Tyrosine in Position 4 Is The Key Amino Acid for The Binding of Angiotensin II to Human Arteriolar Receptor

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Abstract

In order to detect the portion of the angiotensin II molecule binding to the human arteriolar receptor, angiotensine II-(1-5)-pentapeptide, angiotensin II-(1–4)-tetrapeptide and angiotensin II-(1–3)-tripeptide were infused intravenously as saline solutions into the same 5 normal men from 0900 h at a rate of 30 nmol (20 μg)/kg·min (1.2 ml/min) for 15 minutes, at a rate of 90 nmol (54.5 μg)/kg·min (3.0 ml/min) for 6 minutes and at a rate of 140 nmol (54.5 μg)/kg·min (3.0 ml/min) for 6 minutes, respectively, on different occasions. At the end of the infusions average increases in blood pressure were 20/22 mmHg (p<0.001) and 6/7 mmHg (p<0.001), respectively, with the former 2 peptides, while the last peptide showed no pressor action at all. It had previously been found by our research group that angiotensin III, angiotensin II-(3–8)-hexapeptide, angiotensin II-(4–8)-pentapeptide, angiotensin II-(2–7)-hexapeptide, angiotensin II-(1–7)-heptapeptide and angiotensin II-(1–6)-hexapeptide have some pressor action but that angiotensin II-(5–8)-tetrapeptide has no pressor action in normal men. When these previous results are combined with the present result, it is evident that only tyrosine-containing molecules show pressor activity and that tyrosine-deleted molecules do not show pressor activity at all. It is concluded that tyrosine in position 4 is the key amino acid for the binding of angiotensin II to human arteriolar receptor.

The principal biological actions of angiotensin II (ANG II) are pressor, renin-suppressing and steroidogenic actions. However, it is not known which parts in the ANG II molecule are involved in the binding to the arteriolar, renal and adrenal ANG II receptors in man. In order to detect the portions of ANG II molecule binding to human ANG II receptors, various ANG II fragments had been infused by our research group into normal men and patients...
with Bartter's syndrome (Kono et al., 1975; Kono et al., 1978; Kono et al., 1982; Kono et al., 1983; Kono et al., 1985a; Kono et al., 1985b; Kono et al., 1985d; Kono et al., 1986). This time large amounts of ANG II-(1-5)-pentapeptide [ANG-(1-5)], ANG II-(1-4)-tetrapeptide [ANG-(1-4)] and ANG II-(1-3)-tripeptide [ANG II-(1-3)] were given to normal men mainly for their pressor action.

Materials and Methods

Five normal men were examined in the present study. Their ages were 26 to 35 years, and their body weights were 52 to 55 kg. Consent was obtained from each subject for the experiment. Sodium intake was not restricted until the night before the examination. However, it was estimated to be 170 to 200 mEq/day from sodium excretion data for 24-hour urine samples on the day before the examination. ANG-(1-5) and ANG-(1-4) were synthesized by one of the authors (M. C. K.) and ANG-(1-3) was synthesized by another one of the authors (Y. K.). These peptides were dissolved in physiological saline and filtered through a Millipore filter. The resulting sterile solutions were placed in sterile ampoules and stored at 4°C at a concentration of 1.0 mg/ml until use. The chemical purity of the freshly prepared and stored solutions was confirmed by thin-layer chromatography, amino acid analysis, acid hydrolysis and high performance liquid chromatography. The normal men were kept recumbent in a fasting condition after 0800 h on the day of the study. The

![ANG-(1-5) 30 nmol/kg·min infusion](Fig. 1. Effects of an intravenous infusion of 30 nmol/kg·min of ANG-(1-5) on blood pressure (BP), plasma renin activity (PRA) and plasma aldosterone (PA) in 5 normal men. The closed circles with vertical bars in Figs. 1 and 2 express the mean±SEM and asterisks express statistically significant changes compared with pretreatment values (* p<0.02, ** p<0.01 and *** p<0.001, respectively).)
following 3 experiments were done on different occasions.

**Experiment 1:** ANG-(1–5) was infused intravenously into the 5 normal men from 0900 h to 0915 h at a rate of 30 nmol (20 μg)/kg·min (1.2 ml/min).

**Experiment 2:** ANG-(1–4) was infused intravenously into the same 5 normal men from 0900 h to 0906 h at a rate of 90 nmol (54.5 μg)/kg·min (3.0 ml/min).

**Experiment 3:** ANG-(1–3) was infused intravenously into the same 5 normal men from 0900 h to 0906 h at a rate of 140 nmol (54.5 μg)/kg·min (3.0 ml/min).

