Effect of Acute Hypoxia on the Pulmonary Conversion of Angiotensin I to Angiotensin II in Dogs

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SUMMARY We studied the effect of acute hypoxia on pulmonary conversion of angiotensin I to II in anesthetized dogs. When arterial Po2 was decreased from 86 ± 14 (SD) to 33 ± 8 mm Hg without changing pH or PCO2, the single passage conversion of intravenous boluses of radiolabeled angiotensin I in tracer doses fell significantly (P < 0.005) from 72 ± 4 to 67 ± 6%. The effect of comparable levels of hypoxemia on the conversion of continuous intravenous infusions of pharmacological doses (1000 times physiological) of angiotensin I was greater: from 55 ± 14 to 33 ± 13% (P < 0.025). There was prompt return of percent conversion ratios to control levels when hypoxemia was reversed. We conclude that acute hypoxia is associated with a reversible decrease in pulmonary angiotensin converting enzyme availability. Circ Res 46: 221-226, 1980

THE conversion of angiotensin I to II takes place at the luminal surface of capillaries and in the brush border of proximal renal tubules (Erdős, 1977). The enzyme peptidyl dipeptidase (EC 3.4.15.1), responsible for both the conversion of angiotensin I and the hydrolysis of bradykinin, is present within surface organelles of endothelial cells, known as caveolae (Ryan and Ryan, 1977). Even though angiotensin I-converting enzyme has been extracted from a variety of organs, the enzyme that is present in lung capillaries is believed to have a special physiological importance. There is more endothelium in lung than anywhere else in the body. In addition, the pulmonary circulation is located strategically so that it could potentially exert control over the quantity of angiotensin II which reaches the systemic circulation. However, there has been no experimental evidence to indicate that such modulating activity on angiotensin II levels is, in fact, exerted by the pulmonary circulation.

The mechanism by which acute hypoxia causes pulmonary vasoconstriction is unknown (Fishman, 1976). Experiments on isolated perfused rat lungs have suggested that the presence of angiotensin II in the perfusate may be required for hypoxic pulmonary vasoconstriction to occur (Berkov, 1974). The systemic pressor response to intravenous bolus injections of angiotensin I has been found to be reduced during acute hypoxia, suggesting the possibility of interference with angiotensin I conversion in the lung (Leuenberger et al., 1978). In addition, the inactivation of exogenous bradykinin has been shown to be reduced by hypoxia in dogs (Stalcup et al., 1979a).

The present series of experiments examines directly the effects of acute hypoxia on the pulmonary conversion of angiotensin I in the pentobarbital-anesthetized dog.

Methods

Experiments were performed in 13 mongrel dogs ranging from 18 to 24 kg in body mass, anesthetized with intravenous sodium pentobarbital (30 mg/kg). The dogs were intubated and ventilated with a Harvard 606 respirator. To investigate the effects of acute hypoxia on angiotensin I conversion in lungs, we used two experimental approaches: (1) comparison of the conversion of bolus injections of tracer doses of radiolabeled angiotensin I administered during normoxia and during acute hypoxia in the same dog; (2) determination of the effects of acute hypoxia conversion and pressor potency of pharmacological doses of intravenous angiotensin I infused continuously. Acute hypoxia was produced by ventilating with an 8% oxygen-92% nitrogen gas mixture for periods of 5-7 minutes. Arterial blood gases were analyzed for pH, PCO2, and Po2 using a standard electrode assembly (Radiometer, model PHM 71, London Co.). Intravascular pressures were measured through polyethylene catheters connected to P23Db Statham transducers and were recorded using a multichannel oscillographic apparatus (Hewlett-Packard HP7758A).

