 Role of Converting Enzyme in the Responses of Rabbit Atria, Aortas, and Adrenal Zona Glomerulosa to [des-Asp¹]angiotensin I

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SUMMARY Conversion of angiotensin I (A I) and [des-Asp¹]angiotensin I (des'-A I) to angiotensin II (A II) and angiotensin III (A III), respectively, was studied in aortic strips, left atria, adrenal zona glomerulosa cell suspensions from rabbits, and with purified rabbit lung converting enzyme. Conversion of A I and des'-A I was estimated in the presence and absence of Bothrops jararaca nonapeptide, converting enzyme inhibitor (CEI), by measuring the changes in peptide-induced tension development in aortas and atra and on steroidogenesis in cell suspensions. The liberation of histidyl-leucine from A I and des'-A I by the enzyme preparation was studied. Angiotensin I and des'-A I possessed 23% and 1% contractile activity (aorta), and 34% and 4% positive inotropic activity (atria), respectively, when compared to A II. Inhibition of aortic and atrial converting enzymes attenuated responses to A I and des'-A I without significantly altering responses to A II and A III. The steroidogenic activity of A I and des'-A I in adrenal cells was dependent on conversion since treatment with CEI specifically abolished aldosterone biosynthesis induced by A I and des'-A I without changing the activities of A II or A III. The K₅₀ values for A I and des'-A I determined with lung enzyme were 80 µM and 30 µM, respectively. The hydrolysis of A I and des'-A I was competitively inhibited by CEI, A II, and A III. Angiotensin III was the most potent CEI among several metabolites of A I. These results indicate that des'-A I was a better substrate than A I for isolated pulmonary converting enzyme. The present investigation clearly indicates that des'-A I is rapidly converted by purified and tissue converting enzymes. The data are consistent with the postulated alternative pathway for formation of A III from des'-A I subsequent to N-terminal degradation of A I.

THE renin-angiotensin system affects arterial blood pressure directly through the pressor effect of angiotensin II (A II, the octapeptide) and indirectly through the release of aldosterone and the subsequent retention of sodium. It generally has been accepted that A II is the only major active peptide of the renin-angiotensin system, and that metabolites of A II have little biological activity. However, angiotensin III (A III), the C-terminal heptapeptide metabolite of A II, has been shown to be a potent steroidogenic agent in the adrenal zona glomerulosa. The potential physiological significance of A III on aldosterone bio-

References

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synthesis has been advanced by many investigators. Peach and Chiu postulated that part of the action of A II on the adrenal zona glomerulosa might be mediated through A III, which can be produced locally by the action of aminopeptidase(s) on A II. Such aminopeptidase(s) which catalyze the hydrolysis of A II have been found in various tissues. It was suggested that aminopeptidase(s) might similarly cleave angiotensin I (A I, the decapeptide) to form [des-Asp¹]angiotensin I (des'-A I, the nonapeptide), and that A III may be generated from des'-A I by converting enzyme. Recently, the conversion of des'-A I to A III by partially purified porcine plasma and lung converting enzymes was reported, and des'-A I was a better substrate than A I for porcine converting enzymes. The present investigation was performed using tissues from rabbits, a species known to respond to A III, and was undertaken to determine (1) if des'-A I was converted...
to A III by intact tissues; and (2) if des-L-A I had direct biological activity in rabbit aortic strips, left atria, and/or adrenal cortical cell suspensions. In addition, highly purified rabbit lung converting enzyme was used to establish the kinetics of the reactions of this enzyme with A I and des-L-A I as substrates.

**Methods**

Aortic strips, atria, and adrenal glands were obtained from male New Zealand White rabbits (2-3 kg).

