CRITICAL CARE MEDICINE

Vol. 21, No. 5
Printed in U.S.A.

Action of carbon dioxide on hypoxic pulmonary vasoconstriction in the rat lung: Evidence against specific endothelium-derived relaxing factor-mediated vasodilation

SIMON V. BAUDOUIN, BA, MRCP, MD; TIMOTHY W. EVANS, MRCP, MD, PHD

Objectives: **The effect of hypercapnia on pulmonary vascular tone is controversial with evidence for both a vasoconstrictor and vasodilator action. The objective of this study was to investigate the possibility that this dual response to CO_2 could be explained by a direct constrictor action on smooth muscle and an indirect dilator action via the release of endothelium-derived relaxing factor. The effect of ventilation with hypercapnia ($FICO_2$ 0.15) on pulmonary pressor response to hypoxia (FIO_2 0.3) was investigated.**

Design: **Prospective, randomized study.**

Setting: **The National Heart and Lung Institute, UK.**

Subjects: **The isolated, blood-perfused rat lung.**

Interventions: **Angiotensin-II and a blocker of endothelium-derived relaxing factor synthesis, N^G-monomethyl-L-arginine (L-NMMA).**

Measurements and Main Results: **The vasomotor effect of hypercapnia depended on pulmonary arterial pressure. Under resting tone, CO_2 acted as a mild constrictor (change in mean pulmonary arterial pressure from 14 ± 2 to 15 ± 2 mm Hg, n = 4; $p < .05$. At increased tone, induced either by hypoxia or Angiotensin-II, CO_2 was a vasodilator. Thus, hypoxia increased mean pulmonary arterial pressure from 17 ± 2 to 32 ± 2 mm Hg (n = 8; $p < .01$), but simultaneous ventilation with hypoxia and hypercapnia reduced this by 16 ± 1% ($p < .01$). Angiotensin-II (1 μg) increased pulmonary arterial pressure from 14 ± 2 to 39 ± 5 mm Hg (n = 8; $p < .01$), but with hypercapnia, this angiotensin-induced pulmonary vasoconstriction was reduced by 18 ± 6% ($p < .001$). The reduction in hypoxic pulmonary vasoconstriction induced by hypercapnia was not significantly different from that seen with Angiotensin-II hypercapnia. Blocking endothelium-derived relaxing factor synthesis using 30 μM N^G-monomethyl-L-arginine did not significantly change either basal pulmonary arterial pressure or the response to hypercapnia, but increased hypoxic pulmonary vasoconstrictor by 24 ± 4% (n = 4; $p < .01$). There was no significant difference between the change in hypoxic pulmonary vasoconstriction induced by hypercapnia after saline control (21 ± 8% decrease) and the change in hypoxic pulmonary vasoconstriction caused by CO_2 after 30 μM L-NMMA (25 ± 10% decrease, $p < .05$, n = 8).**

Conclusion: **Endothelium-derived relaxing factor seems unlikely to specifically modulate CO_2-induced vasodilation in the rat pulmonary circulation. (Crit Care Med 1993; 21:740–746)**

KEY WORDS: **hypoxia; hypercapnia; endothelium-derived relaxing factor, N^G-monomethyl-L-arginine, vascular endothelium; hypoxic pulmonary vasoconstriction; vasoconstriction; vasodilation; angiotensin-II; pulmonary circulation**

Traditional intensive care practice dictates that a normal $PaCO_2$ should be maintained during mechanical ventilation. However, recent reports (1–3) suggested that to avoid barotrauma, particularly in patients with the adult respiratory distress syndrome (ARDS) who require a large tidal volume yet have poor pulmonary compliance, a degree of hypercapnia is permissible. Implicit in this unconventional approach is the assumption that any adverse effects of hypercapnia on the pulmonary circulation are less harmful to gas exchange than those effects of barotrauma. However, hypercapnia has a significant effect on the pulmonary vasculature. Studies (4–12) in man and animals reported both pulmonary constrictor and dilator actions of CO_2 and hypercapnia-induced changes in

From the Department of Anaesthesia and Intensive Care, The National Heart and Lung Institute, London, UK.

Address requests for reprints to: Simon V. Baudouin, MD, Royal Brompton National Heart and Lung Hospital, Sydney Street, London SW3 6LY, UK.

0090-3493/93/2105-0740$03.00/0

pulmonary vascular resistance are likely to have significant effects on pulmonary gas exchange, particularly after lung injury (13).

The vascular endothelium is an important regulator of vascular tone and vasoresponsiveness (14–16). The dual actions of CO_2 on the pulmonary vascular bed could be explained in terms of a direct action on smooth muscle and/or an indirect action via endothelial mediators. Evidence (17) exists in the systemic circulation to support such a hypothesis. Endothelium-derived relaxing factor, an important mediator of pulmonary and systemic vascular tone, is released in response to a variety of stimuli (14–16) and has been shown previously by our group to modulate hypoxic pulmonary vasoconstriction (18). Hypercapnia has been reported (19–21) to reduce hypoxic pulmonary vasoconstriction, an action that some workers have viewed as evidence for a specific antagonism by free hydrogen ion (H^+) of the mechanisms responsible for hypoxic pulmonary vasoconstriction. An alternative explanation for the reduction in hypoxic pulmonary vasoconstriction is that CO_2 induces the release of endothelium-derived relaxing factor.

