Pulmonary Capillary Endothelium-Bound Angiotensin-Converting Enzyme Activity in Humans

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Background—Pulmonary endothelium has metabolic functions including the conversion of angiotensin I to angiotensin II by angiotensin-converting ectoenzyme (ACE). In this study, we have validated an indicator-dilution technique that provides estimations of dynamically perfused capillary surface area (DPCSA) in humans, and we have characterized pulmonary endothelial ACE in vivo.

Methods and Results—In 12 adults, single-pass transpulmonary (one or both lungs) hydrolysis of the specific ACE substrate \(^\text{3H}-\text{benzoyl-Phe-Ala-Pro} (\text{3H-BPAP})\) was measured and expressed as % metabolism (%M) and \(v = -\ln(1-M)\). We also calculated \(A_{\text{max}}/K_m\), an index of DPCSA. %M (70.1±3.2 vs 67.9±3.1) and v (1.29±0.14 vs 1.20±0.12) were similar in both lungs and the right lung, respectively, whereas \(A_{\text{max}}/K_m/\text{body surface area}\) decreased from 2460±193 to 1318±115 mL/min per square meter.

Conclusions—Pulmonary endothelial ACE activity can be assessed in humans at the bedside by means of indicator-dilution techniques. Our data suggest homogeneous pulmonary capillary ACE concentrations and capillary transit times (\(t_c\)) in both human lungs, and similar \(t_c\) within the normal range of cardiac index. \(A_{\text{max}}/K_m\) in the right lung is 54% of total \(A_{\text{max}}/K_m\) in both lungs, suggesting that \(A_{\text{max}}/K_m\) is a reliable and quantifiable index of DPCSA in humans.

Key Words: lung ■ endothelium ■ circulation ■ angiotensin ■ enzymes ■ hypertension, pulmonary

The pulmonary vascular endothelium participates in important physiological and pharmacokinetic processes, including synthesis and degradation of vasoactive peptides.\(^1,2\) Ectoenzymes responsible for these functions are located on the luminal surface of the endothelium.\(^3\) Because these enzymes are directly accessible to blood-borne substrates, their activities may be measured in vivo by means of indicator-dilution techniques.\(^4-6\) In humans, endothelium-bound angiotensin-converting enzyme (ACE; kininase II; EC 3.4.15.1) is responsible for the conversion of angiotensin I to angiotensin II and for bradykinin inactivation and is thus a major regulator of blood pressure in health and disease.\(^3\)

Pulmonary endothelial ACE activity may be assessed in vivo by monitoring the hydrolysis of a synthetic substrate in plasma during a single passage through the lungs.\(^3\) Unlike natural angiotensin I and bradykinin, synthetic substrates of ACE do not alter vessel tone and are not metabolized by other endogenous peptidases and when hydrolyzed by ACE yield products that are easily separated from the parent compound by organic extraction.\(^6\) The first and most widely used ACE substrate to date is the highly specific tripeptide benzoyl-Phe-Ala-Pro (BPAP).\(^6\)

ACE molecules are uniformly distributed along the luminal pulmonary endothelial surface, including the membrane caveolae,\(^3\) suggesting that in addition to its biological activity, pulmonary capillary endothelium-bound ACE (PCEB-ACE) may be a useful indicator of endothelial function and allow estimation of the perfused capillary surface area under physiological conditions.\(^2,5,7\) Moreover, PCEB-ACE dysfunction is an early and sensitive index of lung vascular injury.\(^8,9\) Thus monitoring of PCEB-ACE activity in humans might be used to estimate changes in perfused capillary surface area, to quantify the degree of endothelial dysfunction, and as a biochemical marker to identify early vascular lung injury.

In this study, we have for the first time validated the PCEB-ACE indicator-dilution technique at the bedside with human subjects and correlated it with pulmonary hemodynamics to identify patterns of PCEB-ACE activity under normal lung conditions and obtain insights into human pulmonary endothelial physiology and pharmacology.
Methods

Subjects

Twelve volunteers, 9 men and 3 women (age 34 to 79 years, body surface area [BSA] 1.5 to 2.1 m²), without pulmonary disease or heart failure and undergoing cardiac catheterization for coronary artery disease assessment, participated in a protocol approved by the Research Ethics Committee. Written informed consent was obtained. They were not receiving ACE inhibitor treatment. No patient had an anatomic shunt or patent foramen ovale detectable by 2-dimensional Doppler echocardiography by use of agitated saline as a contrast agent.

