Metabolism of Angiotensin II-1131 in Normotensive and Hypertensive Human Subjects

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BIOLOGICAL ASSAYS of angiotensin in the blood of patients with normal blood pressures, patients with benign essential hypertension, and patients with malignant hypertension indicate that there are significantly greater quantities of angiotensin in the subjects with benign essential hypertension than in the normotensive group and that the greatest quantities of angiotensin are present in the patients with malignant hypertension.1-3 The present investigations were designed to determine the metabolism and rate of turnover of angiotensin in normotensive and hypertensive human subjects.

Methods

Angiotensin II (ileu-5-angiotensin II and val-5-angiotensin II)1 was iodinated by the method of Pressman and Eisen4 as modified by Newerly.5 The angiotensin II-I131 was filtered through a Seitz filter and cultured before use. There was no apparent loss of hypertensive potency in the labeled angiotensin II preparations containing an average of 0.5 to 1.5 iodine atoms per molecule of angiotensin (1,036 molecular weight) as manifested by the hypertensive responses in the dog following intravenous administration of the labeled and unlabeled angiotensin II. Preparations containing less than 1.0 iodine atom per molecule of angiotensin II were used.

Doses of 50 to 150 μc. of angiotensin II-I131 were injected intravenously in human subjects. Urine was collected at 12-hour intervals thereafter. Heparinized blood samples were drawn from the patients at frequent intervals after administration of the angiotensin II-I131. Radioactivity assays were performed on plasma, packed erythrocytes, urine, plasma precipitates, washed with 10 per cent trichloracetic acid (TCA) and plasma precipitates washed with 23 per cent sodium sulfate and ether.6 Blood and urine radioactivities were assayed in a 5 ml.-capacity, well-type, scintillation counter with a sensitivity of 0.92 × 106 counts per minute per μc. I131 above a background of approximately 175 counts per minute. Blood concentrations of radioactivity were plotted as the products of the fraction of the administered dose per liter of plasma and the body weight in kilograms.

Paper electrophoresis was performed in barbital buffer, ionic strength 0.1, pH 8.6. Ten to one hundred microliters of material were applied to the cathode ends of strips of Whatman no. 3 MM filter paper, 37 by 500 mm., stretched across the vertical supports of the buffer vessels. After electrophoresis at 200 to 250 volts for 12 to 24 hours, the strips were dried for 45 to 50 minutes in an oven at 110 to 120 C. The strips were assayed for radioactivity in an automatic strip scanner with use of a thin window gas-flow counter with a sensitivity of 0.6 × 106 counts per minute per μc. I131 over a background of 40 counts per minute. Naphthalene black stain and 10 per cent acetic acid in methyl alcohol were employed to color the proteins in the strips.

Results

The concentration of radioactivity fell rapidly in the plasma and erythrocytes and rose in the urine following the intravenous injection of angiotensin II-I131. The curve of erythrocyte radioactivity closely paralleled the curve of total plasma radioactivity. Approximately one third of the total blood radioactivity was in the erythrocytes and the remaining two thirds was in the plasma. When angiotensin II-I131 was added to plasma...
in vitro, approximately 10 to 15 per cent was TCA precipitable. Plasma radioactivity samples taken up to 7 hours after injection were also TCA precipitable in the same order of magnitude (10 to 15 per cent). Several of the 24-hour samples of plasma had up to 20 per cent of the radioactivity in the TCA precipitate. Only 1 or 2 per cent of the urinary radioactivity was TCA precipitable. The urinary radioactivity migrated with the same mobility as iodine-131 and probably represented the radioactive end product of the metabolism of angiotensin II-\textsuperscript{131}.

Not more than 3 per cent of the radioactivity was present in the globulin precipitate when angiotensin II-\textsuperscript{131} was added to plasma and treated with 23 per cent sodium sulfate and ether. Plasma samples taken after the injection of angiotensin II-\textsuperscript{131} and treated in a similar fashion also revealed similar small quantities of radioactivity in the globulin precipitates.