**RABBIT AORTIC STRIP PREPARATION**

The rabbit was killed by cervical dislocation and the aorta was rapidly removed, cleaned, and placed in oxygenated modified Krebs-Ringer bicarbonate buffer of the following composition (mm): NaCl, 111; NaHCO₃, 25; KCl, 5; Na₂HPO₄, 1; MgCl₂, 0.5; glucose, 11; and CaCl₂, 2.5. Spirally cut rabbit aortic strips (0.25 x 2 cm) were prepared according to the method of Puchgott and Bhadarakom. A strip was mounted in a 5-ml organ bath containing Krebs' buffer at 37°C, gassed with 95% O₂-5% CO₂. The strip was placed under an initial 2-g passive tension and allowed to equilibrate for approximately 2 hours. During this equilibration period, the buffer was changed every 10 minutes. The final resting tension was adjusted to 1 g and the isotonic contractile responses of the aortic strip to angiotensins were recorded with a Grass force-displacement transducer (FT-03C) and a Brush Mark 220 recorder. Cumulative dose-response curves were determined from the data obtained for A I, A II, A III, and des-L-A I. In studies which evaluated the role of converting enzyme in the generation of responses, the peptide inhibitor of the enzyme, Bothrops jararaca nonapeptide, was administered 5 minutes prior to the agonists. Between exposures to an agonist, the preparation was washed repeatedly and allowed to return to basal resting tension.

**RABBIT ATRIAL PREPARATION**

Before removal of the aorta from the rabbit, the heart was excised quickly and placed in oxygenated (95% O₂-5% CO₂) modified Krebs' solution at 21-22°C with the following composition (mm): NaCl, 119; CaCl₂, 2.54; KCl, 4.74; MgSO₄, 1.18; Na₂HPO₄, 1.18; NaHCO₃, 24.9; and glucose, 5.5. The preparation of the isolated left atria was according to the method described by Blumberg et al. After the left atrium was dissected from the heart, the left atrium was cut in half and each half was mounted in a 10-ml muscle chamber with buffer maintained at 30 ± 1°C and connected to a Grass force-displacement transducer (FT-03C). Isometric contractions were recorded with a Grass model 7 polygraph. The atria were equilibrated in the Krebs' buffer for 60 minutes with bath changes every 10 minutes. Each preparation was paced at a rate of 60 beat/min by field electrodes with a square-wave pulse of 5 ms duration delivered by a Grass stimulator (model S-4). During the first 40 minutes of the equilibration period, the atria were stimulated at threshold voltage. For the remaining 10 minutes of the equilibration period and the duration of the experimental period, the atria were stimulated at twice threshold voltage (three to six volts). Tension-tension curves were determined for each atrium. A resting tension of 50% of that tension which produced maximal contraction to the electrical stimulus was applied (1.5-2.5 g). Contractile responses of the atria to cumulative doses of the angiotensins were recorded. The effects of B. jararaca nonapeptide on positive inotropic responses to A I and des-L-A I were used to evaluate atrial converting enzyme activity.

**CELL SUSPENSION STUDY**

**Preparation of Zona Glomerulosa Cell Suspensions**

The rabbit zona glomerulosa cells were dispersed from the adrenals of the rabbits from which we obtained aortas and atria. After the adrenal glands were removed, the inner zones of the cortex and the medulla were removed and discarded and the capsular layer was minced. Cell suspensions were prepared by the enzymatic (trypsin, DNase, and collagenase) digestion method of Sarstedt et al.

**Incubation Procedure**

The cells were preincubated for 30 minutes at 60 rpm at 37°C in a Dubnoff shaker with an atmosphere of 95% O₂-5% CO₂. Steroidogenic compounds to be tested were added in a volume of 10 µl at the end of the equilibration period. The same amount of vehicle was added to the controls. When utilized, B. jararaca nonapeptide was administered 5 minutes prior to the addition of angiotensins. The experimental incubation period was 1 hour following the addition of steroidogenic peptides.

**Analytical Method**

The LH-20 column purification of aldosterone and the radioimmunoassay of aldosterone were performed according to the method described by Sarstedt et al.

**Statistical Analysis**

The data were tested for significance (P < 0.05) using Student's t-test. All values are expressed mean ± SEM.

