Impaired Angiotensin Conversion and Bradykinin Clearance in Experimental Canine Pulmonary Emphysema

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ABSTRACT Chronic hypoxic lung diseases are associated with abnormal blood pressure regulation. Because the lung is the principal site of angiotensin conversion and because hypoxia decreases converting enzyme activity, we examined whether angiotensin converting enzyme activity was impaired in lung disease. 12 dogs received a 6 wk course of aerosolized and intratracheal papain that produced moderate panlobular emphysema. These dogs and 24 control dogs were anesthetized and sampling catheters were placed under fluoroscopic control. Angiotensin conversion was measured by a blood pressure response bioassay. Pulmonary converting enzyme activity was also assessed by infusing bradykinin (BK) and using radioimmunoassay to measure the instantaneous clearance of BK and the concentration of BK in the pulmonary artery which first produced spillover of BK into left atrial blood. Angiotensin conversion was reduced in the emphysematous dogs to 81.1% (13.2 SD) from 92% (6 SD) in the control dogs ($P < 0.01$). Instantaneous clearance of BK in the emphysematous dogs was only slightly reduced (93%), despite reduction in their Pa_{O_2} to 75 mm Hg, indicating that the greatest proportion of the perfused vascular bed was exposed to alveolar P_{O_2} of >90 mm Hg. However, the barrier to BK passage provided by the lung, and measured by the spillover level, was reduced ¼ to ½ that observed in control animals. That the defect was promptly corrected by supplemental oxygen indicates that regional pulmonary vascular converting enzyme activity had been impaired by regional alveolar hypoxia, which permitted some peptide to pass through the lungs unmetabolized. Determination of peptide metabolism in the lungs may provide a useful measure of regional alveolar hypoxia and may lead to new ways of assessing lung injury.

INTRODUCTION

Several observations indicate that either hypoxia or lung disease can interfere with physiologic responses to hypotensive stress, and may decrease resting blood pressure as well. The studies of Heistad and co-workers (1) established that acute hypoxia impairs reflex vasoconstrictor responses in normal men. When stressed with lower body negative pressure, men exposed to hypoxic gas mixtures for up to 36 h had impaired reflex vasoconstriction that was promptly restored by return to normoxia (2). In men with lung disease sufficient to reduce arterial P_{O_2} below 60 Torr, the same defects were observed and were reversed when supplemental oxygen was provided (3). Cohn and Luria (4) found that men with pulmonary emphysema failed to demonstrate increased systemic vascular resistance in response to shock, regardless of the precipitating event. Anderson et al. (5) found that the resting blood pressures of 30 men with mild to severe emphysema were significantly lower than controls. Other studies suggest that the defect is not exclusive for emphysema, but extends to other lung disease. In patients with cystic fibrosis, whether mild or severe, Lieberman and Rodbard (6) observed lower blood pressure; these patients also had a lower pressor response to exercise. The underlying mechanisms contributing to all of these conditions remain unknown, but they raise the possibility of an interplay between disease of the lung parenchyma and control of systemic blood pressure in the whole organism.

We have shown that acute hypoxia reduces the activity of angiotensin converting enzyme (7–9). The

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pressor response to injected angiotensin I, as well as pulmonary bradykinin clearance, was reduced in hypoxic animals. We speculated that injury to the pulmonary vascular bed, occurring as a consequence of the development of pulmonary emphysema, could interfere with converting enzyme activity and blood pressure regulation in two ways. First, enzyme activity could be reduced because of reduced vascular surface in the injured lung. Second, regional hypoxia within the lung could yield regional impairment in converting enzyme activity. To address these questions we studied angiotensin converting enzyme activity in a canine model of experimentally induced pulmonary emphysema.

METHODS

Experimental canine pulmonary emphysema. The method used to induce experimental pulmonary emphysema derives from two methods (10, 11). Papain powder, 300,000 U/mg (Sterling Winthrop Labs., Rensselaer, N. Y.), was reconstituted in normal saline to a 16% solution. 12 dogs received 2.5 ml by nebulized aerosol four times with a 1-wk interval between each nebulization. Following this, 2.3 mg/kg papain, diluted in 3 ml of normal saline, was installed intratracheally. Each animal was studied 5–6 d after this installation, i.e., ~6 wk after the beginning of the papain treatment. After the studies described below, the lungs were then removed for gross examination, light microscopy, determination of the average distance between alveolar walls (mean linear intercept), and biochemical analysis of elastin content. Mean linear intercept was calculated on lungs fixed with 10% formalin at a transpulmonary pressure of 20 cm, using the method of Thurlbeck (12). Lung tissue taken for analysis of elastic content was processed as described (13), to isolate the elastin fraction from crude connective tissue. Desmosine and isodesmosine, specific amino acids of elastin, were used as markers. Elastin content in lung tissue was determined by comparing desmosine and isodesmosine concentrations in the elastin fraction with concentrations found in the crude connective tissue. Elastin content was expressed as percent elastin in the crude connective tissue fraction of lung parenchyma.