In view of recent support for a policy of "permissive" hypercapnia during mechanical ventilation, particularly in patients with lung injury and ARDS and in whom hypoxic pulmonary vasoconstriction may be abolished (13, 19, 21), we investigated the effect of increased P_{CO_2} on the rat pulmonary circulation and tested the hypotheses that CO_2 reduces hypoxic pulmonary vasoconstriction through the release of endothelium-derived relaxing factor using the specific blocker of endothelium-derived relaxing factor synthesis N^G-monomethyl-L-arginine. We investigated whether any reduction in hypoxic pulmonary vasoconstriction caused by hypercapnia is nonspecific by comparing the effects of CO_2 on hypoxic pulmonary vasoconstriction with those effects on the pressor response to the smooth muscle vasoconstrictor angiotensin-II.

MATERIALS AND METHODS

Tissue Preparation. The study protocol was approved by the Home Office (UK) licensing authority, and the care and handling of animals were in accord with Home Office (UK) guidelines. The isolated, blood-perfused, and ventilated in situ preparation of rat lungs originally described by Hauge (22) and modified by Emery et al. (23) was used. Adult, male Wistar rats (weighing 300 to 350 g) were anesthetized with diazepam (0.6 mg/kg, intraperitoneally) and hypnorm (fentanyl 0.315 mg/mL and fluanisone 10 mg/mL) 0.15 mL intramuscularly. Before each experiment, one rat was exsanguinated, and the blood was placed in a reservoir that was maintained at 40°C by a surrounding water bath and used to fill a perfusion circuit. This method consisted (in order) of a left atrial cannula, reservoir, roller pump (MHRE 200; Watson Marlow 2, Falmouth, UK); connecting tubing, bubble trap, side arm pressure transducer (Bio Medical Systems, Strathclyde, UK); and a pulmonary artery cannula. The pressure transducer was connected to a recorder (Multitrace 2; Ormed, Welwyn Garden City, Herts, UK) to permit constant recordings of changes in pulmonary arterial pressure.

After anesthesia and cannulation of the trachea, the experimental animals were ventilated with normoxic gas (21% oxygen, 5% CO_2) at a tidal volume of 4 mL and a frequency of 16 breaths/min. Animals were heparinized and exsanguinated via the abdominal aorta, and the lungs were exposed through a median sternotomy. A purse-string suture was placed around the left atrium to secure a catheter inserted through an atriotomy, which drained freely into the reservoir, thus ensuring that the left atrial pressure was maintained at zero. A second cannula was inserted into the main pulmonary artery by a right ventriculotomy and tied in place. The lungs were perfused with whole blood at a constant flow rate of 18 mL/min. The changes in perfusion pressure were recorded. Because the flow rate was constant, changes in perfusion pressure represented changes in pulmonary vascular resistance. Blood gas tensions were measured at the beginning of each experiment and after changes in ventilatory gas mixtures, by collecting blood anaerobically from the left atrial cannula and analyzing it immediately (178 pH/blood gas analyzer, Corning, Essex, UK). Blood base excess was maintained between 0 and –2.0 mmol/L by adding small volumes of sodium bicarbonate ($NaHCO_3^-$) (Braun Medical, Aylesbury, UK) to the reservoir. After institution of the perfusion circuit, a 15- to 20-min equilibrium period was allowed to establish a stable pulmonary arterial pressure before starting any experiment.

Experimental Protocols: Protocol 1—Effect of Carbon Dioxide on Pulmonary Vascular Resistance and Hypoxic Pulmonary Vasoconstriction (n = 8). After normoxic ventilation, eight separate preparations were exposed to hypoxic challenges (3% oxygen/5% CO_2) for 5 to 10 mins until two consistent changes in pulmonary arterial pressure (±10%) were obtained. The lungs were then exposed to normoxia/hypercapnia (21% oxygen/15% CO_2) for two separate periods of 10 mins, followed by repeated challenges with hypoxia/hypercapnia (3% oxygen/15% CO_2) until two consistent changes in pulmonary arterial pressure were recorded.

Protocol 2—Effect of Carbon Dioxide on the Angiotensin-II Pressor Response (n = 8). Preparations were ventilated with a normoxic gas mixture, and angiotensin-II (1 μg in 40 μL 0.9% sodium chloride [NaCl]) was

added to the circuit reservoir, producing a circulating concentration of 40 nM. This process was repeated until two reproducible angiotensin-II induced increases in pulmonary arterial pressure were obtained. The lungs were then ventilated for 10 mins with a normoxic/hypercapnic gas mixture before angiotensin-II was added to the circuit. After a return of tension to baseline, this procedure was repeated until two consistent changes in pulmonary arterial pressure were recorded. Finally, the lungs were ventilated with the normoxic gas mixture, and after 10 mins, a further challenge with angiotensin-II was made.

