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Reduced Availability of Energy Initiates Pulmonary Vasoconstriction (42665)

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Abstract. The mechanism responsible for initiating hypoxic pulmonary vasoconstriction (HPV) remains controversial. In this study, reversible constriction of the pulmonary artery was produced when the ratio of carbon monoxide to oxygen was increased, demonstrating that HPV could be imitated even in the presence of a constant oxygen tension. Isolated rat lungs from 14 adult rats were perfused with a 5% albumin–physiological salt solution and ventilated with 21% O_2, 5% CO_2, balance N_2. At the end of a 30-min stabilization period, the lungs were injected with a bolus of angiotensin II (0.2 μg). They were then challenged three times for 5 min with an hypoxic gas mixture alternating with 5 min of normoxia. The ventilatory circuit was then changed to one in which 10% O_2, 5% CO_2 and varying concentrations of CO (balance N_2) could be administered. The concentration ratios of $CO:O_2$ were 0.5:1, 1:1, 2:1, 4:1, and 8:1. These were randomly administered for 6 min interspersed with 6 min of normoxia; a final angiotensin II challenge was given. The results show a slight but significant vasodilation with $CO:O_2$ of 0.5:1, 1:1 with mean depressor responses of -0.7 ± 0.1, -0.7 ± 0.1 cm H_2O and progressive vasoconstriction with $CO:O_2$ of 2:1, 4:1, 8.5:1 with mean pressor responses of 0.11 ± 0.3, 4.0 ± 0.4 and 6.2 ± 0.5 cm H_2O. The pressor response to angtiotensin II remained unchanged from beginning to end (9.5 ± 0.5, 8.1 ± 0.7 cm H_2O. The pressor responses, therefore, are consistent with reduced function of the cytochromes of the electron transport chain. These results suggest that HPV may be initiated by a reduction of energy state of the vascular smooth muscle and that postulation of a specific oxygen receptor is not necessary.

The precapillary vessels of the pulmonary artery, both *in vivo* and *in vitro,* constrict when the oxygen tension in the alveolar gas or the mixed venous blood is decreased (1). This response is termed hypoxic pulmonary vasoconstriction (HPV). Numerous studies have attempted to identify how hypoxia is sensed to produce this vasoconstriction. Suggestions have included a specialized oxygen receptor or an indirect action based on the energy state of the vascular smooth muscle or desaturation of the lung cytochrome P_{450} (2–4). The purpose of the present study was to examine these possibilities using an *in vitro* ventilated and perfused rat lung model with a well-developed response to HPV and in which the relevant variables could be controlled. The role of oxygen receptors was tested by maintaining the oxygen tension at 70 mm Hg throughout with ventilatory gas mixtures containing 10% oxygen, 5% carbon dioxide, and varying nitrogen and carbon monoxide concentrations. In this way, if a specific oxygen receptor were essential to initiate HPV, then no constriction would occur but, if a constriction occurred, then the particular metaloporphyrin involved would be suggested from the characteristics of the $CO:O_2$ ratio.

Materials and Methods. Fourteen adult female Wistar rats were anesthetized with 40 mg pentobarbital/kg body weight intraperitoneally. The preparation has been described in detail elsewhere (5) and is summarized below. A tracheostomy was performed and the animals were ventilated with 21% oxygen, 5% carbon dioxide, balance nitrogen at the rate of 180 ml/min by means of a Harvard rodent ventilator with 2 cm H_2O of PEEP applied. A midline sternotomy was performed and the ribs were retracted exposing the heart and lungs. Heparin (200 IU) was injected into the left ventricle to prevent coagulation of the blood. A small incision was made in the right ventricle into which a "T"-shaped cannula was inserted and secured in the pulmonary artery. Another incision was made in the apex of the left ventricle into which another cannula was tied. The lungs were removed from the chest cavity,

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suspended in a heated chamber, and perfused by means of a Harvard peristaltic pump with a 5% albumin–physiological salt solution at the rate of 0.06 ml/min/g. The left atrial pressure was maintained at zero. The first 30 ml of perfusate was discarded to ensure a blood-free perfusate.