Physiologic preparation. Animals weighing an average of 11.3 kg (2.3 SD) were anesthetized with intravenous pentobarbital (25–30 mg/kg) and maintained with periodic injections throughout each experiment (average dose 2 mg/kg per h). The dogs were paralyzed with succinylcholine (priming dose 0.1 mg/kg, followed by constant infusion of 1.2 mg/kg per h) for complete relaxation of the ventilatory muscles, and ventilated in an oblique lateral decubitus position through a cuffed endotracheal tube on a respiration pump (model no. 607, Harvard Apparatus Co., Inc., S. Natick, Mass.). Approximately every 10 min the lungs were hyperinflated to 15 cm water pressure by applying positive expiratory pressure for five breaths. Blood gases and acid-base status were monitored with pH, P_{CO_2}, and P_{O_2} electrodes (Radiometer, Copenhagen, Denmark). We placed catheters under fluoroscopic control in the right atrium, pulmonary artery, left atrium, and ascending aorta. The tip of the aortic catheter was located ~3–4 cm distal to the aortic valve. Mean pressures were monitored with Statham transducers (P23b, Statham Instrument Co., Oxnard, Calif.) and recorded continuously on a direct writing polygraph (model 7, Grass Instrument Co., Quincey, Mass.). We determined cardiac output by thermodilution (5F KMA thermodilution catheter and KMA cardiac output computer, model 3500, Kimray Medical Assoc., Oklahoma City, Okla.). Pulmonary vascular resistance and systemic vascular resistance were determined from duplicate values of cardiac output and vascular pressures measured simultaneously. Pulmonary vascular resistance was calculated as mean pulmonary arterial pressure − mean left atrial pressure ÷ cardiac output; systemic vascular resistance was calculated as mean systemic arterial pressure − mean right atrial pressure ÷ cardiac output. The infusion rates of normal saline and lactated Ringer's solutions were adjusted so that they would not exceed calculated fluid requirements (100 ml/kg per 24 h), including catheter flushes and thermodilution boluses.

Assessing angiotensin conversion. In vivo conversion of angiotensin I (AI)[1] was studied in six emphysematous adult beagle dogs using a systemic blood pressure response technique (14, 15) that we have previously described (7, 16). $Ileu^5$-AI and $Ileu^5$-AII (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) were reconstituted in distilled water containing 0.1% heat-inactivated lysozyme (Mallinckrodt, Inc., St. Louis, Mo.). Stock solutions were stored in plastic containers at −70°C; the day of the study they were thawed at room temperature and fresh dilutions were made.

Bolus injections of AI in the right atrium and of AII in the aorta were given at random. A dose-response curve was obtained for each peptide in each period of determination of AI conversion. The blood pressure response to AI and AII was measured as the peak change in mean systemic arterial pressure. Subsequent boluses were given only when all the circulatory variables had returned to within 10% of base-line steady-state conditions. Doses of peptides ranged from 500 to 2,000 ng of AI or AII. The log dose-response relationships for AI and AII were found to be linear in all conditions studied; therefore a regression analysis was used to standardize the way of drawing the log dose-response lines. The mean of the lowest and the highest response to either AI or AII was taken as reference and used to determine the equipotent pressor response; equipotent pressor doses of AI and AII were determined by interpolation (16). Conversion of AI was calculated in molar equivalents and expressed as percent of AI converted into AII. AI conversion was usually achieved within 45 min. In six dogs, conversion of AI was determined under normoxia, and during exposure to moderate ($n = 6$) or severe ($n = 3$) hypoxic gas mixtures (from 21 to 8% O_2, balance N_2). The sequence of exposure to normoxia and hypoxia was reversed several times. As controls, nine healthy beagles were studied under normoxic and moderate hypoxic conditions; four of these were also studied under conditions of severe hypoxia. Although it was difficult to manage anesthesia in the emphysematous dogs, the determination of angiotension conversion was accomplished in approximately the same amount of time and using the same range of concentrations of AI and AII as with normal beagles. This discounts the possibility of altered sensitivity of the emphysematous dogs to the pressor effects of angiotensin. Additional control data were taken from 15 other normal dogs studied under normoxic conditions (16).

Assessing bradykinin degradation. Pulmonary converting enzyme activity was assessed in six dogs with emphysema by measuring bradykinin concentrations in simultaneously drawn pulmonary arteries and left atrial blood specimens. The dogs were anesthetized and catheterized as described above. The methods used to assess converting enzyme activity by the bradykinin infusion technique have been described in detail (8). In brief, dilutions of bradykinin (Peninsula Laboratories,

[1] *Abbreviations used in this paper*: AI, angiotensin; BK, bradykinin; LA, left atrium; PA, pulmonary artery.

San Carlos, Calif.) were made from frozen stock solutions and continuously infused into the femoral vein at rates from 0.5 to 5.0 μg/kg per min. The dogs were ventilated with 40, 10, and 8% O_2, balance N_2, and samples drawn at 2, 4, 6, 10, 20, and 30 min after beginning each gas mixture.

