

Role of Cytochrome P-450 in Alveolar Hypoxic Pulmonary Vasoconstriction in Dogs

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ABSTRACT Alveolar hypoxia induces pulmonary vasoconstriction by an unknown mechanism. Cytochrome P-450 (C-P450) is found in the lung and may modify pulmonary vascular tone via its sensitivity to changes in oxygen tension or by affecting metabolism of a chemical mediator. Metyrapone and carbon monoxide are both inhibitors of C-P450. We tested alveolar hypoxic pulmonary vasoconstriction (AHPV) in 20 dogs before, during, and after separate administration of each inhibitor. Anesthetized dogs were ventilated through a double lumen endotracheal tube allowing ventilation of one lung with N₂ or CO as a hypoxic challenge and ventilation of the other lung with O₂ to maintain adequate systemic oxygenation. Distribution of lung perfusion was determined with intravenous ¹³³Xenon and external chest detectors. Before infusion of metyrapone, mean perfusion to the test lung decreased 30% with alveolar hypoxic challenge, but decreased only 10% during metyrapone infusion and returned to a base-line mean decrease of 31% after completion of metyrapone infusion. Prostaglandin F₂ α and angiotensin II infusions produced equivalent increases in pulmonary vascular resistance before and during metyrapone infusion. Before CO, mean test lung perfusion decreased 31% with alveolar hypoxia but was reduced only 10% from control when unilateral end-tidal CO% was >75%. Washout of alveolar CO with unilateral N₂ ventilation restored AHPV, with perfusion decreasing 29% from control. Thus, both metyrapone and carbon monoxide can reversibly inhibit AHPV. C-P450 may, therefore, be involved in the transduction process of the vasoconstrictor response to alveolar hypoxia.

INTRODUCTION

Regional alveolar hypoxia elicits regional pulmonary vasoconstriction (1, 2), enabling the lung to divert perfusion from poorly ventilated to better ventilated areas of the lung. Current attention has focused on a local intrapulmonary mechanism for this response, devoid of substantial influence from the autonomic nervous system or from systemic humoral substances (3, 4). Whether hypoxia induces vasoconstriction directly, by affecting cellular events in pulmonary vascular smooth muscle, or indirectly, by releasing a chemical mediator is unknown, although to date no chemical mediator has definitively been identified (3, 4). The potent pulmonary vasoconstrictors, histamine, angiotensin II, and prostaglandin F₂ α have each been suggested as the mediator for alveolar hypoxic vasoconstriction (5, 6, 7). Other investigators, however, have failed to substantiate a role for any of these possible mediators (8–12). If hypoxia affects vascular smooth muscle directly, primary candidates for molecular oxygen sensors are the oxygen using enzymes themselves. These enzymes, comprising the cytochrome system, have already been implicated as oxygen sensors in the carotid body (13, 14). Changes in oxygen tension alter the redox state of the various cytochromes and this alteration may modify pulmonary vascular tone. Alternatively, cytochromes could play a role in metabolism of a chemical mediator.

Cytochrome P-450, although not a member of the classic mitochondrial electron transport chain, is sensitive to oxygen tension and has been identified in the microsomal fraction isolated from lungs (15–17). Metyrapone, known for its use in evaluating the pituitary adrenal axis, acts by competitive inhibition of cytochrome P-450 (18, 19). Carbon monoxide, in high concentrations, also inhibits cytochrome P-450 (15, 20, 21). We have examined the role of cytochrome

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P-450 in regional alveolar hypoxic vasoconstriction in an intact dog model using metyrapone and carbon monoxide separately, as inhibitors. In the present experiments, regional alveolar hypoxic vasoconstriction was tested before, during, and after administration of each inhibitor.

METHODS

Mongrel dogs weighing between 18 and 38 kg were anesthetized with pentobarbital sodium; 30 mg/kg i.v. initially, and 4.5 mg/kg every 2 h thereafter. Succinylcholine was given as necessary to suppress voluntary ventilation. A double lumen endotracheal tube (Rüsch) was passed to the carina where inflation of the balloon separated the two main bronchi. Connected to a volume-cycled dual ventilator (Harvard Apparatus Co., Inc., Millis, Mass.), each lung received a separate tidal volume of 7.5 cm³/kg at a rate of 14–16 cycles/min. Both lungs were ventilated with room air between experimental runs. Airway pressures were monitored continuously from both lungs via pressure transducers connected to a side arm on each tracheal cannula. To prevent atelectasis, both lungs were hyperinflated every 10 min and 2 min before each perfusion study by raising expiratory airway pressure to 30 mm Hg by occluding the expiratory ports of the ventilator. Tracheal division was tested by ventilating one lung with 100% O₂ and the other with 100% N₂ while measuring end-tidal PO₂ from the nitrogen ventilated lung. As described (22), an end-tidal PO₂ of less than 30 mm Hg after 7 min of N₂ breathing was considered adequate division. Adequacy of division was verified before each hypoxic perfusion study.

