Novel catheterization technique for the in vivo measurement of pulmonary vascular responses in rats

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Departments of 1Surgery and 2Pharmacology, Tulane University School of Medicine, 3H. L. Laboratories, Incorporated, and 5Department of Pharmacology, Louisiana State University Medical School, New Orleans, Louisiana 70112; and 4Nu-Med Incorporated, Hopkinton, New York 12940

Hyman, Albert L., Qingzhong Hao, Allen Tower, Philip J. Kadowitz, Hunter C. Champion, Bulent Gumusel, and Howard Lippton. Novel catheterization technique for the in vivo measurement of pulmonary vascular responses in rats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1218–H1229, 1998.—A novel catheterization technique was devised to investigate the pulmonary arterial pressure–blood flow relationship in intact spontaneously breathing rats (ISBR) under physiological conditions with constant left atrial pressure and controlled blood flow within the normal range. Observations using this new technique in vivo were contrasted with data derived with isolated perfused rat lungs in vitro. Unlike results in isolated perfused rat lungs, the pressure–flow curves in vivo were curvilinear, with pulmonary artery pressure increasing more rapidly at low pulmonary blood flows of 4–8 ml/min and less rapidly at higher flow rates. Pressure–flow curves were reproducible and were not altered by 1–1.5 h of arrested perfusion, cyclooxgenase blockade, or perfusion with aortic or mixed venous blood. In contrast to results in in vitro isolated perfused rat lungs, Nω-nitro-L-arginine methyl ester (L-NAME) increased pulmonary arterial pressure at all but the lowest flow rates with a slight effect on the curvilinear pressure–flow relationship. L-NAME reversed pulmonary vasodilator responses to acetylcholine and bradykinin and enhanced the pulmonary vasodilator response to nitroglycerin. The present data suggest that actively induced pulmonary hypertension is under greater control by endothelium-derived relaxing factor (EDRF). Unlike previous results in in vitro perfused rat lungs, results in ISBR demonstrate that the pulmonary vasodilator response to adrenomedullin (13–52) is not mediated by calctonin gene-related peptide receptors, which are not coupled to the release of EDRF. These results indicate that this novel technique may provide a useful model for the study of the pulmonary circulation in the intact chest rat.

pulmonary vascular bed; endothelium-derived relaxing factor; adrenomedullin; nitric oxide; pressure–flow relationship;}

THE MECHANISMS that maintain low vasomotor tone in the pulmonary vascular bed are incompletely understood. Because L-arginine analogs increase contraction of pulmonary arterial rings (4, 11, 25), it has been suggested that endothelium-derived relaxing factor (EDRF) may be important in maintaining this low basal tone. Indeed, in humans, vasoconstrictor tone in both the pulmonary and systemic vascular beds is considered to be regulated by nitric oxide, since Nω-nitro-L-arginine (L-NMMA) increases both calculated pulmonary and systemic vascular resistances. However, comparisons of single points on a curve relating a pulmonary vascular pressure gradient to pulmonary blood flow before and after an intervention may not distinguish actively mediated changes in pulmonary vascular resistance from passively mediated effects (17, 22). Studies in laboratory animals have revealed conflicting data regarding the effect of inhibitors of endogenous EDRF on basal pulmonary vascular tone. In the intact lamb (14–16) and intact fetal sheep (1), inhibition of endothelium-derived nitric oxide production by L-arginine analogs markedly increases pulmonary vascular tone. Nω-nitro-L-arginine methyl ester (L-NAME) has also been reported to significantly increase basal pulmonary vascular tone in the intact adult cat (28, 30) and open-chest rabbit (39). Basal production of EDRF has been reported to contribute to the low basal vascular tone in the canine pulmonary vascular bed (38); however, data to the contrary have also been reported in this same species (33). The ability of L-NAME to influence resting (low) pulmonary vasomotor tone may not be entirely due to inhibition of EDRF formation, since L-NAME has been reported to possess pulmonary vasoconstrictor properties independent of inhibition of EDRF formation (28).

A single study in intact rats has reported that Nω-nitro-L-arginine (L-NNA) decreased cardiac output but did not alter pulmonary arterial pressure (36). Pulmonary vascular resistance was not measured in that study. In the in vitro isolated perfused rat lung, L-arginine analogs have usually been reported to have little or no effect on basal pulmonary vascular resistance (4, 6, 21, 40, 41, 45). However, recent reports suggest that endothelium-derived nitric oxide may minimally contribute to basal pulmonary vascular tone, since larger doses of L-arginine analogs mildly increased basal pulmonary arterial perfusion pressure in the rat (13, 26, 31). Although the in vitro isolated perfused rat lung is widely used as a pharmacological model to study pulmonary vascular responses, the technique has at least four inherent limitations as follows: 1) the in vitro isolated rat lung is susceptible to ischemic injury, and care must be exercised to maintain perfusion while preparing this excised lung model for experimental observation (5); 2) to avoid pulmonary edema, the in vitro isolated rat lung can only be perfused with 10–15% of the normal pulmonary blood flow of the intact rat, and pressure–flow relationships in the physiological range cannot be studied; 3) the doses of agonists required to induce pharmacological responses in the in vitro isolated perfused rat lung are often large; and 4) data obtained from in vitro isolated...
pulmonary blood flow and constant left atrial pressure. Bradykinin, nitroglycerin, and CGRP were also confirmed in vivo. The present study describes, for the first time, the pressure-flow relationship in the intact pulmonary vascular bed of the rat and the effects of L-NAME on that relationship by a novel modification of a technique (23) that permits observation of controlled lobar blood flow in ISBR. The influence of actively and passively induced pulmonary hypertension on the effects of L-NAME on tone were compared, and the effects of L-NAME on pulmonary vascular responses to acetylcholine, bradykinin, nitroglycerin, and CGRP were also studied in intact rats under conditions of controlled pulmonary blood flow and constant left atrial pressure.