Femoral venous and femoral arterial sheaths were inserted as part of the routine cardiac catheterization procedure. A 5-lumen 8F thermodilution catheter (Abbott) was advanced into the pulmonary artery. Cardiac output was measured by the thermodilution technique.

For estimation of the single-pass transpulmonary hydrolysis of 3H-BPAP, all patients received 2 rapid bolus injections through the pulmonary artery catheter, 10 minutes apart. In injection A (both lungs), the bolus was injected through the proximal port of the catheter to the distal end of the catheter had passed into the right pulmonary artery and was carried with the blood flow into the entire vascular bed of one lung, as confirmed by angiographic dye injection and fluoroscopy. In all but 1 subject (No. 5), the distal end of the catheter had passed into the right pulmonary artery. The order of injections was randomly selected before the beginning of each patient study. Cardiac output (CO) was determined immediately before each injection. Blood flow to one lung was estimated as 53% of the corresponding CO when the injection was made into the right pulmonary artery (all subjects except subject 5, see Table) and as 47% of the corresponding CO when the injection was made into the left pulmonary artery.10

To validate our method and to provide a basis for comparison, 9 other patients with precapillary pulmonary hypertension (primary pulmonary hypertension, 2; collagen disease, 3; anorexigen-related, 1; repaired atrial septal defect, 1; sarcoidosis, 2; age range 22 to 68 years, mean pulmonary artery pressure 47.2±13.5 mm Hg) were also studied. These patients had a single injection of substrate into the right atrium, distributed to both lungs.

Determination of Pulmonary Endothelial ACE Activity

One milliliter of a solution of 3H-BPAP (30 μCi/1.2 mL 0.9% saline; 22.2 Ci/mmol) was used for each injection, followed by a 5-mL saline flush. Simultaneously, arterial blood was withdrawn through the femoral artery sheath (40 mL/min, Coleman Instruments peristaltic pump) into a fraction collector (Gilson), advancing at 1 tube per 1.2 seconds (0.8 mL blood/tube, 39 tubes). The tubes contained 1.75 mL of normal saline with 5 mmol/L EDTA and 6.8 mmol/L 8-hydroxyquinoline 5-sulfonic acid to prevent further activity of blood ACE and heparin 1000 IU/L. After centrifugation (3000 rpm, 10 minutes), 0.5 mL of the supernatant was transferred into a scintillation vial containing 5 mL Ecolite, and total 3H radioactivity was measured (H total). For the determination of the radioactivity associated with metabolites, another 0.5 mL of the supernatant was transferred into a scintillation vial containing 2.5 mL HCl (0.12N). After addition of 3 mL of 0.4% Omnifluor in toluene and mixing by inversion, the radioactivity (3H toluene) was measured after 48 hours of undisturbed equilibration in the dark. With this technique, ~60% of the 3H-BPAP metabolite 3H-Benzoyl-Phe (3H-BPhe, f_p) and <10% of the parent 3H-BPAP (f_s) were extracted in the organic phase of the mixture (ie, toluene). The precise values were calculated by identically processing separate standard tubes containing substrate or previously synthesized product.

After correcting for background radioactivity, radioactivity from the product 3H-BPhe was calculated as

\[ 3H-BPhe = (f_t - f_s) \times V \times \Delta t \]

where \( f_t \) and \( f_s \) are the fractional extractions of substrate (3H-BPAP) and product (3H-BPhe), respectively, into the toluene layer.

Calculations of ACE Activity Parameters

Under first-order reaction conditions ([S] « K_m), the Henri-Michaelis-Menten equation becomes

\[ -d[S]/dt = V = (V_{max}/K_m) \times [S] \]

with \( K_m \), \( V \), and \( V_{max} \) being the Michaelis-Menten constant, the reaction velocity, and maximal velocity, respectively. Because of the minimal contribution of both the extracapillary endothelium-bound and the plasma soluble ACE to substrate conversion, the reaction time is in practical terms equal to \( t_c \) (ie, capillary transit time). By integrating from time 0 to \( t_c \) and from \( [S]_0 \) (ie, initial substrate concentration) to \( [S]_t \) (ie, concentration at time \( t \)), the reaction rate becomes

\[ \int_0^{t_c} V \, dt = \int_0^{t_c} (V_{max}/K_m) \times [S] \, dt \]

\[ V \, t_c = (V_{max}/K_m) \times \int_0^{t_c} [S] \, dt \]

\[ V \, t_c = (V_{max}/K_m) \times \int_0^{t_c} [S]_0 \, dt \]

\[ V \, t_c = (V_{max}/K_m) \times [S]_0 \cdot t_c \]

\[ V \, t_c = (V_{max}/K_m) \times [S]_0 \cdot t_c \]

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Data were further analyzed with the use of the integrated Henri-F一步一步和方程5 7,13 to incorporate the applied Equation 5 7,13 to incorporate the

where $t$ and # 

(10) $Q_{3H} = F_p \times \bar{t}$

where $t$ calculations were corrected for catheter transit time.

