10^{-5} M) and NO (80 parts/million) reversed the effects of L-NNA. Total pulmonary vascular resistance fell with increasing flow, due to a fall in precapillary resistance and dynamic resistance, and was significantly lower than mean total resistance. L-NNA increased the resistances but did not alter the pattern of the pressure-flow relationships. It is concluded that, in isolated pig lungs, the effect of endogenous NO seems to be dependent on flow in the arterial segment and independent of flow in the precapillary segment, but variation of its release does not appear to be fundamental to accommodation to changes in steady flow.

arterial and venous occlusion technique; double occlusion technique; N^G-nitro-L-arginine; hypoxic pulmonary vasoconstriction

THE ABILITY TO ACCOMMODATE increases in flow with little change in pressure and the constrictor response to hypoxia are physiological characteristics of the pulmonary circulation. It has been speculated that endogenous vasodilators may play a role in mediating these phenomena (7). The importance of basal nitric oxide (NO) release in the regulation of pulmonary vascular tone has generated a considerable amount of work. In some mammalian species (6, 34), including humans (6), basal endothelial nitric oxide (eNO) release has been shown to be an important determinant of low normoxic pulmonary vascular tone, whereas in other species there is little evidence of eNO release under normoxic conditions (6, 18, 31). In the systemic circulation, eNO release is stimulated by longitudinal shear stress (4, 38) and contributes to the adaptation of the diameter of blood vessels to changes in pressure and flow. A similar role for eNO may also exist in the pulmonary circulation of certain species. Evidence of flow-induced eNO release in the pulmonary circulation has been demonstrated at birth (1), and inhibition of NO synthase causes pulmonary hypertension in exercising sheep (21).

Contrasting data have been reported regarding the part played by eNO during hypoxic vasoconstriction. A number of studies have shown that pulmonary hypoxic vasoconstriction is enhanced when eNO release or action is inhibited (2, 26, 39), suggesting that eNO may be released under hypoxic conditions, thereby modulating the vasoconstrictor response. Other studies have shown a decrease in eNO production in pulmonary vessels during hypoxia, suggesting that eNO may be directly mediating hypoxic vasoconstriction (20, 42).

Little is known of the regional distribution of eNO production in adult animals. Studies on regional pulmonary vascular resistance have focused mainly on the effects of exogenous NO on preconstricted lungs of animals exhibiting no basal eNO release (24, 37). In the present study, we have performed arterial (AO), venous (VO), and double occlusion (DO) maneuvers on isolated pig lungs to compare the sites of action of the NO synthase inhibitor N^G-nitro-L-arginine (L-NNA), (19), of exogenous NO, and of acute hypoxia. We have also investigated the effects of L-NNA on the flow-related effects in the different segments of the pulmonary vascular bed. Pig lungs were chosen because they exhibit a vigorous constrictor response to acute hypoxia (32, 40) and because NO secretion is an important determinant of their basal pulmonary vascular tone (6).

METHODS

Preparation of In Situ Perfused Lungs

All experiments were undertaken under the guidelines of a project license granted by the Home Office under the Animals (Scientific Procedures) Act of 1986. Fifteen adult male and female pigs weighing between 30 and 40 kg were sedated with intramuscular droperidol (0.5 mg/kg; Droleptan, Janssen Pharmaceutical, Oxon, UK) and midazolam (0.3 mg/kg; Hypnovel, Roche, Welwyn, UK) and were anesthetized with intravenous pentobarbital sodium (up to 25 mg/kg). A tracheostomy was performed, and the animals were intubated and ventilated by a Manley ventilator (Blease Medical, Bucks, UK) with 40% O_2-60% N_2. Systemic arterial blood pressure was monitored through a cannula inserted into the left carotid artery and connected to a transducer (model P50 Spectramed, Coventry, UK). After median sternotomy was performed, the pericardium was opened and heparin (1,000 U/kg) was administered through the right atrium. Two cannulas (ID 5 mm, Portex, UK) were placed, respectively, in the inferior vena cava and in the right ventricle through an incision in the right atrium. The animal was exsanguinated via the cannula in the inferior vena cava while 1–2 liters of buffered Krebs-Ringer solution containing 40 g/l Dextran 70 were concurrently infused into the right ventricle. The rate of infusion was adjusted to keep

systemic arterial blood pressure stable until 3 liters of blood were obtained.

The heart was then stopped by an intracoronary injection of potassium chloride (10^{-3} M), and a stiff cannula (ID 13 mm) was placed in the main pulmonary artery. Through an incision in the left ventricle, another cannula (ID 16 mm) was retrogradely inserted into the left atrium and secured by heavy ties that prevented ballooning of the atrial appendage. The cannulas were then connected to an external perfusion system (Fig. 1). The time from cardiac arrest to the start of perfusion was never more than 20 min.

The perfusion circuit involved collection of autologous blood from the pulmonary veins draining passively into a jacketed reservoir that kept the perfusate temperature at 38°C. From the reservoir the perfusate was pumped into the pulmonary artery by means of a roller pump (Watson Marlow model 5001R, UK). A 150-ml reservoir with a small cushion of air was interposed between the pump and the arterial cannula. This reservoir acted as a pulse damper as well as a bubble trap. Perfusate temperature was monitored with a thermistor in the inflow cannula.