Bradykinin was extracted from blood using our modifications (8) of the method of Mashford and Roberts (17). Blood was drawn into syringes containing kallikrein and kininase inhibitors and the bradykinin was immediately extracted into ethanol. Bradykinin was assayed using the radioimmunoassay methods of Goodfriend and Odya (18) that we modified (19). The standard deviation of replicates from 10 standard curves was 0.24%, the sensitivity of the assay is 20 pg/ml, and the coefficient of variation of 25 samples assayed in different runs was 7.20% (0.95 SD). The antiserum that we used cross-reacted fully with active forms of kinin (kallidin and methionyllysyl-bradykinin) but <0.1% with the [des-Arg^9] bradykinin or [des-Phe^8-Arg^9] bradykinin metabolites. When blood was handled in this manner, the lowest concentration of bradykinin detectable in the catheterized dog described above was 0.11 ng/ml (0.005 SD).

Pulmonary converting enzyme activity was assessed by calculating the instantaneous clearance of bradykinin (BK) across the lung (8).

$$\frac{[BK]_{PA} - [BK]_{LA}}{[BK]_{PA}} \times 100 = \text{instantaneous clearance across the pulmonary circulation.}$$

The "spillover level" of BK (8) was also determined. This is the concentration in the pulmonary artery, $[BK]_{PA}$, which was first associated with a significant rise in the concentration of BK in the left atrium $[BK]_{LA}$, i.e., 0.15 ng/ml or greater. This was done by plotting the simultaneous values of $[BK]_{PA}$ and $[BK]_{LA}$ at varying time intervals after infusing BK into the femoral vein and extrapolating the curves back to the time when the $[BK]_{LA}$ equaled 0.15 ng/ml (3 SD above the lower limit of detectable BK in this preparation by this method). The spillover level reflects the capacity of the lung to clear elevated BK concentrations in venous blood, and is a measure of the barrier to the passage of BK provided by the lung. Note that the spillover level is defined in terms of the pulmonary artery concentration of BK that must be achieved before the amount of peptide escaping pulmonary degradation reaches a level in the left atrium sufficient to be detected by radioimmunoassay. Thus, a high spillover level implies an effective barrier to the passage of pulmonary artery BK through the lung, whereas a low or reduced spillover level implies impaired barrier function, i.e., only a low concentration is necessary to exceed the pulmonary barrier.

The dogs studied by the BK-infusion technique were lightly anesthetized (15–20 mg/kg pentobarbital) and did not receive succinylcholine. To assess the effect of reduced vascular surface area on converting enzyme activity, apart from hypoxia, three dogs were studied after 1 h of assisted ventilation with 40% O_2. They were also studied while ventilation was assisted with hypoxia gas (8 and 10% O_2). The remaining three dogs were studied with spontaneous ventilation, breathing room air and subsequently 40% O_2.

RESULTS

Experimental canine pulmonary emphysema. In the emphysematous dogs, we found a nonuniform destruction of lung parenchyma predominantly in the ventral caudal lobes. The appearance of these lungs is compared with normal lungs in Fig. 1. Alveolar mean linear intercepts were determined in 14 lobes from 6 normal lungs and in 10 lobes of 6 emphysematous lungs. The results were 7.97×10^{-2} mm (±1.12) and 12.80×10^{-2} mm (±3.67) in the normal and the emphysematous lungs, respectively ($P < 0.001$). Elastin contents of lungs from three normal and five emphysematous animals are shown in Table I. Mean elastin