Polyethylene catheters were placed in each femoral artery for measurement of arterial pressure and cardiac output. Catheters were inserted through both femoral veins into the inferior vena cava for all injections. A Swan Ganz catheter was passed through the jugular vein into the main pulmonary artery, using pressure tracings, for determination of pulmonary artery and pulmonary capillary wedge pressure. During the carbon monoxide study, a second Swan Ganz catheter was passed into the main pulmonary artery for mixed venous blood sampling. PO₂, PCO₂, and pH in blood samples were determined with appropriate electrodes at 38°C (models 127 and 213, Instrumentation Laboratory, Inc., Lexington, Mass.). Arterial O₂ contents were determined with an oxygen analyzer (Lex-O₂-Con, Lexington Instruments Corp., Waltham, Mass.). Carboxy-hemoglobin levels were determined with a CO oximeter (model 182, Instrumentation Laboratory, Inc.). Alveolar (end-tidal) carbon monoxide concentrations were measured with a gas chromatograph (model 3920, Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.).

Distribution of pulmonary perfusion was determined with ¹³³Xenon and external chest detectors, as described (23). Four sodium iodide (TI) crystals 32 mm in diameter and 25 mm in height with 10-cm-deep divided lead collimators, two for each lung, were positioned over the anterior thorax in the supine animal. The counters were at a 7° angle of divergence from the mid-sagittal plane, which previous isocount curves have shown to result in less than a 5% overlap of counts between the two lungs. 1 mCi of ¹³³Xenon in 1 ml of saline was injected intravenously during a 15-s breath-hold at tidal inspiration. Counts from each detector, represented by a plateau of activity, were recorded simultaneously on magnetic tape and transcribed sequentially on an X-Y recorder. Counts from upper and lower zones of each lung were summed and expressed as a percentage of total counts to both lungs. This

fraction was then taken to represent relative perfusion to that lung.

Control. Base-line relative perfusion to each lung was determined while each dog was breathing room air. Before ¹³³Xenon injection, arterial blood gases, airway pressures from both lungs, arterial blood pressure, and pulmonary artery and pulmonary capillary wedge pressure were measured and recorded. Cardiac output was measured by dye-dilution technique with a cardiac output computer and clinical densitometer (model RLD, Lexington Instruments Corp.). For the carbon monoxide experiments, additional measurements of mixed venous blood gases and arterial oxygen content were made.

As a hypoxic challenge, 100% nitrogen was then administered to the test lung with 100% oxygen delivered to the other lung to maintain adequate systemic oxygenation. The test lung was chosen randomly. Blood sampling, cardiac output, and pressure measurements were performed as before, after 7 min of unilateral nitrogen ventilation. Relative perfusion to each lung was then determined by the ¹³³Xenon technique. Relative perfusion to the test lung was expressed both as a percentage of total counts, and as a percentage of decrease in perfusion from the room air control value. Previous investigations, with this model, have shown the hypoxic vasoconstrictor response to be maximal at 7 min and stable through 30 min after initiation of unilateral nitrogen ventilation (24). Hence, all hypoxic challenge relative perfusion measurements were made after 7 min of unilateral nitrogen ventilation.

The animals were then returned to bilateral room air breathing for at least 10 min before another experimental run. This entire procedure was repeated at least three times. Only those animals who established a stable base-line room air perfusion distribution and a reproducible relative perfusion decrease with unilateral hypoxia were used in the study.

Metyrapone. After the control procedure outlined above, an intravenous infusion of metyrapone was begun at a mean dose rate of 2.9 mg/kg per min (± 0.2 SEM). Metyrapone (CIBA-Geigy Corp., Pharmaceuticals Div., Summit, N. J.), supplied as metyrapone ditartrate, was dissolved in water and diluted in normal saline to a mean vol of 250 ml. During the infusion, although hemodynamics were monitored, no measurements of relative perfusion were made for the first 45 min. Thereafter, all control measurements, including relative perfusion distribution to the two lungs, were determined serially, while alternating room air and unilateral nitrogen ventilation as before the metyrapone infusion. Thus, steady-state conditions were verified with variable total doses of metyrapone. Because metyrapone might have altered the timing rather than the magnitude of the hypoxic response, additional measurements of all parameters were made, in most dogs, at varying time intervals after initiating the hypoxic challenge. After a total metyrapone dose of 100–350 mg/kg, the infusion was stopped. After a mean recovery time of 25 min, all control measurements, including relative perfusion on room air and with unilateral hypoxia, were repeated at least twice.

