Vascular Angiotensin II Receptors in Renal and DOCA-Salt Hypertensive Rats

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SUMMARY To determine the contribution of receptor number and affinity to changes in vascular reactivity to angiotensin II in hypertensive rats, we have investigated the binding of $^{125}$I-AII to a particulate fraction of the rat mesenteric artery of hypertensive rats. In two-kidney, one clip hypertensive rats, receptor concentration ($B_{max}$) was 83 ± 13 fmol/mg and the dissociation constant ($K_d$) 0.6 ± 0.1 nM vs 75 ± 5.3 fmol/mg and 0.6 ± 0.1 nM in normotensive controls, although PRA was much higher in the former. $B_{max}$ was reduced in these hypertensive rats after sodium depletion, as in normal rats. One-kidney, one clip hypertensive rats ($B_{max}$ 88 ± 17 fmol/mg, $K_d$ 0.6 ± 0.1 nM) did not differ from uninephrectomized control rats (96 ± 9 fmol/mg, $K_d$ 0.5 ± 0.1 nM). In DOCA-salt hypertensive rats, binding capacity was increased (125 ± 2 fmol/mg, $K_d$ 0.7 ± 0.0 nM) vs uninephrectomized salt-loaded rats ($B_{max}$ 95 ± 6 fmol/mg, $K_d$ 0.6 ± 0.1 nM), although PRA was suppressed comparably in both groups. The salt-loaded rats did not differ from uninephrectomized controls drinking water. We conclude that changes in the circulating renin-angiotensin system do not explain all the variations in receptor number in hypertensive rats. Our results suggest a role of mineralocorticoids in the regulation of vascular AII receptors. (Hypertension 5 (supp V): V-16–V-21, 1983)

KEY WORDS • mesenteric artery angiotensin II receptors • renovascular hypertension • DOCA-salt hypertension

THE response of smooth muscle to angiotensin II (AII) depends on the interaction with a membrane receptor to activate the contractile mechanism.1 Response of blood vessels to AII may therefore be modified by changes at the level of the receptor or by postreceptor events. The interpretation of vascular responsiveness to AII thus demands an understanding of the status of AII receptors in vascular smooth muscle. We have therefore investigated the binding of AII to membranes obtained from a resistance-type vessel, the mesenteric vascular bed, in rats with renal and mineralocorticoid hypertension, with the object of gaining a better understanding of the regulation of AII receptors in hypertension and the possible contribution of derangements at the angiotensin receptor level to the physiopathology of high blood pressure.

Materials and Methods

Iodination of Angiotensin II

Angiotensin II (Peninsula, San Carlos, California) was iodinated by a modification of the technique of Freedlender and Goodfriend.2 Specific activity (measured by radioimmunoassay) was 1000–1200 Ci/mmol. $^{125}$I-AII could be precipitated in excess of 95% by an anti-AII antibody and comigrated with AN when subjected to thin-layer chromatography on cellulose plates (Bakerflex, Baker Chemical Company, Phillipsburg, New Jersey) developed with tert-butyl alcohol/3% ammonium (105:35). Biochemical purity under these conditions was 99%.

Preparation of Mesenteric Artery Membranes

A modification of the technique of Wei et al.3 was employed, similar to that used by Gunther et al.4 In brief, rats were sacrificed by decapitation. Three or four rats were used per group in each individual experiment to obtain enough material for the saturation binding curves. The mesenteric artery was sectioned from the aorta at its origin, and the mesentery was dissected from the mesenteric border of the intestine and immersed in cold phosphate buffered saline. Adipose tissue was removed by blunt dissection and the cleaned arteries transferred to a 0.25 M sucrose solution. Remaining fat was removed by 10 strokes in a Heidolph
Digital 2000 homogenizer (Wiarton, Ontario, Canada). Arteries were resuspended in new 0.25 M sucrose solution, finely minced with scissors, then homogenized in a Polytron (Kinematica, Lucerne, Switzerland, setting 8; 10 seconds twice). The homogenate was centrifuged at 1,500 × g for 10 minutes at 4°C; the supernatant was decanted and recentrifuged. The final supernatant was filtered through cheesecloth, then centrifuged at 104,000 × g for 30 minutes. Electron microscopy of the pellet showed a preparation that contained vesicles exclusively. The pellet was resuspended in a 0.05 M Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 2 mM MgCl₂, and 1 mM EDTA. Proteins were measured by the Coomassie blue method. After this, bovine serum albumin was added at a concentration of 0.2%, and the membranes were diluted to a protein concentration of 1 mg/ml in the Tris buffer containing 0.2% albumin ("assay buffer").

