The Effects of Agents that Bind to Cytochrome P-450 on Hypoxic Pulmonary Vasoconstriction

J.T. SYLVESTER AND C. McGOWAN

SUMMARY The relationship between pulmonary arterial pressure (Ppa) and blood flow (Q) was determined during normoxia and hypoxia in isolated cat lungs perfused with blood at constant flow. Ventilation of the lungs with 100% nitrogen caused Ppa to increase. Since the same result had been obtained with other so-called “inert” gases, such as hydrogen and neon, the authors concluded that the pressor response to nitrogen was due to lack of oxygen. Ventilation with 100% carbon monoxide, however, caused Ppa to decrease. Since the major difference between carbon monoxide and the inert gases is the ability of CO to bind to metalloporphyrins, Duke and Killick concluded that the depressor response to CO resulted from binding of CO to one of these compounds. Hemoglobin probably was not the metalloporphyrin involved, however, because a depressor response to CO could be elicited in lungs perfused with dextran solutions instead of blood. Neither did cytochrome oxidase seem likely, because depressor responses to CO could be obtained with gas mixtures having CO/O2 ratios as low as 0.1. At this CO/O2 ratio, cytochrome oxidase should not be inhibited. Duke and Killick also observed that the pressor response to nitrogen or to low concentrations of oxygen was reduced or reversed when CO was added to the inspired gas. Since the nitrogen response could be “superimposed” in this manner on the CO response, they thought it unlikely that both types of response had a common cause. However, precisely the opposite interpretation is also possible. We speculated that oxygen and carbon monoxide may have been competing for a common binding site on the metalloporphyrin responsible for the CO response and that occupation of this binding site by oxygen or by carbon monoxide could somehow decrease pulmonary vascular tone. Conversely, if the binding site were unoccupied, as it would be in the absence of oxygen and carbon monoxide, pulmonary vascular tone would increase. Since it is unlikely that this hypothetical metalloporphyrin is hemoglobin or cytochrome oxidase, we wondered if it might be cytochrome P-450.

Cytochrome P-450 is the name given to a group of hemoproteins described in 1958 by Klingenberg and Garfinkel. These hemoproteins have several properties that indicate they could act in the manner we propose. They are found in lung and they bind both oxygen and carbon monoxide reversibly. Half-saturation with oxygen occurs at an oxygen tension which may be as high as 20–100 mm Hg, although much lower values have also been reported. Thus, a component of P-450 may undergo significant desaturation over the range of O2 tensions known to elicit pulmonary vasoconstriction.

The present study was designed to test the hypothesis that the pulmonary vascular response to hypoxia is caused by desaturation of lung cytochrome P-450.

Methods

If our hypothesis is correct, increasing the saturation of lung cytochrome P-450 during hypoxia should lead to a reduction in pulmonary vascular tone. Saturation could be increased by any agent which, like oxygen, binds to the cytochrome’s heme iron. There are many such agents available, of which carbon monoxide is the obvious example. Another is the drug, metyrapone. There are also agents, such as proadifen (SKF 525-A), which are...
thought to bind to the cytochrome's protein moiety rather than to its heme iron. The effect of this type of agent on pulmonary vascular tone during hypoxia is not predictable from our hypothesis; however, McMurtry et al. recently demonstrated that proadifen can inhibit the pulmonary pressor response to hypoxia in isolated rat lungs. Although they explained this result in terms of calcium exchange across the plasma membrane of the vascular smooth muscle cell (which proadifen is thought to block), it is also possible that proadifen acted by binding to P-450. Consequently, in the present study we examined pulmonary pressure-flow relationships in blood-perfused lungs during normoxia and hypoxia before and during the administration of carbon monoxide, metyrapone, and proadifen. The effects of these agents on the pressure-flow response to prostaglandin F₂α, another potent pulmonary vasoconstrictor, also were examined.