MATERIALS AND METHODS

One hundred twenty-two male Charles River rats weighing 260–340 g were anesthetized with pentobarbital sodium (30 mg ip). Protocols were in conformity with the Institutional Animal Care and Research Advisory Committee of the Tulane Medical School. Rats breathed air enriched with oxygen through an endotracheal tube inserted by tracheostomy. In an initial study, the influence of L-NAME on calculated basal pulmonary vascular resistance was studied under conditions of free, uncontrolled pulmonary blood flow in six rats. The anesthetized animals were strapped in a supine position to a fluoroscopic table, and catheters were inserted into the femoral blood vessels. The venous catheters were passed to the right atrium under fluoroscopy. A 1-F thermistor catheter was passed from the left internal jugular vein into the main pulmonary aorta under fluoroscopy, and a PE-50, 150-mm plastic catheter in the ascending aorta (Fig. 1). Cardiac output was obtained in triplicate by delivering 0.1 ml normal saline at room temperature into the femoral venous catheter at the right venoatrial junction and sensing temperature change in the ascending aorta. Cardiac output determinations were also made in six rats by delivering 0.1 ml of normal saline into the left atrium under fluoroscopic guidance because that vein enters the right atrium through a persistent left superior vena cava that passes to the right atrial posterior wall near the inferior vena cava. Mean pressures in the femoral artery, pulmonary artery, and pulmonary vein at the left venoatrial junction were measured with pressure transducers (Gould P23 ID) and recorded on a Grass model 7 polygraph. Cardiac output was obtained in triplicate by delivering 0.1 ml normal saline at room temperature into the femoral venous catheter over a 5- to 10-min period. Repeat pressure and cardiac output determinations were obtained 30–40 min after infusion of L-NAME.

Because L-NAME significantly decreased cardiac output in these studies, another novel technique was designed to permit observation of the effects of this analog under conditions of constant pulmonary blood flow. Specifically, a previously described technique used in larger animals was modified for studies in the intact rat (23). A specially designed triple-lumen balloon perfusion catheter was constructed (Nu-Med, Hopkinton, NY). This catheter is 145 mm in length and 1.1 mm in OD with a specially curved tip to facilitate passage through the right heart and main pulmonary artery into the artery supplying the right lower lung lobe. At the distal tip of the catheter is a pressure port through which a 0.25-mm soft-tip coronary artery angioplasty guide wire is inserted. Five millimeters proximal to this port is a perfusion port that permits easy passage of a 0.34-mm soft-tipped coronary guide wire. A plastic nondistensible balloon is affixed to a third port just proximal to the perfusion port. When fully distended with contrast material, the balloon is 4.0 mm in diameter and 3.5 mm in length. Before introduction, this catheter curve is initially straightened with 0.45-mm straight wire in the pressure port to facilitate passage from the right jugular vein into the right atrium at the tricuspid valve. As the straight wire is removed, the natural curve permits easy entry into the right ventricle. The catheter is then passed over a 0.25-mm soft-tipped guiding catheter to the main pulmonary artery and then into the right lower lobe artery (Fig. 1). Mean pressures in the right lower lobe and the aorta were continuously recorded. Under fluoroscopy, the balloon is then distended with radiopaque material until lobar arterial pressure falls to pulmonary capillary wedge pressure. In five rats in which both the perfusion catheter and transseptal catheter could be passed from the right jugular vein, pulmonary
capillary wedge pressure was always within 1 mmHg of simultaneously measured left arterial pressure. The distal portion of the right lower lung lobe was then perfused with blood removed from a carotid artery with an extracorporeal pump (Masterflex Quick-Load, Rotary Pump model 7021–24). The volume of the extracorporeal tubing was 1.0 ml. At a perfusion rate of 14.0 ± 0.62 ml/min (n = 24), pressure in the perfused lobar artery approximated that in the main pulmonary artery, and this perfusion rate was taken as the control blood flow rate. Because this catheter perfuses approximately one-sixth of the lung, as determined by measuring lung weight, this perfusion rate approximates physiological flow for that lung area, i.e., at least 15–20% of the 75–85 ml/min normal total pulmonary blood flow of the rat.