**ASSAY OF RABBIT LUNG CONVERTING ENZYME ACTIVITY**

A rabbit pulmonary converting enzyme purified from acetone powder extract of lung was used for studying the conversion of des-L-A I to A III. The enzyme was prepared by a modification of the method of Cheung and Cushman. This purified enzyme was homogeneous under stained disc gel electrophoresis. The purification procedures and additional characterization of this enzyme will be published elsewhere. When A I was used as substrate, the incubation conditions for determining converting enzyme activity were 0.25 µg of enzyme protein in 100 µm potassium phosphate buffer containing 90 mM NaCl (pH 7.5; 37°C) in a final volume of 0.25 ml. When des-L-A I was used as substrate, indental incubation conditions were used as for A I except that the buffer contained 30 mM NaCl. All of the reactions were started by the addition of converting enzyme after preincubation of the sub-
strate(s) in the medium for 5 minutes. A linear rate of reaction was established for the substrate concentrations employed. The released dipeptide product, histidyl-leucine, was quantified by the O-phthaldehyde condensation method of Yang and Neff. When the inhibitory effects of B. jararaca nonapeptide, A II, A III, and ethylenediaminetetraacetic acid (EDTA) were evaluated, these inhibitors were preincubated with the substrate for 5 minutes before initiation of the enzymatic reaction. The concentration of A I and des'-A I was 5 \times 10^{-8} \text{ M}, and the reaction rate was linear during the 4-minute period studied.

Materials

[des-Asp']angiotensin I was obtained from Dr. Khosla, Research Division, Cleveland Clinic, [Asp',Ile 5]angiotensin II, [Asp',Ile 5]angiotensin II, [des-Asp',Ile 5]angiotensin II, and [des-Asp',Arg 2]angiotensin II from Schwarz/Mann; recrystallized bovine serum albumin, lima bean trypsin inhibitor, and deoxyribonuclease from Sigma; crude collagenase from Worthington; \( \alpha \)-aldosterone 1,2-\( ^3 \text{H} \) (specific activity 53.6 Ci/mmol) from New England Nuclear; O-phthaldehyde, histidyl-leucine, and 18,21-dihemisuccinyl aldosterone antiserum from Calbiochem; B. jararaca nonapeptide from Spectrum Medical Industries; acetone powder of rabbit lung from Pel-Freez Biologicals; and Sephadex LH-20 from Pharmacia.

Results

RABBIT AORTIC STRIP

Angiotensin II (1 \times 10^{-9} \text{ M} to 5 \times 10^{-8} \text{ M}) and A III (1 \times 10^{-9} \text{ M} to 2.5 \times 10^{-7} \text{ M}) produced concentration-dependent contractions of the aortic strip (Fig. 1, panels A and B). The time to the onset of response (approximately 30 seconds) and the time to reach the peak of tension development (3-4 minutes) were identical for A II and A III. Both peptides induced the same maximal contractile response. The half-maximal responses to A II and A III were obtained with concentrations of 7.5 \times 10^{-9} \text{ M} and 4.5 \times 10^{-8} \text{ M}, respectively. Based on the pD2 values which represent the negative log of the dissociation constants (Kd) and are a measure of a drug's affinity for a certain receptor, the affinity of A III for the angiotensin receptor is approximately 6 times lower than that of A II.

Angiotensin I (5 \times 10^{-9} \text{ M} to 5 \times 10^{-7} \text{ M}) and des'-A I (7.5 \times 10^{-8} \text{ M} to 5 \times 10^{-6} \text{ M}) produced concentration-dependent responses in the aortic strip (Fig. 1, panels A and B). Angiotensins I, II, III, and des'-A I produced the same maximal responses. The onset of tension development was delayed in response to the deca- and nonapeptides and was between 0.6 and 1.2 minutes and the time to the peak response was 6-10 minutes. The delay in the onset and the time until peak response with A I and des'-A I was apparently due to the conversion of these peptides to A II and A III, respectively. As shown in Figure 1, pretreatment with B. jararaca nonapeptide (1 \times 10^{-5} \text{ M}) produced a parallel shift to the right of the dose-response curve of A I and des'-A I; while pretreatment with this inhibitor of converting enzyme did not alter the responses of the aortic strip to A II or A III (data not shown). Since higher concentrations of the converting enzyme inhibitor were not studied, the contractions induced by the deca- and nonapeptides after treatment with B. jararaca nonapeptide may be due to residual converting enzyme activity or may reflect the direct contractile activities of these peptides. In view of the high concentration of B. jararaca nonapeptide used, the latter seems more likely.

