Evidence for a Dilator Action of Carbon Dioxide on the Pulmonary Vessels of the Cat

By Peter H. Viles, M.D., and John T. Shepherd, M.D., M.Ch., D.Sc.

ABSTRACT

Isolated cat lungs perfused at constant flow (75 ml/min per kg total body weight) were ventilated with gases of varying CO₂ content (0 to 10%), 20% O₂, and the balance N₂. Tidal volume was constant, and airway pressure did not change. Left atrial pressure was held constant, and changes in pulmonary artery pressure (PPA) were taken to reflect changes in pulmonary vascular resistance. Progressive hypercapnia (Pco₂ = 0 to 60 mm Hg) resulted in an increase in PPA as pH decreased. Equivalent degrees of acidosis, produced by the infusion of 0.3 N lactic acid, resulted in a higher PPA. Changes in PPA produced by lactic acid were reversible with 0.89 M sodium bicarbonate. With Pco₂ constant, and pH changed by lactic acid or sodium bicarbonate infusion, PPA was higher in lungs ventilated with room air (Pco₂ = 0) than in those ventilated with 5 or 10% CO₂ (Pco₂ = 30 or 60 mm Hg) at the same hydrogen ion concentration of the perfusate. These findings can be explained by two opposing actions of CO₂ on pulmonary vessels: a dilator action due to the direct effect of CO₂ and a constrictor action caused by the increase in hydrogen ion concentration.

ADDITIONAL KEY WORDS
respiratory acidosis metabolic acidosis pulmonary vascular resistance isolated perfused lungs pulmonary vasomotor activity

Ventilation of the isolated perfused lungs of cats with high concentrations of carbon dioxide causes the pulmonary vessels to constrict (1, 2). Increased hydrogen ion concentration in the perfusate has also been shown to cause constriction in the pulmonary vascular bed of various animals (3-5). Recent workers (6, 7) have suggested that the vasoconstrictor effect of hypercapnia is due to the acidosis produced.

The present paper presents evidence that there are two opposing actions of carbon dioxide on the pulmonary vessels: a dilator action due to the direct effect of the carbon dioxide molecule, and a constrictor action due to the increase in hydrogen ion concentration caused by the hypercapnia.

Methods

The isolated perfused lung preparation described by Donald (8) was adapted for cats. Adult cats weighing 1.8 to 2.6 kg were anesthetized with intravenous chloralose (80 mg/kg) or pentobarbital (30 mg/kg), and the trachea was cannulated. After heparinization, and exsanguination from the carotid artery, the trachea was clamped and the chest was opened bilaterally in the fifth intercostal space. A cannula 4 or 5 mm in diameter was secured in the main pulmonary artery. A cannula 7 or 8 mm in diameter was passed through the apex of the left ventricle and placed in the left atrium; it was secured at the mitral valve orifice in order not to obstruct the blood flow into the atrium. The chest was then closed, and the entire rib cage with its contents was removed by dissection and transsection of the vertebral column at the fifth cervical and second lumbar vertebrae. The liver was left attached to the diaphragm and supported by a metal plate tied over its peritoneal surface; the rest of the abdominal viscera were removed.

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metal pin inserted through the spinal canal served to suspend the thoracic block during perfusion; this maintained the normal anatomic relationships and gravitational forces within the lung.

The lungs were inflated rhythmically with positive pressure 14 times/min by means of a constant-volume Harvard pump. Tidal volume was constant throughout each experiment and was adjusted to give initial peak airway pressures of 15 cm H₂O. End-expiratory pressure was held at 2 cm H₂O. Airway pressure was continuously monitored by a strain gauge or water manometer.

Lung perfusion with autologous cat blood by means of an occlusive roller pump was kept constant at 75 ml/min per kg total body weight. The blood coming from the left atrium drained into a reservoir whose height was adjusted to the level of the mitral valve; this level served as the zero reference point for pressure measurement. Blood was pumped from this reservoir through a bubble trap and heat exchanger before it re-entered the lung. Blood temperature was maintained at 37.5 ± 0.5°C. Pulmonary artery pressure was measured with a Statham P23 B pressure transducer.

