Clinical and Experimental Studies on Angiotensin II Receptors in Arterial Wall and Adrenal Cortex

Studies with Angiotensin II and Its Competitive Antagonist

Kenji Mizuno, MD, Kazumi Haruyama, MD, Katsuo Nakajima, MD, and Soitsu Fukuchi, MD

The effects of 1-sarcosine, 8-isoleucine-angiotensin II (AIIA) on blood pressure (BP), plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were evaluated in 20 patients with hypertension or water and electrolytes disorders. Also the effects of AIIA were tested in the patients receiving a constant administration of angiotensin II (AII). The biological activities of AII and AIIA were estimated by a radioreceptor assay using 3H-AII and AII receptor isolated from bovine adrenal cortex. AIIA rose both systolic and diastolic BP in patients with low-renin levels, but did not in normal- and high-renin groups. AIIA significantly increased PAC correlated with pre-infusion PRA (r = -0.593, p<0.01). AIIA inhibited the biological activities of AII on BP and PAC, but did not on PRA. The constant rates of association (Ka) and dissociation (Kd) of AII for AII receptor in bovine adrenal cortex were; Ka 1.09x10^-7M^-1, Kd 9.16x10^-11M, respectively. However, with addition of AIIA, the binding of AII to the adrenal receptor was apparently blocked. These findings demonstrate the identity of the binding site in the adrenal gland and vascular wall for AII and AIIA, and that AIIA might be of value to assess the angiotensin II-receptor interaction in hypertension.

Key Words: Angiotensin II receptor, Vascular wall, Adrenal cortex, Renin, Angiotensin II, Aldosterone, 1-sarcosine, 8-isoleucine-angiotensin II, Radioreceptor assay, Hypertension, Water and electrolytes disorders

Although angiotensin II has a variety of pharmacologic and biological effects, it is widely recognized that the vascular smooth muscle and the adrenal gland are two main sites of action. However, little is known about the comparative characteristics of angiotensin II receptors in these two tissues.

An especially useful approach is thought to be obtained by examining an effect of competitive inhibitors. The development of angiotensin II analogue that is a competitive inhibitor provides a tool for comparing the angiotensin II receptors in vascular smooth muscle and adrenal glomerulosa cells. Such analogues have already been used to assess the role of angiotensin II in the maintenance of blood pressure and to discriminate angiotensin II receptors in other tissues.1,2)

The present study was made in order to clarify the activities of angiotensin II and its analogue upon the arterial wall in vivo and upon the adrenal gland in vitro experiments.

MATERIALS AND METHODS

(1) In vivo experiments

From the Third Department of Internal Medicine, Fukushima Medical College, Fukushima, 960, Japan.
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Reprint request to: Dr Kenji Mizuno, The Third Department of Internal medicine, Fukushima Medical College, 4-45, Sugitsuma-cho, Fukushima, 960, Japan.