Blood gas values were expressed as means ± SE for n = 12 and were as follows: aortic blood, pH 7.32 ± 0.01, arterial partial CO₂ pressure (Pa₉), 43.0 ± 3 mmHg, arterial partial O₂ pressure (Pa₈), 177 ± 21 mmHg; mixed venous blood, pH 7.26 ± 0.1, Pa₉, 58.0 ± 3.0 mmHg, Pa₈, 50.4 ± 5.0 mmHg. Blood gases were checked hourly and maintained within this range. In some experiments, both the perfusion and transseptal catheters could be inserted from the left jugular vein. In others, the perfusion catheter could be inserted from the left jugular vein. In still other experiments in which simultaneous pressures could not be obtained, left atrial pressures were always recorded separately in a parallel group of rat experiments to determine the effect on left atrial pressure of each of the experimental groups and agents used.

The constant blood flow experiments were performed in five groups. An initial study investigated the relation of pressure to blood flow in the lung of the ISBR. A second group studied the effect of L-NAME on the pulmonary vascular responses to EDRF-dependent and EDRF-independent vasodilator substances.

In an initial group of six ISBR, the effects of changing pulmonary blood flow on baseline hemodynamics were studied. After baseline pressures in the constantly perfused lobar artery and the aorta had stabilized, a pressure-flow curve was obtained in the perfused lobe commencing at zero blood flow and increasing flow at selected rates to 22 ml/min, the maximal capacity of the system. Pulmonary arterial perfusion pressure stabilized for at least 3–5 min at each level before advancing to a higher flow level. In these six ISBR, a control study was also performed to determine the reproducibility of the pressure-flow curve over time. After the control pressure-flow curve was obtained, lobar arterial blood flow was maintained at 14.0 ml/min for 1–2 h before a pressure-flow curve was determined. In six other ISBR, pressure-flow curves were also repeated after perfusion of the right lower lobar artery had been arrested for 1 h. In these studies, reperfusion at basal flow rates was commenced, and pressures stabilized for 5–10 min before repeat pressure-flow curves were done. To assess the effects of arrested perfusion on pulmonary vascular reactivity in four rats, vasoconstrictor responses to injections of adenosine, norepinephrine, and U-46619 were compared before and after perfusion of the right lower lobar artery had been arrested for 1 h. In these studies, reperfusion at basal flow rates was commenced, and pressures stabilized for 5–10 min before repeat pressure-flow curves were done. To assess the effects of arrested perfusion on pulmonary vascular reactivity in four rats, vasoconstrictor responses to injections of adenosine, norepinephrine, and U-46619 were compared before and after perfusion of the right lower lobar artery had been arrested for 1 h. In these studies, reperfusion at basal flow rates was commenced, and pressures stabilized for 5–10 min before repeat pressure-flow curves were done.
edema. The effects of L-NAME on the pressure-flow relationship were studied in another 11 ISBR. Because the effects of nitric oxide synthase blocking agents derived from L-arginine have been reported to be dose dependent in some rat experiments (13, 26) and to possess vascular activity unrelated to their inhibitory effects on nitric oxide production (3, 28), L-NAME was given intravenously in increasing doses. Pressure-flow relationships were obtained in the control period and 30–40 min after each subsequent L-NAME administration in each ISBR at total levels of 30, 100, and 300 mg/kg.

A third group of experiments was designed to contrast the effects of L-NAME on pulmonary arterial pressure elevated to equivalent levels passively by increasing flow and actively by constricting pulmonary blood vessels with U-46619 infusion. In six ISBR in which initial pulmonary arterial perfusion pressure was 12.0–13.0 mmHg at a blood flow of 14.0 ml/min, pulmonary artery pressure was increased by increasing blood flow to the maximum of 22 ml/min and was permitted to stabilize. L-NAME (100 mg/kg) was then given intravenously, and pressures were observed for 30–40 min. In a contrasting group of six other ISBR under conditions of constant lobar blood flow of 14 ml/min, pulmonary arterial perfusion pressure was increased from a control value by carefully administering U-46619 (0.5–1.5 µg/min) in four ISBR or prostaglandin F3, (0.3–1.0 µg/min) in two rats as an intralobar arterial infusion to increase pulmonary arterial perfusion pressure to approximately the same level as observed in the high-flow group above. After pressures had stabilized at the elevated level, L-NAME (100 mg/kg) was given intravenously, and pressures were observed for 30–40 min.