Before, during and after the infusion of the 3 peptides, blood pressure was measured every 2 or 3 minutes. In Experiments 1 and 2, the duration of the pressor action after the cessation of the infusion of the peptides (T) was estimated. In Experiment 1 plasma renin activity (PRA) and plasma aldosterone (PA) were determined before and at the end of the infusion of ANG-(1–5). The radioimmunoassay methods for PRA and PA were described previously (Kono et al., 1975; Kono et al., 1980). Statistical analysis of the experimental data was done by analysis of variance (Wallenstein et al., 1980) for blood pressure and T, and by Student's t-test for PRA and PA.

### Results

**Experiment 1:** The results are shown in Fig. 1. Blood pressure rose significantly. Average increases in blood pressure were 9/12, 18/19, 20/21 and 20/22 mmHg at 2,
5, 10 and 15 minutes, respectively. Average T was 15 minutes. PRA decreased significantly from 0.78±0.09 (SEM) to 0.55±0.14 (SEM) ng/ml·h. PA changed from 8.0±1.1 (SEM) to 9.1±1.7 (SEM) ng/100 ml. However, this change was not statistically significant.

**Experiment 2**: The results are shown in the upper panel of Fig. 2. Blood pressure rose only slightly but significantly. The average increases in blood pressure were 3/5, 6/8 and 6/7 mmHg at 2, 5 and 6 minutes, respectively. Average T was within 3 minutes for systolic and 4 minutes for diastolic.

**Experiment 3**: The results are shown in the lower panel of Fig. 2. Blood pressure did not rise at all.

**Discussion**

It had been reported that there is a specific receptor for angiotensin III (ANG III) in rat adrenals (Devynck et al., 1977), and it had been assumed that ANG III might have arteriolar and adrenal receptors which are different from those for ANG II in man (Carey et al., 1978a; Carey et al., 1978b). However, our research group recently concluded that ANG II (Kono et al., 1975; Kono et al., 1978; Kono et al.; 1985b; Kono et al., 1985c), ANG III (Kono et al., 1975; Kono et al., 1978; Kono et al., 1985a), ANG II-(3–8)-hexapeptide [ANG-(3–8)] (Kono et al., 1982; Kono et al., 1985a), ANG II-(4–8)-pentapeptide [ANG-(4–8)] (Kono et al., 1983), ANG II-(2–7)-hexapeptide [ANG-(2–7)] (Kono et al., 1985d), ANG II-(1–7)-heptapeptide [ANG-(1–7)] (Kono et al., 1986) and ANG II-(1–6)-hexapeptide ANG-(1–6]) (Kono et al., 1986) exert some pressor action while ANG II-(5–8)-tetrapeptide [ANG-(5–8)] (Kono et al., 1983) has no pressor action in normal men and that ANG II and pressor ANG II fragments have the same arteriolar receptor in man. It was also assumed that steroidogenic ANG II fragments might have the same adrenocortical receptor in man (Kono et al., 1985a). From this standpoint we undertook further studies on the biological activities of hitherto untested ANG II fragments—ANG-(1–5), ANG-(1–4) and ANG-(1–3) in normal men.

ANG-(1–5) showed a significant pressor action. The increases in blood pressure during the infusion of this pentapeptide at the rate of 30 nmol/kg·min were similar to those during an infusion of isoleucine5-ANG II (Ile5-ANG II) at the rate of 5 pmol/kg·min in normal men (Kono et al., 1985b; Kono et al., 1985c). Therefore, the pressor action of ANG-(1–5) is thought to be 0.017% of that of Ile5-ANG II. The average T of ANG-(1–5) was longer than that of ANG-(1–6) (10 minutes) (Kono et al., 1986) and shorter than that of ANG-(1–7) (20 minutes for systolic and 30 minutes for diastolic) (Kono et al., 1986) or Ile5-ANG II (20 minutes) (Kono et al., 1985b). PRA showed a significant decrease at 15 minutes of infusion. This may be due to negative feedback resulting from the blood pressure rise or/and direct inhibition of renin secretion via short loop feedback. In order to detect the direct renin inhibition, ANG-(1–5) should be infused into patients with Bartter’s syndrome. However, no more ANG-(1–5) was available this time. PA did not show a statistically significant change. This may be due to the absence of phenylalanine, because the non-steroidogenic nature of ANG-(1–7) and ANG-(1–6) had already been shown (Kono et al., 1986).

