Altered angiotensin I conversion in pulmonary disease

SUZANNE OPARIL, J. LOW AND T. J. KOERNER
Department of Medicine, Pritzker School of Medicine, University of Chicago, Chicago, Illinois, U.S.A.

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Summary
1. A specific method is described for the measurement of angiotensin I converting enzyme activity in plasma with 125I-labelled angiotensin I used as substrate.
2. Converting enzyme activity in plasma from fifteen normal subjects, eleven patients with sarcoidosis, twelve patients with chronic obstructive pulmonary disease and three patients with shock lung was assayed by this technique.
3. Patients with sarcoidosis had increased plasma converting enzyme activity whether or not they were receiving steroid therapy.
4. Patients with chronic obstructive pulmonary disease and shock lung had decreased plasma converting enzyme activity, but extent of conversion did not correlate with the severity of the lung disease.
5. Converting enzyme activity in normal plasma could be completely inhibited by addition of exogenous angiotensin I in 0.5–2.5 × 10^-7 times physiological concentration. Twice as much exogenous angiotensin I was needed to inhibit conversion completely in plasma from patients with sarcoidosis; one tenth as much in chronic obstructive pulmonary disease. These results indicate that plasma has a high capacity for angiotensin I conversion even in patients with pulmonary parenchymal disease.
6. Results suggest that plasma converting enzyme activity may be a reflection of pulmonary conversion and can be altered by pulmonary disease.
7. Measurement of plasma converting enzyme activity may be useful in studies designed to characterize the regulatory role of converting enzyme in the renin-angiotensin system and in cardiovascular homeostasis.

Key words: angiotensin, converting enzyme, pulmonary disease, renin, sarcoidosis.

Introduction
Since large amounts of angiotensin I converting enzyme are found in plasma and in a number of organs and since angiotensin I infused in supra-physiological concentration is rapidly converted into angiotensin II in vivo, the conversion step has not generally been regarded as rate-limiting for angiotensin II production. Accordingly, relatively little attention has been given to the regulation of converting enzyme activity in vivo. Recent studies by non-specific analytical techniques have suggested that converting enzyme activity can be altered in the normal animal by changes in oxygenation (Molteni, Zakheim, Mullis & Mattioli, 1974) and in the isolated perfused dog lung by changes in pulmonary haemodynamics (Fanburg & Glazier, 1973). In addition, abnormalities in plasma converting enzyme activity have been reported in patients with sarcoidosis and chronic lung disease of other aetiologies (Lieberman, 1975; Oparil, Low & Koerner, 1975; Silverstein, Friedland, Lyons & Kitt, 1975b). To test the hypothesis that plasma converting enzyme activity is altered in patients with sarcoidosis and other kinds of lung disease and therefore that plasma conversion may parallel
intrapulmonary conversion, specific methods were
developed for quantifying converting enzyme activity
in human plasma with angiotensin I used as substrate.
Plasma from patients with sarcoidosis, chronic
obstructive pulmonary disease or 'shock' lung and
plasma from normal volunteer subjects was assayed for
converting enzyme activity.