In a fourth study of 16 ISBR, the effects of L-NAME on the pulmonary vasodilator response to acetylcholine, bradykinin, and nitroglycerin were studied in ISBR under conditions of constant pulmonary blood flow and left atrial pressure. After cardiac catheterization was completed and constant pulmonary blood flow was established in the right lower lung lobe, pulmonary vasomotor tone was raised by a continuous intralobar arterial infusion of U-46619 (1.5–25 µg/min) from 12.8 ± 1.1 to 34.8 ± 14.4 mmHg. After pressures were stabilized, the effects of intralobar bolus injections of acetylcholine (1.0 and 3.0 µg; Sigma) were studied. Doses of acetylcholine >3.0 µg usually caused cardiac arrest. In six of these eight rats, nitroglycerin (1.0 µg; Sigma) was also injected in a similar manner under conditions of elevated pulmonary vasomotor tone. Lobar arterial pressure was then lowered to ~25 mmHg by decreasing the rate of infusion of U-46619. L-NAME (100 mg/kg) was then administered intravenously as described above. After all pressures had stabilized for 30 min, the pressure in the perfused lobar artery was carefully readjusted to 35.1 ± 1.4 mmHg with a significantly reduced infusion rate of U-46619 (0.3–0.5 µg/min). After stabilization of pressures, the bolus injections of acetylcholine and nitroglycerin were repeated. In eight other rats, the effects of intralobar injections of bradykinin (1 and 3 µg; Sigma) were similarly studied before and after L-NAME administration. Larger doses of bradykinin frequently produced a biphasic response with initial pulmonary vasodilation, whereas higher doses of bradykinin (>10 µg) promoted instability of the preparation and were not studied. In six of these eight rats, a bolus injection of nitroglycerin (1.0 µg) into the perfused lobar artery was also administered before and after L-NAME.

In each of four additional ISBR, similar injections of acetylcholine, bradykinin, and nitroglycerin were administered into the right atrium under conditions of free flow. Systemic arterial pressure was decreased in a dose-dependent manner, whereas left atrial and pulmonary arterial pressures were unchanged by these injections.

The last series of experiments was divided into three groups and was designed to investigate the nature of the pulmonary vascular response to CGRP (Phoenix Pharmaceuticals) and adrenomedullin (ADM)-(13–52) (Phoenix) in ISBR under conditions of actively increased pulmonary vasomotor tone and to determine if specific CGRP receptor-mediated vasodilation in lungs of ISBR is altered by L-NAME administration. In the first two groups of experiments, pulmonary vascular responses to intralobar injections of CGRP and ADM (13–52) were obtained immediately before and 20 min after administration of CGRP-(8–37) (Phoenix), a selective CGRP receptor antagonist (43). The dose of CGRP-(8–37) was determined in pilot experiments and was 10 µg·kg⁻¹·min⁻¹ for 30 min. In the third group of experiments, pulmonary vascular responses to CGRP were obtained immediately before and 30–45 min after administration of L-NAME.

Data are presented as means ± SE. Statistical analysis was done by Student’s t-test for paired analysis or by one- or two-tailed analysis of variance with an F-test.

RESULTS

Pressure-flow experiments in the ISBR indicate that pulmonary arterial pressure rises as pulmonary blood flow is increased. Pulmonary arterial pressure rises more rapidly at flow rates of 4–8 ml/min and more slowly as higher pulmonary blood flow rates are reached (Fig. 2A). These pressure-flow curves were reproducible with respect to time, and curves obtained in the control period and after periods of 1–1.5 h were similar if not identical (Fig. 2A). The effect of a 1.5-h period of interruption of blood flow was investigated, and pressure-flow curves were not changed after lobar arterial perfusion was arrested for 1–1.5 h in ISBR (Fig. 2B). In addition, a period of arrested perfusion did not alter the subsequent pulmonary vasoconstrictor responses to intralobar injections of adenosine, norepinephrine, and U-46619 (Table 1) or pulmonary vasodilator responses to intralobar injections of acetylcholine, bradykinin, and nitroglycerin (Table 1). The pressure-flow curves with right atrial blood perfusion and with aortic blood perfusion were similar at blood flow rates up to 14 ml/min (Fig. 2C). In addition, meclofenamate in a dose of 2.5 mg/kg iv did not change the pressure-flow curve in the ISBR (Fig. 2D). In contrast, L-NAME in doses of 30–300 mg/kg iv significantly increased perfusion pressure at all rates >4 ml/min and mildly altered the curvilinear relationship of the pressure-flow curve at the higher blood flows (Fig. 3A). L-NAME (100 mg/kg) increased control perfusion pressure at 8 ml/min flow by 3.7 ± 0.65 mmHg but at 22 ml/min by 5.8 ± 0.97 mmHg (P < 0.05). In 11 other ISBR pretreated with L-NAME (100 mg/kg iv), arrest of lobar arterial perfusion for periods of 1.5 h did not alter the pressure-flow curve (Fig. 3B).

In contrast, in the six ISBR studied under free-flow conditions, administration of L-NAME in a dose of 100 mg/kg iv did not alter pressure in the pulmonary artery or left atrium but significantly decreased cardiac output. Calculated pulmonary vascular resistance was not significantly increased by L-NAME, whereas systemic
arterial pressure and systemic vascular resistance were significantly elevated (Table 2).

In six additional ISBR in which a moderate lobar arterial hypertension had been induced by increasing flow to the maximal value of 22 ml/min, L-NAME (100 mg/kg iv) caused a small but significant increase in lobar arterial perfusion pressure. In contrast, L-NAME promoted a markedly greater increase in lobar arterial pressure when baseline lobar arterial perfusion pressure was increased to similar levels by intralobar infusion of U-46619 or prostaglandin F2α (Fig. 4).