**Binding Assay**

The binding assay was performed with ³²P-I-angiotensin II (0.06 to 2 nM) and 100 μg of receptor protein per tube at 22°C for 45 minutes since a plateau is achieved after 30 minutes. All assays were performed in duplicate. Nonspecific binding was determined by incubation in the presence of 1 μM unlabeled All for each point of saturation binding curves. Separation of bound and free radioactivities was achieved by rapid filtration through Whatman GF/C filters prewetted with 0.5 ml of "assay buffer." The filters were washed twice with 3 ml of 0.9% NaCl, then allowed to dry and counted in an Rackgamma LKB counter with 85% efficiency. Total binding to membranes was 4% to 5% of total radioactivity in the presence of 0.1 nM ³²P-I. Specific binding (total binding minus nonspecifically bound, in the presence of 1 μM unlabeled All) was 90%-95% of total binding. Binding to filters in the absence of membranes was 0.1%-0.2% of total radioactivity.

**Animal Experiments**

Two-kidney, one clip hypertensive rats were prepared by placing a silver clip with an internal diameter of 0.2 mm on the left renal artery of 200 g male Sprague-Dawley rats (Charles River Laboratories) under ether anesthesia. Blood pressure was taken weekly by the tail-cuff method under light ether anesthesia and recorded on a Grass model 7 polygraph (Grass Medical Instruments, Quincy, Massachusetts) fitted with a 7P8 preamplifier and a model 1010 Grass crystal microphone as a pulse detector. The average of three pressure readings was recorded. Two-kidney, one clip rats that did not become hypertensive (blood pressure above 150 mm Hg was considered hypertension) served as controls. Experiments were performed on the fourth week after development of hypertension. Sodium depletion was achieved by feeding a sodium-deficient diet (Hartroft Test Diet, ICN Nutritional Biochemicals, Cleveland, Ohio containing less than 5 mmoles of sodium per kg diet) for 10 days and injecting furosemide, 10 mg/kg, intraperitoneally, on the first 2 days of sodium depletion. One-kidney, one clip rats were prepared similarly. Most developed hypertension, and uninephrectomized rats served as controls.

DOCA-salt hypertension was induced by the method of Ormsbee and Ryan. Rats were uninephrectomized. Silicone rubber impregnated or not with deoxycorticosterone acetate (DOCA), 100 mg per rat, was implanted in all experimental groups, and they were offered 1% saline to drink. Rats were studied within 2 weeks of becoming hypertensive.

All groups compared were studied on the same experimental day. Filtration was performed within 6 hours of decapitation and within a period of 90 minutes from the first filtration to the last.

**Biochemical Methods**

Blood was obtained from the trunk within the first 5 seconds after decapitation, on EDTA, on ice, and centrifuged at 4°C. Plasma from rats in each group in individual experiments was pooled and plasma renin activity (PRA) was measured, as previously described. Plasma aldosterone concentration was measured by radioimmunoassay.

**Analysis of Data**

Bmax and Kd were calculated from Scatchard plots of saturation binding isotherms. The linear regressions of all Scatchard plots had a correlation coefficient between 0.9 and 1.0. Results are means ± SEM. Statistical analysis was performed by the paired (within experiment) Student's t test, and results were considered significant when p < 0.05.

**Results**

Two-kidney, one clip hypertensive rats had similar All receptor density (Bmax) and apparent dissociation constant (Kd) calculated from Scatchard plots of saturation binding isotherms (fig. 1) to two-kidney, one clip rats which did not develop hypertension. This occurred in spite of the fact that PRA was significantly elevated (p < 0.01) in the two-kidney, one clip hypertensive rats (table 1). Plasma aldosterone was significantly higher (p < 0.05) in the two-kidney, one clip hypertensive rats.

Sodium depletion produces down-regulation of vascular angiotensin II receptors. In our hands, after sodium depletion, Bmax was reduced in normal rats from 100 ± 4 fmol/mg (Kd 0.7 ± 0.1 nM) to 70 ± 5 fmol/mg (Kd 0.7 ± 0.1 nM) (p < 0.01, n = 10 experiments). Since in sodium replete two-kidney, one clip hypertensive rats, receptor density is not reduced in accordance with the increased PRA (table 1), we tried to see whether sodium depletion could determine down-regulation of mesenteric artery All receptors in these hypertensive rats.