Protocol 3—N^G-Monomethyl-L-Arginine and Hypoxic Pulmonary Vasoconstriction (n = 4). Separate hypoxic challenges were performed until two consistent changes in pulmonary arterial pressure were obtained. A total of 30 μM of N^G-monomethyl-L-arginine was then added to the reservoir (18), and after 10 mins, further hypoxic challenges were performed until two consistent changes in pulmonary arterial pressure were recorded.

Protocol 4—Hypercapnia and N^G-Monomethyl-L-Arginine (n = 4). Preparations were challenged with normoxia/hypercapnia for two separate 10-min periods, and pulmonary arterial pressure was recorded. The lungs were ventilated normoxically between exposures. After 10 mins of normoxic ventilation, 30 μM N^G-monomethyl-L-arginine was added to the reservoir, and after a further 10 mins, two further hypercapnic challenges were performed.

Protocol 5—Hypoxia With Hypercapnia and N^G-Monomethyl-L-Arginine (n = 8). The preparations were first challenged with hypoxia and then hypoxia/hypercapnia until two consistent changes in pulmonary arterial pressure were obtained. A total of 30 μM of N^G-monomethyl-L-arginine was then added to the reservoir, and after 10 mins, further hypoxic/hypercapnic challenges were performed until two consistent recordings were obtained.

Drugs. Angiotensin II and N^G-monomethyl-L-arginine were obtained from (Sigma, Poole, Dorset, UK). All drugs were dissolved in saline or distilled water and diluted with saline.

Calculations and Statistical Analysis. Changes in pulmonary arterial pressure, resulting from hypoxia, hypercapnia, or angiotensin-II, were expressed as percentage change in baseline pressures. The vasodilator response to hypercapnia was calculated as the pulmonary arterial pressure at maximum constriction (induced by either hypoxia or angiotensin-II) during normocapnic ventilation, minus the pulmonary arterial pressure at maximum constriction during hypercapnic ventilation, divided by pulmonary arterial pressure at maximum constriction during normocapnia, expressed as a percentage. Single comparisons of changes in pulmonary arterial pressure in the same animal were made by Student's paired *t*-test, and multiple comparisons were made by analysis of variance; $p < .05$ was considered significant. Results are expressed as mean ± SEM.

RESULTS

Effect of Carbon Dioxide on Pulmonary Vascular Resistance and Hypoxic Pulmonary Vasoconstriction. During ventilation with normoxia, mean $Paco_2$ was 36 ± 7 torr (4.8 ± 0.9 kPa) and pH was 7.40 ± 0.05 (n = 32). During ventilation with hypoxia, $Paco_2$ was 33 ± 7 torr (4.4 ± 0.9 kPa) and pH was 7.37 ± 0.07 (n = 8) (Fig. 1*A*). Ventilation with normoxia/hypercapnia produced a $Paco_2$ of 60 ± 12 torr (8 ± 1.6 kPa) and a pH of 7.24 ± 0.06 (n = 8). Hypoxic/hypercapnic ventilation produced a $Paco_2$ of 59 ± 11 torr (7.9 ± 1.5 kPa) and a pH of 7.20 ± 0.06 (n = 8). Baseline pulmonary arterial pressure was 17 ± 2 mm Hg (n = 8). Hypoxia increased pulmonary arterial pressure to 32 ± 2 mm Hg ($p < .01$). The maximal response was generally observed within 6 mins of the initiation of hypoxic ventilation. Hypercapnia alone did not significantly alter pulmonary arterial pressure. The addition of hypercapnia to the hypoxic challenge significantly reduced the pulmonary pressor response to 27 ± 2 mm Hg (16% reduction in hypoxic pulmonary vasoconstriction; $p < .01$).

Effect of Carbon Dioxide on the Angiotensin-II Pressor response. $Paco_2$ was 66 ± 12 torr (8.8 ± 1.6 kPa) before and 63 ± 16 torr (8.4 ± 2.1 kPa) (n = 8) after the addition of angiotensin-II (Fig. 1*B*); pH was 7.19 ± 0.06 before and 7.20 ± 0.06 (n = 8) after angiotensin-II. Angiotensin-II increased mean pulmonary arterial pressure from 14 ± 2 to 39 ± 5 mm Hg ($p < .01$; n = 8). Ventilation with normoxia/hypercapnia significantly reduced the constrictor response to 32 ± 10 mm Hg (18% reduction; $p < .001$). Returning to normoxic ventilation restored the angiotensin-II constrictor response to control values (pulmonary arterial pressure 36 ± 2 mm Hg).