To determine if metyrapone affects pulmonary vascular tone non-specifically, prostaglandin F₂ α (PGF₂ α)¹ was infused into the inferior vena cava before and during metyrapone infusion. PGF₂ α is a potent pulmonary vasoconstrictor (25). Before the metyrapone infusion, while the

¹ *Abbreviations used in this paper:* AHPV, alveolar hypoxic pulmonary vasoconstriction; C-P450, cytochrome P-450; PA, pulmonary artery; PCW, pulmonary capillary wedge; PGF₂ α , prostaglandin F₂ α ; PVR, pulmonary vascular resistance.

animal was breathing room air, continuous infusions of $\text{PGF}_2\alpha$ in saline were begun at doses of 3–5 $\mu\text{g}/\text{min}$. The dose rate was constant for each animal. Mean pulmonary artery pressure, pulmonary capillary wedge pressure, and cardiac output were recorded before and during $\text{PGF}_2\alpha$ infusion. After a stable increase in pulmonary vascular resistance (PVR) was established, the infusion was stopped and followed by a rapid return to base-line pressures. During the metyrapone infusion, after relative perfusion measurements were completed, the same measurements were made before and during identical dose rates of $\text{PGF}_2\alpha$. In three dogs, the same protocol was applied with 3 $\mu\text{g}/\text{min}$ of angiotensin II in saline as the vasoconstrictor instead of $\text{PGF}_2\alpha$. At least two measurements of pressures and cardiac output were made for each experimental condition. Dose rates for both constrictors were chosen to duplicate the percentage of increase in calculated PVR observed in the hypoxic challenged lung.

Carbon monoxide. In another series of dogs, the same control procedure was performed as in the metyrapone experiments, except that each animal was maintained on unilateral hypoxia after the third alveolar hypoxic challenge. During a 10-sec breath-hold, 100% carbon monoxide ventilation was substituted for the 100% nitrogen ventilation to the test lung, while the other lung remained on 100% oxygen. Thus, unilateral hypoxia was maintained during the transfer to CO. Each animal breathed CO for 2.5 min before nitrogen ventilation was reinstituted to the test lung. 15 s before stopping the unilateral CO ventilation, all control measurements, including relative perfusion to the test lung, end-tidal PO_2 , and end-tidal CO concentration, were repeated. After 6 min of recovery from CO ventilation, on sustained unilateral hypoxia with nitrogen ventilation, all measurements, including relative perfusion distribution, were made at least twice. To determine if CO affects pulmonary vascular tone non-specifically, $\text{PGF}_2\alpha$ was infused in three dogs during CO administration in a manner similar to the metyrapone experiments. Hemodynamic values and relative perfusion to both lungs were determined twice, before and during $\text{PGF}_2\alpha$ infusion, while the animal was breathing room air. Without stopping the $\text{PGF}_2\alpha$ infusion, a mixture of 80% CO and 20% oxygen was then administered to the test lung, while the other lung remained on room air. Thus, each lung was constantly ventilated with 20–21% oxygen, avoiding any hypoxic challenge. After 2.5 min of unilateral CO ventilation, hemodynamic values and relative perfusion were determined for a final time. Maintenance of equal relative perfusion to both lungs in the face of bilateral pulmonary vasoconstriction from $\text{PGF}_2\alpha$ would suggest preservation of vascular responsiveness to an agonist in the CO ventilated lung. Increased relative perfusion to the CO ventilated lung would suggest nonspecific inhibition of vascular reactivity by the high alveolar CO concentration with a loss of pulmonary vasoconstriction in response to the intravenous $\text{PGF}_2\alpha$ infusion.

Statistics. All values, where appropriate, were examined by the *t* test for paired data.