Pulmonary artery and left atrial pressures were measured by matched transducers (model P50 Spectramed) connected to side ports placed near the tips of the cannulas. Pressures were referenced to the top of the lungs. Inflow and outflow were measured by Doppler flow probes placed on the inflow and outflow cannulas (model T101D, Transonic Systems, Ithaca, NY). Pressures and flow were recorded continuously on a chart recorder (model 404, W&W Scientific Instruments, Basel, Switzerland).

The perfusate consisted of autologous blood mixed with Dextran 70 to give a hematocrit of 19–25%. Perfusion was instituted at 10 $ml \cdot min^{-1} \cdot kg^{-1}$ and slowly increased by 10 $ml \cdot min^{-1} \cdot kg^{-1}$ steps over an hour until a flow rate of 100 $ml \cdot min^{-1} \cdot kg^{-1}$ was reached. Oxygen and carbon dioxide tensions as well as pH were checked periodically (Instrumentation Laboratory, Milan, Italy). The pH was maintained between 7.3 and 7.4 by the addition of small volumes of sodium bicarbonate (1 M).

The lungs were ventilated with 21% O_2-74% N_2-5% CO_2 at a tidal volume of 12 ml/kg and a frequency of 8–12 breaths/min. An air-filled pressure transducer attached to the side port of the endotracheal tube was used to measure airway pressure. A positive end-expiratory tracheal pressure of 2 mmHg was applied, and a deep inspiration was periodically simulated to prevent atelectasis.

Occlusion Maneuvers

The arterial and venous cannulas were held firmly in place by clamps to minimize vibration. The flow rate was adjusted to the desired level, and the arterial and venous pressures were allowed to reach a steady state. Ventilation was stopped in end expiration before each occlusion maneuver. AO was performed by clamping the inflow tubing proximal to the pulmonary artery cannula by means of a hemostat and diverting the flow back to the reservoir (Fig. 1). The occlusion was maintained for 10 s. VO was performed by clamping the outflow tubing just distal to the venous cannula for 2 s. DO was carried out by simultaneously occluding the inflow and outflow for 6 s. During occlusion maneuvers, the analog outputs of the three pressure transducers, the ultrasonic flowmeter, and the occlusion markers were digitized at a sample rate of 500 Hz (MP100, Biopac Systems, Goleta, CA), and data were stored on a personal computer (Macintosh SE 30, Apple Computer, Cupertino, CA). For each AO and DO, 15 s of data were sampled that included several preocclusion cycles and ~10 s of the signal after occlusion. Around 10 s of data were sampled for VO, which included the 2 s after occlusion.

Analysis of Occlusion Tracings

To minimize the effect of interference by the clamps and the roller pump on the pressure signals, the digitized pressure signals were filtered via software (Acqknowledge, Biopac) by using low-pass Bessel filters with a cut-off frequency of 50 Hz. Because the analysis of the occlusion experiments required comparisons between pressures measured before and after blood flow had been stopped, pulmonary arterial (Ppa) and venous (Ppv) pressures measured before occlusion were corrected to zero flow to correct for the kinetic energy component. The value of this component was very small (0.3–0.7 mmHg) but was included for accuracy by means of the formula

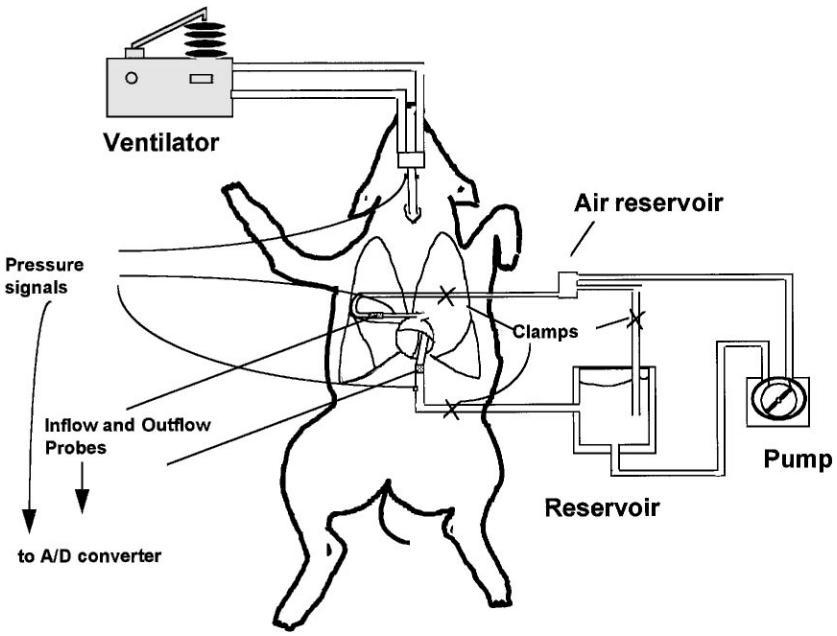

Fig. 1. Experimental setup for constant flow perfusion of pig lungs in situ. Details are explained in text. A/D, analog-to-digital.

$$E_{kin} = (m\dot{Q}^2W^2d^{-4})0.0075 \text{ mmHg}$$

where E_{kin} is the pressure due to kinetic energy, m is the mass of blood in kilograms per cubic meter, $\dot{Q}$ is the flow in $ml \cdot min^{-1} \cdot kg^{-1}$, W is the weight of the animal in kilograms, d is the vessel diameter in millimeters, and 0.0075 converts pascals to millimeters of mercury.