The pig was chosen as the experimental animal because its pulmonary circulation responds vigorously to hypoxia. Pigs weighing 16–33 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and ventilated through a tracheal cannula with room air by a respirator (Harvard model 614). Tidal volume and respiratory frequency were adjusted to maintain end tidal CO₂ tension between 35 and 40 mm Hg. After a mid-sternal thoracotomy and exposure of the heart, heparin (10,000 U) was injected through a 22-gauge needle into the left ventricular chamber. The left atrium and pulmonary artery were cannulated and connected to an extracorporeal perfusion system (Fig. 1). This system, which had a volume of approximately 1 liter, was filled with 200 ml of 6% dextran in normal saline and 800 ml of the animal's blood. Blood entering the left atrium from the lungs drained by gravity into a 250-ml Plexiglas reservoir. From the reservoir, the blood was pumped with a roller pump (Sarns model 3500) through a heat exchanger (Travenol "Miniprime"), blood filter (Pall "Ultipor"), and electromagnetic flow probe to the pulmonary artery. Just before perfusion, the pig was killed by bleeding. Obstructing ligatures were then placed on the root of the aorta and the atrioventricular groove of the heart to prevent loss of blood from the system during perfusion. The time from the induction of anesthesia to the beginning of perfusion was 30–60 minutes.

Blood flow (Q) was measured with an electromagnetic flowmeter (Biotronix model 610). Except during pressure-flow determinations, Q was constant at 0.5 liter/min. Pulmonary artery and left atrial (P₉ₐ) pressures were measured with strain gauges (Statham P23) zero-referenced to the level of the right atrioventricular valve. Left atrial pres-
sure was maintained at -20 mm Hg by adjusting the level of the reservoir. This ensured that the pulmonary veins would always be collapsed and thus that outflow perfusion pressure was always zero. Blood temperature, measured with a thermistor (Yellow Springs Instruments model 402) in the left atrium, was kept between 38.5 and 39.5°C by the heat exchanger. Blood samples from the left atrium were analyzed for pH and oxygen and carbon dioxide tensions, using standard electrode techniques (Radiometer BMS3 MK2). Blood pH was kept above 7.35 by periodic addition of 1 n NaHCO₃ to the perfusate. Usually, this was not necessary after the first 30 minutes of perfusion. Blood pH was never higher than 7.45. Hematocrit was measured with a microhematocrit centrifuge.

After perfusion was begun, the lungs were ventilated with a normoxic gas mixture (see below) at a rate of 6-7 breaths per minute and a tidal volume of 300-500 ml. To ensure that ventilation was constant during perfusion and that inspired gas was not leaking from the ventilation circuit into the room, tidal volume was monitored continuously by electrical integration of the expiratory flow signal from a pneumotachygraph (Fleisch 1) and strain gauge (Statham PM15E). Airway pressure was measured at the trachea with a strain gauge (Statham P23). End-expiratory tracheal pressure was kept at 3-4 mm Hg with an underwater tube attached to the expiratory port of the respirator. The composition of the inspired gas was controlled by calibrated rotameters (Scientific Gas Products models FT2-DO2, 601, 603) and checked by measuring tracheal oxygen, carbon dioxide, and nitrogen tensions with a mass spectrometer (Perkin-Elmer 1100). During normoxia the inspired gas consisted of 13.0% O₂, 5.4% CO₂, and 81.6% N₂. During hypoxia it consisted of 7.0% O₂, 5.4% CO₂, and 87.6% N₂. These mixtures resulted in a tracheal PO₂ of 90-100 mm Hg during normoxia and 50-55 mm Hg during hypoxia. Tracheal PCO₂ was always 38-40 mm Hg.