When two-kidney, one clip hypertensive rats were sodium-depleted, PRA rose further, as expected (table 2). Bmax was significantly reduced (p < 0.05) in these
two-kidney, one clip hypertensive rats after sodium depletion compared to two-kidney, one clip hypertensive sodium-replete rats (table 2), indicating that the capacity to down-regulate vascular All receptors after a negative sodium balance was preserved.

To determine whether the absence of ‘‘down-regulation’’ of All receptors in sodium-replete hypertensive rats in spite of high plasma renin activity (with respect to sodium-replete normotensive rats, table 1) was caused by the high blood pressure per se, we examined a model of normal renin renal hypertension, the one-kidney, one clip rat. Neither receptor density (Bmax) nor Kd were different in one-kidney, one clip hypertensive and normotensive rats with similar renin level (table 3).

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**Table 1. Angiotensin II Receptors in the Mesenteric Artery of Two-Kidney, One Clip Rats**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Bmax (fmol/mg)</th>
<th>Kd (nM)</th>
<th>PRA (ng AI·ml⁻¹·hr⁻¹)</th>
<th>Plasma aldosterone (ng/dl)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-K, 1-C normotensive</td>
<td>75 ± 5</td>
<td>0.6 ± 0.1</td>
<td>2.3 ± 0.6</td>
<td>8.7 ± 3.6</td>
<td>102 ± 2</td>
</tr>
<tr>
<td>2-K, 1-C hypertensive</td>
<td>83 ± 13</td>
<td>0.6 ± 0.1</td>
<td>16.9 ± 4.4†</td>
<td>30.7 ± 12.1*</td>
<td>169 ± 4†</td>
</tr>
</tbody>
</table>

Results are means ± SEM; n = 5 experiments, 37 rats; PRA = plasma renin activity; AI = angiotensin I.

* p<0.05
† p<0.01

**Table 2. Angiotensin II Receptors in the Mesenteric Artery of Sodium-Replete and Sodium-Depleted Two-Kidney, One Clip Hypertensive Rats**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Bmax (fmol/mg)</th>
<th>Kd (nM)</th>
<th>PRA (ng AI·ml⁻¹·hr⁻¹)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na replete</td>
<td>103 ± 18</td>
<td>0.9 ± 0.2</td>
<td>12.8 ± 0.1</td>
<td>170 ± 6</td>
</tr>
<tr>
<td>Na-depleted</td>
<td>74 ± 12*</td>
<td>0.9 ± 0.2</td>
<td>25.9 ± 5.3*</td>
<td>168 ± 7</td>
</tr>
</tbody>
</table>

Results are means ± SEM; n = 3 experiments, 22 rats; PRA = plasma renin activity; AI = angiotensin I.

* p<0.02 vs Na replete.

**Table 3. Angiotensin II Receptors in the Mesenteric Artery of One-Kidney, One Clip Hypertensive Rats**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Bmax (fmol/mg)</th>
<th>Kd (nM)</th>
<th>PRA (ng AI·ml⁻¹·hr⁻¹)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102 ± 13</td>
<td>0.7 ± 0.0</td>
<td>1.9 ± 0.4</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>Uni-Nx</td>
<td>96 ± 9</td>
<td>0.5 ± 0.1</td>
<td>1.5 ± 0.8</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>1-K, 1-C hypertensive</td>
<td>88 ± 17</td>
<td>0.6 ± 0.1</td>
<td>2.7 ± 0.7</td>
<td>172 ± 5*</td>
</tr>
</tbody>
</table>

Results are means ± SEM; n = 3 experiments, 18 rats; PRA = plasma renin activity; AI = angiotensin I; Nx = nephrectomized.

* p<0.01 vs control and Uni-Nx.
We also examined a model of mineralocorticoid hypertension, the DOCA-salt hypertensive rat, and compared these with salt-loaded uninephrectomized rats (table 4). Both groups had suppressed PRA, but DOCA-salt hypertensive rats had a significantly higher Bmax than the salt-loaded rats \((p < 0.01)\). The latter were not different from uninephrectomized rats drinking water, although PRA was suppressed in the former and normal in the rats on a normal sodium balance (table 4 and fig. 2).