Table 1. Effect of 1-sar, 8-ile Angiotensin II on blood pressure

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Initial B.P. and PRA (ng/mL/μg)</th>
<th>PAC (ng/dl) (level)</th>
<th>Dose (ng/kg/min)</th>
<th>1-sar, 8-ile All Effect (ng/mL/μg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>F</td>
<td>Essential hypertension</td>
<td>153</td>
<td>96</td>
<td>0.7 (normal)</td>
<td>6.2</td>
<td>200</td>
<td>161</td>
</tr>
<tr>
<td>45</td>
<td>M</td>
<td>Essential hypertension</td>
<td>154</td>
<td>104</td>
<td>3.7 (high)</td>
<td>10.9</td>
<td>200</td>
<td>158</td>
</tr>
<tr>
<td>45</td>
<td>F</td>
<td>Essential hypertension</td>
<td>208</td>
<td>107</td>
<td>1.7 (normal)</td>
<td>7.5</td>
<td>200</td>
<td>228</td>
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<tr>
<td>51</td>
<td>M</td>
<td>Essential hypertension</td>
<td>151</td>
<td>87</td>
<td>2.5 (normal)</td>
<td>8.6</td>
<td>200</td>
<td>186</td>
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<tr>
<td>22</td>
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<td>Essential hypertension</td>
<td>162</td>
<td>68</td>
<td>2.5 (normal)</td>
<td>11.5</td>
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<td>160</td>
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<tr>
<td>66</td>
<td>F</td>
<td>Essential hypertension</td>
<td>159</td>
<td>90</td>
<td>2.6 (normal)</td>
<td>14.4</td>
<td>200</td>
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<td>F</td>
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<td>146</td>
<td>0.9 (normal)</td>
<td>10.5</td>
<td>200</td>
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<td>30</td>
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<td>Essential hypertension</td>
<td>158</td>
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<td>1.5 (normal)</td>
<td>9.7</td>
<td>200</td>
<td>189</td>
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<td>35</td>
<td>M</td>
<td>Chronic glomerulonephritis</td>
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<td>87</td>
<td>1.5 (normal)</td>
<td>6.8</td>
<td>200</td>
<td>144</td>
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<tr>
<td>54</td>
<td>M</td>
<td>Chronic glomerulonephritis</td>
<td>224</td>
<td>128</td>
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<td>5.5</td>
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<td>250</td>
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<tr>
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<td>Chronic glomerulonephritis</td>
<td>192</td>
<td>98</td>
<td>0.5 (low)</td>
<td>5.9</td>
<td>200</td>
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<td>36</td>
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<td>Chronic glomerulonephritis</td>
<td>137</td>
<td>104</td>
<td>5.4 (high)</td>
<td>15.9</td>
<td>200</td>
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<td>M</td>
<td>chronic glomerulonephritis</td>
<td>151</td>
<td>94</td>
<td>5.7 (high)</td>
<td>12.4</td>
<td>200</td>
<td>168</td>
</tr>
<tr>
<td>67</td>
<td>M</td>
<td>chronic renal failure</td>
<td>151</td>
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<td>16.5</td>
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<tr>
<td>50</td>
<td>M</td>
<td>Chronic renal failure</td>
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<td>136</td>
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<td>7.8</td>
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<td>144</td>
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<tr>
<td>38</td>
<td>M</td>
<td>Renovascular hypertension</td>
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<td>121</td>
<td>13.0 (high)</td>
<td>21.5</td>
<td>800</td>
<td>175</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>Renovascular hypertension</td>
<td>190</td>
<td>129</td>
<td>5.5 (high)</td>
<td>20.7</td>
<td>800</td>
<td>181</td>
</tr>
<tr>
<td>54</td>
<td>F</td>
<td>Cushing's syndrome</td>
<td>173</td>
<td>122</td>
<td>0.5 (low)</td>
<td>5.8</td>
<td>200</td>
<td>207</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>Bartter's syndrome</td>
<td>122</td>
<td>64</td>
<td>26.0 (high)</td>
<td>31.7</td>
<td>800</td>
<td>111</td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>Renal tubular acidosis</td>
<td>124</td>
<td>93</td>
<td>1.5 (normal)</td>
<td>20.4</td>
<td>200</td>
<td>111</td>
</tr>
</tbody>
</table>

SBP, DBP, PRA and PAC mean systolic, diastolic blood pressure, plasma renin activity and plasma aldosterone concentration, respectively.

Subjects
The clinical data are given in Table 1.

1) Essential hypertension (8 patients)

The diagnosis was established with the following criteria; diastolic blood pressure 90 mmHg or higher after 2 hours of bed rest, the absence of papilledema or rapidly accelerating retinopathy, blood urea N below 20 mg/dl, and the absence of any known causes of hypertension. All 8 patients had normal intravenous pyelogram, and normal urinary 17-KS and 17-OHCS excretion. In 4, renal arteriography was done and was normal. Two patients had intermittent diastolic hypertension, but were studied while normotensive in diastolic blood pressure.

2) Chronic glomerulonephritis (5 patients)

The diagnosis was based on renal biopsy. All 5 patients had blood urea N below 35 mg/dl for at least seven months. None had edema or significant salt wastage.

3) Chronic renal failure (2 patients)

Both had stable renal insufficiency with blood urea N above 50 mg/dl and serum creatinine concentration above 4.0 mg/dl.