Figure 1 summarizes the plasma radioactivity at various intervals of time following the intravenous administration of angiotensin II-\textsuperscript{131} in all subjects. The curves illustrate the relatively high radioactivity remaining in the plasma of hypertensive subjects. This group was composed of two patients with essential hypertension and one patient (S.W.) with malignant hypertension with grade-IV retinopathy. A more rapid disappearance of plasma radioactivity was observed in the five normotensive control patients. The rate of
disappearance of plasma radioactivity appeared to be correlated, therefore, with the presence or absence of hypertensive disease.

When small quantities of angiotensin II-\textsuperscript{131}, either alone or mixed with 20 to 50 \(\mu\)l of normal human plasma or serum in vitro were analyzed by paper electrophoresis, the radioactivity migrated approximately 5 to 7 cm. further than the albumin (fig. 2). The distribution of radioactivity formed a smooth, symmetrical peak without skewing or distortion. Similar results were obtained on radiochromatography effected by opening the top of the electrophoretic apparatus and running the voltage supply at 600 volts. The electrophoretic and chromatographic behavior of the plasma radioactivity following the intravenous administration of angiotensin II-\textsuperscript{131} was identical with that described in the in vitro control experiments, and this material represented, therefore, angiotensin II. There was also no difference in the electrophoretic mobility of tracer amounts of angiotensin II-\textsuperscript{131} alone or in a mixture of 50 \(\mu\)l of urine before and after incubation at 37 C. for 24 and 48 hours.

Table 1 summarizes the "apparent spaces of distribution" 30 to 45 minutes after angiotensin II-\textsuperscript{131} injection and the half time of degradation of plasma radioactivity. Characteristic differences were observed between the hypertensive and the control groups. The five normotensive subjects had smaller apparent spaces of distribution and more rapid degradation rates of angiotensin II than the two subjects with benign essential hypertension or the one subject with malignant hypertension.

**Discussion**

The manipulations involved in the labeling of angiotensin II did not alter the electrophoretic, chromatographic, or biologic proper-
ties of the peptide. The time curves of plasma radioactivity were, therefore, a manifestation of the distribution and metabolism of the angiotensin II-I\(^{131}\). These data may be utilized to approximate various parameters of angiotensin II-I\(^{131}\) metabolism. The average whole blood concentrations of angiotensin II have been roughly assayed by Kahn and his associates with an average recovery of 50 per cent.\(^3\) These results indicated a rough plasma angiotensin II concentration of 0.018 \(\mu g\) per liter in normotensive subjects, 0.038 \(\mu g\) per liter in subjects with benign essential hypertension, and 0.32 \(\mu g\) per liter in malignant hypertension subjects. The mean values for the apparent volumes of distribution of angiotensin II were approximately 23 liters in the normotensive subject and 29 liters in subjects with benign and malignant hypertension. The total amounts of angiotensin II that were present in the body and that were readily exchangeable with the blood angiotensin II averaged approximately 0.41 \(\mu g\) in the normotensive subject, 1.10 \(\mu g\) in the two subjects with benign essential hypertension, and 9.3 \(\mu g\) in one malignant hypertensive subject. Although these values may appear low, it should be remembered that intravenously administered injections of angiotensin II in a dosage of 1 to 2 \(\mu g\) produced a detectable blood pressure elevation.\(^7\)

The rate of degradation of angiotensin II lies between the rapid rates of hydrocortisone, adrenocorticotrophic hormone, and insulin (with half times of approximately 30 minutes) and thyroxin, which has a half time of approximately 7 days.\(^8\)-\(^11\) A relatively slow degradation rate of angiotensin II insures a steady angiotensin II blood concentration.

The slower rate of degradation of angiotensin II-I\(^{131}\) from the circulation of hypertensive subjects than from that of normotensives indicates that the degradation rate is a limiting factor governing the concentration of angiotensin II in the body fluids. Since the endogenous blood concentration of angiotensin II remained essentially unchanged throughout the course of the experiments, steady-state conditions may be assumed, and therefore the rate of synthesis was equal to the rate of degradation of angiotensin II. The present study indicated that the high concentrations of angiotensin II in the blood and the large total exchangeable pools of angiotensin II in hypertensive subjects were related to a slow rate of degradation.