The ratio of doses of des'-A I to A III producing a half-maximal response was 9:1, while this ratio for A I and A II was 4:1. These ratios would appear to indicate a 2-fold greater conversion of A I than of the nonapeptide homolog, but one cannot forget that the sensitivity of the bioassay is highly dependent on the activity of the peptide. The 6-fold difference in potencies of A II and A III on the aortic strip must be taken into account in assessing the relative amounts of A I and des'-A I hydrolyzed.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** The contractile force induced by angiotensins on rabbit aortic strips. Each point represents the mean ± SEM obtained from 10-15 aortic strip preparations. Panel A, angiotensin II alone (■■■■); angiotensin I alone (••••); angiotensin I in the presence of Bothrops jararaca nonapeptide (O—O). Panel B, angiotensin III alone (●—●); [des-Asp']angiotensin I alone (△—△); [des-Asp']angiotensin I in the presence of B. jararaca nonapeptide (△—△-△). B. jararaca nonapeptide (1 \times 10^{-5} \text{ M}) was administered 5 minutes prior to the addition of angiotensin.
RABBIT ATRIAL PREPARATION

Angiotensin II (1 x 10^{-10} M to 1 x 10^{-7} M) and A III (1 x 10^{-9} M to 5 x 10^{-7} M) caused dose-dependent positive, inotropic responses in field-stimulated rabbit left atrial preparations (Fig. 2, panels A and B). The threshold dose of A II was 1 x 10^{-10} M, whereas 1 x 10^{-9} M A III was required to increase atrial contractility. For A II and A III, the times for onset (approximately 30 sec) of the inotropic effect and the times to reach peak response (3-5 minutes) were identical. Angiotensins II and III induced the same maximal response in the atria. From the pD2 values, the affinity of A II for the myocardial angiotensin receptor was calculated to be approximately 3.5 times better than A III.

Angiotensin I and des-Asp' angiotensin I alone (A A); des-Asp' angiotensin I in the presence of B. jararaca nonapeptide (O O); angiotensin I in the presence of B. jararaca nonapeptide (O--O); angiotensin I alone (O--O); angiotensin I in the presence of B. jararaca nonapeptide (O--O); angiotensin III in the presence of B. jararaca nonapeptide (O--O); des-Asp' angiotensin I alone (A--A); des-Asp' angiotensin I in the presence of B. jararaca nonapeptide (A--A). B. jararaca nonapeptide (3 x 10^{-6} M) was administered 5 minutes prior to the addition of angiotensin.

30 µM (Fig. 3). The maximal enzyme activity was 8 µmol/min-per mg of protein with A I as the substrate and 2.6 µmol/min-per mg of protein with des'-A I as the substrate. The Vmax/Km value for the decapetide was 1.0 and for the nonapeptide, 0.86. The hydrolysis of A I and des'-A I was competitively inhibited by B. jararaca nonapeptide, A II, A III, and the C-terminal hexapeptide of A II. In the presence of these peptides, the percent inhibition of converting enzyme activity obtained using A I and des'-A I as substrate is presented in Table 2. The percent inhibition of converting enzyme activity determined with A I as substrate was always greater than that with des'-A I as substrate with various concentrations of inhibitors studied.

Angiotensin III was a stronger converting enzyme inhib-
An important finding is that A III at the same molar concentration as A I inhibited the hydrolysis of the latter by 73%. The $K_a$ values obtained from the Lineweaver-Burk plot (Fig. 3) were 16 $\mu M$ when A I was the substrate and 28 $\mu M$ when des'-A I was the substrate. The converting enzyme activity with both the deca- and nonapeptides as the substrate was inhibited by EDTA.

**Discussion**

The effect of A III on aldosterone biosynthesis has been demonstrated in adrenal cell suspensions, in vivo studies of conscious rats, sheep, and man and in anesthetized dogs and rats. In fact, the steroidogenic activity of A III on isolated glomerulosa cells was found to be either equal or more potent than that of A II. These findings suggest that A III may mediate responses produced by the renin-angiotensin system in the adrenals.

Angiotensin III may be generated from A II by the action of aminopeptidases. In view of the low concentration of A III in the arterial blood of sheep and humans, it was proposed that A III might be formed locally from circulating A II. Alternatively, A III may be generated via a pathway with des'-A I as its immediate precursor. The nonapeptide could be formed from A I by aminopeptidases and then converted to A III by the converting enzyme.

