Vascular Angiotensin II Receptors and Sodium Balance in Rats

ROLE OF KIDNEYS AND VASCULAR RENIN ACTIVITY

By John D. Swales, John D. Tange, and Herbert Thurston

ABSTRACT

The relationship between the vascular response to angiotensin II and sodium balance was studied by measuring the volume of specific antiserum needed to block the pressor response to a standard dose of angiotensin II in rats. In normal rats, sodium depletion reduced the volume of antiserum required to prevent a pressor response to 50 ng of angiotensin II from 0.32 to 0.22 ml; sodium loading increased the volume required to 0.65 ml. After bilateral nephrectomy, there was a progressive increase in the antibody requirement to a maximum of 0.63 ml at 6 hours. At this stage, there was no change in the blocking requirement with sodium depletion by either pretreatment with a low-salt diet (0.60 ml) or peritoneal dialysis (0.62 ml). The pressor response to a 50-ng bolus of angiotensin II was closely correlated with the antibody requirement in all experiments (r = + 0.93). These observations indicate that sodium-induced changes in the vascular response to angiotensin II require the presence of kidney tissue. We suggest that such changes in response are mediated by alterations in vascular renin which generates angiotensin II at an arteriolar site that is not accessible to antibody molecules. This locally formed angiotensin II reduces the number of receptors free to respond to circulating angiotensin II and raises the threshold of the pressor response.

The interrelationship between the sodium ion and the renin-angiotensin system is of fundamental importance in the pathogenesis of hypertension (1). One of the likely sites of interaction is the vascular angiotensin II receptor. The pressor response to angiotensin II is impaired by salt depletion and enhanced by salt loading in the rat (2, 3), rabbit (4), and man (5, 6). However, the pressor action of other agents such as norepinephrine is not modified by changes in sodium balance (2, 6). There are a number of possible explanations for the effect of sodium ions on the pressor response to angiotensin II. An inverse relationship has been demonstrated between the blood pressure response to angiotensin II and the plasma renin level (2, 7, 8). This relationship could be fortuitous, however, with changes in sodium balance independently modifying both vascular responsiveness and renin secretion. Brunner et al. (9) have suggested another possibility. They have reported that the amount of specific angiotensin II antibody required to block the pressor response to an angiotensin II infusion is greater than normal in salt-depleted rats and have postulated that, since vascular receptors compete with antibody for angiotensin II, vascular affinity for angiotensin II varies directly with sodium balance. It follows from this concept that the vasoconstrictor effect of increased angiotensin II formation in salt depletion would be diminished or even reversed by the decrease in vascular affinity. The converse effect would be present with salt loading. A third explanation is based on the generation of angiotensin II by renin at a local vascular site that is inaccessible to antibody (10-12). This hypothesis predicts that changes in sodium balance will influence angiotensin II antiserum requirements only through changes in renin secretion. To examine the validity of this hypothesis, we measured the pressor responses to angiotensin II and the blocking requirements for angiotensin II antiserum in a variety of experimental situations in which renin secretion and sodium balance could be varied independently. We also studied the changes in angiotensin II responses and antiserum requirements with the passage of time after bilateral nephrectomy to distinguish this effect from one due to a fall in circulating renin.

Methods

Production of Antiserum.—Antiserum to 5-Val-angiotensin II amide (Hypertensin), (Ciba) was prepared in a single rabbit according to the method of Goodfriend et al. (13). The titer of this antibody was tested both in vitro and in vivo. A 0.1-ml sample of antiserum diluted 1 in 20,000 bound 50% of 62.5 pg of 125I-labeled angiotensin II; binding of 125I-labeled angiotensin I was less than 50% at...
a dilution of 1 in 10. Serial dilutions of this antiserum was also added to a saline solution of 5-Val-angiotensin II amide and titrated in a bilaterally nephrectomized rat (14); 1 ml of antiserum prevented a pressor response to 16,000 ng of angiotensin II. Cross-reactivity with rat angiotensin (prepared by incubating rat plasma) was tested by comparing the pressor response to equipressor doses of synthetic angiotensin II amide and rat angiotensin following the infusion of graded doses of antiserum. An equal reduction of the pressor action of the two angiotensins was observed at all levels of blocking.