In eight ISBR in which the pulmonary arterial pressure was actively increased to 34.8 ± 1.4 mmHg by intralobar infusion of U-46619, intralobar injections of acetylcholine in doses of 1.0 and 3.0 µg decreased lobar perfusion pressure in a dose-dependent manner (Fig. 5). After L-NAME, the pulmonary vasodilator response to acetylcholine became biphasic, and a small secondary pulmonary vasoconstrictor component was unmasked (Fig. 5). After L-NAME administration in eight other ISBR, the pulmonary vasodilator response to bradykinin in doses of 1.0 and 3.0 µg was changed to a biphasic response (Fig. 5). The initial pulmonary vasodilator response to bradykinin was significantly decreased after L-NAME administration, and a large terminal pulmonary vasoconstrictor response was observed in response to bradykinin. In contrast, the pulmonary vasodilator response to nitroglycerin (1.0 µg) was significantly increased after L-NAME administration (Fig. 5).
Although the nadir of the systemic vasodepressor response to acetylcholine, bradykinin, and nitroglycerin was not affected by L-NAME, the duration of the decrease in systemic arterial pressure as assessed by the half-time of the response to acetylcholine and bradykinin was significantly decreased, whereas the half-time of the systemic vasodepressor response to nitroglycerin was significantly increased (Table 3).

In a separate group of ISBR, under conditions of increased pulmonary vascular tone induced by intralobar infusion of U-46619, intralobar injections of CGRP, in doses of 0.1–1.0 µg, decreased lobar arterial pressure in a dose-dependent manner (Fig. 6). After administration of the CGRP receptor antagonist CGRP-(8–37), infused in a dose of 10 µg·kg$^{-1}$·min$^{-1}$ for 30 min, pulmonary vasodilator responses to CGRP were significantly reduced (Fig. 6). Unlike the pulmonary vasodilator responses to CGRP, the pulmonary vasodilator responses to intralobar injections of ADM-(13–52) in doses of 1.0–10.0 µg in a separate group of ISBR under similar elevated-tone conditions were not altered by administration of CGRP-(8–37) (Fig. 7). In the final group of ISBR under similar conditions, the pulmonary vasodilator responses to intralobar injections of CGRP were not altered by administration of L-NAME (Fig. 8).

**DISCUSSION**

The present experiments describe for the first time pressure-flow curves in the pulmonary vascular bed of ISBR as shown in Fig. 2A. Beginning at zero blood flow, lobar arterial pressure rose at flow rates up to 22 ml/min with a steeper slope at flows of 4–8 ml/min in the hemodynamically isolated intact right lower lung lobe (Fig. 2A). Moreover, as the flow rate increased toward the physiological range in the perfused lung lobe, pulmonary arterial pressure increased more slowly as pulmonary blood flow was increased. Similar pressure-flow curves have been described in the intact dog, and the modulation of pulmonary arterial pressure as blood flow increases may serve as a protective mechanism to decrease the tendency toward pulmonary edema formation when cardiac output is increased (22, 37). As blood flow increases, the more gradual rise in pulmonary arterial pressure has generally been attributed to distension of perfused vessels and recruitment of previously unperfused vessels (22, 27, 37). The pressure at zero flow at the origin of the pressure-flow curves reflects the pulmonary arterial wedge pressure and correlates with left atrial pressure (Fig. 2A). Cyclooxygenase products probably do not contribute to the hyperbolic nature of the curve, since pretreatment with meclofenamate in the present study did not alter the

**Table 1. Influence of 1-h period of ischemia-reperfusion on pulmonary vascular responses**

<table>
<thead>
<tr>
<th></th>
<th>Decrease in Pulmonary Arterial Pressure</th>
<th>Increase in Pulmonary Arterial Pressure</th>
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<tbody>
<tr>
<td></td>
<td>Acetylcholine, µg ia</td>
<td>Bradykinin, µg ia</td>
</tr>
<tr>
<td>Control</td>
<td>−2.5 ± 0.2</td>
<td>−4.5 ± 0.1</td>
</tr>
<tr>
<td>Postischemia</td>
<td>−2.4 ± 0.3</td>
<td>−4.3 ± 0.4</td>
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Values are means ± SE; n = 4 experiments. Units are mmHg. Data for decrease in pulmonary arterial pressure reflect values under elevated tone conditions.