**Discussion**

Previous studies performed on the vascular AT receptor have suggested that infusion of AT or increased plasma renin activity after sodium depletion, acting through the generation of AT, down-regulate these receptors.\(^9\)\(^{10}\) Our results demonstrate that the circulating levels of renin, via the production of AT, may not be the only factor involved in the regulation of AT receptors, since we find a dissociation between the AT receptor density in the mesenteric artery and plasma renin activity.

Vascular AT receptor density was normal or increased in two-kidney, one clip hypertensive rats, normal in one-kidney, one clip rats, and increased in DOCA-salt hypertensive rats. These results agree with reported vascular reactivity to AT in hypertensive rats. McGregor and Smirk,\(^11\) Finch,\(^12\) and Collis and Alps\(^13\) have shown the response of the mesenteric vasculature to AT to be increased in renin-dependent renal hypertension. A normal or increased response in human renovascular hypertension has been described by Brown et al.\(^14\) The increased pressor response to AT returned to normal after surgery. Bean et al.\(^15\) have shown an altered relation between arterial pressure and plasma AT concentration after prolonged infusion of AT in the dog, and Brown et al.\(^16\) have provided similar evidence in the rat. This altered relationship suggests that AT receptors are not down-regulated after prolonged infusion of AT or, alternately, that postreceptor phenomena are altered so that an enhanced response occurs by mechanisms that remain to be determined. In the case of primary hyperaldosteronism reported in a study of low renin hypertension in humans,\(^17\) and in mineralocorticoid hypertension in experimental animals,\(^18\) pressor reactivity to AT is increased, in agreement with our findings of a greater vascular AT receptor density in DOCA-salt hypertensive rats.

Gunther et al.\(^9\) and Aguilera and Catt\(^10\) have provided evidence showing that AT produces down-regulation of vascular AT receptors. This mechanism appears to operate after sodium depletion and AT infusion. Aguilera and Catt\(^10\) infused AT at relatively low pressor doses to obtain reduction of the number of AT receptors. We have also observed that low doses of AT infused intravenously produce a reduction of vascular AT receptors.\(^19\) When a high pressor dose \((200 \text{ ng/kg per min})\) was infused intravenously for 6 days, the density of vascular AT receptors was normal or increased, without change in affinity.\(^19\) This suggests that our results in two-kidney, one clip rats may be

![Figure 2. Scatchard plots of data from saturation binding isotherms (as in fig. 1) performed simultaneously on receptor protein from DOCA-salt hypertensive rats. 2 weeks after implantation of DOCA-impregnated silicone rubber and uninephrectomy, uninephrectomized rats receiving 1% NaCl or drinking tap water. The two latter groups were implanted with silicone rubber without DOCA. Results are means ± SEM.](http://hyper.ahajournals.org/)

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Bmax (fmol/mg)</th>
<th>Kd (nM)</th>
<th>PRA (ng Al·ml⁻¹·hr⁻¹)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89 ± 3</td>
<td>0.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>Uni-Nx</td>
<td>99 ± 5</td>
<td>0.8 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>Uni-Nx + NaCl</td>
<td>95 ± 6</td>
<td>0.6 ± 0.1</td>
<td>0.1 ± 0.1*</td>
<td>109 ± 4</td>
</tr>
<tr>
<td>DOCA + Uni-Nx + NaCl</td>
<td>125 ± 2*</td>
<td>0.7 ± 0.0</td>
<td>0.0 ± 0.0*</td>
<td>171 ± 4*</td>
</tr>
</tbody>
</table>

Results are means ± SEM; \(n = 4\) experiments, 31 rats; PRA = plasma renin activity; Al = angiotensin I; Nx = nephrectomized.

\(^*p<0.01\) vs control.
caused by the chronic action of the large amount of circulating All, producing up-regulation of the receptors. The mechanism for this action of All remains to be determined. Since large concentrations of All given chronically will raise aldosterone concentration in plasma while smaller doses will only do so transiently, increased aldosterone may play a role in the effect that large doses of All have on All receptors. This possibility is further supported by the expected high plasma aldosterone of two-kidney, one clip rats in which receptor density was surprisingly not reduced in spite of the high plasma renin. Furthermore, we have recently found that aldosterone infusion up-regulates vascular All receptors in vivo in the rat and that in vitro exposure of cultured mesenteric artery smooth muscle cells to aldosterone results in a significant increase in All receptors.