The results obtained from the in vitro studies of rabbit aortic strips and atria indicated that des'-A I is converted rapidly to A III by converting enzyme. Angiotensin I and des'-A I had some direct biological activity in arterial smooth muscle or the myocardium. In the presence of CEI, the activity of the deca- and nonapeptides was greatly attenuated. These studies indicate that conversion and subsequent accumulation of A II and A III are required for A I and des'-A I to induce contractile responses. Prerequisite conversion is in agreement with the slow onset of induced tension development and the longer times to peak response in aortas and atria after administration of the deca- and nonapeptides. The mechanism responsible for the decrease in maximal response of the atrium to the nonapeptide by pretreatment with CEI is unknown at the present time.

In adrenal cell suspensions pretreated with *B. jararaca* nonapeptide, A I and des'-A I displayed little steroidogenic activity. It was obvious that the increased aldosterone biosynthesis in response to these peptides also was dependent on conversion to A II and A III by the cell suspensions. However, it is not clear whether the converting enzyme was present in the rabbit adrenal cells or was introduced by dispersion of the cells with crude collagenase. It has been reported that the cat adrenal gland contained very little converting enzyme activity, and crude collagenase may contain converting enzyme activity.23

![Figure 3: Double-reciprocal plot of the activity of purified converting enzyme of rabbit lung (0.25 $\mu g$ in 250 $\mu l$ of phosphate buffer) in the absence or presence of angiotensin III. The curves depicted represent angiotensin I as the substrate (O--O); conversion of angiotensin I in the presence of $1 \times 10^{-5} M$ angiotensin III (O--O); [des-Asp']angiotensin I as the substrate (A--A); the hydrolysis of [des-Asp']angiotensin I in the presence of $1 \times 10^{-5} M$ angiotensin III (A--A).](image)

**Table 1: Effect of Angiotensin I and [des-Asp']angiotensin I on the Aldosterone Biosynthesis of Rabbit Adrenal Zona Glomerulosa Cells**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Concentration (M)</th>
<th>CEI (5 x 10^{-6} M)</th>
<th>Aldosterone (ng/10^5 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None*</td>
<td>None</td>
<td>-</td>
<td>11.76 ± 1.24 (5)</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>+</td>
<td>12.18 ± 1.44 (5)*</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>5 x 10^{-8}</td>
<td>-</td>
<td>23.77 ± 2.79 (5)*</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>5 x 10^{-8}</td>
<td>+</td>
<td>21.95 ± 2.70 (5)#</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>5 x 10^{-7}</td>
<td>-</td>
<td>16.04 ± 1.58 (5)#</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>5 x 10^{-7}</td>
<td>+</td>
<td>13.17 ± 1.52 (5)#</td>
</tr>
<tr>
<td>None§</td>
<td>None</td>
<td>-</td>
<td>10.94 ± 1.30 (5)</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>+</td>
<td>10.52 ± 2.00 (4)†</td>
</tr>
<tr>
<td>Angiotensin III</td>
<td>5 x 10^{-8}</td>
<td>-</td>
<td>26.5 ± 4.26 (4)‡</td>
</tr>
<tr>
<td>Angiotensin III</td>
<td>5 x 10^{-8}</td>
<td>+</td>
<td>27.62 ± 4.87 (4)‡</td>
</tr>
<tr>
<td>[des-Asp']angiotensin I</td>
<td>1 x 10^{-6}</td>
<td>-</td>
<td>16.59 ± 1.40 (4)‡</td>
</tr>
<tr>
<td>[des-Asp']angiotensin I</td>
<td>1 x 10^{-6}</td>
<td>+</td>
<td>13.17 ± 2.23 (4)‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM; numbers in parentheses represent the number of experiments performed. Statistical significance is determined by Student's *t*-test.