4) Renovascular hypertension (2 patients)

The diagnosis was made on the basis of hyper-reninemia, hyperaldosteronemia and positive renal arteriography. Both had unilateral stenosis in either the right or the left main renal artery.

5) Cushing's syndrome (1 patient)

The diagnosis was established with determination of urinary 17-OHCS and serum hydrocortisone concentration, and positive adrenal scintigram.

6) Bartter's syndrome (1 patient)

The diagnosis was made on the basis of hypokalemic alkalosis, normotension in spite of hyperactivity in the renin-angiotensin-aldosterone system, very low response of blood pressure to exogenous angiotensin II and hyperplasia of the juxtaglomerular
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7) Renal tubular acidosis (1 patient)

The diagnosis was established with stable hypokalemic acidosis and low urine pH below 5.0, and renal biopsy.

Test procedure

For at least 10 days before the study, the patients were given daily a diet containing approximately 120 or 154 mEq sodium. An intravenous administration of 5% glucose in water was begun at a rate of 0.2 ml/min and continued until the blood pressure was stable for 15 to 20 minutes. Without disturbing the patients, the infusion was then changed to one containing 10 μg of 1-sar, 8-ile AII in each ml of 5% glucose in water. This was started at a constant rate of either 200 or 800 ng/kg/min (see Table 1) for 30 minutes. Serial blood pressure was measured before, during and after the infusion. Blood samples were taken for determination of PRA and PAC before and after the infusion.

Over 24 hours after the infusion, 1-sar, 8-ile AII was tested again for 30 minutes at the same rate as described above in the patients receiving a constant infusion of synthetic AII (Hypertensin, Ciba) at a rate of 5 ng/kg/min (except a patient with Bartter's syndrome) or 10 ng/kg/min (a patient with Bartter's syndrome). Serial blood pressure was recorded before, during and after the infusion of 1-sar, 8-ile AII. Blood samples were taken for measurements of PRA and PAC before and after the infusion.

2) In vitro experiments

1) Tissue preparation

Bovine adrenal was placed in cold saline, and subsequent operation was performed at 4°C. The adrenal was carefully cleaned of adherent fat and decapsulated. The outer cortex was delivered from the decapsulated adrenal, weighed and homogenized in 20 volumes of 0.4 M-KCl buffered with 0.01 M-Tris (pH 7.5). The homogenates were centrifuged at 10,000 G for 20 minutes and the supernatants (hereafter referred to as the KCl extract) were used for radioreceptor assay. Protein determination was performed according to the method of Lowry et al.7

2) Procedure of radioreceptor assay

To siliconized glass tubes were added 0.1 ml of standard angiotensin II or 1-sar, 8-ile angiotensin II (0-313.6 pg) in 0.4-KCl (pH 7.5) diluent, 0.1 ml of 3H-angiotensin II (about 2500 c.p.m.) in 0.4 M-KCl (pH 7.5) diluent and 0.5 ml of the KCl extract (600 μg protein) in an ice bath. This gave a total volume of 0.7 ml in each tube. The tubes were incubated at 25°C for 5 minutes. Then, the tubes were placed in an ice bath. 0.5 ml of dextrancoated charcoal was added to each of the tubes, which were shaken for a few seconds, and were centrifuged at 2500 rev./min for 10 minutes. The dextran-coated charcoal was diluted for 4 times by 0.4 M-KCl (pH 7.5) from the stock solution which had been made previously by mixing an equal volumes of 0.5% dextran 40 and 5% charcoal. 1.0 ml of the supernatants were collected in glass vials and mixed with 15 ml of scintillation solution and were counted in a scintillation detector.

RESULTS

1) Effects of 1-sar, 8-ile AII on blood pressure, PRA and PAC

Result of blood pressure response to
Fig. 1. Relation between pre-infusion PRA and change of mean blood pressure by administration of (1-sar, 8-ile) angiotensin II.


PRA (ng/ml/h)

\[ E.H. \quad C.G.N. \quad R.V.H. \quad R.T.A. \quad C.S. \quad B.S. \quad C.R.F. \]

\[ r = -0.959, \ p < 0.001 \]

\( (n = 20) \)

Fig. 2. Relation between pre-infusion PRA and change of PRA by administration of 1-sar, 8-ile All.


\[ r = -0.999, \ p < 0.0001 \]

\( (n = 20) \)

Fig. 3. Relation between pre-infusion PRA and change of PAC by administration of 1-sar, 8-ile All.


