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Decrease in Angiotensin I Conversion by Acute Hypoxia in Dogs (40252)

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Converting enzyme is a dipeptidylcarboxypeptidase principally found on the surface of pulmonary endothelial cells (1, 2) and is responsible for the conversion of angiotensin I (AI) to angiotensin II (AII) (3) and the inactivation of bradykinin (4–6). This enzyme is of possible importance in arterial blood pressure regulation (7), and its inhibition with synthetic peptides has been useful in reducing elevated blood pressure due to overactivity of the renin-angiotensin system (8). It remains unknown whether pathophysiologic processes affecting the lung can alter angiotension conversion. This study was designed to determine whether one such insult, acute alveolar hypoxia, affects the activity of converting enzyme *in vivo*. Using a systemic blood pressure response technique to assay conversion (9–11), we compared conversion of AI under conditions of normal alveolar PO_2 and varying degrees of acute hypoxia. We found a striking decrease in the capacity of the lung to activate angiotensin during acute hypoxia.

Materials and methods. Beagle dogs, weighing from 9 to 15 kg, were anesthetized with pentobarbital, paralyzed with succinylcholine, and ventilated through a cuffed endotracheal tube on a Harvard ventilator with ambient air and hypoxic gas mixtures (from 21 to 8% O_2, balance N_2). The lungs were periodically hyperinflated to 15 cm H_2O pressure. Blood gases and acid-base status were monitored with pH, PCO_2 and PO_2 electrodes (Radiometer, Copenhagen, Denmark). We placed catheters in the right atrium, pulmonary artery, left atrium and ascending aorta under fluoroscopic control and recorded mean pressures continuously on a direct writing polygraph (Grass Model 7, Grass Instrument Co., Quincy, MA). Cardiac output was measured by thermodilution (5F KMA thermodilution catheter and KMA cardiac output computer model 3500, Kimray Medical Assoc., Oklahoma City, OK). We calculated pulmonary and systemic vascular resistances from duplicate values of cardiac output and vascular pressures measured simultaneously. Normal saline and lactated Ringer's solutions were administered in an amount equivalent to 100 ml/kg/24 hr, which included fluid boluses for thermodilution studies and to flush catheters. $Ileu^5$-AI and $Ileu^5$-AII (Schartz-Mann, Orangeburg, NY) were reconstituted in distilled water containing 0.1% lysozyme (Mallinckrodt, St. Louis, MO); stock solutions were stored in plastic containers at −70° and fresh dilutions made the day of study. Injections of AI in the right atrium and of AII in the aorta were given as boluses and at random order and dose so that a dose-response curve was obtained for each peptide at each level of arterial oxygen tension (PaO_2) studied. The sequence of exposure to ambient air and acute hypoxia was reversed several times. The blood pressure response to AI and AII was measured as the peak change in mean systemic arterial pressure. Subsequent boluses were given when all the circulatory variables had returned to within 10% of baseline steady state conditions. The log dose-response relationships were linear during ventilation with both ambient air and hypoxic mixtures. The mean of the lowest and the highest responses to either AI or AII was taken as reference and used to determine the equipotent pressor response; equipotent pressor doses of AI and AII was determined by interpolation (12). Conversion of AI was calculated in molar equivalents and expressed in percent of AI converted into AII (see Fig. 1). Blood pressure response to the same dose of angiotensin II injected into the aorta was not different between breathing room air or hypoxic gas mixtures.

Results. By the use of different inspired O_2–N_2 mixtures, PaO_2 was varied between 24 and 104 mm Hg. At a mean PaO_2 of 87 mm Hg (SD = 9) conversion of AI was 94% (SD

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= 7). The effect of acute alveolar hypoxia on conversion of AI is shown in Fig. 2. For each dog exposed to hypoxia, there was a progressive fall in conversion of AI with decreasing PaO_2. At a PaO_2 of 30 mm Hg, conversion of AI decreased to one half of control values. Inversion of the sequence of exposure to ambient air and acute hypoxia did not influence the results. Mean left atrial pressure, arterial pH and PCO_2 in the nine dogs studied were in the normal range: 4.6 mm Hg (SD = 2.5), 7.39 (SD = 0.05), 32.0 mm Hg (SD = 6.3), respectively. The preparations were stable for the length of the study, as demonstrated by the minimal mean changes in each of these variables from the beginning to the end of each experiment: 0.9 mm Hg, 2.8 nanoEq $[H^+]$ and 2.1 mm Hg, respectively. The hemodynamic status of the dogs during ambient air breathing and severe acute hypoxia is described in Table I and is similar to that previously described (13, 14). No consistent effect of acute hypoxia on cardiac output, systemic arterial pressure and systemic vascular resistance was seen while pulmonary hypertension developed in each instance. There was no correlation between the magnitude of the changes in conversion of AI induced by acute hypoxia and the magnitude of the hypoxic changes in pulmonary arterial pressure and pulmonary vascular resistance. Gross examination and light microscopy of the lungs showed no abnormalities.