The lungs were ventilated and perfused for a 30-min stabilization period, during which time they were ventilated with a normoxic gas mixture (21% oxygen, 5% carbon dioxide, balance nitrogen). They were then stimulated with 0.2 μg angiotensin II and injected as a 0.1-ml bolus into the pulmonary artery cannula, followed by three 5-min hypoxic gas challenges (3% oxygen, 5% carbon dioxide, balance nitrogen) interspersed with 5 min of ventilation with the normoxic gas. At this point, an additional ventilatory circuit was added to the system by which varying concentrations of carbon monoxide were added to the inspired gas. This circuit consisted of an H. Westholl gas-mixing pump which allowed the mixing of carbon monoxide with 10% oxygen, 5% carbon dioxide, balance nitrogen, resulting in gas mixtures with $CO:O_2$ ratios of 0.5:1, 1:1, 2:1, 4:1, and 8.5:1.

These gas mixtures were administered in random order to the isolated lungs for 6 min, with 6 min of 10% oxygen, 5% carbon dioxide, balance nitrogen administered between each carbon monoxide challenge. After the final challenge, another 0.2 μg of angiotensin II, was injected as a 0.1-ml bolus into the pulmonary artery cannula. The composition of the gas mixtures, including the carbon monoxide concentrations, was monitored by means of a Perkin–Elmer mass spectrometer. Measurements were recorded of pulmonary artery pressure, airway pressure, pH, temperature, alveolar and blood PO_2, PCO_2, animal body weight, and the wet–dry/dry weight of the lung.

The data were analyzed statistically by analysis of variance and specific differences between means were sought with the Neuman–Kuels test. The null hypotheses were rejected at $P < 0.05$.

Results. Lungs isolated from 14 rats (body weight 343.2 ± 15.5 g) were studied. The general conditions were maintained constant throughout for all rats (means ± SE): temperature, 36.5 ± .02°C; perfusate, pH 7.36 ± 0.1; airway pressure, 5.3 ± 0.1 cm H_2O; wet-dry/dry weight 5.37 ± 0.2 ml water/g dry tissue. The results of this study are given in Table I. For each challenge, the baseline pulmonary artery pressure was obtained as the mean of the pressures immediately before and after the challenge stimulus. These baseline pressures are shown in Table I and did not change significantly during the course of the study.

The results show that with the lowest $CO:O_2$ ratios (0.5:1 and 1:1), the pulmonary artery pressure decreased significantly, indicating vasodilation. At higher carbon monoxide concentrations (2:1, 4:1, and 8.5:1), the pulmonary vessels constricted progres-

TABLE I. PRESSOR RESPONSES TO ANGIOTENSIN II AND TO INCREASING CARBON MONOXIDE:OXYGEN RATIOS (n = 14)

	Baseline pressure[a] (cm H_2O ± SE)	Pressure response[b] (cm H_2O ± SE)
Initial 0.2 μg angiotensin II	10.9 ± 0.9	9.5 ± 0.5
3% Hypoxia	11.6 ± 0.8	9.1 ± 0.9
Carbon monoxide:oxygen ratio		
0.5:1	11.2 ± 0.8	−0.7 ± 0.2
1:1	11.4 ± 0.8	−0.7 ± 0.2
2:1	11.3 ± 0.8	0.1 ± 0.3
4:1	10.8 ± 0.9	4.0 ± 0.4
8.5:1	10.2 ± 1.1	6.2 ± 0.5
Final 0.2 μg angiotensin II	11.3 ± 0.7	8.1 ± 0.7

[a] Baseline pressure is the average of the pulmonary artery pressures immediately before and after the indicated challenge.

[b] Pressure response is the peak change of pressure from the baseline during a challenge.

sively and the pulmonary artery pressure increased significantly above baseline by 0.1 ± 0.3, 4.0 ± 0.4, and 6.2 ± 0.5 cm H_2O, respectively. These last two values are significantly different from each other and both are significantly different from the first three carbon monoxide values.