A significant fall was observed in 2 patients with renovascular hypertension, in a patient with Bartter's syndrome and in a patient with renal tubular acidosis. On the contrary, a significant rise was observed in 3 of 8 patients with essential hypertension and in a patient with Cushing's syndrome. A relatively significant rise was observed in 5 of 8 patients with essential hypertension, in all 5 patients with chronic glomerulonephritis and in patients with chronic renal failure.

The fall of mean blood pressure significantly correlated with preinfusion levels of PRA \( (r = -0.642, \ p < 0.01, \text{Fig. 1}) \).

PRA in 4 cases having responded to 1-sar, 8-ile All by a fall more than 10 mmHg in diastolic blood pressure was \( 11.45 \pm 10.82 \) (mean ± standard error) ng/ml/h. On the other hand, PRA in the other groups was \( 2.11 \pm 1.72 \) (mean ± standard error) ng/ml/h. The difference was significant \( (p < 0.01, \text{Table 1}) \). Correlation between pre-infusion PRA and change of PRA by the infusion of 1-sar, 8-ile All was also significant \( (r = -0.959, \ p < 0.001, \text{Fig. 2}) \).

An increase in PAC by the i.v. administration of 1-sar, 8-ile All inversely cor-

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Fig. 4. Influence of 1-sar, 8-ile AII against effect of AII on blood pressure.

Influence of 1-sar, 8-ile AII against effect of AII on blood pressure is summarized in Fig. 4. The depressor effect of 1-sar, 8-ile AII against AII induced hypertension was observed in the group of essential hypertension, chronic glomerulonephritis, in the group of renovascular hypertension and in a patient with Cushing's syndrome. The depressor effect of 1-sar, 8-ile AII against hypertension induced by AII became manifest within 5 to 10 minutes after the i.v. infusion of 1-sar, 8-ile AII. The blood pressure gradually elevated after secession of 1-sar, 8-ile AII, and reached to the initial level produced by AII only within 90 minutes. However, in the group of chronic renal failure, 1-sar, 8-ile AII failed to block the elevation of diastolic blood pressure, while it showed the depressor effect against AII in the systolic hypertension induced by AII. In a patient with Bartter's syndrome, AII did not increase the blood pressure, but the diastolic blood pressure was reduced by the administration of 1-sar, 8-ile AII concomitant with AII. In a pa-

2) Influence of 1-sar, 8-ile AII on blood pressure, PRA and PAC against effects of AII.

Influence of 1-sar, 8-ile AII against pre-infusion PRA (r = -0.593, p < 0.01, Fig. 3).
3) Effect of unlabelled AII and AIIA on binding of \(^3\)H-AII to the KCl extract

The results obtained by increasing concentration of unlabelled AII and AIIA on binding of \(^3\)H-AII and the KCl extract indicated that AIIA inhibited the binding of labelled AII more actively than unlabelled AII at a range from 0 to 313.6 pg of both the peptides (Fig. 6). The binding activities of AII and AIIA were analyzed by Scatchard's plot, and the constant rates of association (Ka) and dissociation (Kd) were; Ka 1.09×10^{-8}M^{-1} and Kd 9.16×10^{-12}M for AII, Ka 1.83×10^{-12}M^{-1} and Kd 5.46×10^{-12}M for AIIA, respectively.

DISCUSSION

Of late years, the role of renin-angiotensin system has been investigated with various anti-angiotensin agents in diverse hypertension and water or electrolytes disorders\(^8,9\). For example, angiotensin II analogue, especially, 1-sar, 8-ile angiotensin II is an active and long-lasting blockade of angiotensin II, whose antagonism against angiotensin II was first demonstrated by Türker et al.\(^10\).