Blood samples were obtained from the pulmonary artery cannula and analyzed immediately for pH, P O₂, and P CO₂ with an Instrumentation Laboratory Model 113 meter at 37.6°C. Appropriate corrections to actual blood temperature were made (9, 10). Blood samples drawn simultaneously from the pulmonary venous return and the pulmonary artery had identical values.

In all experiments, the fraction of oxygen in the inspired air was 20 to 21%; the P O₂ of the blood was in excess of 100 mm Hg (range, 100 to 136 mm Hg). In those experiments in which P CO₂ was changed, the ventilating gas mixtures were room air, and 2.5, 5.0, 7.5, and 10% CO₂ with 20% O₂; P CO₂ of the blood was zero to 60 mm Hg with these mixtures. In all experiments

\[ Flow = 75 \text{ cc/min/kg} \]
\[ P O_2 = 120 \text{ mm Hg} \]
\[ Temp. = 37-38 \degree C \]

**FIGURE 1**
Pulmonary artery perfusion pressure at varying pH with progressive hypercapnia (open circles) or lactic acid infusion (solid circles) in four isolated perfused lung preparations with 20% oxygen in the ventilating gas mixture. At any given pH, pressure is lower in the presence of carbon dioxide than in its absence. In two preparations, after partial neutralization of the lactic acidosis by sodium bicarbonate, the PPA decreased from the maximum value of 21 mm Hg (bottom, left panel) and 25 mm Hg (bottom, right panel) to 15 mm Hg and 14 mm Hg, respectively (crosses).
the balance of the ventilating gas mixture was nitrogen. Gases were warmed and humidified before they entered the lung.

In the experiments in which \( \text{Pco}_2 \) was held constant, the \( \text{pH} \) of the perfusate was changed by the infusion of 0.89 M sodium bicarbonate (0.5 ml/min) or 0.3 N lactic acid (0.2 ml/min) between the reservoir and the pump. With these rates of infusion \( \text{pH} \) would change 0.1 unit in approximately 2 minutes. Infusions were continued for 6 to 12 minutes. As noted by Barer and her associates (7), faster rates of acid infusion occasionally result in abrupt increases in perfusion pressure.

Blood samples were drawn and pressures measured after a steady state had been achieved. This occurred within a few minutes of stopping the infusion of acid or base and within 7 to 10 minutes of changing the ventilating gas mixture. The delay between change in gas mixture and stabilization of pressure is attributed to the large volume of the respirator, humidifier, and connecting tubes.

The time from the death of the animal to the reestablishment of perfusion was 20 to 30 minutes. Like Donald and Ferguson (11), we have found this preparation to be stable and reactive for at least 4 hours. Stability was marked by constancy of perfusion pressure as long as \( \text{pH} \), \( \text{Po}_2 \), and \( \text{Pco}_2 \) did not vary, and by constancy of the blood volume in the reservoir, which, except for sampling losses, remained at its initial value (~50 ml) throughout the experiment. The lung continued to show changes in perfusion pressure as \( \text{pH} \) and \( \text{Pco}_2 \) were altered over the course of the perfusion, which lasted 2 to 3 hours. At the conclusion of each perfusion, all lung preparations showed an increase in pulmonary artery pressure when ventilated with gas mixtures containing 2.5 to 5% oxygen, thus demonstrating the viability of the preparation.

**Results**

Airway pressure did not increase by more than 2 cm H\(_2\)O during any experiment and did not change during the administration of carbon dioxide or the infusion of lactic acid or sodium bicarbonate. The increases in pulmonary artery pressure during hypercapnia were reversible by ventilation with room air; similarly, the increases with infusion of lactic acid were reversed by the addition of bicarbonate. The infusion of an equivalent amount of 0.3 M sodium lactate with no change in \( \text{pH} \) did not cause a change in pulmonary artery pressure.