The constricture responses to angiotensin II were 9.5 ± 0.5 cm H_2O before the carbon monoxide was begun and 8.1 ± 0.7 cm H_2O after carbon monoxide had been eliminated.

Discussion. The present study shows that the pulmonary vessels do not constrict when the oxygen tension is maintained at 70 mm Hg, but that constriction can be induced, even at this PO_2, by increasing the carbon monoxide concentration. This constriction is reversible and is dose dependent, and the pulmonary vessels are viable at the end of the study. With the lowest concentrations of carbon monoxide (i.e., $CO:O_2$ ratios of 0.5:1 and 1:1), the pulmonary vessels dilated slightly but, as the ratios increased, there was a dose-dependent increase in the pulmonary vascular tone. When carbon monoxide was eliminated, constriction was abolished. Not only were the effects of carbon monoxide reversible, but the pulmonary vessels responded as well at the end of the experiment as they did at the beginning to a bolus injection of 0.2 μg angiotensin II.

It was hypothesized that if specific oxygen receptors are essential to initiate HPV, then no constriction would occur if an adequate oxygen tension (70 mm Hg) were maintained. However, if HPV is due to changes in the saturation of metaloporphyrins, then constriction would occur and the associated ratio of carbon monoxide to oxygen would indicate which system was the source of the stimuli because the relative affinities of these enzymes for oxygen and carbon monoxide vary systematically. This property is conveniently expressed as the $CO:O_2$ ratio at which 50% of the function of the metalprotein is inhibited and values have been reported from a variety of cellular systems. For hemoglobin (6, 7) and myoglobin (8), this occurs at ratios much less than 0.1. For the cytochrome P_{450} enzyme values of 0.5–1.5 are observed (9), while for cytochrome oxidase, the ratio is 5-15 (7, 10).

Previous studies in the isolated rat lung have shown that, with an oxygen tension of 70 mm Hg, the lungs do not constrict. This level of oxygenation (carbon dioxide tension 35 mm Hg and pH 7.36) was maintained throughout the whole experiment while the intracellular metaloporphyrins were successively influenced by the varying concentration of carbon monoxide. The ratios of carbon monoxide to oxygen selected were those which are known to preferentially bind to specific metaloporphyrins. A $CO:O_2$ ratio of 0.5:1 reduces the ability of hemoglobin and myoglobin to take up oxygen. In this study, a perfusate free of hemoglobin was used to eliminate any influence hemoglobin might have on the results.

A ratio of 1:1 inhibits most of the P_{450} enzymes. In studies undertaken by Sylvester *et al.* (3), perfused isolated pig lungs were ventilated with a gas mixture containing 11.5% CO. This gave a $CO:O_2$ ratio of 0.9 and 1.5 during normoxic and hypoxic ventilation, respectively, and, as in the present study, they observed vasodilation of the vessels with their low levels of CO which they attributed to a desaturation of the lungs' cytochrome P_{450}. Some of the first studies undertaken by Duke and Killick (11) in the 1950s in which isolated cat lungs were ventilated with mixtures of CO of 1–20% in air also observed vasodilation. The pH, however, in those studies was alkalotic, which decreases the vasoconstrictor response to hypoxia (12). While they did state that adding CO_2 did not affect the pulmonary artery pressure, the paper does not provide details of the experiment, so it is not possible to compare that work with this. It is also to be expected that vasodilation will be observed when vascular smooth muscle is poisoned by exposure to 100% CO. Subsequent investigation of the oxygen dependent function of some specific cytochrome P_{450}-mediated reactions have demonstrated that inhibition occurs only when the oxygen tension is one or two orders of magnitude less than that necessary to initiate HPV (13, 14).