We have followed the method described by Hakim et al. (12) to analyze AO and VO tracings. After AO, the arterial pressure trace shows an initial rapid drop, followed by a more gradual fall (Fig. 2, *A* and *B*). The mean Ppa between 8 and 10 s after the occlusion (Ppa_{00}) was determined, and this value used as the asymptote in the equation describing the fall in Ppa after AO because there was little variation in pressure at this time. The first 0.3 s after the occlusion was discarded because of noise caused by the maneuver, and a monoexponential relationship was fitted to the following 1.5 s of data by using a standard software package (Igor, Wavemetrics, CA).

$$Ppa_t = Ppa_0 \cdot e^{-k_\tau} + Ppa_\infty$$

where Ppa_t is the pressure change with time (t), Ppa_0 is the initial pressure on the exponential, and k is the reciprocal of the time constant (τ). The fitted curve was extrapolated to the instant of occlusion, defined as the time when inflow reached zero. The instant when flow reached zero coincided with the point at which the extrapolated line intercepted the rapid phase in pressure change. The intercept was taken as the pressure at the distal end of the arterial segment (Pa′). Subtraction of Pa′ from Ppa yielded the pressure drop across the relatively noncompliant arterial segment (ΔPa). The choice of these intervals was based on previous work by Hakim et al., who showed that in this time interval, selection of the begining and end of the data for extrapolation had little effect on the slope of the exponential and the extrapolated occlusion pressure.

VO tracings showed a rapid rise immediately after occlusion, followed by a more gradual rise (Fig.2, *C* and *D*). As in AO, the first 0.2 s was discarded because of noise and a linear relationship was used to fit the first 1.5 s of the gradual rise in Ppv. This was extrapolated back to the time of occlusion, and the intercept was taken as the pressure at the proximal end of the downstream segment (Pv′). The pressure gradient across the downstream veins was calculated as ΔPv = Ppv − Pv′. From DO tracings, the mean pressure for 2 s after equilibration was measured and taken as capillary pressure (Pc) (8).

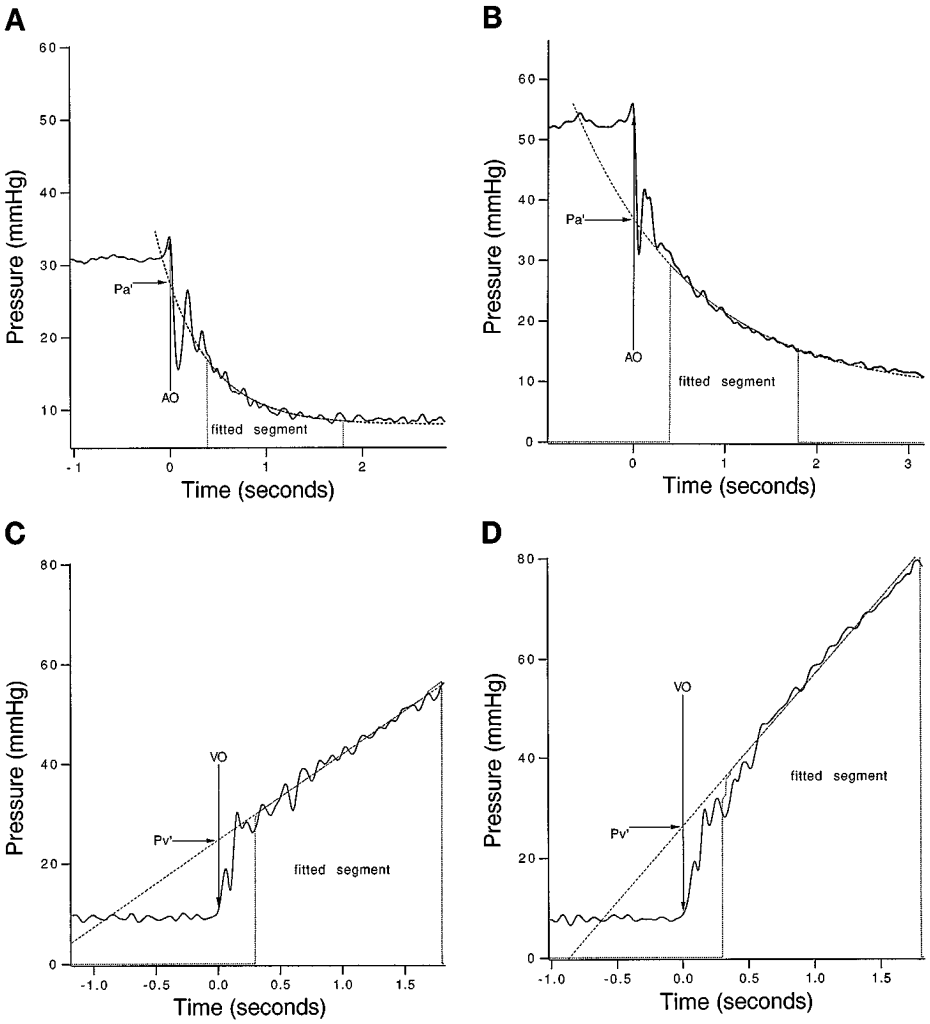

Fig. 2. Actual tracing of pulmonary arterial pressure during arterial occlusion (AO) before (*A*) and after (*B*) N^G-nitro-L-arginine (L-NNA) (10^{-5} M) and of pulmonary venous pressure during venous occlusion (VO) maneuvers before (*C*) and after (*D*) L-NNA (10^{-5} M). From these tracings, changes in pressure across arterial and venous segments were determined, as described in text. Pa′, pressure at the distal end of arterial segment; Pv′, pressure at proximal end of downstream venous segment.