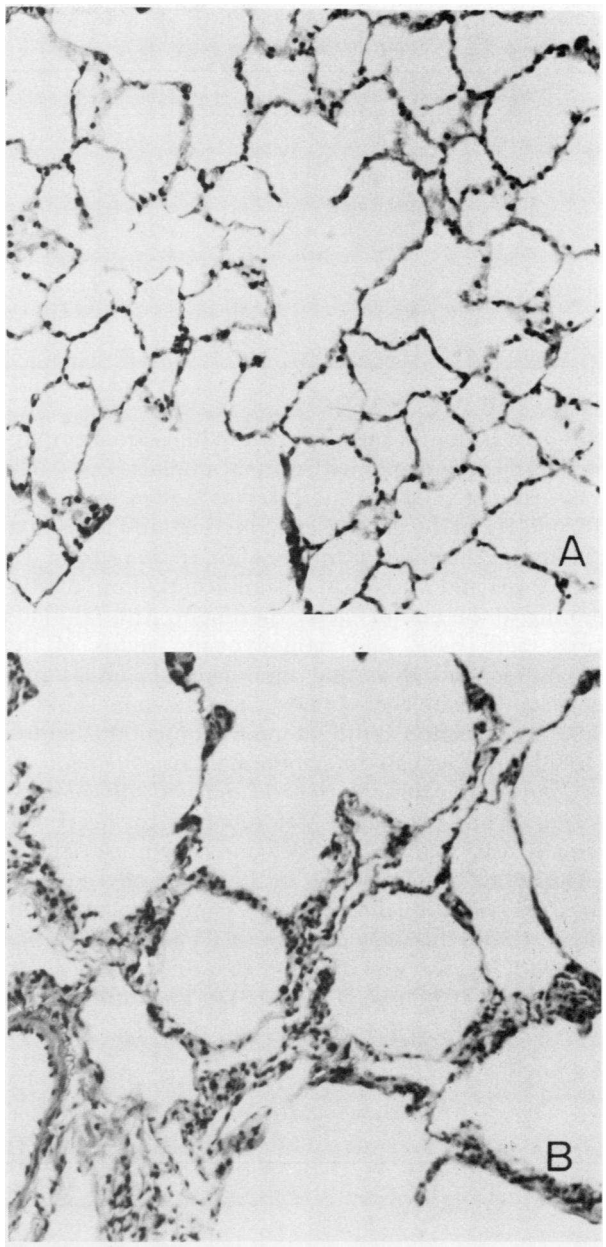

FIGURE 1 Hematoxylin-eosin stain of normal (A) and emphysematous (B) lungs (×250). Both sections came from the anteriorbasal segment of the left lower lobe. The parenchymal destruction shown (B) is a representative sample of moderate emphysema.

TABLE I
Elastin Content

Normal dogs		Emphysematous dogs	
	%		%
1	16.26	1	7.50
3	10.97	2	3.23
4	18.47	3	4.39
		5	4.29
		6	11.95
Mean	15.24	Mean	6.27
SD	3.86	SD	3.55

content was significantly reduced in lungs from emphysematous as compared with normal animals ($P < 0.02$).

By varying F_{IO_2}, arterial P_{O_2} was shifted from 24 to 102 mm Hg in the emphysematous dogs and from 24 to 104 mm Hg in the control dogs. In neither group did arterial pH and P_{CO_2} change significantly from the beginning to the end of each experiment. Arterial blood gases, acid base, and hemodynamic status of the emphysematous dogs and their controls under normoxia and severe acute hypoxia are shown in Table II. Gross examination and light microscopy of the control normal lungs revealed no abnormalities.

AI conversion under normoxia and acute alveolar hypoxia. When ventilated with room air, mean AI conversion was 81.0% (13.2 SD) and mean arterial P_{O_2} was 92.6 mm Hg (10.7 SD) in the six emphysematous dogs studied with the blood pressure response technique. Under the same conditions, 24 normal dogs had a mean AI conversion of 92.0% (6.0 SD) and mean arterial P_{O_2} of 101.8 mm Hg (16.0 SD). The difference in AI conversion between emphysematous and normal dogs breathing room air was statistically significant ($P < 0.01$). Difference in mean P_{aO_2} was not statistically significant ($P = 0.16$). In the emphysematous dogs studied while breathing room air, AI conversion tended to be lowest in the animals with the lowest P_{aO_2}.

The results of AI conversion studies of each emphysematous dog under room air and hypoxic conditions are shown in Table III with their corresponding arterial P_{O_2}. Exposure to acute alveolar hypoxia decreased AI conversion in all emphysematous dogs. Fig. 2 compares the decrease in AI conversion with hypoxia observed in the six emphysematous dogs and in the nine normal control dogs. The effect of alveolar hypoxia on AI conversion was determined in each animal of both groups. Comparison of the mean slopes by *t* test showed no statistically significant intergroup difference, and indicated that angiotensin conversion was decreased by hypoxia to the same extent in both normal and emphysematous lungs (Fig. 2).

BK degradation in emphysematous dogs. The first three emphysematous dogs studied with this technique were studied after being ventilated with 40% O_2 for periods of up to 1 h. There were no differences between the clearance of BK across the lungs of these dogs and the normal dogs that we have previously described (18). The spillover levels were also similar to those seen in normal dogs, i.e., these lungs cleared BK from venous blood until an elevation in concentration of 5 to 6 ng/ml in the PA was achieved. At this point an elevation in [BK]LA concentration was first detected. The decline in BK clearance and the progressively lower spillover level with decreasing P_{O_2} were also similar to these values in normal dogs. These

TABLE II
Acid-Base and Hemodynamic Status of Normal and Emphysematous Dogs during Study of AI Conversion under Normoxia and Severe Acute Hypoxia

	Normal dogs		Emphysematous dogs	
	Normoxia	Severe hypoxia	Normoxia	Severe hypoxia
n	6	4	6	3
pHa	7.39 (7.36–7.66)	7.37 (7.29–7.45)	7.38 (7.34–7.42)	7.42 (7.42–7.46)
P_{aCO_2}, *mm Hg*	31.9 (6.3)	33.5 (8.7)	31.1 (5.9)	26.7 (3.8)
P_{aO_2}, *mm Hg*	86.8 (8.9)	31.5* (6.6)	91.3 (11.1)	35.0* (9.8)
$\dot{Q}$, *ml/min per kg*	130.1 (48.2)	170.7 (78.5)	133.7 (9.3)	160.4 (27.7)
$\overline{PAP}$, *mm Hg*	15.9 (2.9)	35.9* (4.2)	15.4 (3.5)	25.3 (11.2)
PVR, *mm Hg/liter per min*	9.5 (2.7)	19.7‡ (5.0)	10.0 (1.7)	12.7 (2.9)
$\overline{SAP}$, *mm Hg*	146.0 (16.2)	140.8 (26.8)	143.2 (24.3)	124.2 (2.9)
SVR, *mm Hg/liter per min*	122.4 (32.2)	91.2 (31.8)	110.6 (29.8)	70.1 (21.3)

n, number of dogs; pHa, arterial pH; P_{aCO_2}, arterial P_{CO_2}; P_{aO_2}, arterial P_{O_2}; $\dot{Q}$, cardiac output; $\overline{PAP}$, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; $\overline{SAP}$, mean systemic arterial pressure; SVR, systemic vascular resistance. pH values are calculated pH for mean $[H^+] \pm SD$; all other values are means ± SD (shown in parentheses).
* $P < 0.01$.
‡ $P < 0.02$.