Methods
Samples of blood (10 ml) were drawn from the
antecubital veins into iced glass tubes containing
heparin (143 USP units). The plasma was separated
within minutes at 4°C by centrifugation. A portion
(1 ml) of each plasma sample was diluted (1:3:5)
with Tris/acetate buffer (0.1 mol/l, pH 7.4) con-
taining NaCl (0.1 mol/l). Because converting enzyme
has a higher affinity than angiotensinases for the
substrate angiotensin, dilution of plasma favours
conversion over breakdown. 251-labelled angiotensin
I (1
ml) was added to 4.5 ml of
diluted plasma. The mixture was incubated at 37°C
and aliquots (250 µl) were removed at 0, 2.5, 5,
7.5, 10, 15 and 30 min. A portion (50 µl) was im-
mediately spotted on Whatman 3MM paper and
subjected to electrophoresis (Savant Co., Hicksville,
N.Y., U.S.A.) at 4000 V for 1 h in pyridine/acetate
buffer, pH 3.55, for identification and quantification
of angiotensin I, angiotensin I1 and their metabolites.
The paper was dried, cut into 1 cm strips, and the
radioactivity was counted in a gamma counter
immediately spotted on Whatman 3MM paper and
subjected to electrophoresis (Savant Co., Hicksville,
N.Y., U.S.A.) at 4000 V for 1 h in pyridine/acetate
buffer, pH 3.55, for identification and quantification
of angiotensin I, angiotensin I1 and their metabolites.
The remaining 200 µl was treated with 8-hydroxy-
quinoline (3.4 mmol/l final concentration) to
inhibit plasma converting enzyme and angio-
tensinases. These samples were subjected to column
chromatography as an independent means of
identifying and quantifying the angiotensins and
their metabolic products. The stored samples were
thawed and passed through a 0.9 cm x 100 cm
column of DEAE-Sephadex A-25, which was
developed with sodium phosphate buffer (0.1
mol/l, pH 7.4) at room temperature. A sample
(1 ml) of each 3 ml fraction of column eluate was
counted for radioactivity.
Generated angiotensin II was distinguished from
angiotensin I and from the products of other
circulating enzymes by comparison with standard
peptides, which had been prepared by enzymatic
digestion of 125I-labelled angiotensin I and 125I-
labelled angiotensin II. The mono-iodinated
angiotensins were incubated with trypsin,
chymotrypsin or leucine aminopeptidase (Worth-
ington Biochemical Corp., Freehold, N.J.,
U.S.A.) under conditions in which the reactions
went to completion (Oparil, Koerner, Tregear,
Barnes & Haber, 1973). Peptide fragments of known
sequence generated by these procedures were
subjected to high-voltage paper electrophoresis and
column chromatography on DEAE-Sephadex. When
analysed by both techniques, all of the small
peptide fragments occupied positions distinguishable
from one another and from angiotensin I and II.
The only fragments encountered after incubation of
human plasma were the labelled product of chymo-
tryptic digestion, which presumably consisted of the
(I-4) sequence, and mono-iodinated tyrosine.
The effect of substrate inhibition was assessed by
adding graded amounts of unlabelled angiotensin
I to tracer quantities of 125I-labelled angiotensin
I and incubating with diluted plasma, as described
above. Converting enzyme was considered to be
completely inhibited by substrate when no conversion
of 125I-labelled angiotensin I was detected over a 5
min incubation period. Plasma samples from one
normal subject, one patient with chronic obstructive
lung disease and two patients with sarcoidosis were
examined.

Blood samples from a total of fifteen normal
subjects (nine male and six female), eleven patients
with sarcoidosis (seven male and four female),
twelve patients with chronic obstructive lung disease
(six male and six female) and three male patients with
'shock' lung were assayed for converting enzyme
activity. Converting enzyme activity was assessed by
computing percentage conversion [(AII/AI
+ AII) x 100], for each time-point and plotting percentage
conversion against time.

Sarcoidosis was diagnosed from a typical chest
X-ray picture (bilateral reticular infiltrates with or
without hilar adenopathy) in the absence of evidence
for tuberculous or fungal infection by skin test and
culture. No patient had evidence of pulmonary or
extrapulmonary malignancy. The arterial Po2 of
all patients with sarcoidosis was >9.5 kPa (>70
mmHg) on room air. Pulmonary function tests were
normal or showed only mild restrictive abnormalities.
In seven of eleven cases, non-caseating granulomata
were found on liver or lymph node biopsy. Of the
four patients from whom biopsies were not obtained,
one had uveitis and skin lesions typical of sarcoidosis.
and three had shown resolution of pulmonary infiltrates and hilar adenopathy with steroid therapy. The Kveim test was not used because of the difficulty in obtaining high-quality antigen and because of the high incidence of false negatives.

Chronic obstructive lung disease was diagnosed on clinical grounds and by pulmonary function testing. All patients had a chronic cough and progressive exertional dyspnoea without evidence of cardiac disease. Chest X-ray films showed no evidence of infiltration, mass lesions or hilar lymph adenopathy. All patients had moderately severe to severe obstructive abnormalities on pulmonary function tests. Forced vital capacity in litres (FVC, l) ranged from 1.31 to 2.90 l (mean 2.03 l), a range of 35–81% of predicted (mean 53.1% of predicted). The forced expiratory volume in 1 s, expressed in litres (FEV₁,₀), a measure of upper airway resistance, ranged from 0.60 to 2.12 l (mean 1.30 l), a range of 19–70% of predicted (mean 65.4% of predicted). The FEV₁,₀/FVC ratio, an index of airway obstruction, ranged from 0.41 to 0.79 (mean 0.65) (values <0.80 are considered compatible with obstructive pulmonary disease). Mid-maximal expiratory flow, expressed in litres/s, a measure of lower airway resistance, ranged from 0.32 to 2.09 l/s (mean 1.03 l/s), a range of 9–70% of predicted (mean 36.4% of predicted). The arterial PO₂ of all patients with chronic lung disease was <7 kPa (<50 mmHg) on room air.