Measurement of the relationship between pulmonary artery pressure and flow over a wide range of values has been used to assess pulmonary vascular responses by Hall21 and by Dawes.22 The main advantage of this approach is the avoidance of possible ambiguities that can arise if only a single point on the relationship, such as Ppa at a constant flow, is measured. In the present study, pulmonary pressure-flow relationships were determined in the following manner. After stopping ventilation at end expiration, blood flow was gradually increased from the control level of 0.5 liter/min to a maximum of 3 liters/min, decreased to zero, and then returned to control over a period of 45-60 seconds. During this procedure, Ppa (x) and Q (y) were recorded on a x-y recorder (Hewlett-Packard 7004A). Subsequently, these data were transferred to magnetic tape with a coordinate digitizer (Gerber model S-10-E-X-Y) to permit linear regression analysis by least squares to the equation, \[ Ppa = Ppa_{\infty} + QR_p \] where Ppa_{\infty} is the Ppa-axis intercept and Rp, the slope (dPpa/dQ), of the regression line. This analysis is the same as that used by Dawes.22 Pulmonary artery pressure at a blood flow of 1 liter/min (Ppa Q=1), determined from the regression line, was used to make quantitative comparisons among the curves. Since outflow pressure was always zero in our preparation, Ppa_{\infty} is algebraically equivalent to the more commonly used parameter, pulmonary vascular resistance (PVR), defined as \( (Ppa-P_{pl})/Q \).

In each pig, the first pressure-flow relationship was measured during normoxia following a 50- to 60-minute stabilization period. On completion of measurements, when oxygenation was normal, the lungs were exposed to the hypoxic condition, and after 10 minutes the pressure-flow determination was repeated. Ventilation with the normoxic gas mixture was then resumed. After 10 minutes, a second Ppa-Q relationship at normal oxygenation was determined. Prostaglandin F₂α was then infused into the pulmonary artery at 0.01 mg/min. The F₂α infusate was prepared by diluting a stock solution (5 mg of the tromethamine salt per ml of 95% ethanol) 50-fold with normal saline. After 10 minutes of F₂α infusion, the pressure-flow relationship was again measured. This sequence (normal oxygenation, hypoxia, normal oxygenation, F₂α) was repeated four times in each pig, except in those exposed to carbon monoxide (group 2), in which it was repeated five times. In all cases, the 10-minute period of exposure to hypoxia or F₂α was sufficient for pulmonary artery pressure at the control level of flow to reach a steady state.

Four groups of pigs were studied. Group 1 was a control group composed of six pigs, each of which received a pulmonary artery infusion of normal saline (0.4 ml/min) for 120 minutes, beginning on completion of the second exposure to F₂α (approximately 120 minutes after the beginning of perfusion). The other three groups were composed of five pigs each. Group 2 received carbon monoxide in addition to the saline infusion. Carbon monoxide was administered in the inspired gas at a concentration of 11.5%. This concentration was chosen because it was high enough to yield a CO/O₂ ratio of 0.9 during normoxia and 1.5 during hypoxia, which cytochrome P-450 should be half-saturated with CO.23 Also, this concentration was never achieved. Since CO and N₂ have the same mass number, the nitrogen channel of the
mass spectrometer was used to confirm that the desired airway CO tension was being delivered. In group 2, pressure-flow responses to hypoxia and F2 were determined not only during but also 20-50 minutes after administration of the P-450 binding agent was stopped. Airway CO tension at this time was less than 1 mm Hg. Group 3 received metyrapone ditartrate, infused into the pulmonary artery at 10 mg/min, instead of normal saline. The infusate was made by buffering the original 100 mg/ml solution (Ciba Pharmaceutical Co.) with 1 N NaOH and 0.2 M phosphate buffer to a pH of 7.4 and then diluting with deionized water to a final concentration of 25 mg/ml. Pigs in group 4 received proadifen hydrochloride, infused into the pulmonary artery at 1 mg/min. In this case, the infusate was prepared by dissolving 125 mg of the drug in 50 ml of normal saline.

Analysis of variance was used for statistical comparisons. Differences were considered significant when P was less than 0.05.