From this measurement, another two gradients, namely, precapillary ($\Delta Pa' = Pa' - Pc$) and postcapillary ($\Delta Pv' = Pc - Pv'$) venous gradients, were obtained. In this way the pressure drop across the pulmonary vasculature was partitioned into four functional segments, defined for convenience as arterial, precapillary, postcapillary, and venous segments (11).

Experimental Protocols

Dose-dependent effects of exogenous NO and sodium nitroprusside. The dose-dependent effects of NO and sodium nitroprusside on total pulmonary vascular resistance (PVR) were studied in four pig lungs perfused at 100 ml·min^{-1}·kg^{-1} and with Ppv set at 6–8 mmHg by adjustment of the height of the venous reservoir. These were first preconstricted by addition of L-NNA (Sigma Chemical) to the venous reservoir (10 mg in 2 ml saline to give a final concentration in the perfusate of 10^{-5} M). NO was subsequently added to ventilation mixture such that concentrations of 10, 40, 80, and 160 parts/million (ppm) NO measured by chemiluminescence (model 42, Thermoelectron, Warrington, UK) were delivered to the lungs for 10 min. This was followed by cumulative doses of sodium nitroprusside (from 100 μg to 100 mg in 2 ml vehicle, final concentrations = 10^{-7} to 10^{-4} M).

Inhibition of NO synthesis and the effects of exogenous NO. The effect of inhibition of NO synthesis on segmental pressure gradients was studied in five lungs. After 1 h of perfusion, flow was set at 100 ml·min^{-1}·kg^{-1} and Ppv at 6–8 mmHg. After the pressures had stabilized, AO, VO, and DO were performed three times. The order of the maneuvers was interchanged in consecutive measurements. After control measurements were taken, L-NNA was added to the venous reservoir (10 mg in 2 ml saline to give a final concentration in the perfusate of 10^{-5} M) and, after a stable Ppa tracing was achieved, usually ~5 min, AO, DO, and VO maneuvers were repeated. NO was subsequently added to the ventilation mixture such that a final concentration of 80 ppm NO measured with a chemiluminescent NO analyzer (model 42, Thermoelectron) was delivered to the lungs. AO, DO, and VO were performed after 10-min ventilation with NO, after 10 min without NO, and after addition of sodium nitroprusside (10 mg in 2 ml vehicle, final concentration = 10^{-5} M). The dose of L-NNA selected was one that had given maximal effects in previous experiments (6).

Effects of hypoxia. The effects of hypoxia on the segmental pressure gradients was investigated in five lungs. After 1 h of perfusion, flow was set at 100 ml·min^{-1}·kg^{-1} and Ppv at 5–6 mmHg, and AO, VO, and DO were performed during normoxic conditions. Hypoxic vasoconstriction was induced by ventilation with a gas mixture containing 5% O_2-5% CO_2-90% N_2 for 5–10 min (Table 1).

Effects of changing flow at constant outflow pressure. To identify the nature of the resistances in the different segments of the pulmonary vasculature, AO, VO, and DO were performed at three flow rates (100, 75, and 50 ml·min^{-1}·kg^{-1}) and at a constant outflow pressure of 5–6 mmHg. The flow rates were chosen because of the linear relationship between flow and pressure in this range (41). The lungs were subsequently treated with L-NNA (10^{-5} M), and the occlusion maneuvers were repeated at the same three flow rates.

Statistical Analysis

PVR in the different segments was calculated from the pressure gradients divided by the flow. The changes in PVR during inhibition of NO synthesis and administration of exogenous NO were analyzed by one-way analysis of variance by using Scheffé's correction for multiple comparisons. To compare the effects of L-NNA and hypoxia on segmental PVR, the data were normalized by expressing the change in PVR in each segment as a percentage of the change in total PVR across the pulmonary vascular bed

$$\text{change in PVR(\%)} = \frac{\Delta\text{segmental PVR}}{\Delta\text{total PVR}} \times 100$$

In the experiments at varying flow, linear-regression analyses of pressure gradients as functions of flow were performed on the data for each experimental protocol. From the regression data, dynamic resistance was calculated as the slope of the relationship between pressure gradient and flow ($dP/d\dot{Q}$) and was compared with the mean of the PVR values at different rates of flow ($\overline{PVR}$). This was carried out to avoid extrapolation beyond the range of the data. A significantly greater value of $\overline{PVR}$ indicated that the regression line would extrapolate to a positive intercept on the pressure axis. The differences between $dP/d\dot{Q}$ and $\overline{PVR}$ and between PVR values at maximum and minimum flows were analyzed by Student's *t*-test for paired data. Results are presented as means ± SE. *P* values < 0.05 were considered significant.