RESULTS

There was no statistical difference in any measurement among the multiple room air runs in each animal before metyrapone or carbon monoxide administration. Similarly, there was no difference among the multiple alveolar hypoxia runs. Therefore, all values were averaged to give a single room air and alveolar hypoxia value for each animal before further statistical

TABLE I
Relative Perfusion to Test Lung: Metyrapone and Carbon Monoxide Experiments

		Metyrapone experiment relative perfusion	Carbon monoxide experiment relative perfusion
		%	%
Control	Air	48±2	47±2
	N ₂	33±2*	32±1
Drug	Air	47±2	
	N ₂	43±2*†	43±2*†
Recovery	Air	49±2	
	N ₂	33±2*	33±3*

Relative perfusion to test lung ±SEM is shown before, during, and after metyrapone and carbon monoxide administration while the animal was breathing room air or unilateral 100% N₂.

* Significant ($P < 0.01$) change from room air value.

† Significant ($P < 0.01$) change from control N₂ value.

analysis (Tables I, II, and IV). Airway pressures were equal in both tracheal cannulae (6.4 ± 0.3 mm Hg SEM) and did not change in either airway with hypoxia or any other experimental maneuver.

Metyrapone. In 11 dogs breathing room air, mean perfusion to the test lung was 48% (± 2 SEM) of cardiac output (Table I). After 7 min of unilateral nitrogen ventilation, perfusion to that lung was reduced to 33% (± 2 SEM) of cardiac output, a decrease of 31% ($P < 0.01$) from room air control (Fig. 1). Mean end-tidal PO_2 from the hypoxic test lung was 23 mm Hg (± 1 SEM). Pulmonary capillary wedge (PCW) pressure, cardiac output (Table II), and mean arterial blood pressure did not change with unilateral hypoxia. Mean pul-

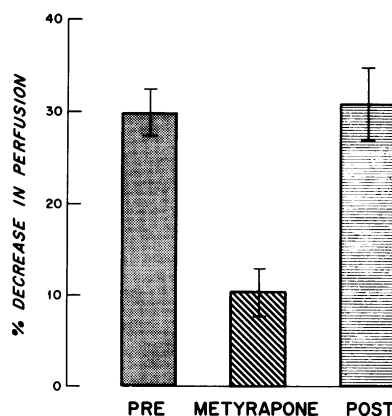


FIGURE 1 Metyrapone: changes in perfusion with alveolar hypoxia. The percentage of decrease in perfusion to the test lung with alveolar hypoxic challenge in 11 dogs ±SEM is shown before, during, and after metyrapone infusion. Metyrapone caused a significant ($P < 0.01$) and reversible inhibition of the perfusion decrease to the alveolar hypoxic test lung.

TABLE II
Metyrapone Experiments: Control and Metyrapone Data

	Mean PA pressure	PCW pressure	Cardiac output	Arterial PO ₂	Arterial PCO ₂	Arterial pH
	mm Hg	mm Hg	liter/min	mm Hg	mm Hg	
Control						
Air	19±1	11±1	1.9±0.1	81±4	38±1	7.34±0.02
N ₂	21±1*	11±1	2.0±0.1	64±4*	38±2	7.34±0.02
	n = 11	n = 11	n = 7	n = 10	n = 10	n = 10
Metyrapone						
Air	24±2	12±1	2.2±0.2	79±9	38±1	7.29±0.01
N ₂	22±2	13±1	2.3	54±3*†	38±1	7.29±0.01†
	n = 11	n = 11	n = 10	n = 10	n = 10	n = 10

Hemodynamic and arterial blood gas values±SEM are shown in anesthetized dogs before and during metyrapone infusion while the animal was breathing room air bilaterally and with unilateral alveolar hypoxic challenge breathing 100% N₂.

* Significant ($P < 0.01$) change from respective room air value.

† Significant change ($P < 0.01$) from predrug control value with hypoxic challenge.

monary artery (PA) pressure rose significantly from 19 mm Hg on room air to 21 mm Hg with alveolar hypoxia ($P < 0.01$, Table II). Thus, PVR increased from 4.1 to 4.9 U. The lack of more striking increases in PA pressure and PVR with unilateral hypoxia can be attributed to a reduction in PVR in the lung on 100% O₂. All pressures measured are influenced by the PVR in both lungs, and increased perfusion, as seen in the oxygenated lung, has been shown to decrease PVR (26), thus offsetting the rise in PVR in the hypoxic lung. Using percentage of perfusion to each lung to determine the flow to each lung, PVR increased 72% in the test lung with hypoxic challenge.

Arterial blood gases are shown in Table II. PO₂ was 81 mm Hg on room air and fell to 64 mm Hg with unilateral hypoxia.