* Control values for experiments of angiotensin I and angiotensin II.
† Not significantly different from control values ($P > 0.1$).
‡ Significantly different from control values ($P < 0.05$).
§ Not significantly different from values obtained in the presence of angiotensin II or angiotensin III alone ($P > 0.1$).
¶ Control values for experiments of [des-Asp']angiotensin I and angiotensin III.
A comparison of the dose ratios of des\(^1\)-A I to A III and A I to A II at 50% of the maximal response in aortic strips suggested that the conversion of the decapetide was 2 times greater than that of the nonapeptide. However, the data do not permit an evaluation of the avidity of the substrates for converting enzyme. As in most bioassay systems, the accuracy and/or sensitivity of the bioassay performed in the aortic strip is dependent on at least four factors: (1) activity of the individual reaction products and, in this case, A II is 6 times more potent than A III; (2) the susceptibility of the individual reaction products to degradative enzymes present (e.g., A III has been shown to be degraded by the aorta much faster than A II); (3) the amount of converting enzyme present; and (4) the V\(_{\text{max}}\) of each substrate with converting enzyme. Conversion of angiotensins was studied in the isolated atrium which has been shown to contain very little angiotensinase activity\(^4,28\) and in vivo in the rat.\(^\text{28}^\text{26}\) The results from these studies indicated that the amounts of A I and des\(^1\)-A I converted were approximately equal. Hence, responses of bioassay organs which are dependent on the actions of converting enzyme may be an inadequate means of comparing substrates.

To circumvent the problems encountered with isolated tissues, purified converting enzyme was used to compare the preference of the enzyme for A I or des\(^1\)-A I. The lower K\(_m\) values obtained for the nonapeptide compared to the decapetide with pulmonary converting enzyme indicated that the nonapeptide had a better affinity for the enzyme. This higher affinity was substantiated by the observations that conversion of des\(^1\)-A I was much more resistant than A I to inhibition with competitive inhibitors such as \(B. \text{jararaca}\) nonapeptide, A II, A III, and the C-terminal hexapeptide. The greater affinity of the nonapeptide for converting enzyme made it an effective competitive inhibitor of the hydrolysis of A I. These results are in complete agreement with the lower K\(_m\) values for des\(^1\)-A I determined with porcine plasma and lung converting enzymes.\(^7\)

According to Angus et al.\(^\text{27}\) the value of V\(_{\text{max}}\)/K\(_m\) is proportional to the percentage rate of hydrolysis of the substrate when substrate concentration is far below the K\(_m\) value. For converting enzyme, which normally functions in the presence of very low concentrations of A I (approximately 5 orders of magnitude lower than K\(_m\)), the percentage rate of hydrolysis may be more representative of the true rate of hydrolysis under physiological conditions. The V\(_{\text{max}}\)/K\(_m\) value for the A I obtained with the rabbit enzyme is about the same as the value calculated for des\(^1\)-A I. These ratios indicated that the rate of hydrolysis of A I and des\(^1\)-A I will be approximately the same if each peptide is present at the same concentration.

Although pulmonary converting enzyme is a determinant of circulating arterial titer of A II, converting enzyme localized in target tissues (e.g., kidney, aorta, heart, and adrenal cortex) may play a significant role in the formation of the octa- and heptapeptides by effector organs. Ryan et al.\(^\text{28}\) reported that 20% of A I was metabolized to A II in one passage through the isolated perfused lung. This observation was confirmed by Freer and Stewart\(^\text{29}\) who showed that the pulmonary circulation in the rat was not a major site for the conversion of A I to A II. Significant conversion of A I has been demonstrated in isolated perfused rat kidney,\(^\text{30}\) perfused canine kidney \(\text{situ}\),\(^\text{34}\) and canine mesenteric vasculature.\(^\text{25}\) In many tissues, converting enzyme has been shown to be distributed primarily in the endothelial cells of blood vessels and, therefore, is readily accessible to the circulating A I and/or des\(^1\)-A I. Since low concentrations of A III are found in the arterial blood, the alternative pathway to A III is more likely to be dependent on aminopeptidases and converting enzyme localized in specific tissues.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration of inhibitor (M)</th>
<th>Angiotensin I (5 x 10(^{-8}) M)</th>
<th>[Des-Asp(^1)-angiotensin I (5 x 10(^{-8}) M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bothrops jararaca nonapeptide</td>
<td>5 x 10(^{-7})</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1 x 10(^{-5})</td>
<td>55</td>
<td>17</td>
</tr>
<tr>
<td>Angiotensin III</td>
<td>1 x 10(^{-5})</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5 x 10(^{-5})</td>
<td>73</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1 x 10(^{-4})</td>
<td>84</td>
<td>58</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>1 x 10(^{-5})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 x 10(^{-5})</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1 x 10(^{-4})</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>Hexapeptide (C-terminal)</td>
<td>5 x 10(^{-5})</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>1 x 10(^{-5})</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 x 10(^{-4})</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

