SUMMARY To assess changes in responsiveness to pressor agents in experimental hypertension, we examined pressor dose-response curves to graded doses of angiotensin II (All) and norepinephrine (NE) in anesthetized normal rats and rats with early (< 6 weeks) or chronic (> 4 months) two-kidney one clip renovascular hypertension and deoxycorticosterone (DOC) salt hypertension. Occupancy of receptors by endogenous All was reduced by converting enzyme inhibition with captopril or bilateral nephrectomy. Rats with DOC-salt hypertension were significantly more responsive to All than normal rats or rats with renovascular hypertension (p < 0.05). Captopril administration had no effect upon All responsiveness in DOC-salt hypertension, but enhanced the responses of normal rats and rats with renovascular hypertension, so that there were no significant differences in the All dose-response curves after captopril. Bilateral nephrectomy also eliminated the differences between the responsiveness of DOC-salt and other groups, although responsiveness after bilateral nephrectomy was lower than that after captopril. Captopril administration to nephrectomized animals had no effect upon responsiveness. It is concluded that receptor occupancy by endogenous All is the major factor that alters pressor responsiveness to All in normal and experimental hypertensive rats. Duration of hypertension seemed to play no role. By contrast, norepinephrine responsiveness was not significantly different between the experimental groups. However, captopril treatment increased the slope of the dose response curves in intact but not nephrectomized groups. This increased sensitivity appears to be due to inhibition of the renin-angiotensin system, since it was also produced by saralasin infusion, but not by bradykinin infusion. The renin-angiotensin system therefore appears to be important in modulating pressor responses to norepinephrine in the intact animal, and this interaction differs from the effect that has been demonstrated in isolated tissues. (Hypertension 4: 238-244, 1982)

KEY WORDS • pressor responses • two-kidney one clip hypertension • bilateral nephrectomy • captopril • saralasin • norepinephrine • angiotensin II • deoxycorticosterone hypertension

Despite extensive study, the mechanisms by which blood pressure is maintained in the various forms of clinical and experimental hypertension are still obscure. Theoretically, hypertension could be due to excessive activity of a neural or humoral pressor system, to enhanced responsiveness to such systems, or to a combination of these two factors. Alterations in responsiveness to infused pressor substances can be demonstrated in hypertensive patients,1,2 and changes in the relationship between plasma All levels and the resultant blood pressure have been implicated in the pathogenesis of renovascular hypertension. Thus, it is postulated that the renin-angiotensin system may maintain elevated blood pressure even when plasma All is normal.3 The complexity of this situation is increased by the possible influence of vascular hypertrophy induced by hypertension: thus, changes in the geometry of the resistance vessel wall may increase responsiveness.4 Since this is a consequence of hypertension it cannot be an initiating cause, although it may be of importance in maintaining established hypertension.
Changes in vascular reactivity can be demonstrated in isolated tissues from hypertensive animals. Thus, some groups have demonstrated hyperreactivity in arterial strips taken from rats with spontaneous, DOC, and renovascular hypertension. Other groups have demonstrated increased responsiveness of aortic strips obtained from spontaneously hypertensive rats, but not rats with Goldblatt two-kidney hypertension. Such changes ("true hypersensitivity") cannot be due to vascular hypertrophy. Hypersensitivity to norepinephrine and AII has been demonstrated in the isolated mesenteric vasculature of rats with one-kidney figure-of-8 hypertension and hypersensitivity to AI but not norepinephrine has been observed in both kidneys of rats with Goldblatt two-kidney hypertension. However, hypersensitivity to norepinephrine was also demonstrated in the pig isolated hindlimb which had been protected from the effects of increased perfusion pressure produced by DOC hypertension. It has been suggested by Jones that abnormalities in sodium transport in vascular smooth muscle might give rise to enhanced responsiveness to such vasoconstrictor stimuli as norepinephrine in spontaneously hypertensive rats.

In vivo conditions are likely to modify such changes substantially, and phenomena demonstrated in isolated tissues, may be overridden in the whole animal. Nevertheless, increased pressor responsiveness to AII develops as a result of infusion of subpressor doses of AII for prolonged periods, and rats with Goldblatt two-kidney hypertension show an increased blood pressure response to infusions of renin, AII, or NE after the constricting clip has been removed from the renal artery.

We have previously reported a series of experiments in which the role of receptor occupancy by endogenous AI in determining pressor responsiveness was examined by using a converting enzyme inhibitor to prevent the generation of AII. These experiments indicated that administration of converting enzyme inhibitor to rats with high endogenous renin levels restored depressed sensitivity to normal and supported the role of receptor occupancy as a major factor in determining pressor sensitivity to AI. The present studies were designed to evaluate the role of receptor occupancy in determining pressor responsiveness to AI in renovascular and mineralocorticoid hypertension. To achieve this, we have reduced the generation of endogenous AI in two ways: by converting enzyme inhibition and by bilateral nephrectomy. These methods may clearly influence the cardiovascular system in other ways and so each has been used separately and in combination. Because mechanisms of hypertension may alter with passage of time (perhaps in association with the development of vascular hypertrophy), we have studied the early and late stages of two-kidney one clip renovascular hypertension. Finally, NE responsiveness has been examined in each model at the same time as angiotensin responsiveness, to determine whether changes in reactivity are similar for these two pressor substances that have different mechanisms of action.