During metyrapone infusion, relative perfusion to the test lung, while ventilated with air, was unchanged from control (Table I). Inhibition of the alveolar hypoxic response, represented by a substantially reduced ability to decrease perfusion in the nitrogen-ventilated lung, was obtained after an average of 62 min of metyrapone infusion, at a mean total dose of 180 mg/kg. Once inhibition of the hypoxic response was observed, while infusion of the metyrapone continued, a steady state of all recorded variables was achieved, verified by multiple testing during room air and unilateral nitrogen ventilation. Therefore, as with the control data, all values were averaged to give a single room air and alveolar hypoxia value for each animal before further statistical analysis (Tables I and II).

With alveolar hypoxic challenge during metyrapone infusion, perfusion to the test lung fell to 43% (±2 SEM) of cardiac output (Table I), a decrease in relative perfusion of only 10% (Fig. 1) compared to a 31% fall

before metyrapone infusion. Arterial PO₂ fell to 54 mm Hg with hypoxic challenge during metyrapone infusion, a significantly greater drop ($P < 0.01$) than with control hypoxic challenge when there was less relative perfusion to the alveolar hypoxic lung. PCO₂ was unchanged from control, but pH decreased from 7.34 to 7.29 ($P < 0.01$, Table II).

During air or unilateral nitrogen ventilation, metyrapone caused no significant changes by paired *t* testing in PA pressure, PCW pressure, or cardiac output (Table II) as compared to control, despite the fall in mean arterial PO₂ to 54 mm Hg during hypoxic challenge. Mean systemic blood pressure fell by 21 mm Hg to 124 mm Hg (±6 SEM) with metyrapone ($P < 0.01$).

At an average of 26 min (±4 SEM) after metyrapone infusion, relative perfusion to the test lung while breathing room air was unchanged from control (Table I) and alveolar hypoxic vasoconstriction was fully recovered as seen by a 31% decrease in perfusion to the test lung (Fig. 1). Arterial pH remained low at 7.30, unchanged from during the metyrapone infusion, and the blood pressure reduction noted with metyrapone was sustained through recovery of the hypoxic response.

In 10 dogs, PGF₂ α was infused before and during metyrapone infusion. Before metyrapone infusion, PA pressure increased from 18 to 25 mm Hg with PGF₂ α infusion without any significant change in PCW pressure or cardiac output (Table III). Thus, PVR increased 78% from 4.1 to 7.3 U comparable to the 72% increase with hypoxic challenge in test lung PVR. During metyrapone infusion, at the time of maximum inhibition of alveolar hypoxic vasoconstriction, PGF₂ α effected a PA pressure rise from 20 to 27 mm Hg, again without change in PCW pressure or cardiac output. Calculated PVR increased 79% from 3.9 to 7 U.

TABLE III
Hemodynamic Changes with PGF₂ α

	Mean PA pressure	PCW pressure	Cardiac output	PVR
	mm Hg	mm Hg	liter/min	mm Hg/liter/min
Premetyrapone infusion				
Control	18±1	11±1	1.9±0.2	4.1±0.7
PGF ₂ α	25±2*	11±1	2.0±0.2	7.3±1*
During metyrapone infusion				
Control	20±2	12±1	2.3±0.2	3.9±0.7
PGF ₂ α	27±2*	12±1	2.3±0.3	7.1±0.1*

Hemodynamic changes±SEM are shown in 10 dogs with infusions of PGF₂ α before and during infusion of metyrapone.

* Significant ($P < 0.01$) change from pre-PGF₂ α control. There was no significant difference in the response to PGF₂ α during metyrapone infusion as compared to premetyrapone values.

In three dogs, angiotensin II, infused before metyrapone, induced an increase in calculated PVR from 3.8 to 5.9 U, an increase of 55%, whereas the same dose of AII during metyrapone effected a 78% rise in PVR.

After recovery, fluid volume control studies were performed on three dogs to assess any effects of intravenous infusion on the hypoxic vasoconstrictor response. 400 ml of saline were infused at the same rate as the metyrapone infusion in these animals. A fluid load in excess of the maximum metyrapone volume (250 ml) caused no significant changes in any measurement, including perfusion shift with hypoxic challenge.

To exclude any influences of adrenocortical suppression by metyrapone on the hypoxic vasoconstrictor response, hydrocortisone was administered to three dogs. Each dog received 5 mg/kg of hydrocortisone before the metyrapone infusion. There was no difference in any measurement from the other metyrapone treated dogs, including degree of hypoxic vasoconstrictor response inhibition.