ANG-(1–4) showed a very slight but significant rise in blood pressure. The increases in blood pressure during the infusion of this tetrapeptide were similar to those during an infusion of Ile5-ANG II at the rate of 1 pmol/kg·min in man (Kono et al., 1985c). Therefore, the pressor action of ANG-(1–4) is thought to be 0.001% of that of Ile5-ANG II. The average T of ANG-(1–4) was much shorter than that of
Table 1. Approximate relative biological activities, on a molar basis, of various fragments and substituted analogues of angiotensin II in normal men (Kono et al., 1975–1986)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Pressor action (%)</th>
<th>T (min)</th>
<th>Direct renin suppression</th>
<th>Aldosterone stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>H · Asp-Arg-Val-Tyr-Ile-His-Pro-Phe·OH 1985b, 1985c</td>
<td>100</td>
<td>20</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>H · Arg-Val-Tyr-Ile-His-Pro-Phe·OH (1975, 1978, 1985a)</td>
<td>20</td>
<td>5</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>H · Val-Tyr-Ile-His-Pro-Phe·OH 1982, 1985a</td>
<td>&lt; 0.20</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H · Tyr-Ile-His-Pro-Phe·OH 1983</td>
<td>&lt; 0.12</td>
<td>60</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H · Ile-His-Pro-Phe·OH 1983</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H · Arg-Val-Tyr-Ile-His-Pro·OH 1985d</td>
<td>&lt; 0.004</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H · Asp-Arg-Val-Tyr-Ile-His-Pro·OH 1986</td>
<td>&lt; 0.028</td>
<td>s 20 d 30</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>H · Asp-Arg-Val-Tyr-Ile-His·OH 1986</td>
<td>&lt; 0.024</td>
<td>10</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>H · Asp-Arg-Val-Tyr-Ile·OH (present study)</td>
<td>0.017</td>
<td>15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H · Asp-Arg-Val-Tyr·OH (present study)</td>
<td>0.001</td>
<td>s &lt; 3 d 4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H · Asp-Arg·OH (present study)</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H · Asn-Arg-Val-Tyr-Val-His-Pro-Phe·OH (1975, 1978, 1985b)</td>
<td>50</td>
<td>5</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>H · Sar- Arg-Val-Tyr-Ile-His-Pro-Phe·OH 1985b</td>
<td>150</td>
<td>40</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>H · Sar- Arg-Val-Tyr-Ile-His-Pro-Ile·OH 1985c</td>
<td>&lt; 0.83</td>
<td>s 100 d 110</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H · Asp-Arg-Val-Tyr-Ile-His-Pro-Ile·OH 1985c</td>
<td>&lt; 0.83</td>
<td>s 70 d 80</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H · Arg-Val-Tyr-Ile-His-Pro-Ile·OH 1976</td>
<td>0.40</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

s : systolic  d : diastolic

ANG-(1–5). This indicates that the metabolic degradation of the tetrapeptide is faster than that of the pentapeptide.

ANG-(1–3) showed no pressor action. Because of the limited amounts of ANG-(1–4) and ANG-(1–3) available, these 2 peptides could be infused for only 6 minutes. This is sufficient time in which to assess their pressor activity but is too short to assess the renin-suppressing and aldosterone-stimulating actions. At least 15 minutes is necessary for the latter 2 actions. Therefore, PRA and PA were not determined in Experiments 2 and 3. ANG-(1–3) infusion at a rate of more than 140 nmol/kg-min also was not tried because of the lack of available amounts. Even if this were tried, such a high protein concentration would be toxic in man.

The results of our previous studies on the biological activities, on a molar basis, of various fragments and substituted analogues of ANG II in normal men were combined with the results of the present study and are summarized in Table 1. From this table it is evident that only tyrosine-containing molecules show pressor activity and tyrosine-deleted molecules do not show pressor activity at all. From these results it is concluded that tyrosine in position 4 in the ANG II molecule is the key amino acid for the binding to human arteriolar ANG II receptor. It was reported that alanine4-ANG II showed no pressor activity in the rat (Peach et al., 1969). This indicates the importance of the presence of tyrosine in position 4 for pressor action. Our conclusions do not conflict with this report.

For the direct renin-suppressing action, it is thought at present from this table that...
at least the structure of Val-Tyr-Ile-His or Val-Tyr-Val-His is necessary. Regarding the aldosterone-stimulating action, ANG II fragments without valine or phenylalanine are all inactive. Therefore, it is assumed that valine in position 3 or/and phenylalanine in position 8 are key amino acid(s) for the binding of ANG II to the receptor in the zona glomerulosa cells of the human adrenal cortex. However, as some position 8-substituted ANG II analogues are more or less steroidogenic (Kono et al., 1976; Kono et al., 1985c), valine in position 3 is thought to be more important.

Fig. 3 is a putative schema of the binding of ANG II to the human arteriolar ANG II receptor. Tyrosine is probably the binding portion, and there are 2 acting portions namely, Asp-Arg-Val-Tyr on N-terminal side and Tyr-Ile-His-Pro-Phe on the C-terminal side. The latter is more active than the former (Kono et al., 1983). Arteriolar ANG II receptor probably recognizes these 2 acting molecules. It is thought that both molecules co-operate to reinforce the pressor activity and especially, as reported previously (Kono et al., 1975; Kono et al., 1978; Kono et al., 1982; Kono et al., 1986), aspartic acid in position 1, arginine in position 2 and phenylalanine in position 8 contribute to a great extent to the enhancement of the pressor action of the ANG II molecule.

References