In DOCA-salt hypertensive rats, renin suppression by mineralocorticoids may be the mechanism for increase in Bmax of vascular All receptors. Salt-loaded rats, with comparably suppressed renin (table 4), however, did not present up-regulation. Thus renin suppression does not appear to be the mechanism for increase of vascular All receptor concentration in DOCA-salt hypertensive rats. Hypertension by itself, perhaps via smooth muscle cell hyperplasia, is not involved, since similarly hypertensive normal renin one-kidney one-clip rats have no change in receptor density. The effect of mineralocorticoids is probably the main mechanism involved in our findings in the DOCA-salt hypertensive rat.

In recent studies, it has been shown that increases in potassium down-regulate uterine All receptors. However, in another study, the same author has found no relationship between serum potassium and the density of uterine receptors in DOCA-salt postnephrectomy, although after nephrectomy, elevated plasma aldosterone may counterbalance the action of potassium. This possible direct up-regulating action of aldosterone is further supported by a subsequent report in which aldosterone infusion increased the number of vascular All receptors in vivo in the rat and that in vitro exposure of cultured mesenteric artery smooth muscle cells to aldosterone results in a significant increase in All receptors.

A role of the sodium ion in our findings should be considered. However, the results obtained in DOCA-salt hypertensive rats vs sodium-loaded rats suggest that sodium-loading per se does not result in the up-regulation of vascular All receptors. This is in contrast to the findings of Gunther et al. and Aguilera and Catt. These investigators demonstrated that excess sodium increased the binding of All to mesenteric artery membranes and to a urinary bladder homogenate. We cannot explain the reason for these differences with our results. In our study, rats were uninephrectomized and their plasma renin activity was suppressed, indicating adequate sodium-loading. A direct action of sodium at the moment of performing in vitro binding, as shown by Wright et al., was impossible, since we diluted membranes in an assay buffer containing 120 mM NaCl. Finally, we found an absence of down-regulation in two-kidney, one clip hy-

### Table 5. Effect of Different Procedures on Vascular Angiotensin II (All) Receptors

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Plasma All</th>
<th>Plasma aldosterone or DOC</th>
<th>Plasma K</th>
<th>Bmax</th>
<th>Kd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na depletion</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>High sodium intake</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineralocorticoid infusion</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DOC or aldosterone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All infusion suppressor</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(200 ng/kg per min i.p.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All infusion pressor</td>
<td>↑</td>
<td>↔</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>(200 ng/kg per min i.v.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-K, 1-C hypertension</td>
<td>↑</td>
<td>↓</td>
<td>↔</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>1-K, 1-C hypertension</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>DOC-salt hypertension</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

**DOC** = deoxycorticosterone; i.p. = intraperitoneally; i.v. = intravenously. From studies reported in this paper in refs. 19 and 20 and unpublished observations.
pertensive rats (except after sodium depletion), which have been shown to present a sodium deficit, and up-regulation (same direction as the former) in the DOCA-salt hypertensive rats, which present sodium retention. Thus, a qualitatively similar effect resulted from opposing phenomena involving the sodium ion, suggesting that per se it does not play an important role in our findings.

When the results of tables 1 and 3 are compared, it may be seen that changes in the order of 30% in Bmax and slight differences in K0 occurred between sets of experiments. This may be explained by inaccuracies in the determination of the specific activity of 125I-AII between batches. Furthermore, there is a rather large interassay coefficient of variation (22.5%, n = 31 experiments, 116 control rats), but results within experiment have a remarkable consistency and reproducibility. This explains the use of a paired (within experiment) Student’s t test for evaluation of the data.

In conclusion, all may produce two effects. Acutely (such as after sodium depletion), the down-regulating action of all predominates. Chronically, in small amounts and in the absence of increase of plasma aldosterone, the density of all binding sites is also reduced. After infusion of large amounts of angiotensin, or in renin-dependent hypertension (two-kidney, one clip Goldblatt hypertension), vascular all receptors are inappropriately normal instead of down-regulated. In DOCA-salt hypertension, vascular all receptors are up-regulated beyond what can be attributed to suppression of PRA. Table 5 summarizes these concepts on regulation of vascular all receptors from these and other data. It is important to mention that, in contrast to what has been shown with uterine all receptors, we did not find significant changes in the affinity of the vascular all receptor after any of the procedures we have employed.

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