Carbon monoxide. In nine dogs breathing room air, perfusion to the test lung was 47% (±1 SEM) of cardiac output (Table I). After hypoxic challenge, relative perfusion was reduced to 32% (±1 SEM), a decrease of 31% ($P < 0.01$) from room air control (Fig. 2). Mean end-tidal PO₂ from the test lung was 21 mm Hg (±2 SEM). As with metyrapone controls, there were no significant changes in PCW pressure, cardiac output (Table IV), or systemic blood pressure during hypoxic challenge. PA pressure, however, did increase significantly (Table IV). Control arterial PCO₂ was 40 mm Hg (±2 SEM) and pH was 7.32 (±0.01 SEM); neither changed with hypoxic challenge.

After a reproducible decrease in perfusion with alveolar hypoxia was obtained, CO ventilation was

substituted for N₂ ventilation. After 2.5 min of unilateral hypoxia with CO ventilation, perfusion to the test lung increased to 43% (±2 SEM) of cardiac output (Table I), a decrease of only 10% from room air control (Fig. 2).

Mean PA pressure, PCW pressure, blood pressure, and cardiac output were unchanged after 2.5 min of CO breathing (Table IV). Arterial PCO₂ and pH were unchanged; arterial O₂ content fell from a control value of 21 to 5.4 vol % (±1 SEM). Mixed venous PO₂ fell from 37 to 10 mm Hg (±2 SEM). After 2.5 min of CO, arterial carboxyhemoglobin saturation was 80% (±3 SEM). In six dogs, end-tidal (alveolar) gas analysis at the same

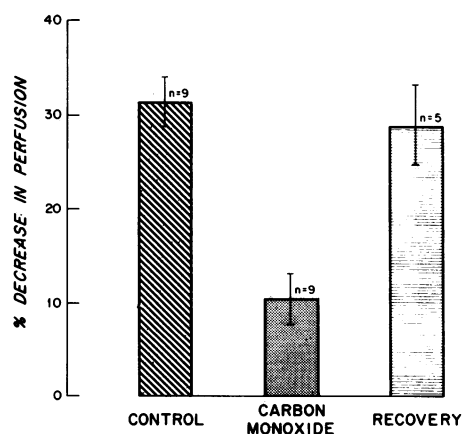


FIGURE 2 Carbon monoxide: changes in perfusion with alveolar hypoxia. The percentage of decrease in perfusion to the test lung with alveolar hypoxic challenge in nine dogs±SEM is shown before, during and after CO administration. Substitution of unilateral carbon monoxide for nitrogen ventilation as the hypoxic challenge caused a significant ($P < 0.01$) and reversible inhibition of the perfusion decrease in the alveolar hypoxic test lung.

TABLE IV
Carbon Monoxide Experiments: Control, Carbon Monoxide,
and Recovery Data

	Mean PA pressure	PCW pressure	Cardiac output	Mean systemic blood pressure
	mm Hg	mm Hg	liter/min	mm Hg
Air	20±1 n = 9	12±1 n = 9	2.5±0.2 n = 6	146±8 n = 9
Control N ₂	21±2* n = 9	12±1 n = 9	2.3±0.2 n = 8	152±7 n = 9
Carbon monoxide CO	22±2 n = 9	12±1 n = 9	2.5±0.4 n = 5	136±9 n = 9
Recovery N ₂	22±2 n = 5	10±2 n = 5	2.3±0.4 n = 5	132±5 n = 5

Hemodynamic values ± SEM are shown. Control data refers to values obtained while the animal was breathing room air or 100% nitrogen as a unilateral alveolar hypoxic challenge. CO refers to breathing 100% CO as a unilateral alveolar hypoxic challenge and recovery N₂ refers to values after reinstitution of N₂ ventilation.

* A significant ($P < 0.01$) change from room air control. All other values are unchanged under the different experimental conditions.

time from the test lung showed 80% carbon monoxide and an alveolar PO₂ of 8 mm Hg (±1 SEM).

In five dogs, N₂ ventilation was reinstituted as the alveolar hypoxic challenge to the test lung. After 6 min of recovery from CO, the decrease in perfusion with alveolar hypoxia was fully restored, dropping to 33% (±3 SEM) of cardiac output (Table I), a decrease of 29% from control (Fig. 2). Thus, restoration of alveolar hypoxic vasoconstriction occurred as the alveolar partial pressure of CO fell. All hemodynamic measurements remained without significant change (Table IV), as did arterial PCO₂ and pH. Arterial O₂ content rose slightly to 7.7 vol % (±1 SEM) and mixed venous PO₂ remained at the same reduced level of 11 mm Hg (±2 SEM). Recovery carboxyhemoglobin saturation was still markedly elevated at 70%.