Discussion. This study demonstrates that conversion of AI *in vivo* is substantially reduced under acute alveolar hypoxia. Additional support demonstrating that substrate conversion by converting enzyme is decreased by acute hypoxia has been derived from experiments using radioimmunoassay for bradykinin in this laboratory (15, 16). There is convincing evidence that AI conversion and bradykinin degradation are functions of the same converting enzyme (17). In both *in vivo* using a similar dog preparation to that described in the present study (15) and *in vitro* using cultured endothelial cells from human umbilical cord (16), a marked de-

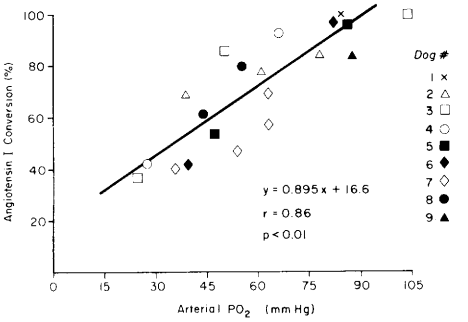

FIG. 2. Decrease in angiotensin I conversion during acute alveolar hypoxia in closed chest anesthetized dogs ($n = 9$). Each dog is represented by an individual symbol.

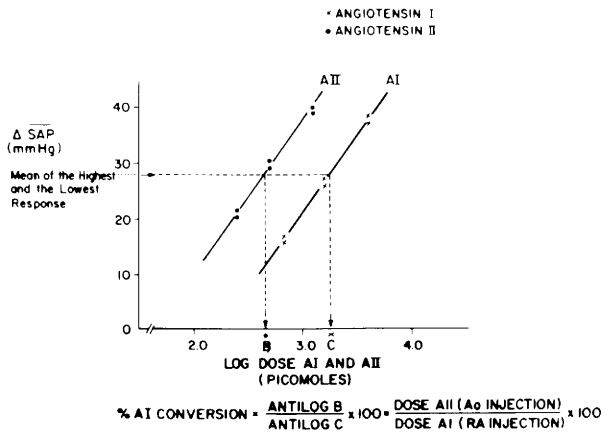

FIG. 1. Method of determination of angiotensin I conversion using a systemic blood pressure response technique. AI = angiotensin I; AII = angiotensin II; $\Delta \overline{SAP}$ = blood pressure response to angiotensin II = peak change in mean systemic arterial pressure; dots = responses to angiotensin II bolus injections; solid line = regression line for the angiotensin II responses; open circles = responses to angiotensin I bolus injections; dashed line = regression line for the angiotensin I responses; Ao = aorta; RA = right atrium. Interpolation (→) of equipotent pressor doses of angiotensin I (C) and II (B) was done at the mean of the lowest and the highest responses to angiotensin I or II.

TABLE I.[a]

Number of dogs	PaO_2 (mm Hg)	$\dot{Q}$ (ml/min/kg)	$\overline{PAP}$ (mm Hg)	PVR (mm Hg/l/min)	SAP (mm Hg)	SVR (mm Hg/l/min)
7	87 ± 9	131 ± 48	18 ± 5	12 ± 6	146 ± 16	122 ± 32
4	32 ± 7	171 ± 78	36 ± 4**	20 ± 5*	141 ± 27	91 ± 32

[a] Hemodynamic status of anesthetized, mechanically ventilated dogs under ambient air and severe acute hypoxia. PaO_2 = arterial oxygen tension; $\dot{Q}$ = cardiac output; $\overline{PAP}$ = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance; $\overline{SAP}$ = mean systemic arterial pressure; SVR = systemic vascular resistance. Values are mean ± SD. * $P < 0.05$; ** $P < 0.01$.

crease in substrate degradation for bradykinin was produced by acute hypoxia.

There are several possible mechanisms to explain these observations. Fanburg and Glazier have shown that the percentage conversion of AI in isolated perfused dog lung is independent of the amount of AI administered and the perfusate transit time when pulmonary converting enzyme is not saturated (18). At most, we administered 500-fold less AI than has been shown to saturate the enzyme. The absence of correlation between the decrease in conversion with hypoxia and either the percent change in pulmonary arterial pressure or pulmonary vascular resistance suggests that hypoxia-induced hemodynamic factors are not directly responsible for the decrease in converting enzyme activity during hypoxia. Hypoxia could have a direct effect on enzyme kinetics by decreasing the affinity of converting enzyme for AI or the velocity of the enzymatic reaction; this seems unlikely to have any influence on the conversion of AI *in vivo* because of the large excess of converting enzyme in the lung. However, hypoxia might induce intrapulmonary fixation of AI or AII, stimulate alternate pathways for their degradation, or produce competitive inhibitors of converting enzyme. One appealing hypothesis is an alteration of the structural configuration of the surface of the endothelial cells by acute hypoxia. The caveolae intracellulares of the endothelial cells represent a specialized adaptation of the pulmonary endothelial surface which vastly increases the amount of converting enzyme in contact with the vascular luminal surface (19); a reduction of their size or number in response to acute hypoxia would eliminate this increased surface and permit streaming of substrate past the enzyme. The rapid reversibility of the phenomenon which we observed upon return to ambient air excludes the possibility of profound structural changes in the lung.

Despite the marked effect of alveolar hypoxia to decrease conversion of AI to AII no significant effect could be demonstrated on systemic blood pressure or cardiac output in these experiments.

Summary. Conversion of AI to AII and inactivation of bradykinin are regulated by converting enzyme. In this study, conversion of AI *in vivo* is decreased during acute alveolar hypoxia. At a PaO_2 of 30 mm Hg, conversion of AI is decreased to one half control values. The decrease in AI conversion could not be related to hemodynamic factors in the pulmonary vasculature induced by hypoxia. It may be related to the effects of hypoxia on endothelial cell function or conformation.

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