N^G-L-Monomethyl-Arginine and Hypoxic Pulmonary Vasoconstriction. Normocapnic ventilation produced a $Paco_2$ of 34 ± 2 torr (4.5 ± 0.27 kPa) and a pH of 7.42 ± 0.03 before N^G-monomethyl-L-arginine and a $Paco_2$ of 33 ± 7 torr (4.4 ± 0.93 kPa) and a pH of 7.39 ± 0.03 (n = 4) after the addition of N^G-monomethyl-L-arginine (Fig. 2). Baseline pulmonary arterial pressure was 15 ± 1 mm Hg (n = 4). Hypoxia significantly increased pulmonary arterial pressure to 32 ± 4 mm Hg ($p < .01$). The addition of saline vehicle had no effect on the hypoxia-induced increase in pulmonary arterial pressure. The addition of N^G-monomethyl-L-arginine did

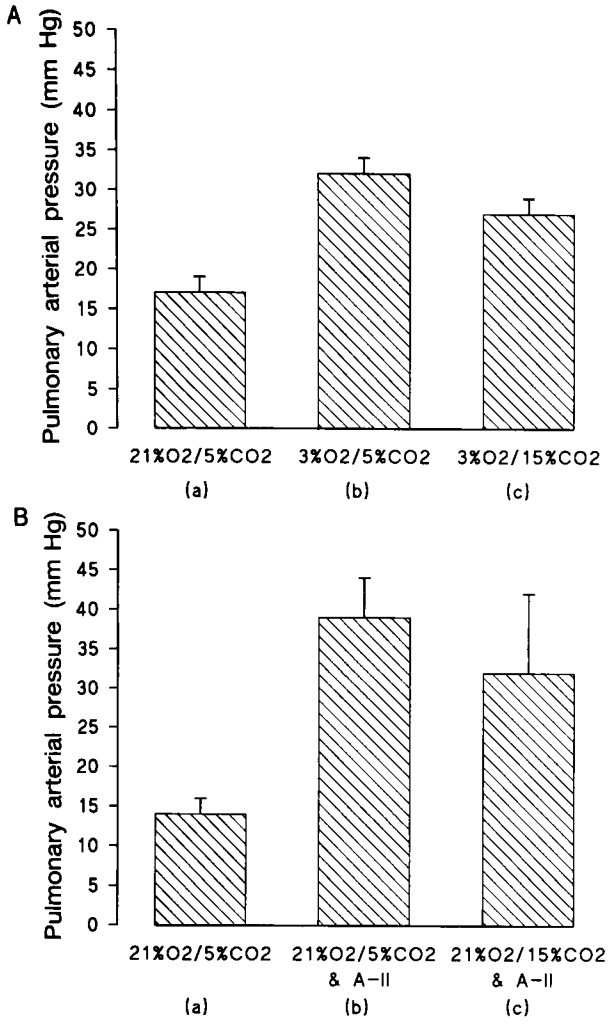

Figure 1. *A*) Pulmonary arterial pressures (mean ± SEM, mm Hg) during *(a)* normoxic (F_{IO_2} 21%, F_{ICO_2} 5%), *(b)* hypoxic (F_{IO_2} 3%, F_{ICO_2} 5%), and *(c)* hypoxic/hypercapnic (F_{IO_2} 3%, F_{ICO_2} 15%) challenges (n = 8). The pulmonary pressor response to hypoxia alone was significantly (*c* vs. *b*; $p < .01$) greater than the response to hypoxia with hypercapnia. *B*) Pulmonary arterial pressures (mean ± SEM, mm Hg) after angiotensin-II administration during ventilation with *(a)* normoxic (F_{IO_2} 21%, F_{ICO_2} 5%), *(b)* hypoxic (F_{IO_2} 3%, F_{ICO_2} 5%), and *(c)* hypoxic/hypercapnic (F_{IO_2} 3%, F_{ICO_2} 15%) gas mixtures. The constrictor response to angiotensin-II was significantly (*c* vs. *b*; $p < .001$; n = 8) less during hypercapnic ventilation.

not significantly alter baseline pulmonary arterial pressure, but significantly augmented hypoxic pulmonary vasoconstriction to 40 ± 4 mm Hg (24% increase compared with control hypoxic pulmonary vasoconstriction; $p < .01$ vs. hypoxia with saline control).

Hypercapnia and N^G-Monomethyl-L-Arginine. Hypercapnia produced a P_{ACO_2} of 57 ± 14 torr (7.6 ± 1.9 kPa) and a pH of 7.25 ± 0.13 with saline vehicle and a P_{ACO_2} of 58 ± 14 torr (7.7 ± 1.9 kPa) and a pH of 7.23 ± 0.05 with N^G-monomethyl-L-arginine (n = 8). Baseline