**Results**

In all four groups, blood temperature, pH, and CO2 tension were constant throughout the study. Hematocrit exhibited a slight but statistically significant decrease; e.g., in group 1, hematocrit decreased from 23.6% at 54 minutes of perfusion to 21.8% at 247 minutes. This change was not different from that in the other groups. Since tidal volume and end-expiratory tracheal pressure were constant, peak inspiratory tracheal pressure was followed as an index of alveolar and airway stability. In group 1, peak tracheal pressure increased from 13.2 mm Hg at 54 minutes of perfusion to 17.1 mm Hg at 247 minutes. Similar increases were seen in groups 2 and 3. In group 4, however, the increase was greater: from 14.6 mm Hg at 50 minutes of perfusion to 23.5 mm Hg at 240 minutes. Although the chest was widely opened in every pig, parts of the lung were still contained within the thorax; thus, some of the increase in peak tracheal pressure probably was due to rigor and the progressive distention of the abdomen by intestinal gas which occurred after death. Intra-alveolar edema, as evidenced by foam in major airways, was not observed in any preparation on dissection of the lungs at the end of the study.

Pressure-flow recordings typical of those obtained in group 1 are shown in Figure 2. Hypoxia and prostaglandin F2 shifted the pressure-flow relationship to the right and decreased its slope, indicating pulmonary vasoconstriction. The magnitude of these responses increased with time. To quantify the responses, linear regression analysis was performed for each curve, as described above. Since hypoxia and F2 consistently increased both the intercept (PpaQ=0) and the slope (R به) of the pressure-flow line determined from the regression analysis, we quantified the response by calculating pulmonary artery pressure at a flow of 1 liter/min (PpaQ=1), which is simply the sum of PpaQ=0 andRp. In Figure 3, mean values of PpaQ=1 in group 1 are plotted as functions of time after the beginning of lung perfusion. After an initial increase at 75 minutes, PpaQ=1 during normoxia remained constant at about 20 mm Hg. Values measured during

**Figure 2**  Typical pressure-flow recordings from a pig in group 1. NS = normal saline. N = normal oxygenation. H = hypoxia. N-F2 = constant infusion of F2 during normal oxygenation.

**Figure 3**  Group 1. Time course of pulmonary arterial pressure at a blood flow of 1 liter/min (PpaQ). NS = normal saline. Brackets = ±1 SE.
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Figure 4. Pressure-flow recordings from a pig in group 2. This animal had the greatest response to CO. In the other pigs, the effects of CO were qualitatively similar, but less dramatic. N = normal oxygenation. H = hypoxia. N-F2O = constant infusion of F2O during normal oxygenation.

hypoxia and F2O infusion always were greater than the normoxic values, and progressively increased until about the 150th minute of perfusion. Thereafter, they remained constant at about 40 mm Hg. These data, which represent the normal time courses of the flow-resistive properties of our preparation, were compared to data obtained from each of the other three groups.

Group 2 was given 11.5% carbon monoxide. The effects of this agent on pulmonary pressure-flow relationships are illustrated in Figure 4, which shows recordings obtained from the pig having the greatest response to CO. Curves recorded before CO administration were similar to those recorded in group 1 before saline infusion. During CO administration, however, two effects were noted. First, the curves at normal oxygenation were steeper and displaced to the left, indicating pulmonary vasodilation. Second, the right shifts and decreases in slope caused by hypoxia and F2O were decreased. Both effects were reversed when CO was removed from the inspired gas. Mean data for the entire group are shown in Figure 5. Before CO, values of PpaQ-1 followed time courses similar to those in group 1. During CO, however, all values of PpaQ-1 were reduced dramatically. In addition, both the hypoxic responses (the distance between the normal and hypoxic points) and the F2O responses (the distance between the normal and F2O points) were significantly less than in group 1 (P < 0.03 and 0.05, respectively). After CO was removed from the inspired gas, normal PpaQ-1 promptly returned to its pre-CO level. The increases in PpaQ-1 caused by hypoxia and F2O now were greater than those measured either during or before CO administration.

Group 3 was given metyrapone. As shown in

Figure 5. Group 2. Effect of CO on PpaQ-1. NS = normal saline. Brackets = ±1 SE.

Figures 6 and 7, the effects of this agent on pulmonary pressure-flow relationships were similar to those of CO. The pressure-flow curve at normal oxygenation was left-shifted and increased in slope. The right shifts and decreases in slope caused by hypoxia and F2O were diminished. In comparison with group 1, all values of PpaQ-1, as well as the increases in PpaQ-1 caused by hypoxia and F2O, were
significantly reduced. The reversibility of these effects was not tested.