In three separate dogs, PGF₂ α was infused into the inferior vena cava during unilateral test lung ventilation with 80% CO in oxygen. Relative perfusion to the test lung was 51% before PGF₂ α infusion was begun. PVR increased 70% from 3.8 to 6.45 U with PGF₂ α at a mean dose rate of 5.7 μg/min and relative perfusion distribution between the two lungs during room air ventilation was unchanged. After 2.5 min of CO in oxygen ventilation, without stopping the PGF₂ α infusion, PVR remained elevated at 6.25 U. Relative

perfusion to the test lung, with an end-tidal CO concentration of 70%, was unchanged at 49%. Thus, CO ventilation, while inhibiting hypoxic vasoconstriction did not nonspecifically block reactivity of the pulmonary vessels.

DISCUSSION

Unilateral pulmonary vasoconstriction in response to unilateral alveolar hypoxia was demonstrated in these intact dogs by a relative decrease in perfusion to the alveolar hypoxic or nitrogen ventilated lung. The pulmonary vasoconstriction elicited was primarily caused by alveolar hypoxia, because arterial oxygen tensions were reasonably well maintained by ventilation of the other lung with oxygen. During metyrapone infusion, alveolar hypoxic pulmonary vasoconstriction (AHPV) was significantly inhibited ($P < 0.01$) (Fig. 1) but was completely reversible after cessation of metyrapone infusion. Blood pressure fell significantly during metyrapone infusion as has been previously noted (27). This hypotensive effect, however, persisted through recovery of AHPV, suggesting that the hypotension per se was not responsible for AHPV inhibition. Arterial pH also fell slightly during the metyrapone infusion (Table II). This mild degree of acidosis might be expected to enhance AHPV rather than inhibit it (4). In addition, the mild acidosis seen with metyrapone infusion persisted through recovery of AHPV. Volume control studies and concomitant administration of hydrocortisone showed that neither the fluid load of the metyrapone infusion nor cortisol suppression were mechanisms responsible for inhibiting AHPV. Likewise, the metyrapone infusion seemed to inhibit AHPV without nonspecifically reducing pulmonary vascular reactivity as responsiveness to PGF₂ α and angiotension II was not affected.

Intravenous administration of 75 mg/kg of metyrapone in dogs results in a marked reduction in plasma cortisol levels within 15 min (26). Therefore, because metyrapone blocks cortisol production by inhibition of substrate binding to cytochrome P-450 (C-P450) (18, 19), and because in the present experiments 100–350 mg/kg were used, enough metyrapone was probably administered to inhibit C-P450 significantly. A slow infusion rate of metyrapone was used to avoid changes in cardiac output seen at higher infusion rates, which in itself might affect AHPV quantitatively (27).

Carbon monoxide, administered in concentrations >75%, also inhibits C-P450 (15, 20, 21). Inhalation of CO to an alveolar concentration of 80% in the hypoxic lung significantly inhibited AHPV (Fig. 2). This inhibition was accomplished without significant change in arterial pH, PCO₂ or any hemodynamic parameter measured (Table IV). 6 min after cessation of CO breathing, AHPV was fully restored (Fig. 2). Recovery

of AHPV was observed despite a persistently elevated carboxyhemoglobin level and continued evidence of severe systemic hypoxia with a mixed venous PO_2 of 11 mm Hg. The specificity of AHPV inhibition by CO was demonstrated by a preservation of $\text{PGF}_2\alpha$ -induced vasoconstriction in the test lung ventilated with 80% CO in oxygen.

Previous investigators have also sought to implicate C-P450 in pulmonary hypoxic vasoconstriction (28, 29). Duke, in 1950, noted that CO ventilation of the isolated cat lung blocked hypoxic vasoconstriction and also decreased PVR below base line, suggesting CO may have been a nonspecific vasodilator. More recently, Sylvester and McGowan (29), using isolated pig lungs, attempted to demonstrate inhibition to AHPV by CO, metyrapone, and another inhibitor of C-P450, SKF525. Their data, however, also have several significant limitations. They report initial vasodilation, using only 11% CO, and a reduction in the absolute increase in PVR induced by hypoxia or $\text{PGF}_2\alpha$ although the percentage of increase in PVR was actually greater with either hypoxia or $\text{PGF}_2\alpha$ as compared to control. Metyrapone was also reported to inhibit AHPV and $\text{PGF}_2\alpha$ response but no steady state was achieved and inhibition of both suggests a toxic level of metyrapone may have occurred in the isolated lung and had some nonspecific effect on vascular reactivity. SKF525 inhibited AHPV without a change in base-line PVR but unfortunately SKF525 also affects calcium transport into smooth muscle, which is known to block AHPV (30).