The patients with 'shock' lung had all developed progressive intrapulmonary arteriovenous shunting after a period of hypotension and decreased cardiac output. None was able to maintain an arterial PO₂ >8 kPa (>60 mmHg) even with assisted ventilation via an endotracheal tube; none survived.

Statistical analyses were performed by comparing results of incubation of normal and patient plasma for each time of sampling by the unpaired Student's t-test. Results for grouped data are presented as the mean values ± SEM.

Results

Results from a representative experiment with a normal subject, a sarcoidosis patient and a patient with chronic lung disease are summarized in Fig. 1. Conversion of angiotensin I into II was both more extensive and more rapid in the sarcoidosis patient than normal; conversion was less extensive and slower in the patient with chronic obstructive lung disease than normal. The rate of generation of tyrosine and the (1–4) peptide, metabolic products of angiotensin I and II, appeared to be greater in the sarcoidosis patient than in the normal or in the patient with chronic lung disease. In all individuals, the inactive metabolites accounted for a substantial proportion (>25%) of total radioactivity counts only after 10 min of incubation. Results obtained with column chromatography on DEAE-Sephadex A-25 confirmed those obtained with paper electrophoresis.

The data for normal subjects and patients with sarcoidosis or chronic obstructive lung disease studied are in Fig. 2. Mean percentage conversion for patients with sarcoidosis was significantly (P<0.01 to <0.001) greater than normal at all time-points except at 0 and 30; mean percentage conversion for patients with chronic obstructive lung disease was significantly less than normal only at 2.5 (P<0.005) and 7.5 (P<0.05) min. Steroid treatment of sarcoidosis did not appear to alter plasma converting enzyme activity. Both treated and untreated patients are included in Fig. 2. The rate of conversion was more variable in chronic obstructive lung disease than in either sarcoidosis or the normal population. Mean percentage conversions at 7.5, 10 and 15 min for patients with chronic obstructive lung disease were 36 ± 5% (SEM), 48 ± 7% and 65 ± 9% respectively; for patients with sarcoidosis, 71 ± 3%, 86 ± 2% and 98 ± 1%; for normal subjects, 48 ± 3%, 58 ± 2% and 72 ± 2%. The greater variability in the obstructive lung disease group may be attributed to heterogeneity in aetiology and severity of the lung disease. The three patients with shock lung had less converting enzyme activity than either normal subjects or patients with chronic lung disease. Mean percentage conversions at 7.5, 10 and 15 min were 22 ± 7%, 31 ± 5% and 29 ± 6%. These are significantly (P<0.001) below normal for each time-point.

Table I summarizes the results of experiments in which graded amounts of unlabelled angiotensin I were added to the incubation mixture in order to assess the capacity of the system for conversion. The patient with obstructive lung disease required less (50 nmol/ml vs. 300 nmol/ml) of angiotensin I than the normal subject to inhibit conversion completely; the two sarcoidosis patients required twice as much unlabelled angiotensin I as the normal subject. These results indicate that the plasma from sarcoidosis patients has an increased
capacity for conversion of angiotensin I into II and that the plasma from patients with obstructive lung disease has a decreased capacity for conversion. In normal plasma the rate of conversion of $^{125}$I-labelled angiotensin I was completely inhibited by unlabelled angiotensin I only at concentrations $10^{-25} \times 10^6$ times those achieved physiologically (assuming a normal circulating angiotensin I concentration of $10^{-90}$ fmol/ml; Walle, 1973). The very high capacity of plasma converting enzyme can be attributed to a high concentration of enzyme in plasma, to rapid turnover of angiotensin I at the active site of the enzyme or to both. The present experiments do not allow us to distinguish between the three possibilities.

Discussion

Several reports have suggested that alterations in angiotensin I conversion occur in pulmonary disease and when abnormalities in pulmonary function are experimentally induced. Molteni et al. (1974) showed that serum and lung converting enzyme activity (as assessed by the spectrophotometric method of Cushman & Cheung, 1971) and renal renin content were elevated in mice within 2 weeks of
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FIG. 2. Angiotensin I (AI) conversion into angiotensin II (AII) in treated plasma. Conversion (\%) refers to [(AII/AI + AII) x 100]. Symbols represent mean values for each group; bars represent ± 1 SEM. ●, Normal subjects (n = 15); ○, sarcoidosis patients (n = 11); Δ, patients with chronic obstructive lung disease (n = 12).