Statistics
Data are presented as mean±SEM. To compare means and individual values, the paired $t$ test, Student’s $t$ test, and Pearson $r$ test were used where appropriate. Correlations were determined with the use of least-squares linear regression. A value of $P<0.05$ was considered significant.

Results
All subjects (Table) had atherosclerotic coronary artery disease with the exception of subject 2, who had a normal coronary angiogram. Cardiac indexes (CI), arterial blood pH, $PCO_2$, and $PO_2$ values were within the normal range.

Representative arterial outflow concentration curves of total-$^3$H and the surviving $^3$H-BPAP after a single passage through both lungs and the corresponding hydrolysis ($v$) of $^3$H-BPAP by ACE are presented in Figure 1. Sample-to-sample substrate hydrolysis ($v$) fluctuates slightly around 1.47, denoting that in this particular individual, $\approx 77\%$ of the injected BPAP was metabolized to BPhe.

ACE Activity in One Versus Both Lungs
No differences were observed between injections A (both lungs) and B (one lung) in CO ($5.73\pm0.28$ vs $6.21\pm0.34$ L/min, respectively) or $F_p$ ($3.62\pm0.20$ vs $3.91\pm0.22$ L/min). There were no differences (Figure 2) in either $^3$H-BPAP %M ($70.1\pm3.2\%$ vs $67.9\pm3.1\%$) or $v$ ($1.29\pm0.14$ vs $1.20\pm0.12$), whereas a 46% reduction occurred in $A_{max}/K_m$ when $^3$H-BPAP was injected into the right lung ($2449\pm249$ mL/min) versus both lungs ($4564\pm425$, $P<0.01$). An identical 46% difference was observed between the right lung and both lungs when $A_{max}/K_m$ values were normalized to the BSA of each subject ($2460\pm193$ vs $1318\pm115$ mL/min per square meter, $P<0.01$).

ACE Activity and Hemodynamics in Both Lungs
As $A_{max}/K_m$ (Figure 3) increased from $1639$ to $3793$ mL/min per square meter, $^3$H-BPAP %M increased linearly from $57.9\%$ to $91.7\%$, with a strong positive correlation between the two parameters ($r=0.89$, $P<0.01$).

$^3$H-BPAP %M and $v$ (Figure 4) correlated inversely with mean pulmonary artery pressure (mPAP) ($r=0.68$, $P<0.05$ and $r=0.644$, $P<0.05$, respectively), whereas $A_{max}/K_m$/BSA was not related to mPAP ($r=0.42$, $P=NS$). In addition, $^3$H-BPAP transpulmonary hydrolysis expressed as %M and $v$, as well as $A_{max}/K_m$/BSA, were independent of both CI and pulmonary vascular resistance index (PVRI). The independence of $A_{max}/K_m$/BSA versus mPAP, CI, and PVRI was maintained even when $A_{max}/K_m$ was not normalized to BSA (data not shown). Similar patterns were observed in the aforementioned relations when the enzyme activity parameters were plotted against PVRI instead of PVRI (data not shown).

There was a trend toward a negative linear relation between transit time and CI (Figure 5, $r=0.51$). There was no correlation between $Q_{3H}$ and CI ($r=0.17$).

Patients with Pulmonary Hypertension
In the pulmonary hypertension patient group (Figure 6), mean values for %M ($37\%\pm9\%$), $v$ ($0.47\pm0.13$), and $A_{max}/K_m$/BSA
(641±297) were markedly reduced as compared with the 12 subjects with normal pulmonary arterial pressures (P<0.01 in all cases).

