Role of the Lung in Angiotensin Metabolism

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ABSTRACT

The detection and properties of the enzyme which converts angiotensin I to angiotensin II (converting enzyme) are reviewed. Converting enzyme, which is found principally in the lung, is probably responsible in the intact animal for the conversion of angiotensin I to angiotensin II under physiological conditions, the lung thus being the principle site of conversion. The enzyme is distributed in the endothelial cells of the pulmonary capillaries. Conversion of angiotensin I to angiotensin II depends on the area of the vascular bed and the rate of blood flow through the pulmonary circulation.

The lungs, in addition to their central role in respiration, play an important part in the metabolism of some vasoactive substances. As long ago as 1925, Starling and Verney\(^5\) showed that the isolated kidney could not be perfused using a simple circuit because of vasoconstriction produced by the defibrinated blood. As the effect could be overcome by perfusing the kidney from a heart-lung preparation, they suggested that blood was “detoxicated” in the lungs. The vasoconstrictor was later identified as 5-hydroxytryptamine;\(^4\) about 98 percent of 5HT infused intravenously disappeared in a single passage through the lungs of the anesthetized dog.\(^8\)

The protective action of the lungs against the potentially harmful effects on the arterial system of vasoactive chemicals has been demonstrated for other substances. These are destroyed by enzymes or stored in reticuloendothelial cells and later released in small amounts. Noradrenaline,\(^2\) bradykinin,\(^1\) and prostaglandins E\(_1\), E\(_2\) and F\(_{2\alpha}\)\(^9\) are removed to the extent of 30 percent, 80 percent and 95 percent, respectively, in one passage through the pulmonary circulation.

In contrast, the lung has an activating effect on angiotensin, the almost inactive angiotensin I being almost completely converted into the highly potent vasoconstrictor angiotenin II in a single lung passage.\(^8\) The mechanism of the conversion forms the main theme of this paper.

A powerful method of identifying and assaying vasoactive substances is the blood-bathed organ technique,\(^6\) in which blood is superfused,\(^2\) i.e. bathes the outside, over a test organ. Blood, suitably anticoagulated, is pumped from an artery of the animal under investigation and allowed to flow over a series of up to three isolated test organs, selected from rat colon, cat jejunum, chick rectum, rabbit rectum and rat stomach. Organs and blood are kept at 37°C. Contraction or relaxation of the test organs is suitably recorded.
The superfused blood is collected in a reservoir and returned by the circulating pump to the animal. Antagonists to the vasoactive substances can be introduced intraluminally into the test organs. The assay is calibrated by measuring the response of the test organ to known amounts of the substances under investigation. By using several test organs and inhibitors, identification of a vasoactive substance can be made with some confidence. Angiotensin I has no effect on the test organs; angiotensin II causes marked contraction of rat colon, slight contraction of rabbit rectum and is without action on the other test organs. Its action can be inhibited by peptides extracted from the venom of Bothrops jararaca,\(^8,17,36,59\) a very potent inhibitor in it being a pentapeptide.

Angiotensin, which is produced in the plasma by the enzymatic action of renin (made in the kidney) on an \(\alpha\)-globulin (a glycoprotein made in the liver)\(^{23}\) was discovered independently by Page and Helmer\(^{40}\) in 1939 and Braun-Menendez et al in 1940.\(^{11}\) It was named “angiotonin” by the former and “hypertensin” by the latter investigators causing much confusion in the literature. The compromise name “angiotensin” was adopted by the two discoverers in 1958.\(^{12}\)

In the intervening period, Skeggs et al\(^{54}\) had demonstrated the existence in plasma of two hypertensins (now called angiotensin I and angiotensin II). Skeggs et al purified them and showed them to be a decad and octapeptide, respectively, and determined the amino acid sequence of horse angiotensins.\(^{52,53,55,56}\) Naturally occurring angiotensin I was found to be asp-arg-val-tyr-ile-his-pro-phe-his-leu in the horse,\(^{10,58}\) hog\(^{13}\) and man;\(^4\) in the ox,\(^{16}\) valine replaces isoleucine in position 5. A converting enzyme in the plasma was shown by Skeggs et al\(^{21}\) to split off the C-terminal dipeptide histidyl-leucine, the remaining octapeptide being angiotensin II. The early work on angiotensin is reviewed by Page and Bumpus\(^{29}\) and more recent work by Page and McCubbin.\(^{41}\)