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Angiotensin I added (pmol)</th>
<th>Conversion (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Normal</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Sarcoid with steroids</td>
<td>85</td>
<td>—</td>
</tr>
<tr>
<td>Sarcoid without steroids</td>
<td>59</td>
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exposure to hypoxia in a hypobaric chamber. Manipulation of pulmonary vascular surface area and mean transit time through the pulmonary capillary bed by changing pulmonary venous pressure altered conversion of injected angiotensin I in the isolated perfused dog lung (Fanburg & Glazier, 1973). Conversion increased with prolongation of transit time, which caused a lengthening of exposure of substrate to enzyme. These observations are compatible with the hypothesis that alterations in pulmonary function may have regulatory effects on the renin-angiotensin system through the activity of the angiotensin I converting enzyme.

Depressed plasma converting enzyme activity has been reported in patients with several kinds of pulmonary disease, including chronic obstructive pulmonary disease, tuberculosis, carcinoma and cystic fibrosis (Lieberman, 1974; Oparil, Low & Koerner, 1975). The observed depression of con-
converting enzyme activity did not correlate with the severity of the lung disease. In contrast, converting enzyme activity was elevated in plasma (Lieberman, 1975; Oparil, Low & Koerner, 1975; Silverstein, Friedland, Lyons & Kitt, 1975b) and lymph node extracts (Silverstein, Friedland, Lyons & Gourin, 1975a) of patients with sarcoidosis. These observations suggest that plasma converting enzyme activity may be a reflection of the concentration and/or activity of the pulmonary converting enzyme.

Further evidence that converting enzyme activity is increased in sarcoidosis and depressed in chronic obstructive pulmonary disease was provided by those experiments of the current study in which the rate of conversion of $^{125}$I-labelled angiotensin I was slowed by addition of large excesses of unlabelled angiotensin I. More unlabelled substrate was required to slow conversion in plasma from patients with sarcoidosis than in normal plasma; less unlabelled substrate was required to slow conversion in plasma from patients with chronic obstructive pulmonary disease than in normal plasma. Inhibition (or saturation) of converting enzyme was probably due to excess of substrate rather than the accumulation of split products of hydrolytic enzymes, since, at the incubation time chosen, angiotensinase activity was not detectable and \[(\text{AII/AI+ AII}) \times 100\] was $<25\%$. The results suggest that either there are increased amounts of circulating plasma converting enzyme in patients with sarcoidosis and decreased amounts of enzyme in patients with chronic obstructive pulmonary disease or that the enzyme in sarcoidosis is more active and in obstructive pulmonary disease is less active than in the normal subject. These data do not permit us to distinguish between the two possibilities.

The site(s) of origin of plasma converting enzyme remain obscure. Attempts to establish the identity of converting enzyme from plasma and from various organs have been made by comparing kinetics, substrate specificity and antigenic properties of purified preparations. Antibody raised to purified renal converting enzyme cross-reacted with both homologous lung and plasma enzyme (Oshima, Gece & Erdős, 1974). This observation does not allow differentiation among the various organ beds as sites of origin of converting enzyme. It is likely, however, that since pulmonary converting enzyme is found in greatest concentration and in most intimate proximity to circulating blood, the lung is the most important source of the plasma enzyme.

The studies of Lieberman (1975) and Silverstein et al. (1975a, b) utilized a spectrophotometric method in which the rate of release of free hippuric acid from an artificial substrate, hippurylhistidylleucine, was quantified. Since proteolytic enzymes from plasma and lymphoid tissue other than angiotensin I converting enzyme may cleave the artificial substrate, this method is not highly specific when used in unpurified plasma or tissue extracts. Use of the labelled natural substrate for the enzyme provides a more specific assay procedure for converting enzyme. The accelerated rate of appearance of tyrosine and the (1–4) peptide in plasma from patients with sarcoidosis suggests that the activities of proteolytic enzymes which act as angiotensinases as well as converting enzyme are elevated in that disease. The source of the enzymes and their role in the pathogenesis of the sarcoidosis are obscure. Nevertheless, it has been suggested (Lieberman, 1975) that the measurement of plasma converting enzyme may be useful in the diagnosis of sarcoidosis and in the assessment of its severity. In our study and that of Silverstein et al. (1975a) steroid treatment of sarcoidosis did not alter the elevated plasma converting enzyme activity. Further data will be needed before the value of plasma converting enzyme assay in the diagnosis and management of chronic lung disease can be assessed.

The abnormalities in converting enzyme activity observed in lung disease have not been associated with alterations in circulating angiotensin II concentration or renin activity or in cardiovascular homeostasis, but these relationships have not yet been rigorously investigated. The technique for measurement of plasma converting enzyme activity described here is rapid and specific and may be useful in the characterization of the regulatory role of converting enzyme in the renin–angiotensin system and cardiovascular homeostasis.

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