Infusion Studies.—White Wistar rats (150-250 g) of either sex were used throughout these studies. All procedures were performed under ether anesthesia. The jugular vein and the carotid artery were cannulated, and mean arterial blood pressure was measured with a Statham P23gd transducer connected to a Grass polygraph recorder. After base-line pressure had been recorded, 50 ng of 5-Val-angiotensin II dissolved in 0.1 ml of isotonic saline was injected intravenously as a bolus. After the mean blood pressure had returned to basal levels, 0.1 ml of antiserum was injected and the pressor response to 50 ng of angiotensin II was again measured. Repeated 0.1-ml doses of antiserum were administered until the response to 50 ng of angiotensin II was less than 5 mm Hg. Control rats infused with serum from a nonimmunized rabbit showed no reduction in response to angiotensin II.

Experimental Groups.—Groups 1-4 and 6-7 each comprised 6 rats. Subgroups 5a, 5b, 5c, and 5d each contained 6 rats, and subgroup 5e was composed of 11 rats. Group 1 (normal) had free access to tap water and to laboratory chow containing 0.205 mEq/g of sodium and 0.163 mEq/g of potassium for 8-14 days. Group 2 (low salt) was allowed to drink only deionized water and to eat food containing 0.0138 mEq/g of sodium and 0.190 mEq/g of potassium. Group 3 (high salt) was given 1% sodium chloride solution to drink together with free access to laboratory chow for 8-14 days. Group 4 (dialysis) underwent peritoneal dialysis 5 hours after bilateral nephrectomy, and infusion studies were carried out 1 hour after that.

Plasma Renin Activity.—Thirty-three rats were bilaterally nephrectomized, and blood was collected by aortic puncture using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant after intervals ranging from 2 minutes to 24 hours. Plasma renin activity was measured by radioimmunoassay (15).

Results

Blood Pressure

Direct mean blood pressure in both sodium-depleted and nephrectomized rats was somewhat lower than that in normal rats, although only the blood pressure of the nephrectomized, low-salt group (group 6) was significantly lower than normal (P < 0.05) (Table 1). Each group with its kidneys in situ (groups 1-4) showed a fall in blood pressure with antiserum administration, although the change was not significant (P > 0.05). Of the nephrectomized rats (groups 5a-e, 6, and 7), four groups showed a nonsignificant fall and three a nonsignificant rise.

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Blood Pressure before and after Antiserum Blockade and the Blood Pressure Response to Angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Group 1 (normal)</td>
<td>104.9 ± 6.1</td>
</tr>
<tr>
<td>Group 2 (low salt)</td>
<td>105.3 ± 10.1</td>
</tr>
<tr>
<td>Group 3 (high salt)</td>
<td>109.7 ± 5.0</td>
</tr>
<tr>
<td>Group 4 (dialysis)</td>
<td>88.8 ± 5.9</td>
</tr>
<tr>
<td>Group 5 (nephrectomy)</td>
<td></td>
</tr>
<tr>
<td>a (1 hour)</td>
<td>101.5 ± 6.7</td>
</tr>
<tr>
<td>b (1 hour)</td>
<td>97.5 ± 4.5</td>
</tr>
<tr>
<td>c (3 hours)</td>
<td>91.9 ± 2.2</td>
</tr>
<tr>
<td>d (6 hours)</td>
<td>90.8 ± 6.5</td>
</tr>
<tr>
<td>e (24 hours)</td>
<td>89.7 ± 4.8</td>
</tr>
<tr>
<td>Group 6 (nephrectomy, low salt)</td>
<td>82.9 ± 7.8</td>
</tr>
<tr>
<td>Group 7 (nephrectomy, dialysis)</td>
<td>81.6 ± 12.3</td>
</tr>
</tbody>
</table>

All values are means ± SE. The blood pressure response to angiotensin II is the increase in pressure seen on injection of a 50-ng bolus of angiotensin II.
RESPONSE TO ANGIOTENSIN II

Both the high-salt (group 3) and the nephrectomized (groups 5–7) rats showed a greater pressor response to 50 ng of angiotensin II than did the normal rats with the exception of the rats studied 15 minutes after nephrectomy (group 5a) (Table 1). This increased pressor response was statistically significant \((P < 0.05)\) for all of the groups except those studied 3 and 24 hours after nephrectomy (groups 5c and 5e). The pressor response to angiotensin II was significantly reduced \((P < 0.05)\) in the sodium-depleted nonnephrectomized rats (groups 2 and 4). There was a highly significant correlation \((r = +0.93)\) between the pressor response to 50 ng of angiotensin II and the volume of antibody required to block the response.