TABLE III
Conversion of AI to AII in Six Emphysematous Dogs

Dog	PaO_2	AI conversion
	mm Hg	%
1	74	63.6*
	54	32.7
2	49	63.8
	55	66.4
	55	54.5
	82	71.6*
3	78	91.4
	98	100.0*
	38	51.7
4	102	69.9*
	24	47.0
	51	47.4
5	92	87.1*
	82	49
6	100	81.7*
	71	72.6
	43	37.2
	100	93.3*

Dogs were studied under normoxia and acute alveolar hypoxia and listed in the order in which the studies were performed.
* Value obtained during room air breathing.

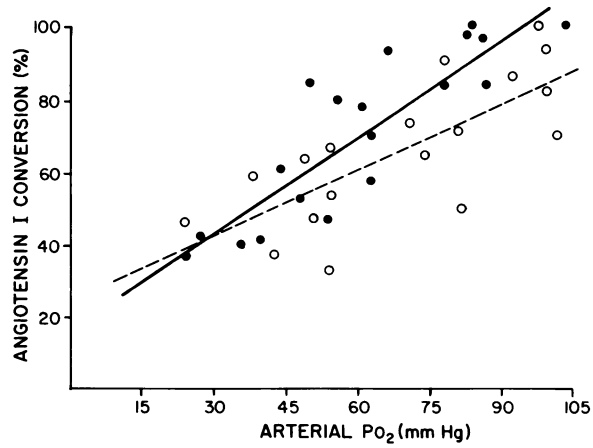

FIGURE 2 In vivo conversion of AI in normal and emphysematous dogs under normoxia and acute alveolar hypoxia. Filled circles, % AI conversion in normal dogs (n = 9); open circles, % AI conversion in emphysematous dogs (n = 6). Each circle represents a single determination of AI conversion. Dashed line, regression line for the AI conversion in the emphysematous dogs ($y = 0.60x + 25.10$, $r = 0.74$, $P < 0.01$); t test comparison of the mean slopes of the decrease in AI conversion with hypoxia among the normal and the emphysematous dogs shows no statistically significant intergroup difference and indicates that the mechanisms responsible for the modulation of converting enzyme activity by hypoxia are intact in both the normal and emphysematous lungs.

data are displayed in Fig. 3 (emphysema, assisted ventilation group).

The second group of three emphysematous dogs was studied during spontaneous ventilation of room air, and subsequently with supplemental oxygen. A strikingly different response to infused BK was immediately apparent during room air breathing. In contrast to the first group, which demonstrated widened pulse pressure but had minimal hypotension when BK was infused at this rate, these animals developed prompt severe hypotension and unstable blood pressure (Fig. 4A). These physiologic changes occurred with spillover of BK. Representative infusion experiments, from which clearance and spillover data were taken, are depicted in Fig. 5A and are contrasted with data taken from normal control dogs being ventilated with enough hypoxic gas to reduce PaO_2 into the same range as the lightly anesthetized spontaneously breathing emphysematous dogs. In dogs with normal lungs in this study (Fig. 5, top panel) and in previous studies (8), PaO_2 values in the range of 70 mm Hg are associated with instantaneous clearance values of 60%. The instantaneous clearance in the emphysematous animals (92–94%) was nearly the same as in normal dogs with PaO_2 values above 90 mm Hg, even though PaO_2 in the emphysematous animals was only 72–73 mm Hg. More striking, as shown, the spillover values in the emphysematous dogs were reduced to final levels, one half to one quarter of those seen in the normal dogs at the same PaO_2 (Fig. 3, spontaneous ventilation). The spillover values approximated those seen in normal dogs made hypoxic in the range of 40–50 mm Hg. Dogs with normal lungs made hypoxic to 70 mm Hg have instantaneous clear-

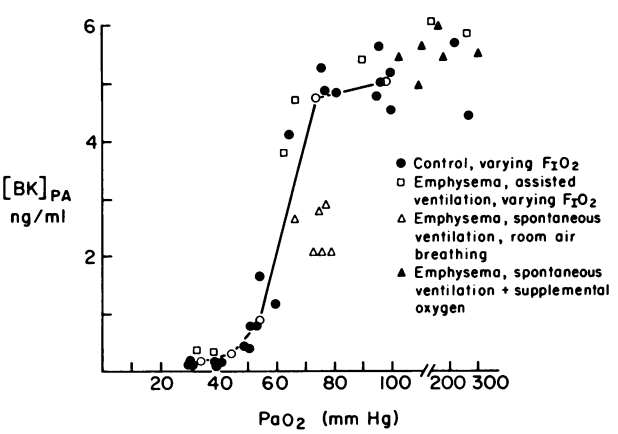

FIGURE 3 BK spillover levels in normal and emphysematous dogs at various levels of P_{O_2} showing the return to normal of spillover levels when oxygen is administered (see text).