Group 4 received proadifen. The effects of this drug on pulmonary pressure-flow relationships were different from those of CO and metyrapone, as shown in Figures 8 and 9. During proadifen administration, the normoxic curve was shifted to the right and decreased in slope, indicating vasoconstriction, whereas vasodilation occurred with CO and metyrapone. Immediately after the beginning of proadifen infusion, hypoxia continued to shift the curve to the right and decrease its slope, but eventually this response was diminished. In contrast, the F2O curve remained to the right of the normoxic curve throughout the study; thus, the F2O response was preserved. As with metyrapone, the reversibility of these effects was not determined.

**Discussion**

Like other hemoproteins, cytochrome P-450 can bind CO as well as O2 to its heme iron.13 By analogy with hemoglobin,25 the amount of desaturated P-450 present in a mixture of oxygen, carbon monoxide, and the cytochrome should equal that which would exist if CO were absent and the O2 tension were equal to P02 + MPco, where M, the relative affinity constant, is the ratio of P02 to Pco when all the cytochrome is bound, half to O2 and half to CO. For P-450, M is probably around 1.0,23 i.e., its affinity for CO is about the same as its affinity for O2. Thus, a unit increase in CO tension should be as effective in decreasing the concentration of desaturated P-450 as a unit increase in O2 tension. If the amount of desaturated P-450 determines the degree of pulmonary vasoconstriction, as our hypothesis states, then CO, like O2, should decrease the vasoconstriction caused by hypoxia.

In group 2 (Figs. 4 and 5), an alveolar carbon monoxide tension of about 80 mm Hg shifted the hypoxic pressure-flow curve to the left and increased its slope, indicating a reduction of pulmonary vascular tone, as predicted by our hypothesis. In addition, CO reduced pulmonary vascular tone
during normoxia. This also would be predicted if a significant amount of desaturated P-450 were present during normal oxygenation. As far as P-450 is concerned, addition of CO in this situation would be equivalent to increasing PO2 from 95 mm Hg (the O2 tension during normoxia) to 175 mm Hg (PO2 + MPco). Barer et al.17 have demonstrated that an increase in O2 tension over this range can indeed cause a significant decrease in pulmonary vascular tone. The curves during normal oxygenation and hypoxia were moved closer together by CO. This would be predicted if the decrease in “effective” oxygen tension (PO2 + MPco) produced by hypoxia occurred on an upper, flatter portion of the cytochrome's dissociation curve, resulting in a smaller increase in desaturated P-450 than would have occurred in the absence of CO.

Although these results are consistent with our hypothesis, they in no way prove that cytochrome P-450 acted in the manner we propose. They do suggest strongly, however, as do the results of Duke and Killick,1 that there is in lung a metalloporphyrin capable of influencing pulmonary vascular tone. This suggestion derives from the fact that CO has not yet been demonstrated to affect biological systems except by means of its ability to bind to such compounds.3,4