RESULTS

Action of L-NNA, Inhaled NO, and Perfused Nitroprusside

The mean arterial and venous pressures at a flow rate of 100 ml·min^{-1}·kg^{-1} as well as the perfusate gas

Table 1. *Hemodynamics, blood gas tension, and pH data in isolated pig lungs*

	Flow, l/min	Ppa, mmHg	Ppv, mmHg	Pa_{O_2}, Torr	Pa_{CO_2}, Torr	pH
			Protocol 1			
Control	3.34 ± 0.04	30.3 ± 1.8	6.3 ± 1.1	109 ± 4	40 ± 2	7.38 ± 0.02
L-NNA	3.30 ± 0.07	48.0 ± 2.0*	6.1 ± 1.0	105 ± 6	40 ± 3	7.34 ± 0.03
NO	3.30 ± 0.07	32.0 ± 1.5	6.1 ± 0.7	111 ± 3	42 ± 3	7.37 ± 0.02
SNP	3.23 ± 0.07	27.0 ± 1.3	7.2 ± 0.9	110 ± 2	41 ± 2	7.36 ± 0.02
			Protocol 2			
Control	3.30 ± 0.03	29.4 ± 1.1	8.2 ± 1.5	111 ± 2	41 ± 1	7.35 ± 0.02
5% O_2	3.30 ± 0.04	37.5 ± 2.1*	8.1 ± 1.2	44 ± 7*	42 ± 2	7.36 ± 0.02

Values are means ± SE; $n = 5$ lungs. Steady-state hemodynamics and blood gases were measured during control and after various treatments. Ppa and Ppv, pulmonary arterial and venous pressures, respectively; Pa_{O_2} and Pa_{CO_2}, arterial P_{O_2} and P_{CO_2}, respectively; L-NNA, N^G-nitro-L-arginine; NO, nitric oxide; SNP, sodium nitroprusside. *Significantly different from control, $P < 0.05$ (paired *t*-test.)

tensions for each experimental condition are shown in Table 1. The mean PVR across the total pulmonary vascular bed under control conditions was 0.243 ± 0.014 mmHg·ml^{-1}·min·kg. The resistance was distributed as follows: arterial 31 ± 1.7%, precapillary 20 ± 2.1%, postcapillary 14 ± 1.8%, and venous 35 ± 1.6%. Inhibition of NO synthesis with L-NNA (10^{-5} M) increased total PVR by 0.19 ± 0.02 mmHg·ml^{-1}·min·kg ($P < 0.05$). Both NO and sodium nitroprusside showed dose-dependent decreases in total PVR with little change at higher concentrations (Fig. 3). The increase was mainly observed in the arterial and precapillary segments, which increased by 35 ± 6.6% ($P = 0.003$) and 39.3 ± 5.1% ($P = 0.001$), respectively (Fig. 4). A smaller but significant increase was also observed in the venous segment (18.3 ± 4.8%, $P = 0.01$), but changes in the postcapillary segment were not consistent. No change in pH was observed after addition of L-NNA (Table 1).

Ventilation with NO (80 ppm) decreased the resistance in the arterial and precapillary segments to control levels but had no effect on the venous segment. After cessation of ventilation with NO, resistances in all segments returned to the high level observed after addition of L-NNA. Addition of sodium nitroprusside (10^{-5} M) decreased the resistances in the arterial, precapillary, and venous segments to levels not significantly different from control.

Effects of Hypoxia

The mean inflow and outflow vascular pressures as well as the gas tensions and pH are given in Table 1. In the five lungs studied, the mean total PVR across the pulmonary vascular bed under normoxic conditions was 0.243 ± 0.024 mmHg·ml^{-1}·min·kg at a flow rate of 100 ml·min^{-1}·kg^{-1}. The PVR was distributed as follows: arterial 31 ± 2.7%, precapillary 20 ± 2%, postcapillary 15 ± 2.4%, and venous 32 ± 1.8%. Ventilation with a hypoxic mixture of 5% O_2 caused an increase of 0.108 ± 0.04 mmHg·ml^{-1}·min·kg in total PVR ($P = 0.01$; Fig. 5). This increase was concentrated in the precapillary (75 ± 10.6%, $P = 0.001$) and venous (26.4 ± 1%, $P = 0.01$) segments, but changes in other segments were not significant.

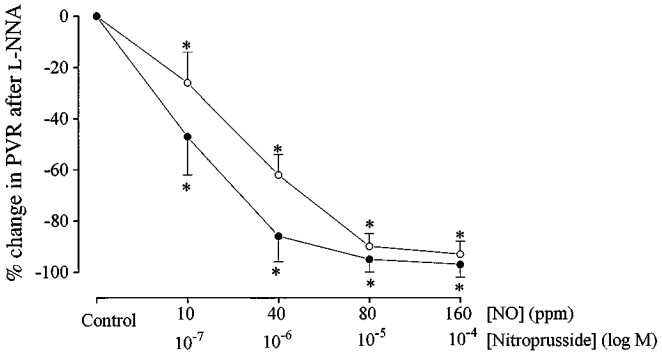

Fig. 3. Effect of nitric oxide (NO) and sodium nitroprusside (SNP) on total pulmonary vascular resistance (PVR). ○, NO; ●, SNP. Dose-response relationships of PVR to NO and SNP after preconstriction with L-NNA (10^{-5} M) are shown. Values are means ± SE. ppm, Parts per million. Brackets denote concentration. *Significantly different from constricted level, $P < 0.05$ (Scheffé's test).