Bolus Injections

Experiments were performed in seven dogs. Catheters were positioned under fluoroscopic guidance in the right atrium, pulmonary artery (Swan-
Ganz, balloon-tipped catheter), and aortic root. Bolus injections of 25 ng (25 pmol, or $2.5 \times 10^7$ counts/min) of $^{125}$I-angiotensin I ($^{125}$I-AI) (New England Nuclear) were given through the right atrial catheter while arterial blood was sampled continuously at a steady rate (300 ml/min) using a Masterflex pump (Cole Parmer) and delivered into test tubes mounted on a turntable-collector. The test tubes contained dimercaprol, 8-OH-quinoline, and EDTA as described previously (Oparil et al., 1974). The speed of the collector was adjusted so that each test tube was exposed to 1 second of pump output. Four injections were given to each dog, two during normoxia (breathing room air) and two after 5–7 minutes of hypoxia. The sequence of normoxic and hypoxic periods was chosen randomly, and a minimum interval of 30 minutes was allowed to elapse between injections to avoid interference by residual radioactive material. This dose of $^{125}$I-AI gave a peak concentration of labeled peptide in aortic plasma that approached physiological levels ($1 \times 10^9$ courts/min per ml or 0.1 pmol/ml) and did not produce a systemic pressor response.

An aliquot of plasma from each test tube was placed in a $\gamma$ counter (model 1185 Searle) to determine the concentration of total labeled material. The remainder of the sample was used for the quantification of angiotensin I and II as described below. Time-concentration curves for total radioactivity and for angiotensin II-associated radioactivity were plotted for each experimental run. Cardiac outputs and mean transit times were calculated from the plots of total radioactivity according to formulas from indicator dilution theory, after correction of hematocrit. Corrections for delays in transit through tubing were made according to Milnor and Jose (1960).

To verify the assumption that all $^{125}$I-labeled material remained in the vascular compartment during a single passage through the pulmonary circulation, two additional dogs were given four bolus injections each of angiotensin I and indocyanine green dye contained in the same syringe. Sampling and measurement of $^{125}$I-labeled material were the same as described above. Concentrations of indocyanine green were measured spectrophotometrically. No significant differences were observed between cardiac outputs and mean transit times computed from radioactivity or from indocyanine green curves, confirming previous observations (Fanburg and Glazier, 1973) in isolated perfused dog lungs.

Pulmonary vascular resistance was computed as the quotient of the difference: mean pulmonary artery pressure minus mean wedge pressure over cardiac output ($PA - LA/CO$). The conversion ratio of each sample was expressed as the percentage of angiotensin II-associated radioactivity relative to total radioactivity: $\% AII = 100 \cdot AII/(Al + AII)$. Conversion ratios of angiotensin I to II for each experimental run were obtained by averaging percent conversions for all individual samples located before recirculation was evident on the downslope of the time-concentration curve, as illustrated in Figure 1. The significance of differences in mean conversion ratios during control and hypoxia was assessed using the paired $t$-test (Bahn, 1972).

**Continuous Infusions**

Catheters were positioned in the main pulmonary artery, left atrium, right atrium, and femoral artery in six open-chest dogs. After obtaining control measurements of pressure and arterial blood gases, an infusion of angiotensin I ([Ile$^5$] angiotensin I, Bachem, Inc.) was initiated. Angiotensin I was dissolved in 0.9% saline (1 mg/100 ml) that contained bovine serum albumin (1 mg/ml) as a protein carrier and was infused at constant rates (from 0.63 to 0.80 $\mu$g/kg per min) for 15 minutes through the right atrial catheter using an infusion-withdrawal pump (Harvard Apparatus Co.). This quantity of angiotensin I gave a concentration of angiotensin II in aortic plasma (20 pmol/ml) that was 1000 times greater than physiological levels, produced a large pressor response, and thus can be considered a pharmacological dose. When angiotensin I had been infused for 5 minutes, ventilation was switched from room air to an 8% $O_2$-92% $N_2$ gas mixture. Acute hypoxia was maintained for 5 minutes, after which ventilation was returned to room air. Angiotensin I infusion was maintained for an additional 5 minutes after returning from the hypoxic gas mixture to room air. Beginning at the 3rd minute of angiotensin I infusion (control state) and extending to 3 minutes after discontinuation of hypoxia, blood samples were drawn manually into plastic syringes at 1-minute intervals from the pulmonary artery and left atrial catheter simultaneously. Blood samples were transferred to test tubes containing dimercaprol, 8-OH-quinoline, and EDTA. The plasma fraction was separated by centrifugation at 4°C and stored as described previously (Oparil et al., 1974).