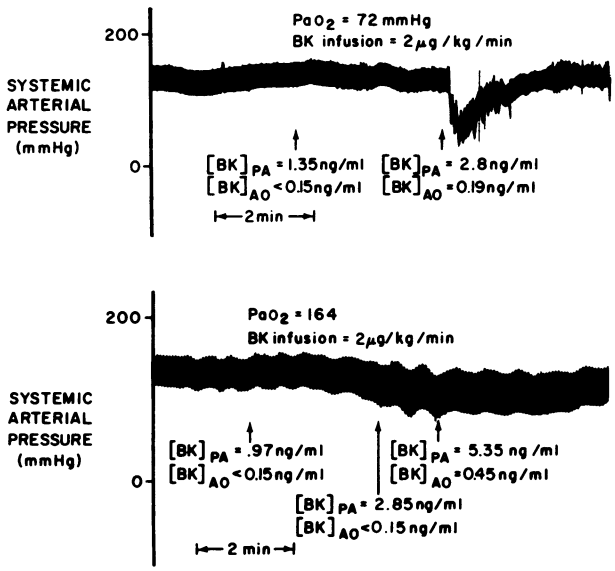

FIGURE 4 (A, above) The blood pressure response to infused BK (2 μg/kg per min) in an emphysematous dog spontaneously breathing room air. The instantaneous clearance of BK is 93%, within the range seen in animals that have a Pa_{O_2} of >90 mm Hg, even though this animal has moderate hypoxia because of emphysema. The PA and AO (aortic) concentrations of BK (determined by radioimmunoassay) before and after the blood-pressure decline are indicated. When hypotension occurs, a significantly elevated $[BK]_{AO}$ is detected, indicating that spillover has occurred when the $[BK]_{PA}$ is only 2.8 ng/ml, a low spillover level. (B, below) The blood pressure response to infused BK in the same dog after spontaneous ventilation with 40% O_2. The abrupt decline in blood pressure seen previously is prevented; instead, widening of the pulse pressure and slight hypotension are seen. The low spillover level seen previously has been fully corrected by oxygen administration.

ance values of 60% and spillover levels of 4 ng/ml (8), whereas the dogs with pulmonary emphysema demonstrated significant deviation from these values. We reasoned that regional impairment in converting enzyme had developed because of regional alveolar hypoxia arising in areas of the lung with low ventilation to perfusion ratios. Higher instantaneous clearance and lower spillover levels than those seen in dogs with normal lungs (made hypoxic to 70 mm Hg) suggested that two compartments developed in the diseased lungs: (*a*) a large area of normal alveolar P_{O_2}, maintaining instantaneous clearance at 92%, and (*b*) a smaller area of low alveolar P_{O_2}, through which AI and BK pass unmetabolized. We conjectured that these reduced the pressor response to angiotensin and calculated spillover level for BK.

We tested this notion by supplying the same animals with enriched oxygen mixtures. As shown in Fig. 5B, the defect in the spillover level was fully corrected by oxygen administration. Also, a slight increase in instantaneous clearance of BK was found. In addition to the correction of the biochemical defect, the oxygen-supplemented dogs did not demonstrate the transient hypotension in response to infused BK (Fig. 4B).

The spillover levels in three types of experiments in emphysematous dogs are summarized in Fig. 3. These data are compared with spillover data taken from 24 studies of 15 normal dogs, some of which we have previously published (8). In emphysematous dogs, ventilated with supplemental oxygen mixtures, regional hypoxia was corrected; spillover and clearance values were similar to those of normal dogs. Emphysematous dogs ventilated with hypoxic gas mixtures also had the same clearance and spillover levels as normal dogs exposed to hypoxic gas mixtures. Spontaneously breathing emphysematous dogs had lower spillover values (while demonstrating clearance values near the normal range) that, as we showed in Fig. 4B, were corrected by oxygen administration.

DISCUSSION

The lung endothelial surface processes vasoactive substances that affect blood pressure (20). In previous studies, we asked whether this lung function could be influenced by physiologic states to meet the physiologic needs of the body. We demonstrated that one example of this lung function, angiotensin converting enzyme activity, is modulated by oxygen tension (7–9). Converting enzyme metabolizes the peptides of two plasma-protease systems with directly opposite effects on blood pressure. BK, the biologically active peptide of the kallikrein-kinin system, is a powerful hypotensive agent. AII is equally potent as a vasopressor peptide. When kinin was infused into hypoxic dogs with normal lungs, we found that systemic arterial vasodilation was more than offset by increased cardiac output, so that hypotension did not occur (8, 21). The increased cardiac output, however, presumably augments nutrient and O_2 delivery to peripheral tissues, and hence may be beneficial. We and others (21, 22) have also found a prompt decline in arterial AII concentrations in acutely hypoxic animals. These declines are associated with decreased systemic vascular resistance, having the effect of reducing left ventricular afterload. From these studies, we concluded that oxygen modulation of converting enzyme activity is a physiologic response to hypoxia, serving to adjust the circulating arterial concentrations and local tissue concentration of AII and BK with secondary effects on cardiac output, vascular resistance, and distribution of blood flow (21).