The present series in the intact dog avoids the problems seen in the isolated lung. No change in base-line PVR occurred during the metyrapone or carbon monoxide inhibition of AHPV and preservation of pulmonary vasoreactivity was verified with angiotensin II and $\text{PGF}_2\alpha$ infusions. Thus, metyrapone and CO seemed to specifically and reversibly inhibit AHPV. We have no direct evidence linking this inhibition with these agents' known blocking effects on C-P450, but the combination of observed effects makes the argument more compelling and inhibition of C-P450 is the only known pharmacological property shared by metyrapone and CO. High concentrations of CO, $\geq 95\%$, inhibit other cytochromes as well (20, 21), but our alveolar levels were only 80%. Carbon monoxide, however, in addition to inhibiting C-P450, may have blocked vasoreactivity by another mechanism.

Knowledge about C-P450 in the lung is limited despite extensive data accumulated on liver and adrenal C-P450 function. C-P450 has been found in lung microsomes and is involved in oxygen-dependent hydroxylation of numerous substrates (15–17). Substrate metabolism involving lung C-P450 occurs within the same time frame as the pulmonary vasoconstrictor response to hypoxia (15). Oxidative demethylation of

p-nitroanisole, for example, a C-P450-linked, mixed-function oxidation, occurs within 2–3 min upon introduction of the substrate into an isolated lung preparation and is inhibited equally rapidly by 75% CO administration (15). Thus, the kinetics of C-P450 activity are consistent with our observed timing of AHPV induction and inhibition. Any theories, however, on how oxygen-dependent substrate hydroxylation by lung C-P450 is translated into vasoconstriction in response to alveolar hypoxia are speculation.

C-P450 is an oxygen-dependent cytochrome (17), and therefore could be the direct sensor of oxygen in the lung. A prerequisite for C-P450 to be involved in oxygen sensing in the lung is a K_m for oxygen allowing redox-state changes in the range of alveolar gas tensions. The oxygen tension that induces vasoconstriction in the present model is not specifically known but lies between that of pulmonary arterial blood (PO_2 34 mm Hg) and that of the nitrogen-ventilated alveolus where diffusion from the blood produces a mean PO_2 of 23 mm Hg. The K_m for most hepatic cytochromes, including C-P450, is near a PO_2 of 1 mm Hg (31). Thus, if lung C-P450 has the same oxygen K_m , it would always be fully oxidized. Other investigations, however, suggest that the oxygen K_m for cytochromes may vary from organ-to-organ (13) and substrate-to-substrate (32). The K_m for cytochrome *c* oxidase in intact rabbit brain, for example, allows redox-state changes between oxygen tensions of 95–100 mm Hg (33). The rate of *N*-ethylaniline hydroxylation by hepatic microsomal fractions drops over a PO_2 range of 140–1 mm Hg, whereas maximal reaction rates are preserved for aniline hydroxylation until PO_2 falls below 4 mm Hg (32). Thus, although the oxygen K_m for lung C-P450 is unknown, it may be distinctly higher than for most hepatic microsomal functions.

Even if we assume an appropriately high K_m for oxygen, another problem with C-P450 as the oxygen sensor initiating AHPV is the paradox of partially reduced C-P450 producing vasoconstriction during alveolar hypoxia, but metyrapone or CO inhibition of C-P450 resulting in the absence of vasoconstriction. Resolution of this paradox must assume that partial reduction of C-P450 by hypoxia may affect only some hydroxylating functions, initiating an increase in vascular tone via a new vasoconstricting metabolite, whereas blockade of substrate binding by metyrapone or complete reduction of C-P450 by CO would then inhibit all hydroxylating function and initiate no change in base-line vascular tone.

Apart from these conjectures, C-P450 is more likely to be involved in the transduction process of AHPV than as an oxygen sensor. Lung C-P450 probably has a low K_m for oxygen and is fully oxidized even with marked alveolar hypoxia. Hypoxia might elicit a yet unknown mediator whose

vasoactive properties are dependent on C-P450 hydroxylation. Inhibition of C-P450 by metyrapone or CO then blocks the formation or activation of the vasoconstrictor metabolite. Thus, although these data do not definitively identify an O₂ sensor or mediator of AHPV, a likely role for C-P450 is suggested. Further research may focus on mediators that require C-P450-controlled hydroxylation.

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