Fig. 3. A: effects of 30 (†), 100 (○), and 300 (△) mg/kg iv NG-nitro-L-arginine methyl ester (L-NAME) on the relationship of pressure to pulmonary blood flow in the right lower lung lobe of intact rats (n = 11). □, Control. *P < 0.05 by 2-tailed analysis of variance. B: influence of L-NAME pretreatment (100 mg/kg iv; ○) on the pressure-flow curve after pulmonary arterial perfusion was arrested for 1.5 h (■). There was no significant difference in pressure at any flow rate tested (n = 11).
pressure-flow relationship (Fig. 2A). Similar data have been previously reported in the isolated rat lung under conditions of constant pulmonary pressure gradient (5). In the present study, the pressure-flow relationship was not changed by perfusion with right atrial or aortic blood (Fig. 2C). Previous experiments in intact cats demonstrated that, under similar experimental conditions, baseline pulmonary arterial perfusion was higher when the lung was perfused with right atrial blood when compared with perfusion with aortic blood (24). This difference may be related to species or to the fact that, in the present experiments, only one level of right atrial blood \( P_aO_2 \) was studied. In the present experiments, the pressure-flow relationship was stable over the course of experiments from 1 to 2 h of continuous perfusion and was not altered by stopping perfusion for periods up to 1.5 h (Fig. 2B). The stability of the pressure-flow relationship even after catheter perfusion (arterial inflow) had been arrested for 1–1.5 h was likely due to the presence of an intact bronchial circulation and to ventilation with air enriched with oxygen. Indeed, under physiological conditions in erect mammals, including humans, gravitational effects may reduce perfusion of apical portions of the normal lung without inducing lung injury with changes in position and perfusion. This is in contrast to the isolated perfused rat lung, which is susceptible to ischemic lung injury, in that arrested perfusion for 1–2 h has been shown to induce acute lung injury with reperfusion (5).

The hyperbolic shape of the pressure-flow relationship in ISBR further differs from that in the isolated perfused rat lung model in which only a linear rise in pulmonary arterial pressure from zero blood flow to flows as high as 80 ml/min has been reported (6, 20, 26). The reason for the differences in the shape of the pressure-flow relationships in rat lungs in vivo and isolated perfused rat lungs in vitro is not clear. The present results in the ISBR indicate that, at the highest flow rate of 22 ml/min into the right lobar artery, lobar arterial perfusion pressure ranged from 16.4 ± 0.6 to 18.2 ± 1.0 mmHg. In terms of the whole lung, this flow rate represents a pulmonary blood flow of \( \sim 130 \) ml/min or \( \sim 1.4–1.5 \) times baseline cardiac output in the ISBR. The pulmonary arterial pressure flow curves were much steeper in the in vitro isolated perfused rat lung, reaching values of 38–40 mmHg for whole lung perfusion rates of 30 ml/min and 60 mmHg for perfusion rates of 80 ml/min (6). In other reports, whole rat lung in vitro perfused at rates of 45–50 ml/min increased pulmonary arterial perfusion pressure linearly to \( \sim 18-25 \) mmHg (20, 26), but these flows could only be maintained for 30 s to prevent overt pulmonary edema. At basal pressure, in these in vitro isolated whole rat

<table>
<thead>
<tr>
<th>Control</th>
<th>L-NAME</th>
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<tr>
<td>SAP, mmHg</td>
<td>111.7 ± 13.7</td>
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<tr>
<td>SVR, mmHg/ml/min</td>
<td>1.5 ± 0.3</td>
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<tr>
<td>PAP, mmHg</td>
<td>11.8 ± 3.4</td>
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<tr>
<td>LAP, mmHg</td>
<td>2.3 ± 0.3</td>
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<tr>
<td>PVR, mmHg/ml/min</td>
<td>0.14 ± 0.05</td>
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<tr>
<td>CO, ml/min</td>
<td>82.5 ± 15.1</td>
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</table>

Data are means ± SE. L-NAME, \( \text{N}^6\)-nitro-L-arginine methyl ester (100 mg/kg iv). SAP, systemic arterial pressure; SVR, systemic vascular resistance; PAP, pulmonary arterial pressure; LAP, left atrial pressure; PVR, pulmonary vascular resistance; CO, cardiac output. *P < 0.05 by paired t-test.

![Fig. 4. Comparison of effects of L-NAME administration (100 mg/kg iv) on passively induced (high flow) and actively induced (preconstriction with U-46619) elevations on pulmonary arterial pressure under conditions of constant flow (n = 6 each group). *P < 0.05 by 2-tailed analysis of variance for different groups.](http://ajpheart.physiology.org/)
lungs, perfusion rates only ranged from 10 to 20 ml/min. These differences suggest that the initial pulmonary vascular resistance in the isolated perfused rat lung in vitro is markedly higher than in the ISBR, and the physiological process of recruitment and distension in rat lungs in vitro with increased flow is altered.

The contribution of nitric oxide to this curvilinear relationship of pressure to flow in the present intact lung model is difficult to assess. The increase in pressure at all but the lowest flow rate caused by L-NAME suggests that, in the physiological range of flow, nitric oxide does contribute to the modulation of basal tone in the pulmonary vascular bed (Fig. 3A). In contrast, in the present experiments, no evidence of modulation of pulmonary vascular tone by L-NAME could be detected in the lung of ISBR when pulmonary blood flow was not maintained constant when a single point on the pressure-flow curve was compared before and after L-NAME (Table 2). Because the pulmonary vasopressor response to L-NAME was somewhat greater at the higher flow rates under conditions of controlled pulmonary blood flow, the present data suggest that the loss of nitric oxide modulation after L-NAME may be greater at higher pulmonary blood flow and shear rates (Fig. 3A). However, at higher flow rates, recruitment and distension within the lung segment tend to minimize the increase in flow, shear rate, and nitric oxide production in vessels initially perfused. Moreover, this recruitment and distension concurrently increase flow, shear rate, and nitric oxide production into lobar vessels that had not been previously perfused at lower flow rates. At much higher flow rates, the contribution of nitric oxide may have been greater. In addition, although the pressor response to L-NAME was greater at higher flows than at the lower flow rates, and the comparison of pressor responses to L-NAME at different control baseline pressures may be difficult. Furthermore, the effects of L-NAME on the pulmonary vessels may not be mediated completely by its blocking effect on nitric oxide production (3, 28).