The present studies are directed to a different question. Can injury to the lung, with losses of vascular surface and development of inhomogeneous distribution of ventilation and perfusion, interfere with the handling of vasoactive substances by the lung, and thereby contribute to some of the pathophysiology of

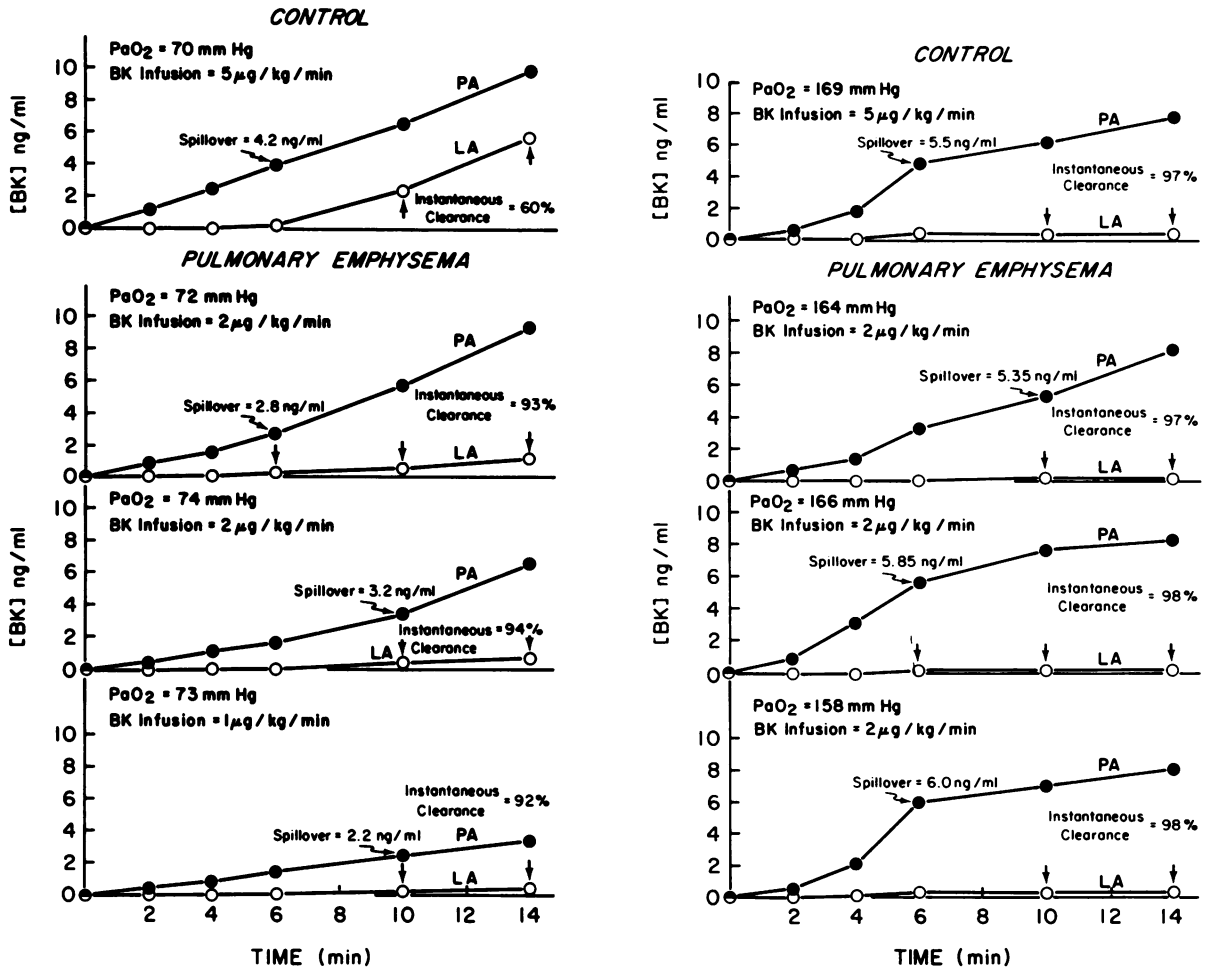

FIGURE 5 (A, left) The comparison of spillover levels and instantaneous clearance of BK in a normal control dog, breathing a hypoxic-inspired gas mixture, and three dogs with papain-induced emphysema, breathing room air. A lower inspired-oxygen mixture was administered to the control dog to produce the same levels of PaO_2 as in the spontaneously breathing emphysematous animals. Spillover occurs at much lower mixed venous concentrations of BK in animals with emphysema, clearance is only slightly reduced. (B, right) Comparison of spillover levels and instantaneous clearance of BK in the control dog and three dogs with papain-induced emphysema shown in Fig. 5A. Alveolar and arterial P_{O_2} were elevated by ventilating all animals with enriched gas mixtures. Increasing the alveolar and arterial P_{O_2} in control and emphysematous animals corrected the defect in spillover and increased the clearance of BK.