It was assumed for some years that the conversion of angiotensin I to angiotensin II occurred in the blood, at the very fast rate required to account for the observation that an increase in blood pressure occurs at about the same time after the intravenous injection of either angiotensin I or angiotensin II. However, Ng and Vane\(^{33,34}\) showed by means of the blood-bathed organ technique applied to the assay of angiotensin II,\(^{44,62}\) that the half-life of angiotensin I in the blood of dogs was about 100 seconds. Intravenous injections of angiotensin I (itself almost inactive) resulted in a pressor response in 15 seconds, far too soon to have resulted from any conversion of angiotensin I in the blood. To demonstrate that conversion occurred during the passage of blood through the pulmonary circulation, Ng and Vane first showed, by bioassay, that angiotensin II passed through the pulmonary circulation unchanged. Comparison was made of the angiotensin II concentrations of femoral arterial blood following the infusion of angiotensin I first intravenously and then into the ascending aorta, close to the left ventricle. Data indicated marked angiotensin II activity after the intravenous infusion of angiotensin I; 80 percent was converted into angiotensin II during one passage through the lungs, and about 30 percent conversion occurred following intraaortic infusion. Further, it was found that angiotensin II and unconverted angiotensin I were destroyed by peptidases (angiotensinases) in the peripheral vascular beds. These findings have been confirmed by other investigators in the dog and other animals using the same and different bioassay techniques.\(^7,10,37,58\) and in
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human subjects undergoing cardiac catheterization for the investigation of cardiac function.

Identification by bioassay is only presumptive: there is always the possibility that the passage of angiotensin I through the lungs might generate or liberate some other vasoconstrictor than angiotensin II, or might in some other way activate angiotensin I. It is desirable to have direct chemical proof of the conversion. This was obtained by Ryan et al.48 who perfused blood-free rat lungs with C14-labelled angiotensin I in Krebs-Henseleit solution and collected the venous effluent from the perfused lungs over the next three minutes. Chromatography on BioGel P2 columns and isolation and identification of derivatives confirmed that angiotensin I was converted into angiotensin II by removal of the C-terminal dipeptide histidylleucine in one step during a single circulation through the rat lung washed free of blood. In addition, a second vasoactive substance, different from angiotensin II, was found. It contracted rat colon and raised arterial blood pressure. This substance, of lower molecular weight than angiotensin II, may be produced from angiotensin I by the action of an aminopeptidase.

Converting enzyme, although most abundant in the lung, is found in many other tissues (table I). Because of the possible role of angiotensin II in the pathogenesis of renal hypertension, evidence for the intrarenal conversion of angiotensin I to II has been sought with conflicting results probably related to the different experimental and assay methods used. Some intrarenal conversion does occur, but its physiological significance is unclear. It is generally agreed that conversion can take place outside the lungs, in the mesenteric circulation and hindlegs of the dog and other animals and in dogs during cardio-pulmonary bypass. However, under physiological conditions in the intact animal, the lung is the most important site of conversion, as it is the first organ to receive the angiotensin I formed in the venous circulation. Angiotensin conversion is slow in other organs and competes with the enzymatic destruction of angiotensin I and II by angiotensinases, which is rapid. About 65 percent of each type of angiotensin is destroyed in the peripheral vascular beds. The residual angiotensin I and II enter the venous circulation, where most is destroyed. Thus, a small amount of "endogenous" angiotensin I and angiotensin II can be found in venous blood.

Recently introduced radioimmunossay methods for angiotensin I and II and assay methods for converting enzyme have been used to increase our knowledge of converting enzyme and its role in angiotensin metabolism. The enzyme appears to be different from bradykininase, although the two enzymes have many properties in common. Plasma and lung converting enzymes may be identical: human and hog lung enzymes differ in molecular weight. Plasma and lung converting enzymes are endopeptidases (dipeptidyl carboxypeptidases). An endopeptidase with a

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Converting Enzyme (mU/mg protein)</th>
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<tbody>
<tr>
<td>Lung</td>
<td>37.2</td>
</tr>
<tr>
<td>Small intestine</td>
<td>4.7</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.2</td>
</tr>
<tr>
<td>Brain</td>
<td>1.5</td>
</tr>
<tr>
<td>Serum</td>
<td>1.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.6</td>
</tr>
<tr>
<td>Liver</td>
<td>0.2</td>
</tr>
<tr>
<td>Heart</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Assayed using carbobenzoxy-phenylalanyl-histidyl-leucine as substrate.
TABLE II