Similar experiments with L-arginine analogs in the in vitro isolated perfused rat lung have yielded conflicting data. At basal whole lung perfusion rates of 10–20 ml/min, perfusion pressure was not altered by L-arginine analogs in some experiments (4, 6, 21, 40, 41), whereas very small increments were reported by others (13, 26, 31). Those experiments may be similar to the present studies, since L-NAME had no vasoconstrictor effect at low nonphysiological flow rates in the lungs.

### Table 3. Effects of L-NAME on half-time of systemic vasodepressor responses

<table>
<thead>
<tr>
<th></th>
<th>Duration of Response</th>
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<tbody>
<tr>
<td></td>
<td>Acetylcholine, µg ia</td>
<td>Bradykinin, µg ia</td>
<td>Nitroglycerin (1 µg ia)</td>
</tr>
<tr>
<td>Control response</td>
<td>17.02 ± 0.4</td>
<td>23.0 ± 0.8</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td>L-NAME</td>
<td>10.0 ± 0.4*</td>
<td>12.2 ± 0.7*</td>
<td>7.0 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>6.0 ± 0.7*</td>
<td>10.7 ± 0.4*</td>
<td>40.3 ± 2.1*</td>
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</table>

Values are means ± SE; n = 4 experiments. Units are seconds. *P < 0.05, paired t-test.
The pulmonary pressor response to L-NAME is not flow dependent. However, in a parallel study from the same laboratory, L-NMMA did not alter pulmonary arterial perfusion pressure in the isolated rat lung in vitro perfused at steady rates of 12–13 ml/min (4). Other reports have also suggested that the failure of L-arginine analogs to increase perfusion pressure of isolated lungs in vitro may have been related to higher PaO2 levels of increased pulmonary arterial pressure, L-NAME induces a greater pulmonary vasoconstrictor response in arteries actively constricted than in pulmonary blood vessels passively distended (Fig. 4). The reasons for this difference are not established but may in part be related to the greater shear rate in constricted pulmonary blood vessels at basal constant blood flow compared with distended pulmonary blood vessels at higher constant blood flow. Reports using much lower perfusion rates in the isolated perfused rat lung in vitro are conflicting. Similar data have been reported in in vitro studies (6, 36), but data to the contrary have also been reported (7, 21). Other mechanisms may contribute to the enhanced response in actively constricted vessels (10), since L-NNA markedly increases the contractile response to phenylephrine in isolated conductance pulmonary arterial rings obtained from rats subjected to chronic hypoxia.

In the present study, the pulmonary vasodilator response to acetylcholine was blocked by L-NAME in the ISBR, suggesting that this response may be mediated by nitric oxide (Fig. 5). Furthermore, the pulmonary vasodilator response to acetylcholine is reversed with the appearance of a pulmonary vasoconstrictor response (Fig. 5). The appearance of a pulmonary vasoconstriction in response to acetylcholine after L-NAME may result from the loss of an opposing more potent pulmonary vasodilator effect (28). It is possible that L-NAME may have increased the activity of muscarinic receptors, obscuring the vasodilator response to acetylcholine. However, other work indicates that L-NAME is a muscarinic receptor antagonist (9, 28). Previous reports of the effects of L-arginine analogs on the response to EDRF-dependent vasodilators in isolated rat lungs perfused at low nonphysiological flow rates in vitro are inconsistent. The pulmonary vasodilator response to acetylcholine was not affected by the L-arginine analog L-NMMA in one study (4) but was decreased in another study (29). The reasons for these differences are not clear but likely represent differences between the present intact lung preparation and the in vitro isolated perfused lung model. Moreover, the low perfusion rates in in vitro experiments may reflect the loss of modulation of pulmonary vascular tone, since prolonged perfusion of the rat lung in vitro at physiological flow induced pulmonary edema (5).

In the present studies in ISBR, the pulmonary vasodilator response to bradykinin was almost completely reversed by L-NAME, resulting in a marked pulmonary vasoconstrictor response (Fig. 5). Inhibition of nitric oxide formation by L-NAME may have completely blocked this pulmonary vasodilator response, thereby permitting the expression of a less potent pulmonary vasoconstrictor response. However, the possibility that the pulmonary vasoconstrictor response to bradykinin may also have been potentiated by L-NAME and thereby
diminished the magnitude of the vasodilator response cannot be excluded (28). Data from the present experiments in ISBR and in hypoxic isolated perfused rat lungs in vitro are similar. In lungs from such experiments, L-NMMA did not increase basal pulmonary perfusion pressure but did greatly decrease the pulmonary vasodilator response to bradykinin and induced a pulmonary vasoconstrictor response (4). On the other hand, in the isolated perfused rat lung in vitro, pulmonary vasodilation in response to bradykinin has not been consistently observed. Russell et al. (41) observed a pulmonary vasodilator response to bradykinin in low doses when pulmonary vasomotor tone was elevated by acute or chronic hypoxia, whereas higher doses of bradykinin produced a pulmonary pressor response. In contrast, in another study in the isolated blood-perfused rat lung, a pulmonary vasoconstrictor response to bradykinin was observed at all doses studied (35). The reason for the differences between the present experiments and results in the isolated blood-perfused rat lung are not clear but may be related to the doses of bradykinin used, experimental techniques employed, and levels of pulmonary vascular tone in each preparation.