**ANTIBODY REQUIREMENT**

**Group 1.**—The pressor response to 50 ng of angiotensin II was blocked after a mean dose of 0.32 ± 0.03 ml of antiserum had been administered (Table 2).

**Groups 2–4.**—The blocking requirement of the salt-loaded rats was significantly greater and that of the salt-depleted rats significantly less than normal \((P < 0.05)\) (Table 2). Peritoneal dialysis lowered the blocking requirement to a value significantly less than normal \((P < 0.02)\) but not significantly different from \((P > 0.4)\) that of the rats on the low-salt diet (group 2).

**Group 5.**—The mean blocking requirement 15 minutes after nephrectomy was less than normal, although the difference was not significant \((P > 0.3)\) (Fig. 1). The dose then progressively increased up to 6 hours after nephrectomy. At 3 and 6 hours, the requirement was significantly greater than normal \((P < 0.001)\). At 24 hours, the blocking requirement had fallen slightly, although it was still greater than normal \((P < 0.001)\).

**Groups 6 and 7.**—After bilateral nephrectomy, the blocking dose of rats on the low-salt diet was identical to that of rats on a free diet 6 hours after surgery. Sodium depletion by dialysis following bilateral nephrectomy did not result in a significantly different blocking dose from that required by nephrectomized rats on the low-salt diet (group 6) \((P > 0.4)\).

**Plasma Renin Activity.**—Following nephrectomy, plasma renin activity declined rapidly from a mean value of 46.5 ± 3.04 ng angiotensin I/ml hour\(^{-1}\) at 2 minutes to 2.5 ± 1.49 ng angiotensin I/ml hour\(^{-1}\) at 60 minutes and 0.37 ± 1.81 ng angiotensin I/ml hour\(^{-1}\) at 24 hours.

**Discussion**

Vascular affinity for angiotensin II was measured by the pressor response to a standard dose of angiotensin II and by the volume of specific angiotensin II antiserum required to block this pressor response (9). Although these two variables occasionally diverged, they were highly correlated with each other. Moreover, neither showed any correlation with the basal blood pressure level (Table 1).

According to our hypothesis (10–12), renin generates angiotensin II at a peripheral vascular site that is inaccessible to antibody. Such locally generated angiotensin II occupies vascular receptors and reduces the pressor response to exogenous angiotensin II since few receptors remain unoccupied. The elevation of threshold for a pressor response to angiotensin II facilitates blocking by antiserum. Conversely, after salt loading or bilateral nephrectomy, the reduction in vascular renin activity reduces local angiotensin II generation and thereby lowers the threshold for a pressor response to an angiotensin II infusion and increases the antibody blocking requirement. The findings of Brunner et al. (9) are not consistent with this hypothesis in one important respect. These workers demonstrated a smaller antibody blocking requirement in salt-depleted rats than they did in normal rats following bilateral nephrectomy, although both groups showed a greater than normal response to angiotensin II. Our work was therefore, in part, designed to study the effect of different methods of inducing salt depletion. It is also important to document the time relationships of the changes in blocking requirements and responsiveness. Theoretically, cir-

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Free diet</th>
<th>High-salt diet</th>
<th>Low-salt diet</th>
<th>Peritoneal dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>0.32 ± 0.03</td>
<td>0.65 ± 0.03</td>
<td>0.22 ± 0.04</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Bilaterally nephrectomized rats</td>
<td>0.30 ± 0.03 (15 minutes)</td>
<td>0.60 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.63 ± 0.04 (6 hours)</td>
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<td></td>
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</tbody>
</table>

All values are means ± sr.
Volume of angiotensin II antiserum required to block the pressor response to a 50-ng bolus of angiotensin II in normal rats (N) and rats at different intervals after bilateral nephrectomy. Six rats were included in each group except for the group nephrectomized 24 hours before the study which comprised 11 rats (numbers in parentheses above the first and last columns).

Calculating rather than peripheral vascular renin could be the relevant factor. If so, the observed changes should occur well within the first hour after nephrectomy.