the disease? In the emphysematous dog, we found that AI conversion, in response to bolus intravenous injection, was impaired, and that BK was able to escape the barrier to its passage that was normally posed by the lung. In contrast to the hypoxic dogs with normal lungs (which appear to benefit from reduced AII and increased BK concentrations as discussed above), the emphysematous dogs became hypotensive when BK was infused, suggesting that the physiologically appropriate and selective modulation of angiotensin converting enzyme activity by oxygen in normal lungs is lost when regional alveolar hypoxia develops in diseased lungs. Even so, converting enzyme activity is still modulated by oxygen in diseased lungs (Fig. 2), indicating that the disease process did not interfere with the responsiveness of endothelial cell-bound converting enzyme to hypoxia. The selective loss of converting enzyme activity in diseased hypoxic areas of lung could reduce the efficiency of the renin-angiotensin system, and expose the animal to hypotensive levels of BK if kallikrein were activated.

Several studies have suggested that lung disease can affect blood pressure by impairing reflex responses to hypotension, lowering systemic blood pressure, and reducing pressor responses to exercise (1–6). The present studies do not establish that impaired converting en-

zyme activity alone is responsible for these defects; it is not possible from these data to correlate the extent of lung injury to the degree of abnormality in blood pressure regulation. Instead, several studies of vasoactive mediators indicate that many known mediator systems interact to produce net circulatory effects especially in pathophysiologic states. In this regard, circulating vasoactive substances such as catecholamines, BK, and AII may exert their effect in part through the production of "second messenger" tissue mediators such as prostaglandins (23) and cyclic nucleotides (24) in target organs. Changing the concentrations of AII and BK can be expected to initiate altered concentrations of other mediator substances; the observed circulatory effects result from a new balance among these various mediators. Acute hypoxia stimulates the release of catecholamines from the adrenal medulla (25) and affects the balance between the parasympathetic and sympathetic limbs of the peripheral autonomic nervous system (26). In hypoxic states there are multiple points of interaction between the kallikrein-kinin and renin-angiotensin systems and autonomic cardiovascular control. Increased epinephrine stimulates renin release (27) and activates kallikrein, thereby leading to increased kinin production (28). In turn, angiotensin has been shown to (*a*) stimulate central vasomotor neurons, (*b*) further increase adrenal medullary catecholamine secretion, and (*c*) to act at the nerve ending to potentiate responses to stimulation of sympathetic nerves (29, 30). Thus, interference with angiotensin converting enzyme activity can be expected to have secondary effects on autonomic responses to hypoxia and on the tissue production of second messenger mediators. In addition, the pulmonary endothelial bed clears, synthesizes, and releases several other types of vasoactive substances (20) that affect blood pressure; the present study did not examine these other systems. It is likely that injury to the lung may affect other pulmonary endocrine functions which affect blood pressure and this remains an important area for further investigation.

These studies suggest that the measurements of pulmonary converting enzyme activity may provide a useful tool for assessing the functional state of the pulmonary vascular bed and the distribution of ventilation. Total lung enzyme activity (as measured by the instantaneous clearance of BK) was higher than expected in the emphysematous dogs since their PaO_2 values were 70–74 mm Hg. Even mild hypoxia of this degree has been shown to reduce BK clearance by dog lungs (8) and specific converting enzyme activity of cultured endothelial cells (9). This suggests that in the greatest proportion of the vascular bed the enzyme was exposed to oxygen tensions above 90 mm Hg. Additionally, spillover levels appeared to provide a sensitive index of lung damage when it was sufficient to produce regional alveolar hypoxia. In the emphysematous dogs, net pulmonary angiotensin conversion and BK degradation result from converting enzyme activity in relatively normoxic areas plus decreased activity in more hypoxic areas. If low spillover values result from the passage of unmetabolized BK through hypoxic areas of the lung, then similarly, AI will pass through these same areas without being converted to AII, with a corresponding drop in the systemic arterial pressor response to prepulmonary injections of AI. In this regard, it is helpful to visualize the effect of angiotensin converting enzyme as resembling a net which captures 96% of the particles presented to it. A small hole in the net will permit small numbers of particles to pass through without grossly impairing the calculated efficiency of the net. In these experiments, the particles that leak through have marked effects on blood pressure. Thus, although the total pulmonary efficiency is essentially intact (reflected in instantaneous clearance of 93%), the diseased lungs perform as if "holes" have developed that permit both AI and BK to pass through (reflected in the low pressor response to injected AI and low BK spillover values). The holes appear to be created in the areas of lung in which converting enzyme is exposed to alveolar hypoxia and are presumably the result of inhomogeneously distributed ventilation, a pathophysiologic hallmark of pulmonary emphysema. Although regional alveolar hypoxia producing regional impairment in angiotensin conversion and BK degradation is a reasonable explanation because supplemental oxygen corrected the deficits, we cannot measure the size or extent of the hypoxic areas using our present methods. Since these biochemical abnormalities result in abnormal blood pressure regulation, our studies suggest that one useful way of following progressive lung injury may be the sequential determination of resting blood pressure, orthostatic changes in blood pressure, and the pressor response to mild exercise.

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