The present experiments in intact rats demonstrate that L-NAME caused a significant enhancement of the pulmonary vasodilator response to nitroglycerin (Fig. 5) that has not been identified in the isolated perfused rat lung in vitro and may result from the removal of nitric oxide and enhanced sensitivity of soluble guanylate cyclase to nitrovasodilators (13, 18, 32).

The nadir of the systemic vasodepressor responses to acetylcholine, bradykinin, and nitroglycerin was not decreased by L-NAME, whereas the duration of responses to acetylcholine and bradykinin were shortened, and the response to nitroglycerin was increased in duration (Table 3). Similar data have been reported in the intact guinea pig after L-NMMA (2) and rats after L-NAME (18). In contrast, in other studies in the rat, the vasodepressor response to acetylcholine and bradykinin was blocked by L-NMMA (44). The reasons for these differences are not clear but may be related to the differences in magnitude of the hypertensive response induced by the L-arginine analogs themselves.

ADM-(13–52) decreases pulmonary vascular resistance and, since CGRP-(8–37) did not alter the pulmonary vasodilator response to ADM-(13–52), the present data suggest that ADM-(13–52) does not act on CGRP receptors to dilate the pulmonary vascular bed of ISBR (Fig. 6). Although ADM has limited homology with CGRP and has been reported to act through CGRP receptors in the mesentery (8) and microvasculature (19), the present data in the lung are in agreement with studies in the kidney (12), demonstrating that ADM does not act on CGRP receptors (Fig. 7). The present in vivo data are in agreement with earlier work in vitro (43) demonstrating that CGRP dilates the rat pulmonary vascular bed by activating specific CGRP receptors (Fig. 6). It has been reported that the pulmonary vasodilator response to CGRP in in vitro isolated perfused rat lungs is inhibited by L-NAME, suggesting that CGRP dilates the rat pulmonary vascular bed by releasing nitric oxide and acts as an endothelial-dependent vasodilator peptide in the rat lung (34). The present data in vivo using the ISBR demonstrate that CGRP acts on specific pulmonary vascular receptors that are not coupled to the release of nitric oxide (Fig. 8). Moreover, the present data in vivo do not agree with recent work in vitro and suggest that CGRP is not an endothelial-dependent vasodilator peptide in the pulmonary vascular bed of the ISBR.

In summary, the present study reports a novel cardiac catheterization technique for studying the pulmonary vascular bed in ISBR at constant left atrial pressure and controlled pulmonary blood flow rates within the physiological range for intact rats. Results with this model are different from results in the in vitro perfused isolated lung preparations in which maximal flows are limited to 15–20% of normal cardiac output because of markedly elevated pulmonary vascular tone. The preparation was stable and not altered by periods of no flow up to 1.5 h. In the intact rat, the pressure-flow relationship differs from the in vitro model in that it is curvilinear. At pulmonary blood flow rates of 4–8 ml/min, pulmonary arterial pressure rises rapidly but does so more slowly as flow increases. These experiments suggest that the curvilinear pressure-flow relationship results predominantly from distension of perfused vessels and recruitment of previously unperfused vessels. EDRF does contribute to modulation of the low pulmonary vascular tone and may contribute to a lesser extent to the curvilinear pressure-flow relationship. Cyclooxygenase products do not modulate the pressure-flow relationship in ISBR. In contrast, experiments with the in vitro isolated perfused rat lung model display a linear pressure-flow curve that, for the most part, is not influenced by L-arginine analogs. Furthermore, at similar levels of pulmonary hypertension, L-NAME causes greater vasoconstriction when the hypertension is induced by active vasoconstriction than by passive distension of pulmonary vessels. In addition, L-NAME reversed the pulmonary vasodilator response to acetylcholine in intact rats in contrast to the in vitro isolated perfused rat lung in which the effects of L-arginine analogs have been inconsistent. The pulmonary vasodilator response to bradykinin was largely abolished by L-NAME, and a vasoconstrictor response is unmasked. Although these changes may have resulted largely from the loss of the vasodilator effect of nitric oxide, the possibility that L-NAME independent of inhibition of nitric oxide formation may have enhanced vasoconstrictor mechanisms cannot be excluded. Vasodilator responses to CGRP but not to ADM-(13–52) are reduced by the CGRP antagonist CGRP-(8–37), and responses to CGRP are not altered by L-NAME. The present results indicate that this novel technique may provide a useful model for studying pulmonary vascular responses in the intact-chest rat.
REFERENCES