In four lung preparations, hydrogen ion concentration was increased first by progressive hypercapnia and then by lactic acid infusion. Results are shown in Figure 1. Ventilation of the lungs with increasing concentrations of carbon dioxide (0, 2.5, 5, 7.5, and 10%) with 20% oxygen resulted in an increase in pulmonary artery pressure (\( \text{P}_{\text{PA}} \)) at constant flow. The increase in \( \text{P}_{\text{PA}} \) was reversed with ventilation with room air. Ventilation of the same lungs with 20% oxygen in nitrogen (room air) during infusion of 0.3 N lactic acid into the blood perfusing the lungs caused a greater increase in \( \text{P}_{\text{PA}} \) at any given \( \text{pH} \).

In two of the lungs the \( \text{P}_{\text{PA}} \) at \( \text{pH} \) 6.8 to 6.9 was 21 and 25 mm Hg, after lactic acid infusion (Fig. 1). The increase in pressure with lactic acidosis was reversible. When sodium bicarbonate was then added to the perfusate and the \( \text{pH} \) increased to about 7.2, the \( \text{P}_{\text{PA}} \) decreased to 15 and 14 mm Hg, respectively—values similar to those found at that \( \text{pH} \) during acid infusion.

**FIGURE 2**

Pulmonary artery perfusion pressure at varying \( \text{pH} \) in two isolated, perfused lung preparations. Pressure is low at \( \text{pH} \) of more than 7.0 with hypercapnia (\( \text{Pco}_2 = 60 \) mm Hg, open circles) as bicarbonate is infused. Progressive increase in \( \text{P}_{\text{PA}} \) occurs at \( \text{pH} \) of less than 7.4 in the absence of carbon dioxide (\( \text{Pco}_2 = 0 \), solid circles) as lactic acid is added to the perfusate. The cross = pulmonary artery pressure and \( \text{pH} \) when lung, represented by solid circles, was ventilated with 10% carbon dioxide-20% oxygen before lactic acid was added. Compare with pressure at similar \( \text{pH} \) in absence of carbon dioxide.

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Fourteen additional lung preparations were perfused at constant alveolar and blood PCO₂: seven were ventilated with 10% carbon dioxide with 20% oxygen, and seven with room air. Hydrogen ion concentration was altered by the infusion of sodium bicarbonate or lactic acid. Typical examples of each type of experiment are shown in Figure 2.

In one experiment (Fig. 2), the isolated lungs of a cat were ventilated with 10% carbon dioxide with 20% oxygen; the blood PCO₂ was 60 mm Hg. Initial blood pH was 6.83 and PPA, 37 mm Hg. As the pH was raised to 6.94 by the infusion of sodium bicarbonate, the pulmonary artery pressure decreased to 19 mm Hg, and at pH 7.63, PPA was 16 mm Hg. The lung vasculature was still reactive at this last pH since an 8-mm Hg increase in PPA occurred when the ventilating gas mixture was changed to 10% CO₂ with 2.5% O₂ (Pao₂ = 25 mm Hg).

In the second experiment (Fig. 2), the isolated lungs from another cat were ventilated with air so that the blood PCO₂ was zero. Infusion of lactic acid caused a progressive increase in PPA as pH decreased below 7.4. When these lungs were ventilated with 10% carbon dioxide with 20% oxygen before the acid infusion, the PPA was 19 mm Hg (Fig. 2), at pH 6.97, as compared with

![Figure 3](image-url)