In this study, we have not performed experiments to rule out the possibility that metalloporphyrins other than cytochrome P-450 were responsible for the responses to hypoxia and CO. Duke and Killick,1 however, were able to elicit depressor responses to CO in lungs perfused with dextran solutions instead of blood. Thus, the possibility that hemoglobin was responsible seems remote. Fisher et al.6 demonstrated that ventilation of the isolated perfused rat lung with 76% CO, 19% O2, 5% CO2 (CO/O2 ratio = 1.5) did not alter the lung’s oxidation-reduction state or change the ratio of lactate to pyruvate in either tissue or perfusate, suggesting that oxidative metabolism was not inhibited. Ball et al.6 found that the CO/O2 ratio required to inhibit the cytochrome oxidase of beef heart by 50% was about 9.0. These results make it unlikely that cytochrome oxidase played a significant role in our experiments, in which the CO/O2 ratio was never greater than 1.5. In contrast, a CO/O2 ratio of 1.5 should cause substantial binding of CO to cytochrome P-450.22 A significant role for myoglobin can be excluded on theoretical grounds. This hemoprotein is almost completely saturated with oxygen at an O2 tension of 3-4 mm Hg,26 whereas the pulmonary vasoconstrictor response to hypoxia is only half-maximum at an O2 tension of about 50 mm Hg.15 Thus, myoglobin does not change its saturation significantly over the range of O2 tensions known to cause the greatest change in pulmonary vascular tone. To assess further the possibility that cytochrome P-450 was responsible for the responses to hypoxia and CO, we studied the pressure-flow effects of metyrapone, an agent which binds avidly to P-450.16,19 Metyrapone had effects qualitatively similar to those of CO: the normoxic and hypoxic curves were moved closer together, shifted to the left, and increased in slope (Figs. 6 and 7). Metyrapone, like CO, is thought to bind to the heme iron of P-450,19 and thus will decrease the concentration of desaturated P-450 present at any given O2 tension. These results, therefore, are consistent with our hypothesis and can be explained in the same manner as the CO results. The possibility that metyrapone acted by some mechanism other than combination with cytochrome P-450 cannot be ruled out, however. Lefer and Nazdam27 showed that metyrapone caused a dose-dependent decrease in mean arterial pressure, heart rate, and aortic flow in anesthetized dogs. In addition, it decreased contractile force in the intact left ventricle of the dog and in isolated cat papillary muscle. Parnham28 found that administration of metyrapone to isolated uteri from pregnant rats increased uterine output of prostaglandins E and F, but inhibited spontaneous contractile activity. The spontaneous activity of isolated rabbit ileum was also inhibited in a dose-dependent manner by this drug. The mechanisms of these effects are unknown. Kahl and Nettet29 recently demonstrated that 5 mM metyrapone (corresponding to 2.63 g of metyrapone ditartrate per liter) could increase the lactate-pyruvate ratio and decrease the oxygen consumption of rat liver slices, suggesting that mitochondrial electron transport was partially blocked. Although this concentration is two times greater than the maximum attained in the present study (1.2 g/liter, assuming that no drug was metabolized) and almost seven times greater than the lowest concentrations at which we observed significant pressure-flow effects (0.4 g/liter), we cannot rule out the possibility that metyrapone blocked mitochondrial electron transport in our preparation. We would emphasize, however, that at the concentrations employed in our study, the only pharmacological property known to be shared by metyrapone and CO is the ability to bind to the heme iron of cytochrome P-450. Insofar as this is true, the metyrapone results strengthen the possibility that desaturation of cytochrome P-450 is the cause of hypoxic pulmonary vasoconstriction.

Both metyrapone and CO decreased the vasoconstrictor response to prostaglandin F2α (Figs. 4-7), suggesting that CO and metyrapone simply may have been toxic to pulmonary vascular smooth muscle. This possibility could be ruled out by demonstrating that CO and metyrapone do not block pulmonary responses to vasoconstrictor agents other than hypoxia or F2α. In the isolated rat lung, McMurtry (personal communication) found that metyrapone virtually eliminated the hypoxic response while reducing the pressor response to angiotension II by only 50%. As far as we know, CO has not been studied in this manner. Another possible explanation for the results with F2α is that F2α caused vasoconstriction by the same mechanism as
does hypoxia. In this respect, it is interesting to note that $F_{2a}$ has been found to bind to cytochrome P-450.