Purified converting enzyme of rabbit lung (0.25 μg) was incubated with angiotensin I and [des-Asp\(^1\)]angiotensin I at a peptide concentration of 5 x 10\(^{-8}\) M in the presence or absence of various concentrations of inhibitors. The inhibitors were preincubated with the substrates for 5 minutes before the addition of enzyme to initiate the reaction. The reaction time was 4 minutes. The values presented are the averages of three triplicate experimental observations.
Angiotensin II has been recognized for some time as a product inhibitor of porcine plasma and lung converting enzymes. More recently, A III and the C-terminal hexapeptide of A II were reported to inhibit the hydrolysis of A I catalyzed by human lung and porcine plasma and lung converting enzymes. The affinity of A III for converting enzyme exceeds that of A II (Kᵢ ~ 120 μM) and the Kᵢ values for these products are comparable to the corresponding Kᵢ values for the precursors (A I and des'-A I). Angiotensins II and III may function via a feedback mechanism to modulate the converting enzyme activity in tissues which have limited amounts of converting enzyme. Such modulation between the alternative pathway and the classical pathway for the production of A III may be a function of the local concentration of these angiotensin homologs as shown in the metabolic scheme (Fig. 4).

The results of the present investigation indicate that after hydrolysis of A I by aminopeptidases, the nonapeptide will be converted rapidly to A III and suggested that this alternative pathway may be operative in vivo. Several other reports lend further support to the hypothetical pathway. In isolated, retrogradely perfused feline adrenal glands, Ackerly et al. found that the major metabolite of ³H-A I was ³H-des'-A I. In fact, 60–65% of the ³H-decapeptide was degraded to ³H-des'-A I during a single pass through the adrenal. Chiu et al. reported that a metabotile of A I in the effluent from perfused rat lungs resembled des'-A I.

The results of the present investigation indicate that the postulated alternative pathway for the formation of A III is feasible and must be considered with regard to the regulation of aldosterone synthesis by the renin-angiotensin system.

Acknowledgments

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References

Intrarenal Hemodynamics in Cross-Circulated Hypervolemic and Isovolemic Rats

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SUMMARY The microsphere technique was used to measure renal blood flow and intrarenal flow distribution in cross-circulated pairs of rats. One rat of each pair was made hypervolemic by infusion of blood (2.3% of body weight), followed by intravenous reinfusion of urine to maintain intravascular expansion. The other rat of each pair, which also received an urine reinfusion, was kept isovolemic throughout the experiment. As shown previously, blood infusion resulted in a large diuretic and natriuretic response in the hypervolemic partner, while a smaller but statistically significant response occurred in the isovolemic partner. Total renal blood flow did not change in the expanded rats and fell slightly in the isovolemic. The transferred natriuresis in isovolemic partners was not associated with any change in microsphere concentration from outer to inner cortex. This shift was not, however, correlated with the magnitude of the renal response. The transferred natriuresis in isovolemic partners was not associated with any change in microsphere distribution. We conclude therefore that redistribution of blood flow to the inner cortex, although a feature of intrarenal hemodynamic changes.

RATS MADE hypervolemic by infusion of donor blood develop a natriuretic activity in their own blood which can be transferred to a normovolemic partner, causing diuresis and natriuresis in the latter. 1,2 Possible mechanisms of this transferred effect include inhibition of active sodium transport in some part of the nephron, and indirect alteration of transport by redistribution of intrarenal blood flow. Increased flow to outer cortex3 or to inner cortex and medulla4 both have been suggested as possible mediators of “volume natriuresis,” the former by shifting filtration from juxtamedullary “salt-conserving” nephrons to superficial “salt-losing” ones, and the latter by washing out the normal medullary solute gradient.

In this study we attempted to determine (1) whether the natriuresis following isotonic and iso-oncotic blood volume expansion in rats was related to changes in intrarenal
Role of converting enzyme in the responses of rabbit atria, aortas, and adrenal zona glomerulosa to [des-Asp1]angiotensin I.

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