**Figure 3**

Record of pulmonary artery pressure (Ppa) and airway pressure (Pt) from isolated cat lung perfused with autologous blood at constant flow. The record is continuous over a 90-minute period. Ventilation with various gas mixtures (balance nitrogen) was as shown below recording. Breaks in Ppa trace occur at time of blood sampling. Pao₂ = 125 to 130 mm Hg, and PCO₂ = 0 except where indicated. The tidal volume was constant at 70 ml and frequency 14/min. Ventilation with 10% carbon dioxide-20% oxygen caused a 4-mm Hg increase in Ppa. Lactic acidosis caused a 7-mm Hg increase in Ppa, greater than after ventilation with carbon dioxide despite greater decrease in pH with the CO₂. Infusion of sodium lactate was without effect on pH or perfusion pressure. Changes due to lactic acid were reversed with sodium bicarbonate, and the preparation was still capable of reacting to alveolar hypoxia. Peak airway pressure was 12 ± 2 cm H₂O throughout the experiment.
DILATOR ACTION OF CO\textsubscript{2} ON PULMONARY VESSELS

Comparison of seven lung preparations ventilated with 10\% carbon dioxide with 20\% oxygen (open circles) and seven ventilated with room air (solid circles). Values represent mean (±1 se) of 10 to 13 observations. Difference at pH 7.0 and 7.6 both statistically significant. (See text and Table 1.)

Table 1 and Figure 4 compare the pulmonary artery pressures at pH ~7.0 and ~7.6 in the seven lung preparations ventilated with 10\% carbon dioxide and the seven ventilated without carbon dioxide. Each value is the mean (±1 se) of 10 to 13 observations from each group. At pH ~7.0 with hypercapnia, the mean PPA was 17.0 mm Hg; without carbon dioxide the PPA was 31.7 mm Hg. There was no overlap of individual observations at the two different carbon dioxide tensions. At pH ~7.6, although the individual values showed some overlap, the lungs ventilated with carbon dioxide had a lower perfusion pressure, which was statistically significant ($P<0.01$). Values for PPA at the two different hydrogen ion concentrations in four additional preparations ventilated with 5\% carbon dioxide (PCO\textsubscript{2}=30 mm Hg) were intermediate between those at 0 and 10\% carbon dioxide.

<table>
<thead>
<tr>
<th>pH</th>
<th>P(A)=6 0 mm Hg</th>
<th>P(A)=0 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO\textsubscript{2} (mm Hg)</td>
<td>117.6±2.0</td>
<td>196.6±3.5</td>
</tr>
<tr>
<td>PPA (mm Hg)</td>
<td>17.0±0.8</td>
<td>31.7±1.6</td>
</tr>
<tr>
<td>P(A)</td>
<td>75.7±0.1</td>
<td>7.60±0.02</td>
</tr>
<tr>
<td>PPA (mm Hg)</td>
<td>108.6±3.5</td>
<td>111.5±3.2</td>
</tr>
<tr>
<td>PPA (mm Hg)</td>
<td>13.2±0.8</td>
<td>18.0±1.0</td>
</tr>
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$P<0.001$ $P<0.01$
The greater increase in Ppa in the absence of carbon dioxide when the pH of the perfusate was decreased from 7.6 to 7.0 might be explained by the fact that the initial Ppa was higher (18 mm Hg compared with 13 mm Hg). A greater initial wall tension in the pulmonary vessels might result in a greater constriction of these vessels as the pH of the perfusate decreased. To examine this possibility, four additional lung preparations were ventilated with 10% carbon dioxide with 20% oxygen. The initial pH was about 7.0, and pulmonary blood flow was 75 ml/min per kg. The flow was increased to 100 and 125 ml/min per kg, and the Ppa was measured. Sodium bicarbonate was then infused to bring the pH to about 7.6, and the Ppa was again determined at the same blood flows (Table 2). At pH 7.6 the mean pressures were 15.5, 19, and 21 mm Hg at the three flow rates, and at pH 7.0 the corresponding pressures were 18, 22, and 23 mm Hg. The increase in pressure with increased flow was the same at both levels of hydrogen ion concentration (Table 2). Thus when the Pco2 is constant and the pH of the perfusate changes from 7.6 to 7.0, the increase in Ppa is independent of the initial pressure at pH 7.6, at least over the range of pressures in the present experiments. Hence the greater Ppa at pH 7.0 shown in Figure 4 and Table 1 at a Pco2 of zero compared with that with a Pco2 of 60 mm Hg can be attributed to the absence of carbon dioxide and not to the small difference in initial pulmonary artery pressures at pH 7.6.