**Discussion**

In this study, pulmonary endothelium–bound ACE activity and mass have been estimated in vivo and correlated with pulmonary hemodynamic parameters in the normal human lung. We also present data from patients with pulmonary hypertension for comparison purposes. ACE function was assessed with indicator-dilution techniques and study of the transpulmonary utilization of the synthetic ACE substrate BPAP. PCEB-ACE dysfunction has been an early and sensitive finding in animal models of lung injury,8,9 and, under physiological conditions, PCEB-ACE assays estimate enzyme mass5 and perfused vascular surface area.7,15 Although ACE is present on all endothelial cells, microvessels, principally capillaries, are responsible for the great majority of single-pass transpulmonary hydrolysis,9 whereas the contribution of plasma ACE is minimal.9 Thus in our studies, measurement of pulmonary ACE activity represents in practical terms PCEB-ACE activity. ACE inhibitors inhibit endothelium-bound ACE,16 and our subjects were carefully questioned about ACE inhibitor usage. Binding of BPAP to serum albumen would also affect the assay.17 However, BPAP binding by albumen does not necessarily predict similar binding by native serum proteins, and serum protein binding of BPAP does not appear to be a factor limiting BPAP hydrolysis in vivo.18

The sample-to-sample variability in BPAP hydrolysis (Figure 1) reflects ACE activity within individual groups of capillaries and may be influenced by substrate transit time, enzyme concentration, and the enzyme kinetic constants, as in Equation 5. The pattern observed suggests the presence of mild heterogeneity in capillary transit times, since capillary enzyme concentrations and ACE catalytic properties should remain unchanged.

The lack of difference in 3 H-BPAP hydrolysis or %M between one and both lungs (Figure 2) indicates homogeneous PCEB-ACE activity between the two lungs. The relatively wide range of individual values may be explained in part by greater population heterogeneity in humans than the average, typically inbred, animal group. PCEB-ACE activity was independent of age (data not shown). Our patients had coronary atherosclerosis, and this might have contributed to the variability, since endothelial dysfunction, an early feature of atherosclerosis, might also affect the lung circulation. Hyperlipidemia might also contribute.19 However, there was no overt evidence of pulmonary vascular disease as assessed by hemodynamics. Subject 2 in our study had angiographically normal coronary arteries, yet her data were similar to the rest of the group. Furthermore, PCEB-
ACE activity in healthy lung donors and patients with lung carcinoma, conditions in which pulmonary endothelial dysfunction is less likely, is similar to that of our coronary disease group. Thus as in other studies, our “normal” population with coronary disease presents a standard to be used in future studies of patients with lung dysfunction. By comparison, our patients with pulmonary vascular disease had markedly reduced PCEB-ACE activity.

$^3$H-BPAP %M and $v$ were independent of CI, consistent with similar capillary transit times among individuals within the normal range of pulmonary blood flow at rest. $^3$H-BPAP hydrolysis, expressed as %M and $v$, decreased with increasing mPAP values. This negative correlation might be related to subtle endothelial dysfunction from the atherosclerosis in our population, manifested by a slight rise in PAP and causing mild decreases in PCEB-ACE activity. The mPAP reflects systolic characteristics of the large pulmonary arteries and diastolic microvascular resistance, representing the pressure characteristics of the entire lung circulation. There was no relation of %M with PVRI. However, PVRI is a derived value (mPAP − PAWP [mean pulmonary artery wedge pressure])/CI. Because $^3$H-BPAP metabolism was independent of CI, it would also be independent of the calculated PVRI regardless of any relation between %M and mPAP.

$A_{\text{max}}/K_m$ has been used as an index of PCEB-ACE mass and consequently dynamically perfused capillary surface area (DPCSA). This assumption is based on Equation 7: when $k_{\text{cat}}$ and $K_m$ remain constant, changes in $A_{\text{max}}/K_m$ reflect changes in E (ie, enzyme mass). For enzymes evenly distributed along the luminal endothelial surface, such as ACE, changes in E should reflect changes in DPCSA. Under normal conditions, $k_{\text{cat}}$ and $K_m$ are not affected by changes in flow and do remain constant. $A_{\text{max}}/K_m$ is an index of DPCSA only (ie, capillaries through which blood is flowing allowing substrate-enzyme interaction) and not of capillaries that are nonperfused or transiently filled with “stagnant” blood.

$A_{\text{max}}/K_m$ and $A_{\text{max}}/K_m$/BSA were reduced by 46% when $^3$H-BPAP was injected into the right lung instead of both lungs (Figure 2). No change in CO, which could contribute to this decrease, was noted between the two injections. There

Figure 4. Relations of mPAP (left), CI (center), and PVRI (right) with BPAP %M (top) and with $v$ (middle) and plot of mPAP with $A_{\text{max}}/K_m$/BSA (bottom) in the 12 study subjects. Lines denote significant linear relation. $r$, Pearson $r$ value.