The expression $\% AII = (AI_{II-A} \cdot AI_{II-P} \cdot 100)/AI_{PA}$ was used to describe percent conversion ratios of angiotensin I in the pulmonary circulation: $AI_{II-A} =$ the concentration of angiotensin II in the left atrium, $AI_{II-P} =$ the concentration of angiotensin II in pulmonary arterial blood, and $AI_{PA} =$ the concentration of angiotensin I in pulmonary arterial blood.

Conversion ratios obtained during the first 3 minutes of the control state, the last 3 minutes of hypoxia, and the last 3 minutes of recovery from hypoxia were pooled. Statistical significance of differences between pooled mean conversion ratios was assessed by paired $t$-test (Bahn, 1972).

Radioactive samples were subjected to high voltage paper electrophoresis for identification and quantification of labeled peptides (Oparil et al., 1973). Angiotensin I and II and the peptide fragments generated by the action of angiotensinases in
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**Figure 1** Effect of acute hypoxia on angiotensin I conversion. Representative curves of total radioactivity (unfilled circles) and angiotensin II-associated radioactivity (filled circles) sampled at the root of the aorta after bolus injections of tracer doses of angiotensin I. Each point represents 1 second of sampling. The left panel illustrates curves obtained during normoxia and the right panel curves obtained during acute hypoxia in the same dog. During acute hypoxia, mean transit times are shorter and conversion ratios smaller. MTT = mean transit time; cpm = counts/min.

Results

**Bolus Injections**

The results of this series of experiments are summarized in Table 1. During acute hypoxia, arterial \( Po_2 \) fell significantly; \( pH \) and \( P_{CO_2} \) remained unchanged.

In keeping with known effects of acute hypoxia in dogs, cardiac output increased significantly and mean transit time across the pulmonary capillary bed decreased significantly during acute hypoxia. Hypoxic pulmonary vasoconstriction resulted in significant elevations of mean pulmonary artery pressure and in significant increases in pulmonary vascular resistance. Conversion rates decreased in all but one experiment, averaging 72 ± 4% (SD) during normoxia and 67 ± 6% during hypoxia. This difference was small but highly significant. There was no demonstrable correlation between decrements in mean transit times and decrease in conversion rates during acute hypoxia. Results of a representative experiment are illustrated in Figure 1, in which the appearance times of total radioactivity and of angiotensin II radioactivity are very

<table>
<thead>
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<th>Table 1</th>
<th>Effect of Acute Hypoxia on Conversion of Tracer Doses of Angiotensin I</th>
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<tr>
<td></td>
<td>PAP (mm Hg)</td>
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<tr>
<td>Control</td>
<td>22±7</td>
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<tr>
<td>Hypoxia</td>
<td>33±11†</td>
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Results are expressed as mean ± se. PAP = pulmonary arterial pressure; LAP = left atrial pressure; CO = cardiac output; PVR = pulmonary vascular resistance; MIT = mean transit time; C = percent conversion ratio of angiotensin I = \( \text{AI} - 100 \times (\text{AI} + \text{AII})^{-1} \).

* \( P < 0.02; † P < 0.01; ‡ P < 0.005; § P < 0.001. \)
close. The shapes of the time-concentration curves are analogous. During acute hypoxia, the peak concentration of total radioactivity decreased, reflecting an increase in flow without a proportional increase in the volume of distribution of the marker. Associated with the decrease in mean transit time of approximately 30% during hypoxia, there was a 10% decrease in conversion of angiotensin I to II in this experiment.