In the present study, constriction began when the $CO:O_2$ ration was 2:1 and increased progressively as the ratio was increased to 4:1 and 8.5:1. At these ratios, the cytochrome oxidase of the electron transport chain is reduced. An hypothesis consistent with all

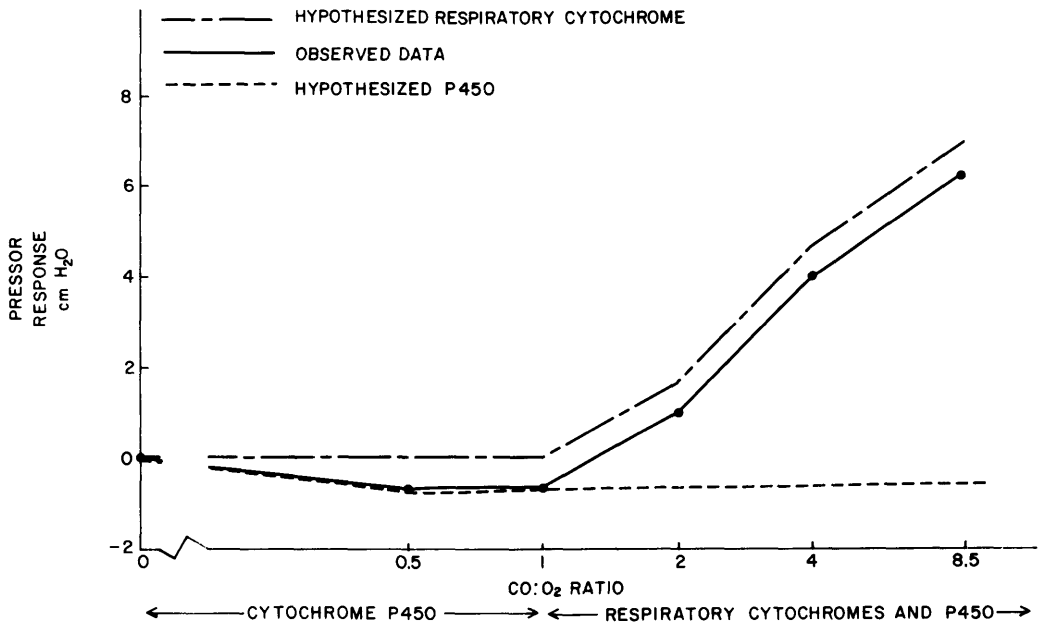

FIG. 1. Pressure responses to increasing $CO:O_2$ ratios plotted on a semilogarithmic scale. The solid line represents the observed values (solid circles), while the other two lines are theoretical lines derived from it as follows. It is assumed that the initial dilation attributable to P_{450} is maximal at a $CO:O_2$ of 1 and hereafter remains constant (dashed line). The constriction hypothesized to be due to reduction of respiratory cytochromes (semidashed line) is derived by subtracting the pressor response (dilatation) hypothesized for P_{450} from the pressor response of the observed data at each $CO:O_2$.

these observations (Fig. 1) is that lower levels of carbon monoxide interfere with the P_{450} system and influence biosynthetic pathways such as those of the prostaglandins or leukotrienes (10, 15), resulting in pulmonary vasodilation, while the higher ratios of carbon monoxide act upon the cytochrome oxidase a_3 of the electron transport chain, resulting in a decrease in the energy state of the cell. Oha and associates (16) have suggested that, during HPV, the increase in cytosolic Ca^{2+} may be due to the cell's inability to pump Ca^{2+} out, as if ATP synthesis becomes limiting, resulting in a decreased Ca^{2+} ATPase activity. Rounds *et al.* (17) and Stanbrook and McMurtry (18) have observed that the addition of glucose to the perfusate of hypoxically stimulated rat lungs reduces the vasoconstrictor response, suggesting that restoration of ATP from anaerobic sources prevents HPV.

In conclusion, these experiments are consistent with the suggestion that HPV is initiated by a reduction in the energy state of the vascular smooth muscle and that stimulation of a specific oxygen receptor is not necessary.

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