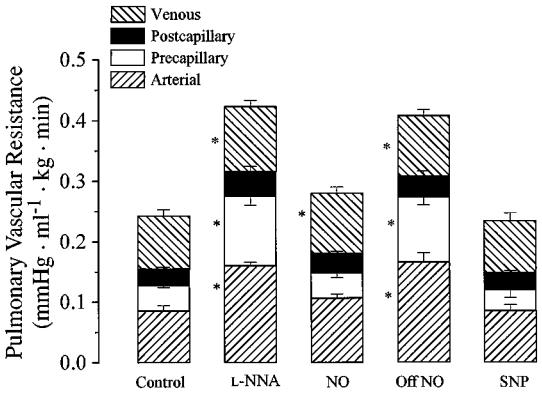

Fig. 4. Effect of L-NNA (10^{-5} M) and subsequent inhaled NO (80 ppm) and SNP on segmental PVR measured by occlusion technique. After termination of ventilation with NO (off NO), resistance returned to elevated levels caused by L-NNA, and this was taken as a 2nd baseline. Values are means ± SE. *Significantly different from control, $P < 0.05$ (Scheffé's test).

Effects of Changing Flow Rate at Constant Outflow Pressure

Under normoxic conditions, there was a linear relationship between the total pressure gradient and flow in the range of flow explored ($r = 0.99$, $P = 0.005$; Fig. 6). The dynamic resistance (measured as dP/dQ̇) was significantly lower than $\overline{PVR}$ (0.147 ± 0.02 and 0.318 ± 0.04 mmHg·ml^{-1}·min·kg, respectively, $P = 0.03$) indicating that there was a positive intercept pressure, and the total PVR was smaller at a flow rate of 100 ml/min than at 50 ml/min as expected (Table 2). The decrease in total PVR was mainly due to the precapillary segment (Table 2.)

L-NNA (10^{-5} M) increased both dP/dQ̇ (0.40 ± 0.04 vs. 0.32 ± 0.04 mmHg·ml^{-1}·min·kg) and $\overline{PVR}$ (0.548 ± 0.06 mmHg·ml^{-1}·min·kg, but $\overline{PVR}$ was still significantly higher than dP/dQ̇ ($P = 0.03$). After L-NNA, total PVR was lower at maximum than at minimum flow (Table 2), due to a significant fall in precapillary segmental resistance (R'_a). The decrease in R'_a with flow was similar before and after L-NNA (42 vs. 43%);

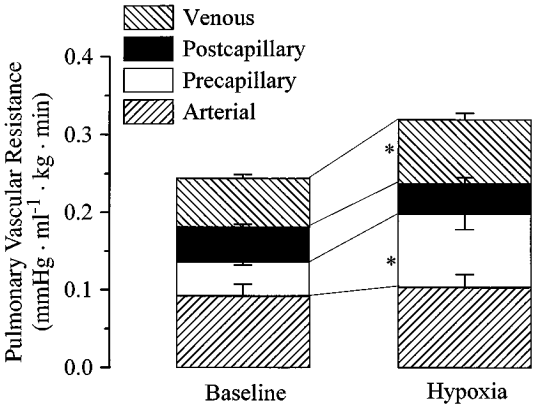

Fig. 5. Effect of ventilation with 5% O_2-90% N_2-5% CO_2 on segmental PVR measured by occlusion technique. *Significantly different from control, $P < 0.05$ (Scheffé's test).

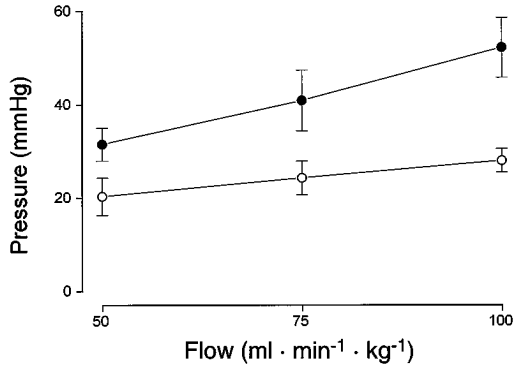

Fig. 6. Effect of flow on total pulmonary gradient across vascular bed of isolated pig lungs (n = 5). ○, Before L-NNA; ●, after L-NNA (10^{-5} M). Results are means ± SE. See text and Table 2 for significant effects.

however, the absolute change was greater after L-NNA (0.08 vs. 0.04 mmHg·ml^{-1}·min·kg, $P < 0.05$). Furthermore, L-NNA increased the slope of the dPa/$\dot{Q}$ line (0.084 ± 0.01 vs. 0.268 ± 0.01 mmHg·ml^{-1}·min·kg, $P < 0.05$) and caused an upward shift in the dPa′/$\dot{Q}$ line (PVR from 0.066 ± 0.01 to 0.139 ± 0.02 mmHg·ml^{-1}·min·kg, $P < 0.05$), with little or no change in dPv′/$\dot{Q}$ and dPv/$\dot{Q}$ lines (Fig. 7).

DISCUSSION

The main findings of this study are that inhibition of basal eNO release increased resistance in the arterial, precapillary, and venous segments and that L-NNA caused an increase in dPpa/$\dot{Q}$ due to arterial segmental resistance and an upward shift of the pressure-flow line due to R'_a. Hypoxia caused a significant increase in R'_a and a small increase in venous segmental resistance.