**Discussion**

The increase in pulmonary artery pressure produced by the administration of carbon dioxide or lactic acid was reversed with room air ventilation or infusion of bicarbonate, respectively; this indicates that these agents had no permanent effect on pulmonary vascular resistance. The absence of response to sodium lactate infusion also indicates that the pressure changes noted with acid or base infusion were not dependent on alteration in lactate or sodium ion concentration.

In the intact animal, carbon dioxide ventilation causes bronchoconstriction, reflexly mediated by the peripheral chemoreceptors (12). The direct action of CO2 on the bronchial musculature, however, appears to result in bronchodilation (13). A similar action has been observed in the isolated perfused cat lung (1, 2, 14), although changes in bronchomotor tone are slight (2) and may be absent (14). There was no change in airway pressure during these experiments and no change in reservoir volume during the perfusion. Hence the changes in pulmonary artery pressure were not a consequence of changes in lung compliance or the development of pulmonary edema. Since pulmonary blood flow and left atrial pressure remained constant, we have concluded that changes in pulmonary artery pressure were due to alterations of vessel caliber somewhere in the pulmonary vascular bed.

The present experiments do not demonstrate the site of the vasomotor action. All the pulmonary vessels were exposed to an increased Pco2 and to increases in hydrogen ion concentration. Hyde and associates (2) have shown in isolated cat lungs that both arteries and veins can constrict in response to an increase in blood or alveolar Pco2.

The observation that a given increase in hydrogen ion concentration when due to carbon dioxide administration causes less vasoconstriction than when due to infusion of lactic acid does not appear to have been
made previously. Barer and her associates (7) perfused the left lung of the cat and studied the effect of hypercapnia and infusions of various acids on the pulmonary vascular resistance. They concluded that increases in resistance during carbon dioxide ventilation were a result of the decrease in pH produced, and they found no evidence for an independent vasoconstrictor action of carbon dioxide. Examination of their data, however, indicates that at any given pH, pulmonary vascular resistance is lower at higher arterial PCO₂. This is similar to our findings and can be explained by two opposing actions of carbon dioxide on the pulmonary vessels: a constrictor action caused by the increase in hydrogen ion concentration and a dilator action due to the direct effect of CO₂.

Bergofsky and associates (6) presented data from three anesthetized dogs in which acidosis was produced either by infusion of 0.3 M lactic acid or by ventilation with 5% carbon dioxide. Similar decrements in arterial pH were associated with equal increments in calculated pulmonary vascular resistance, regardless of the method used to alter the pH of the blood. They concluded that carbon dioxide caused constriction of the pulmonary vessels as a result of the changes in hydrogen ion concentration. Failure to reveal the opposing dilator action of carbon dioxide may have been due to the fact that during the lactic acidosis the carbon dioxide tension was 30 mm Hg, and to concomitant reflex changes in the intact dog. The direct vasodilator action of carbon dioxide can be demonstrated most convincingly in the isolated lung when the pH of the perfusing blood is zero and 60 mm Hg. Under these conditions the pulmonary artery pressure is lower than that observed with a PCO₂ of zero.

The present experiments, like previous studies (1-5), demonstrate that an increase in hydrogen ion concentration of the pulmonary perfusate can cause constriction of the pulmonary blood vessels. If this vasoconstriction is mediated by alterations in extracellular rather than intracellular hydrogen ion concentration, the dilator action of the carbon dioxide molecule may then be greater than indicated in the present study. Adler and associates (15) have shown that intracellular pH is lower with respiratory acidosis than with metabolic acidosis that results in the same extracellular pH. Intracellular hydrogen ion concentration would therefore be higher with hypercapnia than with the infusion of fixed acids even though the pH of the perfusate was the same.

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References

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