Figure 5. Top, Plot of $^3$H transit time with CI. Bottom, Plot of volume of $^3$H distribution with CI. Symbols as in Figure 4.
was an almost identical pattern between $A_{\text{max}}/K_m$ values obtained in the right lung, being 54% of those obtained in both lungs, and the corresponding fraction of lung perfusion reported in supine humans (53% to the right lung and 47% to the left).\textsuperscript{10} $A_{\text{max}}/K_m$ thus appears to be a sensitive and quantifiable DPCSA index in humans, as it is in other animals. We used $A_{\text{max}}/K_m$/BSA because, unlike experimental animal groups, human populations have great variability in BSA and consequently in lung capillary surface area. Normalizing $A_{\text{max}}/K_m$ to BSA reveals the homogeneity of the human population (Figure 2), allowing better comparisons of different human groups in future studies. $A_{\text{max}}/K_m$/BSA has been shown to be a sensitive index of DPCSA changes induced by cardiac output elevations in humans.\textsuperscript{24}

It has been suggested that $A_{\text{max}}/K_m$ cannot be used as a quantitative index of perfused capillary surface area because of the presence of the nonreactive cis isomer BPAP fraction.\textsuperscript{15,25} This fraction was estimated to be \approx 15% in buffer solution,\textsuperscript{26} whereas in vivo it appears to represent 7% of all BPAP,\textsuperscript{13} probably because of the presence of natural isomerases in plasma. However, studies done with different substrates concurrently injected, or correcting for the nonreactive fraction, confirm that $A_{\text{max}}/K_m$ is a quantifiable index of perfused lung capillaries.\textsuperscript{5,7,13}

Assessing PCEB-ACE activity in humans with pulmonary vascular pathologies, such as acute lung injury–adult respiratory distress syndrome or pulmonary hypertension, may help distinguish between abnormalities secondary to endothelial dysfunction per se and decreased pulmonary vascular surface area. If endothelial dysfunction is related either to decreased enzyme mass and consequently decreased enzyme concentrations or to kinetic constant alteration, then substrate hydrolysis would be altered (Equation 5). In such a case, $A_{\text{max}}/K_m$ should be viewed as an index of functional capillary surface area, related to both enzyme quantity and functional integrity. The data from our 9 patients with precapillary pulmonary hypertension show decreased substrate hydrolysis and $A_{\text{max}}/K_m$, suggesting some combination of endothelial dysfunction and loss of DPCSA. If, on the other hand, loss of DPCSA occurs with neither endothelial dysfunction nor changes in capillary transit times, substrate hydrolysis would remain unchanged, whereas $A_{\text{max}}/K_m$ would decrease, since the enzyme mass available for reaction would be decreased (Equation 7). Dupuis et al\textsuperscript{27,28} have provided evidence that the DPCSA available for ACE substrate reaction and norepinephrine and serotonin uptake increases during exercise in dogs in a parallel fashion, which confirms the consistency and validity of the techniques and demonstrates capillary recruitment. Similar studies should be performed in exercising normal humans. Moreover, a failure to increase DPCSA during exercise might prove to be an early and subtle marker of pulmonary vascular disease.

Mean $^3$H transit time is an approximation of mean lung transit time because it also includes the time needed for the blood to travel from the left atrium to the tip of the femoral arterial sheath. Similar considerations apply to the volume of $^3$H distribution. The trend toward a negative correlation among $t$ and CI (Figure 5) confirms that the transpulmonary passage of the probe tends to be quicker when CI is higher. The fact that the CI-related decreases in $t$ are combined with unchanged $^3$H-BPAP %M and $v$ suggests that higher cardiac outputs are accommodated through recruitment of unperfused capillaries with similar $t_c$. The absence of correlation among $Q_{3H}$ and CI may relate to differences in lung blood volume and subsequently $Q_{3H}$ being blunted by BSA normalizations within the range of normal resting pulmonary blood flows. In fact, when $Q_{3H}$ is plotted against CO, the pattern becomes more linear (data not shown) Additional factors may be the differences in hematocrit and the plasma volume inside the aorta.

In summary, our studies demonstrate that pulmonary endothelial ACE activity may be assessed in humans at the bedside by means of indicator-dilution techniques and that it provides a reliable and quantifiable index of DPCSA. Our data establish the metabolic activity values for humans without overt pulmonary vascular disease. Studies performed with other markers, such as the clearance of prostaglandin $E_1$, norepinephrine, propranolol, serotonin, and endothelin-1, have contributed greatly to the understanding of normal pulmonary endothelial function and of endothelial dysfunction in various pulmonary vascular diseases.\textsuperscript{4,22,23,27,28} With the use of the techniques we describe, the assessment of PCEB-ACE activity in these and other disease states will further our understanding of pulmonary vascular metabolism and pathophysiology.
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References
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