The pressure gradient across the pulmonary vasculature perfused at 100 ml·min^{-1}·kg^{-1} in our study was more than twice that reported in dogs (16), although very similar to that reported in isolated pig lungs (36, 40, 41). In the study by Rock et al. (36) on isolated pig lungs by using AO and VO, the arterial segment accounted for 26%, the middle compartment for 39%, and the venous 35% of the total pressure gradient. This is in agreement with our results, although by using AO, VO, and DO we were able to further subdivide the pressure gradient across the pulmonary vascular bed into four functional segments. In this study, the middle compartment (ΔPa′ + ΔPv′) accounted for a similar proportion of the total pressure drop (34%), of which 20 ± 2.1% was due to the precapillary segment. In dogs studied by AO, VO, and DO where occlusion curves were analyzed in the same way, the middle segment accounted for 26% of the pressure drop, but only 9% of this was due to the precapillary segment (11). Pa′ and Pv′ are thought to represent pressures in vessels between 900 and 50 μm (15); whether the same applies in pig lungs is not known, but it is probable. The difference in longitudinal distribution of resistance may be related to the structural differences of the pulmonary vasculature between the two species because in the pulmonary arteries of the pig, unlike in those in the dog, muscularization is normally present down to diameters of about 50 μm (22).

The increase in total PVR with L-NNA observed in this study was similar to that reported previously with other specific inhibitors of NO synthase, such as N^G-nitro-L-arginine methyl ester and N^G-monomethyl-L-arginine (6). Resistance was increased mainly in the arterial and precapillary segments. A small but significant increase was also seen in the venous segment. Endogenous basal production of NO therefore acts principally on the arterial side of the porcine pulmonary vascular bed. In neonatal pig lungs, N^G-nitro-L-arginine methyl ester increased both the resistance upstream and downstream of the double occlusion pressure (29), presumably due to the greater muscularization of small arteries after the first 2 wk of life (35). Similar differences have been observed in lambs; during normoxia, L-NNA increased arterial but not venous segmental resistance at 1 mo of life, whereas at 2 days an increase in both arterial and venous segments was observed (9). Administration of 80 ppm NO by inhalation reversed the effects of L-NNA on the arterial and precapillary segments in agreement with the notion that NO synthesis maintains tone in the pulmonary arterial tree. NO inhalation did not reverse the effects of L-NNA on the venous segment, perhaps due to the greater diffusion distance between airways and the vessels or due to the low tone that was present in the veins. The addition of sodium nitroprusside reversed the effects in all the segments. Very few studies have examined the effects of eNO synthesis inhibition on basal segmental resistance. In the dog lobe, PVR and its distribution change little after L-NNA (13). A number of studies have examined the effects of NO inhalation in preparations at elevated tone. For example, in lungs constricted with endothelin-1, inhaled NO di-

Table 2. *Effects of flow and NO synthase inhibition on segmental PVR*

	Control			After L-NNA		
	PVR_{50}	PVR_{100}	P	PVR_{50}	PVR_{100}	P
Total	0.411 ± 0.08	0.280 ± 0.03	0.03	0.640 ± 0.09	0.522 ± 0.06	0.03
Arterial	0.115 ± 0.03	0.099 ± 0.02	0.12	0.146 ± 0.03	0.200 ± 0.05	0.08
Precapillary	0.086 ± 0.01	0.050 ± 0.01	0.006	0.185 ± 0.02	0.104 ± 0.02	0.002
Postcapillary	0.050 ± 0.02	0.030 ± 0.01	0.12	0.085 ± 0.05	0.043 ± 0.02	0.11
Venous	0.152 ± 0.04	0.101 ± 0.02	0.09	0.214 ± 0.02	0.154 ± 0.01	0.20

Values are means ± SE; n = 5 lungs. Pulmonary vascular resistance (PVR; mmHg·ml^{-1}·min·kg) of whole lung and of different vascular segments at 50 (PVR_{50}) and 100 (PVR_{100}) ml·min^{-1}·kg were measured at control and after L-NNA (10^{-5} M). Significance of difference between groups was determined by paired t-test.