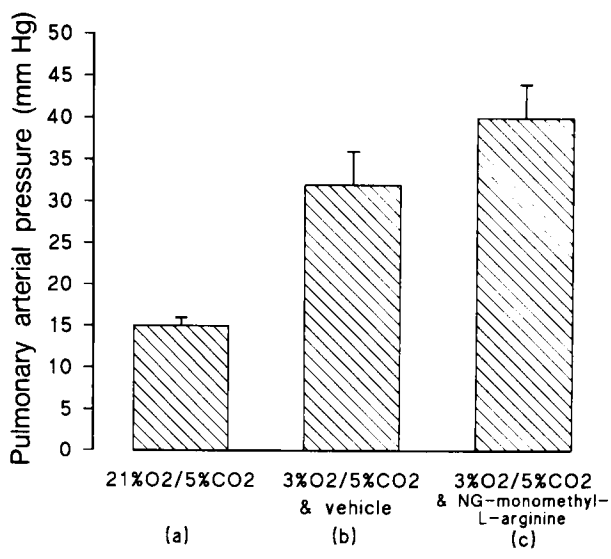

Figure 2. Pulmonary arterial pressures (mean ± SEM, mm Hg) during *(a)* normoxic (F_{IO_2} 21%, F_{ICO_2} 5%) or during hypoxic (F_{IO_2} 3%, F_{ICO_2} 5%) challenges after the addition of *(b)* saline vehicle or *(c)* 30 μM N^G-monomethyl-L-arginine. N^G-monomethyl-L-arginine significantly (*c* vs. *b*; $p < .01$; n = 4) augmented hypoxic pulmonary vasoconstriction.

pulmonary arterial pressure was 14 ± 2 mm Hg (n = 4). Hypercapnia produced a small, but significant, increase in pulmonary arterial pressure to 15 ± 2 mm Hg ($p < .05$). Adding the saline vehicle did not produce a sustained increase in pulmonary arterial pressure. After the addition of N^G-monomethyl-L-arginine, pulmonary arterial pressure did not change significantly (mean pressure 15 ± 2 mm Hg).

Hypoxia With Hypercapnia and N^G-Monomethyl-L-Arginine. Ventilation with normoxia/hypercapnia produced a P_{ACO_2} of 32 ± 1 torr (4.3 ± 0.13 kPa) and a pH of 7.40 ± 0.08 (n = 8) (Fig. 3). Hypoxic ventilation produced a P_{ACO_2} of 29 ± 4 torr (3.9 ± 0.53 kPa) and a pH of 7.35 ± 0.11 (n = 8). Hypercapnic ventilation produced a P_{ACO_2} of 62 ± 4 torr (8.3 ± 0.53 kPa) and a pH of 7.16 ± 0.06 (n = 8). Hypoxic/hypercapnic ventilation produced a P_{ACO_2} of 59 ± 13 torr (7.9 ± 1.7 kPa) and a pH of 7.23 ± 0.03 (n = 8). Baseline pulmonary arterial pressure on air was 17 ± 6 mm Hg (n = 8). Hypoxic challenges increased mean pulmonary arterial pressure to 33 ± 2 mm Hg ($p < .01$). During the hypoxic/hypercapnic challenge, mean pulmonary arterial pressure increased to 26 ± 2 mm Hg ($p < .05$, compared with hypoxia alone). There was no further sustained increase with saline control. The addition of N^G-monomethyl-L-arginine did not significantly increase basal pulmonary arterial pressure, but during challenge with hypoxia/hypercapnia, pulmonary arterial pressure increased significantly to 30 ± 2 mm Hg ($p < .01$ compared with hypoxia/hypercapnia and saline control).

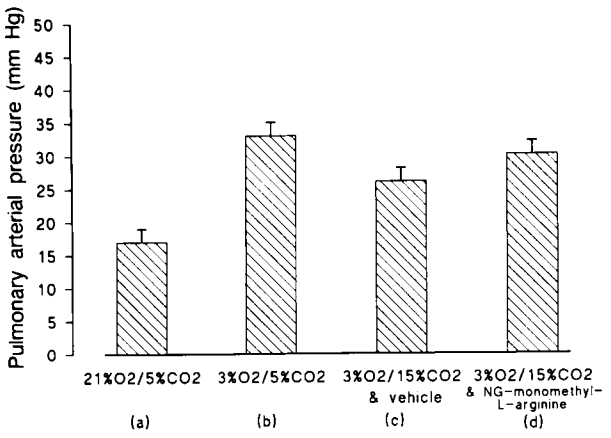

Figure 3. Pulmonary arterial pressures (mean ± SEM, mm Hg) during ventilation with *(a)* normoxia (F_{IO_2} 21%, F_{ICO_2} 5%), *(b)* hypoxia (F_{IO_2} 3%, F_{ICO_2} 5%), hypoxia/hypercapnia (F_{IO_2} 3%, F_{ICO_2} 15%) and with the addition to the perfusate of either saline vehicle *(c)* or 30 μM N^G-monomethyl-L-arginine *(d)*. N^G-monomethyl-L-arginine significantly (*c* vs. *d*; $p < .01$; n = 8) reduced the pulmonary dilator response to hypercapnia.