**Summary and Conclusion**

Many patients with hypertension have greater concentrations of the peptide, angiotensin II, in their blood than normotensive persons.

Angiotensin II-I\(^{131}\) has been intravenously administered to normotensive and hypertensive human subjects.

The plasma radioactivity after injection has been studied by electrophoretic and chemical analysis and indicates that the rate of degradation of angiotensin II-I\(^{131}\) is slower in hypertensive than in normotensive subjects.

**Acknowledgment**

We are indebted to Drs. I. H. Page and F. M. Bumpus for the leu-5-angiotensin II, and to Dr. W. E. Wagner of the Ciba Pharmaceutical Co. for the val-5-angiotensin II used in this study. We are also indebted to Drs. S. Feitelberg and L. Sharney for critical review of the manuscript, and are grateful to Miss Julia Pick for valuable technical assistance, and to Miss Judith Weinberg for secretarial assistance.

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**Table 1**

Space of Distribution and Degradation Rate of Plasma Radioactivity after Angiotensin II-I\(^{131}\) Administration

<table>
<thead>
<tr>
<th>Subject</th>
<th>Diagnosis</th>
<th>Space of distribution per cent body weight</th>
<th>Rate of degradation T (\frac{1}{2}) hr.</th>
<th>% hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. B.</td>
<td>Control</td>
<td>36</td>
<td>10</td>
<td>6.93</td>
</tr>
<tr>
<td>S. T.</td>
<td>Control</td>
<td>42</td>
<td>11</td>
<td>6.30</td>
</tr>
<tr>
<td>P. H.</td>
<td>Control</td>
<td>31</td>
<td>8</td>
<td>8.66</td>
</tr>
<tr>
<td>G. G.</td>
<td>Control</td>
<td>28</td>
<td>12</td>
<td>5.77</td>
</tr>
<tr>
<td>P. C.</td>
<td>Control</td>
<td>24</td>
<td>12</td>
<td>5.77</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>32.2</td>
<td>10.6</td>
<td>6.69</td>
</tr>
<tr>
<td>A. H.</td>
<td>Essential hypertension</td>
<td>45</td>
<td>14</td>
<td>4.95</td>
</tr>
<tr>
<td>I. R.</td>
<td>Essential hypertension</td>
<td>36</td>
<td>17</td>
<td>4.07</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>41</td>
<td>15.5</td>
<td>4.51</td>
</tr>
<tr>
<td>S. W.</td>
<td>Malignant hypertension</td>
<td>42</td>
<td>37</td>
<td>1.87</td>
</tr>
</tbody>
</table>
References


Sir Thomas Browne
1605 - 1682

Critics from Johnson to Walter Pater have put on record their estimate of Browne and of his place in literature. Among these for keenness of appreciation Pater takes the first rank. Lamb and Coleridge dearly loved the old Norwich physician, in whom they found a kindred spirit. In America the New England writers, Ticknor, Fields, Holmes, and Lowell, were ardent students of his works. Lowell in particular is fond of apt quotations from him, and in one place speaks of him as "our most imaginative mind since Shakespeare"... The growing popularity of Browne's writings testifies to the assured position he holds, if not in the hearts of the many, at least in the hearts of that saving remnant which in each generation hands on the best traditions of our literature. We, who are members of his profession, may take a special pride in him. Among physicians, or teachers of physic, there is, perhaps, but one name in the very first rank. Rabelais stands apart with the kings and queens of literature. Among the princes of the blood there are differences of opinion as to rank, but Sir Thomas Browne, Holmes, and John Brown of Edinburgh form a group together high in the circle. Of the three, two were general practitioners; Oliver Wendell Holmes only in the early part of his life, and for forty years a teacher of anatomy; but all three have far closer ties with us than Goldsmith, Smollett, or Keats, whose medical affiliations were titular rather than practical.—Sir WILLIAM OSLER. A Way of Life and Other Selected Writings. New York, Dover Publications, Inc., 1951, p. 57.
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