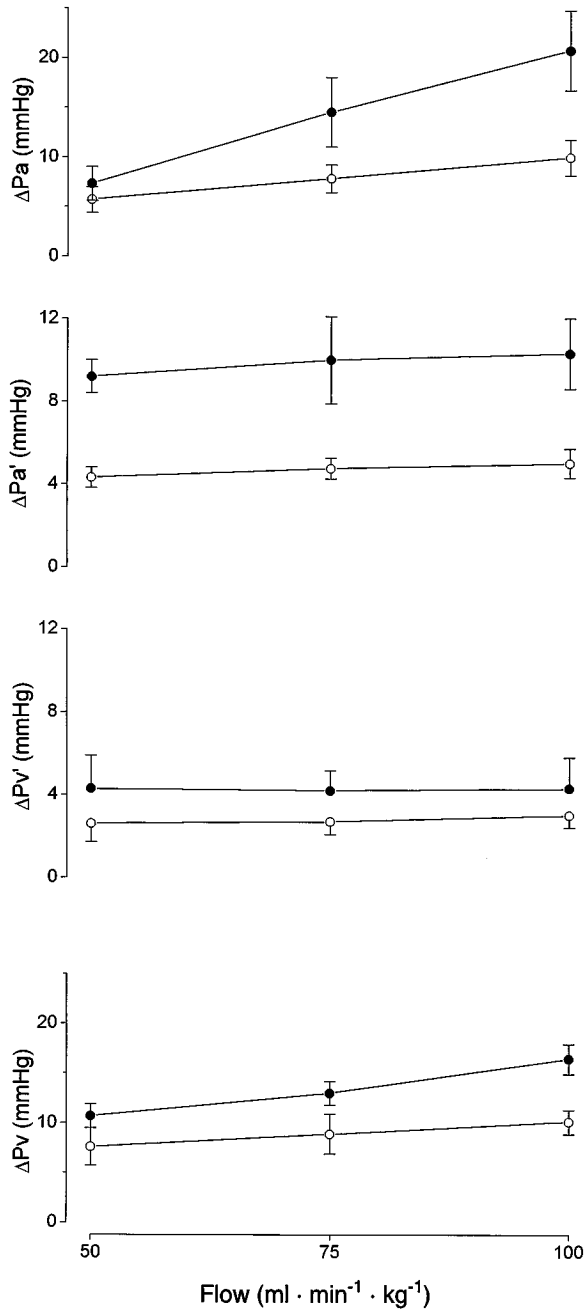

Fig. 7. Effect of flow on segmental pulmonary gradients [arterial (ΔPa), precapillary (ΔPa′), postcapillary (ΔPv′), and venous (ΔPv) segments] measured by arterial, venous, and double occlusion in isolated pig lungs ($n = 5$). ○, Before L-NNA; ●, after L-NNA (10^{-5} M). Results are means ± SE. See text and Table 2 for significant effects.

lated small arteries and veins but had no effect on larger vessels (37). In lungs perfused with Krebs solution, both inhaled NO and sodium nitroprusside affected large vein capacitance. On the other hand, in isolated rabbit lungs perfused with Krebs solution or blood and constricted with the thromboxane analogue U-46619, inhaled NO affected all the resistance segments equally (24). The discrepancy among these findings may be attributed to the difference in animal models used and to the difference in the agents used to constrict the vasculature. In contrast to the pig, the rat and rabbit pulmonary vascular beds show little or no basal eNO release, and inhibition of NO synthesis had little or no effect under normoxic conditions. It may be that NO-induced dilatation may be dependent on the existing tone present in the particular vascular segment. In the pig, veins and arteries follow the branching pattern of the airways and it is likely that the distance across which NO would have to diffuse to reach the arteries and veins is similar.

The main effect of hypoxia was observed in the precapillary segment, although a small but significant increase was also observed in the venous segment. These findings are in agreement with those reported in adult pig lungs (36), canine lungs (11, 17), and neonatal pig lungs (30). The relationship among hypoxia, NO synthesis, and hypoxic pulmonary vasoconstriction is complex. Direct measurement of NO in cultured cells (42) or in expired air (5, 10) has been shown to decrease during acute hypoxia. However, inhibitors of NO synthesis appear to enhance hypoxic pulmonary vasoconstriction (2, 30, 34) and during unilateral alveolar hypoxia cause a reduction in flow to the hypoxic lung (39). Furthermore, in both adult and neonatal pig lungs, hypoxic vasoconstriction still occurred after inhibition of eNO synthesis (6, 29), suggesting that reduced eNO release is not the mechanism directly responsible for hypoxic pulmonary vasoconstriction. In the present study, hypoxia resembled L-NNA in acting predominantly on the precapillary segment. The lack of effect of hypoxia on the proximal arterial segment may reflect a qualitative difference in the action of L-NNA. L-NNA abolishes NO production, whereas hypoxia may merely reduce it.

The pressure-flow relationship in the pig pulmonary vascular bed was linear over the range of flows investigated, in agreement with previously reported studies in this species (28, 41). In this study, it was possible to examine only three flow rates, which precluded a precise extrapolation to zero flow. However, the observed fall in resistance with increasing flow is in accordance with previous reports that attribute it to mechanical expansion of partially collapsed vessels (3, 27, 33). In the present study, resistance was constant with increasing flow in arterial, venous, and postcapillary segments but fell significantly in the precapillary segment. The concentration of sensitivity to flow in the precapillary segment is consistent with the presence of a Starling resistance in the precapillary segment that increased after L-NNA. Similar effects of flow on segmental resistance have been reported in AO and VO studies carried out in pigs (36) and other species (14). Although the results of these studies have been interpreted according to the classic Ohmic-Starling resistor model, alternative models have been proposed that could similarly explain the results (25). The current work does not present data to support any particular model, and for this reason we have purposely chosen to refer to purely descriptive terms such as dynamic resistance and PVR to interpret our results.

In the range of flow rates studied, the localization and persistence of the effects of flow on segmental resistance after L-NNA treatment do not support variation of eNO release by shear stress as a fundamental mechanism of accommodation to flow, although it may be a contributing factor. In sheep (23), NO synthase inhibition raised pulmonary vascular tone equally at rest and during exercise, suggesting that NO release is not enhanced by exercise. In canine femoral arteries, Rubanyi et al. (38) showed that doubling flow rate produced significant relaxation of the perfused vessels even in the presence of indomethacin. The low tone and rapidly branching structure of the pulmonary vascular bed may account for the lack of evidence of shear stress release of NO in our study. This may also explain the results reported by Hakim (13), who found that the decrease in precapillary resistance with flow was attenuated following L-NNA only during hypoxia and with the use of pulsatile flow, suggesting that the increments in shear stress necessary to demonstrate flow-induced NO release in the pulmonary vascular bed must be much larger than those in the systemic circulation.

In conclusion, there appear to be important regional differences in basal eNO production in the segments of the pulmonary circulation. The arterial and precapillary eNO release in pig lungs contribute more than the venous segment to low PVR. NO release does not appear to be fundamental to the mechanism underlying accommodation to increasing steady flow because flow-dependent changes in resistance occurred before and after L-NNA. Because there are important differences among animal species in basal release of eNO in the lung, it is likely that differences among species also occur in terms of regional eNO production.

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