**Continuous Infusions**

In this series of experiments, administration of the hypoxic gas mixture resulted in decreases in arterial Po2 from 75 ± 11 (SD) to 33 ± 7 mm Hg (P < 0.001); pH and PCO2 did not change significantly from 7.31 ± 0.05 (SD) to 7.31 ± 0.4 and from 40 ± 4 (SD) to 39 ± 4 mm Hg, respectively. Changes in hemodynamic variables are illustrated in Figure 2. Infusion of pharmacological doses of angiotensin I resulted in marked elevations of systemic arterial pressures from 117 ± 30 to 173 ± 29 mm Hg (P < 0.001), in elevations of pulmonary arterial pressures from 17 ± 5 to 23 ± 9 mm Hg (P < 0.01), and in elevations of left atrial pressures from 6 ± 3 to 12 ± 7 mm Hg (P < 0.01). Superimposition of acute hypoxia on angiotensin I infusion resulted in significant further increments in pulmonary arterial pressure from 23 ± 9 to 30 ± 9 mm Hg (P < 0.001) without change in either systemic arterial pressure from 173 ± 29 to 174 ± 34 mm Hg or in left atrial pressure from 12 ± 7 to 9 ± 5 mm Hg. The elevations in pulmonary vascular pressures probably were the result of hypoxic vasoconstriction. As shown in Figure 2, these variables showed a tendency to return to control levels as acute hypoxia was discontinued and after the infusion of angiotensin I was stopped. Toward the end of the period of angiotensin I infusion, systemic pressure elevations diminished, consistent with the development of tachyphylaxis.

The recovery of total immunoreactive angiotensin (I + II) in left atrial blood was nearly complete during both normoxia and hypoxia: 94 ± 20% (SD) of that in pulmonary arterial blood. As illustrated in Figure 3, acute hypoxia was associated with a progressive decline in conversion ratio of angiotensin I which was rapidly reversed on return to normoxia. Pooled data for the first 3 minutes of normoxia, last 3 minutes of hypoxia, and last 3 minutes after recovery from hypoxia are illustrated in Figure 4. In contrast to the small decrements in conversion ratios observed during acute hypoxia with tracer doses of angiotensin I, with pharmacological infu-
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FIGURE 4 Average values (bars) and standard errors (brackets) of percent conversion ratios of angiotensin I for experiments using continuous infusions of pharmacological doses of angiotensin I (Fig. 3). During acute hypoxia, there is a significant decrease in conversion of angiotensin I as compared to control (P < 0.025).

Discussion

These experiments have demonstrated impairment of angiotensin I conversion in the lung during acute hypoxia. Using intravenous bolus injections of tracer doses of substrate, we have shown slight (of the order of 5%) but consistent decreases in conversion of angiotensin I in a single passage through the pulmonary circulation during acute hypoxia. In contrast, when substrate was infused intravenously in pharmacological doses (1000 times physiological levels), conversion of angiotensin I to II decreased more drastically, but reversibly, from 55% to 33% during acute hypoxia.

Fanburg and Glazier (1973) related hemodynamic features of the pulmonary circulation to the characteristics of angiotensin I conversion in isolated perfused dog lungs. By changing perfusion pressures and pump flows, they showed the conversion of angiotensin I to increase in proportion to increments in pulmonary vascular surface area and to be related directly to mean transit times across the vascular bed. The angiotensin I-converting system in the lung was conceived as being analogous to a solid phase chromatography system in which the rate of product formation is a function of the quantity of enzyme present in the column and the mass flow of substrate. An increase in mean transit time in such a system would result in increased exposure time of substrate to enzyme and, hence, increased product formation.

We believe our results to be best interpreted as consistent with a decrease in enzyme availability during acute hypoxia. Under physiological conditions, the angiotensin I-converting enzyme in lung operates at extremely low substrate concentration, similar to levels observed during our tracer experiments. Substantial reductions in enzyme availability could be expected to cause only slight reductions in conversion of angiotensin I. However, with increases in substrate concentration (pharmacological dose experiments), the formation of angiotensin II would be expected to become more dependent on the quantity of enzyme available. Reductions in enzyme availability during acute hypoxia in our pharmacological dose experiments would tend to produce more drastic reductions in conversion ratios of angiotensin I than in our tracer dose experiments.