<table>
<thead>
<tr>
<th>Molecular Weight</th>
<th>150,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km (pH 7.2, .05M PO₄)</td>
<td>40 (\mu M) (plasma)</td>
</tr>
<tr>
<td></td>
<td>20 (\mu M) (lung)</td>
</tr>
<tr>
<td>(pH 7.3, .2M PO₄)</td>
<td>48 (\mu M) (plasma)</td>
</tr>
<tr>
<td></td>
<td>5.5 (\mu M) (lung)</td>
</tr>
</tbody>
</table>

Halide activation: concentration for maximum effect
[Cl\(^-\), I\(^-\), Br\(^-\), F\(^-\)]

Activation by anions: order of effectiveness
[Cl\(^-\), HSO\(_4\)^-, CH\(_3\)COO\(^-\), NO\(_3\)^-, CN\(^-\)]

Effect of other substances: (all at 10\(^{-3}\) M)
- Dithiothreitol: No inhibition
- Cysteine
- N-Ethylmaleimide
- p-Chloromercuribenzoate
- Phenylmethylsulfonylfluoride: restored by
- Cation activation
- Cation inhibition
- EDTA inhibition
- Mn\(^{++}\), Ca\(^{++}\) (partly)

Converting enzyme is a metalloprotein (? Zn), dipeptidylcarboxypeptidase, chloride activated, stable in pH range 4–9, denatured at 60°C. It may be stored for at least one month at –20°C. It is not inhibited by 10\(^{-3}\) M 2-mercaptoethanol or 10\(^{-4}\) M 8-hydroxyquinoline. Using AI as substrate, its optimum pH is 7.25, but this varies with the buffer anion. It is completely inhibited by 0.01 M EDTA and the pentapeptide pyr-lys-trp-ala-pro in 0.1 \(\mu M\) solution.

Different substrate specificity has been found in plasma. Properties of lung and plasma converting enzymes are given in table II. Some of these data will require revision when the enzymes have been obtained in the pure state.

Angiotensin I probably has no direct effect on the arterial wall. However, it can act indirectly by local, intramural, conversion to angiotensin II. Whether or not this is physiologically important is not known. There is very much more enzyme in the pulmonary artery wall than in the aortic wall.

The cellular site of action of converting enzyme has been studied by ultracentri-

fugal fractionation of lung tissue and by histochemical techniques. Bakhle found that lung tissue homogenized at pH 6.5 in 0.01 M phosphate buffer yielded after centrifugation at 105,000 g for 60 minutes a pellet containing converting enzyme activity. The supernatant contained “destroying enzymes” (angiotensinases). These enzymes are intracellular; some may be lysosomal.

Using a different ultracentrifugal technique, designed to separate the endothelial cells, Ryan et al. have shown that converting enzyme is located on the luminal surface of pulmonary endothelial cells and in the caveolae intracellulares (“pinocytic vesicles”) open to the vascular lumen. The enzyme isolated from the endothelial cells was very potent, converting more than 90 percent of angiotensin I to angiotensin II in fifteen seconds. No lower homologues were produced, indicating that when these have been detected in the perfusate of lungs, they have resulted from the activity of other enzymes.

The histochemical location of angiotensin activity was achieved by the use of angiotensin II coupled with the histologically demonstrable enzymes cytochrome c and horseradish peroxidase. These complexes were shown to give a pressor response in the mouse and not to be decomposed by the tissues. Following intravenous injection, the complexes were found to be located on the endothelium and not on the smooth muscle during the pressor response. Thus, not only is converting enzyme located on the surface of endothelial cells, but so are the receptor sites for the action of angiotensin II.

Much of the work on angiotensins and converting enzyme has been carried out in animals or in vitro. The role of the lung in angiotensin metabolism has been clearly established in the experimental animal and the human. Although current interest is in the radio-immunoassay of angiotensin I as
an indicator of renin activity, it would seem that assay of angiotensin II in arterial blood might provide information about the pulmonary circulation, particularly in diseases in which the pulmonary bed is reduced in volume. If plasma converting enzyme turns out to be related to lung converting enzyme, another parameter of pulmonary function would be available for measurement. In the few years since pulmonary conversion of angiotensin I has been demonstrated, much knowledge of the processes involved has accumulated. With the ready availability of radioimmunological assay tests, investigation of angiotensin metabolism will probably be added to the armamentarium of pulmonary function tests.

References


58. Starling, E. H. and Verney, E. B.: The


“Those scientists who think they know it all are most annoying to those of us who do.”

Personal communication of Robert P. MacFate to F.W.S.