Since proadifen is thought to bind to the protein moiety of P-450 rather than to the heme iron, its effects on the concentration of desaturated P-450 (and therefore on pulmonary vascular tone) could not be predicted from our hypothesis. McMurtry et al.,20 however, demonstrated that proadifen could significantly reduce the pulmonary pressor response to hypoxia in isolated rat lungs. The effects of this agent in our preparation are shown in Figures 8 and 9. Since proadifen shifted the normoxic curve to the right and decreased its slope, it must be considered a pulmonary vasoconstrictor. To our knowledge, this effect of proadifen has not been described previously. Like McMurtry et al.,20 we found that the pressor response to hypoxia was reduced by proadifen. The pressor response to $F_{2a}$, however, was not reduced. These effects were different from those of CO and metyrapone in that CO and metyrapone caused vasodilation during normoxia and reduced the $F_{2a}$ response as well as the hypoxic response. Possibly, these differences result from the fact that proadifen binds to P-450 at a different site than metyrapone or CO.18 It is also possible, however, that the effects of proadifen were not related to cytochrome P-450. This drug has been found to inhibit autoregulation of renal blood flow, uncouple oxidative phosphorylation, stabilize red blood cell membranes, and block transmembrane ion transport in both nerve and muscle.34-36 The mechanisms for these effects have not been elucidated.

By what mechanism might desaturation of cytochrome P-450 lead to an increase in pulmonary vascular tone? It is unlikely that the mechanism could have anything to do with the hydroxylation reactions which are catalyzed by this cytochrome.18 In these reactions, oxygen is bound to the heme iron, "activated," and then transferred to a substrate. By replacing the oxygen on the heme iron, carbon monoxide inhibits the reaction. The effects of O$_2$ and CO on hydroxylation therefore are opposite. In contrast, the effects of O$_2$ and CO on pulmonary vascular tone were the same: both caused vasodilation. This disparity makes it unlikely that the hydroxylation reaction could be the link between desaturation of P-450 and pulmonary vasoconstriction. This type of disparity, however, is also seen with hemoglobin. Carbon monoxide and oxygen have opposite effects on hemoglobin oxygen transport, but similar effects on hemoglobin structure. The similarity of the effects of O$_2$ and CO on hemoglobin structure is illustrated by hemoglobin S, the hemoglobin of sickle cell anemia. When this hemoglobin is combined with either O$_2$ or CO, the allosteric alterations that result in sickling do not occur.27 By analogy with hemoglobin, we speculate that cytochrome P-450 might influence pulmonary vascular tone by means of its structure, which could change as O$_2$ is bound and released.

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Pressor Responses to Norepinephrine in Rabbits with 3-Day and 30-Day Renal Artery Stenosis

The Role of Angiotensin II

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SUMMARY Pressor responses to norepinephrine (NE) infusions were examined in normal rabbits, in rabbits with renal artery stenosis of over 30 days' duration (chronic renal hypertensive rabbits), and in rabbits with renal artery stenosis of 3 days' duration (3-day clipped rabbits). The 3-day clipped rabbits did not have hypertension, but they showed the same increased pressor responses to NE as did the chronic renal hypertensive rabbits, which was about 2.5 times that of the normal rabbits. Plasma renin activity (PRA) was the same in the 3-day clipped rabbits as in the normal group, but in the chronic renal hypertensive rabbits the PRA was significantly below normal. Infusions of angiotensin II (AII) in either subpressor or pressor amounts potentiated the pressor responses to NE in normal rabbits, whereas, in 3-day clipped rabbits and chronic renal hypertensive rabbits, AII in subpressor or pressor doses did not alter the pressor responses to NE. Infusion of the AII antagonist, [1-sarcosine, 8-isoleucine]angiotensin II, did not alter the pressor responses of normal rabbits to NE, but this AII analogue completely abolished the pressor hyperresponsiveness to NE in the 3-day clipped rabbits and greatly reduced the NE hyperresponsiveness in the chronic renal hypertensive rabbits; this AII antagonist did not alter the control arterial pressure in any of the three groups of rabbits. These studies show that the increased pressor response to NE in rabbits with renal artery stenosis occurs before the onset of hypertension and thus is not merely a result of the hypertension. Also, these results provide evidence that AII plays an important role in the increased pressor responses to NE in hypertensive and prehypertensive rabbits.

INCREASED vascular reactivity to vasoconstrictor substances has been observed both in clinical hypertension and in animal models of hypertension. However, the mechanisms responsible for this increased reactivity are not completely understood. Folkow et al. have provided evidence suggesting that this hyperresponsiveness in hypertension is due to structural changes in the walls of the resistance vessels which result in an increased vessel

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