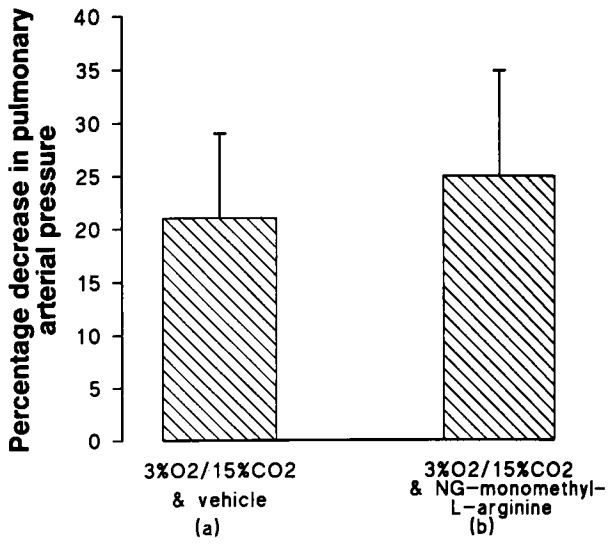

Figure 4. The percentage decrease in pulmonary arterial pressures from maximum constriction with hypoxia during ventilation with hypercapnia and vehicle *(a)* and hypercapnia and N^G-monomethyl-L-arginine *(b)*. There was no significant difference between the reductions in hypoxic pulmonary vasoconstriction.

The reduction in hypoxic pulmonary vasoconstriction induced by hypercapnia after saline control (protocol 5) was compared with the reduction in hypoxic pulmonary vasoconstriction with hypercapnia after N^G-monomethyl-L-arginine (protocols 3 and 5). There was no significant difference between the change in hypoxic pulmonary vasoconstriction with hypercapnia after saline control (21 ± 8% decrease) and the change in hypoxic pulmonary vasoconstriction with hypercapnia after N^G-monomethyl-L-arginine (25 ± 10% decrease; $p > .05$; Fig. 4).

DISCUSSION

In this study, we showed that the vasomotor action of CO_2 on the isolated rat pulmonary circulation depends on basal pulmonary artery tone. At low pulmonary arterial pressure, CO_2 is a mild pulmonary vasoconstrictor; at higher tone, it acts as a dilator. Also, we found that this dilator action appears to be independent of the constrictor stimulus, as the reduction in pulmonary arterial pressure was very similar whether the pressor agent employed was hypoxia or angiotensin-II. This finding suggests that CO_2 is not a specific antagonist of hypoxic pulmonary vasoconstriction as has been hypothesized previously. We found no evidence that endothelium-derived relaxing factor mediates the dilator action of CO_2, as N^G-monomethyl-L-arginine did not alter the mild constrictor response to hypercapnia at low basal tone or reduce the dilator response at higher tone.

The effect of CO_2 on the pulmonary circulation and its interaction with hypoxic pulmonary vasoconstriction remain controversial (4–12, 19, 21). Some reports (5, 6, 8, 9) stressed the pulmonary vasoconstrictor action of hypercapnia, while other workers (4, 6, 8) found hypercapnia to be a pulmonary vasodilator with an action similar to that reported in the systemic circulation, and hypothesized that CO_2 specifically antagonizes hypoxic pulmonary vasoconstriction (8, 9, 19–21). The action of CO_2 in this respect assumes considerable clinical significance. The loss of hypoxic pulmonary vasoconstriction in patients with ARDS probably contributes to ventilation/perfusion mismatching and consequent refractory hypoxemia. Additional changes in pulmonary vascular tone, induced by hypercapnia, could further exacerbate this mismatch and worsen gas exchange.

We found that the vasoactive action of CO_2 on the isolated rat lung is dependent on the basal pulmonary arterial pressure; at low pulmonary arterial pressure, CO_2 is a mild vasoconstrictor, while at high pulmonary vascular resistance, it is a potent vasodilator. In this respect, our observations agree with those observations of Barer and Shaw (6) who also found a dual response of the isolated rat lung to CO_2, depending on the initial pulmonary vascular resistance. In other animal species, CO_2 exerts a stronger constrictor action on the pulmonary circulation (5–9), although a dilator action can be identified if changes in hydrogen ion concentration are prevented from occurring. On the basis of these

studies, it has been proposed that dilation is due to a direct action of CO_2 on smooth muscle while constriction is caused by the intracellular acidosis produced by the dissociation of CO_2 to free hydrogen ions (6–8). The action of hypercapnia on the human pulmonary circulation is less clear. The evidence for a constrictor action has been reviewed (10). Many studies are difficult to interpret, as changes in pulmonary arterial pressures rather than changes in pulmonary vascular resistance were reported and the well-documented alterations in cardiac output that occur during hypercapnia were either not recorded or were measured using indirect techniques. Most of the studies were performed on patients with either chronic chest problems or mitral valve disease and may not be relevant to patients with acute lung injury.

The endothelium plays an important modulatory role in the vasomotor control of both systemic and pulmonary vessels (14–16). This control is typified by the action of acetylcholine on the pulmonary circulation in causing dilation in preparation with an intact endothelium and constriction in those vessels denuded of endothelium. The constriction is caused by a direct action of acetylcholine on the smooth muscle and the dilation by the release of endothelium-derived relaxing factor (now thought to be nitric oxide). Other drugs and nonspecific stimuli, such as an increase in intraluminal pressure, release endothelium-derived relaxing factor, which relaxes vascular smooth muscle by increasing intracellular guanosine 3′, 5′-cyclic monophosphate levels (cyclic GMP). We hypothesized that CO_2 could directly a) constrict vascular smooth muscle in a manner similar to acetylcholine, possibly by increasing intracellular hydrogen ion concentration; and b) simultaneously relax the muscle by releasing endothelium-derived relaxing factor from the endothelium.