Intravenous administration of 1-sar, 8-ile AII elevated blood pressure in 16 patients with low or low-normal plasma renin activity. On the contrary, it lowered blood pressure in 4 patients whose plasma renin activity was significantly higher than that in the other groups. Hence 1-sar, 8-ile AII per se appears to have a pressor effect although less active than AII, but the binding to receptor of AII in the arterial wall is seemed to be more active than AII, and consequently, it appears that 1-sar, 8-ile AII shows an antagonism against AII with depressor effect in the presence of high level of circulating AII, while shows an agonistic effect in the presence of low level of circulating AII. The findings obtained above are of limited value for the characterization of the type of antagonism, because the concentration of agonist or antagonist at the receptor level cannot be measured accurately. However,
the results of these experiments indicate that 1-sar, 8-ile AII inhibit the vasoconstrictive action of exogenous AII in vivo. To define the interaction of agonist and antagonist at the receptor level, the use of isolated cell membrane prepared from the vascular smooth muscle is preferable. Shimada et al\textsuperscript{11)} showed that 1-sar, 8-ile AII was higher active than AII in binding to the receptor in the arterial wall of rabbits in vitro.

An increase in PRA by the administration of 1-sar, 8-ile AII inversely correlated with the pre-infusion levels of PRA. It is possible to consider that 1-sar, 8-ile AII inhibits vasoconstrictive effect of AII in receptor level of the renal afferent arterioles, and consequently, PRA is decreased according to dilatation of the arterioles. Evidence for the supposition described above could be supported by the data that PRA, which was depleted by the infusion of AII, far from decreasing, rather was slightly elevated by the addition of 1-sar, 8-ile AII to AII infusion in our experiment. On the contrary, Pettinger et al\textsuperscript{12)} reported that PRA was increased with elevation of blood pressure by administration of 1-sarcocine, 8-alanine AII in high-renin patients, and they concluded that its increase was a compensatory mechanism for the maintenance of blood pressure regulation.

Intravenous administration of 1-sar, 8-ile AII increased the plasma level of aldosterone in patients whose initial PRA was low or normal, while it decreased plasma aldosterone in high-renin groups.

Furthermore, plasma aldosterone which was significantly increased by the administration of AII was slightly decreased by concomitant 1-sar, 8-ile AII administration. These findings demonstrate that 1-sar, 8-ile-AII per se produces aldosterone as AII, but eliminates the binding of AII to the adrenal receptor and consequently inhibits the production of aldosterone by AII, that is, it shows an antagonism against AII with decreasing the concentration of aldosterone in the presence of high level of circulating AII, while shows an agonism in the presence of low level of AII. This interaction of agonist or antagonist at the receptor level in the adrenal cortex could be defined by our experiments which were performed with radioreceptor assay using \textsuperscript{3}H-AII and AII receptor isolated from bovine adrenal gland. The AII receptor of the bovine adrenal cortex exhibited the properties of high affinity (K_a is about 1.09×10^{-9}M^{-1} for AII). The specific inhibition of binding of the receptor to \textsuperscript{3}H-labelled AII by addition of unlabelled AII or 1-sar, 8-ile AII occurred at a range from 0 to 313.6 pg of both the peptides. These findings demonstrate the identity of the binding site in the adrenal receptor for AII and 1-sar, 8-ile AII. In the present studies, the constant rate of association for 1-sar, 8-ile AII was about 18 times higher than AII, indicating that 1-sar, 8-ile AII does exhibit a significantly increased affinity for AII receptor of adrenal gland. Douglas et al\textsuperscript{13)} reported that K_a for AII measured in canine glomerulosa cells was 3.3×10^{-9}M^{-1}, and 1-sar, 8-ile AII was 3 times higher than for AII in relative binding-inhibition potencies in isolated adrenal cells in vitro in the canine. Our data are similar to Douglas in the results that AII binds to the receptor of adrenal cortex more actively that AII.

From these findings described above, it is concluded that 1-sar, 8-ile AII binds to an identical receptor site as AII in the vascular smooth muscle and adrenal cortex, and that 1-sar, 8-ile AII might be useful adjunct to assess the role of renin-angiotensin-aldosterone system by examining its binding activity to the angiotensin II vascular and adrenal receptors in diverse hypertension or water and electrolytes disorders in man.

**REFERENCES**

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