Our results confirm the finding (9) that the blocking dose of antiserum is significantly decreased in salt-depleted and increased in salt-loaded rats (Table 2). The change in the blocking requirement can be rapid, since it was equally great with acute dialytic salt depletion as it was with depletion from prolonged treatment with a low-salt diet. Since less circulating renin and angiotensin II are present after salt loading, the greater requirement in these rats cannot be explained in terms of saturation of available antibody binding sites.

Our observations indicate that sodium balance influences vascular receptor affinity for angiotensin II only in the presence of intact kidney tissue. When rats maintained on a low-salt diet were nephrectomized group 6), the antibody blocking requirement and the blood pressure response to angiotensin II after 6 hours were identical to those of normal rats which had been nephrectomized 6 hours previously (group 5d). Sodium depletion by peritoneal dialysis after nephrectomy (group 7) also failed to influence the blocking requirement or the angiotensin II response (Table 2), but it did exert a striking influence on these variables in normal rats (group 4). The change in vascular affinity for angiotensin II after nephrectomy is not however immediate. The blocking dose requirement only reached its maximum at 6 hours: the pressor response to angiotensin II, although it was maximum at 6 hours, showed less consistent changes owing to a relatively poor response by the 3-hour group (Table 2).

Enhanced pressor responsiveness to both angiotensin II (16) and renin (17) has been noted after nephrectomy. The response to renin may be partly due to increased angiotensin II formation either because of increased substrate concentration (18) or changes in renin kinetics (19). Such changes cannot, of course, provide an explanation for the enhanced pressor response to angiotensin II or the increased blocking requirement for angiotensin II antiserum. Our results support the view that depletion of endogenous renin rather than a change in sodium balance determines the vascular responsiveness to angiotensin II after bilateral nephrectomy (20). We have previously shown that renin infusion of bilaterally nephrectomized rats dramatically reduces the blocking dose of antiserum (10).

In support of this hypothesis, a specific angiotensin II antagonist lowers blood pressure in renin-induced hypertension, although blockade with angiotensin antiserum has a much smaller depressor effect (11, 12, 21). Furthermore, renin activity can be detected in arterioles (22) and is increased in sodium depletion (23).

The increase in the antiserum blocking requirement continued for 6 hours; however, the half-life of circulating renin activity is much shorter than this figure would suggest. This fact supports the view that circulating renin is not the relevant variable and, using the present hypothesis, it suggests that vascular renin decays much more slowly than does circulating renin. This concept is consistent with the findings of Schaechtelin et al. (24) that the pressor effect of a bolus of renin persists well beyond the time at which circulating pressor material can be demonstrated.

Our studies of salt depletion differ from those of Brunner et al. (9) in that salt depletion in their experiments still modified the blocking requirement even after bilateral nephrectomy. It is noteworthy that, at 24 hours, the blocking requirement in our experiments had fallen significantly compared with the requirement at 6 hours (Fig. 1). Our experience with antiserum studies at even longer intervals after nephrectomy and in sick animals indicates that the blocking requirement is very sensitive to a deterioration in the general condition. It seems possible that prolonged dietetic sodium restriction combined with renal failure of 18 hours duration might lead to a nonspecific reduction in the blocking requirement.

The present work indicates that the apparent sodium dependence of vascular receptor affinity for
angiotensin II masks the real mechanism. It seems more likely that the vascular renin–sodium balance relationship is critically important. If so, renin-induced hypertension need not be directly related to plasma renin levels. The relatively poor blood pressure-lowering effects of angiotensin II antiserum support such a view (11, 12). In the uninephrectomized Goldblatt hypertensive rat, in which circulating renin levels are not elevated (25), the blocking requirement is inappropriately small for the degree of sodium retention shown by this model (10); this finding may indicate an abnormality in vascular renin activity.

It is theoretically possible for the kidney to produce the effects described through other actions on vascular responsiveness, e.g., through the kinin or prostaglandin systems. Against such a hypothesis is the recent observation that chemical inhibition of endogenous angiotensin II formation eliminates the changes in vascular responsiveness to exogenous angiotensin II induced by alterations in sodium balance (26). Although these latter experiments did not differentiate between circulating and vascular renin activity, they are consistent with our explanation.

References


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