We found that N^G-monomethyl-L-arginine, a specific blocker of endothelium-derived relaxing factor synthesis, did not alter the response of the rat pulmonary circulation to CO_2 at low resting tone. If CO_2 was simultaneously releasing endothelium-derived relaxing factor and directly constricting the pulmonary circulation, then the prevention of endothelium-derived relaxing factor release by N^G-monomethyl-L-arginine should have unmasked a pure constrictor effect of hypercapnia. This situation was not observed and therefore failed to support the hypotheses that hypercapnia dilated by endothelium-derived relaxing factor release.

If endothelium-derived relaxing factor mediated the hypercapnia-induced decrease in hypoxic pulmonary vasoconstriction, then N^G-monomethyl-L-arginine would be expected to alter this response. However, as previously reported by Liu and co-workers (18), N^G-monomethyl-L-arginine augments hypoxic pulmonary vasoconstriction. The degree of augmentation of hypoxic pulmonary vasoconstriction was very similar in the present study to the degree reported by Liu et al. (18) at the same concentration of N^G-monomethyl-L-arginine. This effect increased the pulmonary pressor response to hypoxia with hypercapnia after N^G-monomethyl-L-arginine as shown in protocol 5, but there was no significant difference between the effect of CO_2 on hypoxia before and after N^G-L-monomethyl-L-arginine (21% vs. 25% decrease in pressure). At present, it is not possible to measure endothelium-derived relaxing factor release directly, and these experiments employed an agent that blocks its synthesis and release. Although this tact is an inherent limitation in these studies, our results suggest that endothelium-derived relaxing factor is unlikely to be important in the response of the hypoxia-constricted pulmonary circulation to hypercapnia.

In the systemic circulation, CO_2 vasodilates the vascular bed (15, 17, 24), and exposure to 12% CO_2 has been shown to reduce tension in the rat aorta *in vitro* and cause a rightward shift of the noradrenaline dose-response curve (17). Removal of the endothelium did not modify this response but, unlike in the intact preparation, the maximal contractile response was not attenuated. Smooth muscle cGMP levels, which increase in the presence of endothelium-derived relaxing factor, did not change in response to CO_2 and the response was not attenuated by hemoglobin, which binds endothelium-derived relaxing factor, again suggesting that endothelium-derived relaxing factor has no role in CO_2-induced vasodilation. The results of the current study support the view that in the rat pulmonary circulation, CO_2 has an action identical to that in the systemic circulation.

The cellular mechanisms underlying hypoxic pulmonary vasoconstriction remain poorly understood (7, 19–21). One candidate for the sensor/transcription process involved in hypoxic pulmonary vasoconstriction is a decrease in the intracellular hydrogen ion concentration. CO_2 rapidly crosses cell membranes and dissociates to free hydrogen ions. If a decrease in hydrogen ion concentration is the signal responsible for hypoxic pulmonary vasoconstriction, then hypercapnia would be expected to specifically interfere with hypoxic pulmonary vasoconstriction (4). If CO_2 produces a nonspecific antagonism of hypoxic pulmonary vasoconstriction, its action should not be quantitatively different from that of any other constrictor substance. Angiotensin-II constricts vascular smooth muscle both by increasing the influx of ionized calcium from the blood directly and by triggering calcium release from intercellular storage sites (25). A dose of

1 μg angiotensin-II produces a relatively short-lived constriction in the rat pulmonary circulation of the same magnitude as that produced by exposure to 3% oxygen. In this study, ventilation with 15% CO_2 reduced pulmonary arterial pressure by a similar amount independent of whether the pressor agent was hypoxia or angiotensin-II (16% vs. 18%). If CO_2 had specifically altered hypoxic pulmonary vasoconstriction, a significant difference between the constrictor responses to hypoxia and angiotensin-II should have emerged. These results are consistent with an action of CO_2 at, or close to, the tension-generating site in smooth muscle common to both hypoxia and angiotensin-II. This action is likely to involve either changes in intracellular calcium concentrations or direct interactions with contractile proteins (26, 27).

It was proposed (1–3) recently that to avoid barotrauma in mechanically ventilated patients with ARDS, attempts to achieve normocapnia should be avoided. It is assumed that hypercapnia is well tolerated in this group of patients. The present study highlights the fact that CO_2 has important vasomotor effects on the mammalian pulmonary circulation and emphasizes the important interaction of hypercapnia with hypoxic pulmonary vasoconstriction. The loss of hypoxic pulmonary vasoconstriction (7, 13, 19, 21) may contribute to the refractory hypoxia that characterizes ARDS (21, 28). Our study suggests that hypercapnia may reduce hypoxic pulmonary vasoconstriction, and that the vasomotor effects of CO_2 should be further investigated before so-called permissive hypercapnia becomes a widely adopted ventilatory technique.