It is likely that, in our experiments, hemodynamic factors changed the characteristics of the pulmonary capillary circulation. However, the overall effect of these changes on the angiotensin I-converting system is difficult to predict. For example, during experiments with tracer doses of substrate, decreases in mean transit time would have tended to reduce conversion ratios, whereas concomitant increases in pulmonary artery pressures would have tended to increase conversion ratios by recruitment of unperfused capillary vessels. Similar circumstances would prevail during experiments with pharmacological doses when both pulmonary arterial and left atrial pressures increased. Recent experiments by Stalcup and associates (1979) have indicated that hypoxic depression of converting enzyme activity can be shown in endothelial cells in culture. Thus, even though decreased mean transit times potentially could reduce single passage conversion ratios, they are not sufficient to explain the entire phenomenon.

Our experiments are supportive of the observations of Leuenberger (1978) who showed an attenuation of the systemic pressor response to intravenous bolus injections of angiotensin I during acute hypoxia and speculated that it could be due to a decrease of its conversion in the lung. In our experiments, systemic pressor responses during continuous infusions of angiotensin I were not attenuated by acute hypoxia. However, we did not ascertain, as did Leuenberger, whether the doses of angiotensin I that we injected were located in the linear portion of the dose-response relationship. However, we did observe an unexpected lack of further increase in systemic blood pressure when acute hypoxia was superimposed on angiotensin I infusion (Fig. 2). Our experiments are also in agreement with
observations by Stalcup (Stalcup et al., 1979a) who noted severe inhibition of hydrolysis of pharmacological doses of exogenous bradykinin during acute hypoxia in dogs. However, our experiments differ significantly from those of Stalcup in the magnitude of the inhibition of converting enzyme activity with hypoxia. At arterial Po2 levels of 30 mm Hg, Stalcup observed essentially no differences in concentrations of bradykinin across the pulmonary circulation, indicating near total inhibition of hydrolysis. We observed hypoxic depression of angiotensin I conversion to a level of approximately one-half of the values obtained during control. It is unlikely that these differences were due to the different substrates used. Alternatively, it is possible that the marked differences in hemodynamic effects resulting from substrate infusion played a role. As demonstrated by Fanburg in isolated dog lungs, the effective area of the pulmonary capillary bed can play an important role in determining the quantity of substrate converted in a single passage (Fanburg and Glazier, 1973). The infusions of angiotensin I that we used tended to produce large elevations of pulmonary artery and left atrial pressures (Fig. 2) thus recruiting maximal capillary surface. In contrast, bradykinin infusions tended to exert a depressant effect on vascular pressures (Stalcup et al., 1979a).

At pharmacological concentrations of plasma angiotensin I, the attenuation exerted by acute hypoxia on conversion of angiotensin I was rapidly reversible, suggesting that it was not related to hypoxic injury to the endothelial membrane. Moreover, even at plasma concentrations 1000 times greater than physiological levels, the pulmonary circulation was still capable of converting 35% of affluent substrate in a single passage.

Several observations in the past have related acute or chronic hypoxia to angiotensin metabolism in the lung. As mentioned before, Berkov (1974) concluded that angiotensin II had to be added to the perfusate of isolated rat lungs for hypoxic pulmonary vasoconstriction to occur. This observation was confirmed (Alexander et al., 1976) in isolated dog lungs. Weir and Chester (1978) demonstrated attenuation of hypoxic pulmonary vasoconstriction with the infusion of SQ-20881, a competitive inhibitor of angiotensin I conversion. Molteni et al. (1974) showed increased concentrations of angiotensin-converting enzyme in serum and lung tissue of rats subjected to chronic hypoxia. Treatment with SQ-20881 prevented the development of muscularization of small pulmonary arteries of rats during chronic hypoxia (Zakheim et al., 1975).

The mechanism by which acute hypoxia decreases converting enzyme availability in lungs is open to conjecture. Among the more relevant possibilities are: a decrease in mean transit time with hypoxia, the production of a competitive inhibitor of angiotensin-converting enzyme during hypoxia, a change in the configuration of angiotensin-converting enzyme so that access of substrate to receptor sites is denied, or a change in the surface configuration of endothelium (Leuenberger et al., 1978) so that less enzyme is exposed to circulating substrate.

The observations that link hypoxia to angiotensin metabolism in the lung suggest that the hypoxic decrease in conversion, whatever its mechanism, may have physiological significance to the lung.

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