REFERENCES

1. Hickling KG: Ventilatory management of ARDS: Can it affect the outcome? *Intensive Care Med* 1990; 16:219–226
2. Hickling KG, Henderson SJ, Jackson R: Low mortality associated with low volume pressure limited ventilation with permissive hypercapnia in severe adult respiratory distress syndrome. *Intensive Care Med* 1990; 16:372–374
3. Pesenti A: Target blood gases during ARDS ventilatory management. *Intensive Care Med* 1990; 16:349–351
4. Marshall C, Lindgren L, Marshall BE: Metabolic and respiratory hydrogen ion effects on hypoxic pulmonary vasoconstriction. *J Appl Physiol* 1984; 57:545–550
5. Barer GR, Howard P, Shaw JW: Stimulus-response curves for the pulmonary vascular bed to hypoxia and hypercapnia. *J Physiol* 1970; 211:139–155
6. Barer GR, Shaw JW: Pulmonary vasodilator and vasoconstrictor actions of carbon dioxide. *J Physiol* 1971; 213:633–645
7. Barer GR: The physiology of the pulmonary circulation and methods of study. *Pharmcology and Therapeutics, Part B* 1976; 2:247–273
8. Viles PH, Shepherd JT: Evidence for a dilator action of carbon dioxide on the pulmonary vessels of the cat. *Circ Res* 1968; 2:325–332
9. Viles PH, Shepherd JT: Relationship between pH, Po_2, and Pco_2 on the pulmonary vascular bed of the cat. *Am J Physiol* 1968; 215:1170–1176
10. Harris P, Heath D: The human pulmonary circulation. Weir EK, Reeves JT (Eds). Edinburgh, Churchill Livingstone, 1986, pp 456–483
11. Brimioulle S, Lejeune P, Vachiery J-L, et al: Effects of acidosis and alkalosis on hypoxic pulmonary vasoconstriction in dogs. *Am J Physiol* 1990; 258:H347–H353
12. Brimioulle S, Vachiery J-L, Lejeune P, et al: Acid-base status affects gas exchange in canine oleic acid pulmonary edema. *Am J Physiol* 1991; 260:H1080–1086
13. Wagner PD, Rodriguez-Rosin R: State of the art. Clinical advances in pulmonary gas exchange. *Am Rev Respir Dis* 1991; 143:883–888
14. Furchgott RF, Vanhoutte PM: Endothelium-derived relaxing and contracting factors. *FASEB J* 1989; 3:2007–2018
15. Mulvany MJ, Aalkjaer C: Structure and function of small arteries. *Physiol Rev* 1990; 70:921–961
16. Benjamin N, Vallance P: Local control of human peripheral vascular tone: Implications for drug therapy. *Clin Sci* 1991; 80:183–190
17. Fukuda S, Matsumoto M, Nishimura N, et al: Endothelial modulation of norepinephrine-induced constriction of rat aorta at normal and high CO_2 tensions. *Am J Physiol* 1990; 258:H1049–H1054
18. Liu S, Crawley DE, Barnes PJ, et al: Endothelium-derived relaxing factor inhibits hypoxic pulmonary vasoconstriction in rats. *Am Rev Respir Dis* 1991; 143:32–37
19. Voelkel NF: State of art. Mechanisms of hypoxic pulmonary vasoconstriction. *Am Rev Respir Dis* 1986; 133:1186–1195
20. Archer SL, McMurtry IF, Weir EK: Mechanisms of acute hypoxic and hyperoxic changes in pulmonary vascular reactivity. *In:* Pulmonary Vascular Physiology and Pathophysiology. New York, Marcel Dekker, 1989, pp 241–290
21. Cutaia M, Rounds S: Hypoxic pulmonary vasoconstriction. Physiologic significance, mechanism, and clinical relevance. *Chest* 1990; 97:706–718
22. Hauge A: Role of histamine in hypoxic pulmonary hypertension in the rat. I. Blockage or potentiation of endogenous amine, kinins and ATP. *Circ Res* 1968; 22:371–383
23. Emery CJ, Bee D, Barer GR: Mechanical properties and reactivity of vessels in isolated perfused lungs of chronically hypoxic rats. *Clin Sci* 1981; 61:569–580
24. Aalkjaer C: Regulation of intracellular pH and its role in vascular smooth muscle function. *J Hypertension* 1990; 8:197–206
25. Ohya Y, Sperelakis N: Involvement of a GTP-binding protein in stimulating action of angiotensin II on calcium channels in vascular smooth muscle cells. *Circ Res* 1991; 68:763–771
26. Wray S: Smooth muscle intracellular pH: Measurement, regulation, and function. *Am J Physiol* 1988; 254:C213–C225
27. Aalkjaer C, Cragoe EJ: Intracellular pH regulation in resting and contracting segments of rat mesenteric resistance vessels. *J Physiol* 1988; 402:391–410
28. Reeves JT, Grover RF: Blockade of acute hypoxic pulmonary hypertension by endotoxin. *J Appl Physiol* 1974; 36:28–32