Methods

Female Wistar rats weighing 150–250 g were used throughout and maintained on standard chow and tap-water except where stated. Two-kidney one clip renovascular hypertension was produced by applying a constricting silver clip (0.2 mm internal diameter) to the left renal artery under ether anesthesia; the right kidney was left undisturbed. Indirect blood pressures were checked by a light plethysmographic method applied to the tail, and hypertensive animals (blood pressure > 150 mm Hg) were studied either within 6 weeks (early) or > 4 months (chronic) after clipping.

Deoxycorticosterone salt (DOC-salt) hypertension was produced by twice weekly subcutaneous injections of deoxycorticosterone acetate 12.5 mg (Ciba, Hershman, England) into rats that had undergone right nephrectomy. Rats were allowed free access to saline (1 g/100 ml) to drink, and indirect blood pressures were checked twice weekly as above. Hypertensive animals (blood pressure > 150 mm Hg) were studied 1 to 2 weeks after establishment of hypertension.

Bilateral nephrectomy was carried out under ether anesthesia through loin incisions 16 to 24 hours before the pressor response studies.

Pressor Response Curves

Rats were anesthetized with intraperitoneal pentobarbionate (5 mg/100 g body weight). A tracheostomy was performed, and the jugular vein and carotid artery cannulated with polyethylene catheters. Direct blood pressure was monitored continuously with a Statham P23 gb transducer and Grass recorder. When blood pressure had stabilized, we recorded the pressor responses to a series of intravenous bolus doses of AI (Beckman, Geneva, Switzerland) and NE (Sigma, Poole, England). Captopril (250 μg stat i.v., Squibb, Princeton, New Jersey) was then given, and when the blood pressure had restabilized the pressor response tests were repeated. To assess converting enzyme inhibition by this dose of captopril, we recorded pressor responses before and after administration of a series of doses of AI (Beckman) in normal intact animals and responses to a single bolus dose of 25 ng AI in all other animals studied. All agents were dissolved in dextrose solutions (5 g/100 ml) and were administered by microsyringes delivering multiples of 0.01 ml of solution.

Ten groups of rats (seven in each group) were studied: normal, early renovascular, chronic renovascular, and DOC-salt hypertension (Groups 1 through 4 respectively), and similar groups of rats after bilateral nephrectomy (Groups 5 through 8). Two additional groups were studied: Group 9, in which pressor responses to AI and NE were studied in bilaterally nephrectomized normal rats before and during infusion of bradykinin (20 μg/kg/min; Sigma) in dextrose (5 g/100 ml); and Group 10, in which pressor responses to NE were studied in intact early renovascular hypertensive rats before and 30 minutes after starting an infusion of sarcosine* alanine* AI (Saralasin 10 μg/kg/min; Norwich Pharmaceuticals, Norwich, New York).
Plasma Renin Concentration (PRC)

Blood (0.5 ml) was obtained from the tail vein under ether anesthesia the day before the pressor responses were measured. The sample was collected in a precooled tube moistened with a drop of a concentrated solution of dipotassium EDTA. After centrifugation at 4°C, the plasma was stored at −20°C and assayed for PRC using bilaterally nephrectomized rat plasma as a source of substrate. 16

Statistical Analysis

Pressor responses were compared by one-way analysis of covariance. 16 Plasma renin concentration (PRC) is not normally distributed unless logarithmically transformed. This was done before statistical analysis. Student's t test was used to compare baseline PRC and blood pressure.

Results

Baseline Blood Pressure

In intact animals there was no significant difference between blood pressures in the hypertensive groups (Groups 2 through 4); they were all higher than blood pressures in normal rats (Group 1, p < 0.01, fig. 1). After nephrectomy blood pressures fell significantly in each group and there was no significant differences between blood pressures of normal and early and chronic renovascular hypertensive rats (Groups 5 through 7, p > 0.2). Blood pressure of nephrectomized DOC-salt animals (Group 8) was significantly higher than that of the other three groups (p < 0.05).

Captopril produced a significant immediate fall in blood pressure in all intact groups (fig. 1). This fall was greatest in the early renovascular hypertensive rats in which blood pressure became normal. In DOC-salt and chronic renovascular hypertensive rats, blood pressure fell but still remained significantly higher than that of normal animals either before or after captopril. After nephrectomy the maximum effect of captopril was considerably reduced and did not differ in any of the groups, although blood pressure of nephrectomized DOC-salt animals remained significantly higher than that of the other groups. The response to captopril was related to the PRC value (table 1) in all but the intact DOC-salt group. After this acute fall, the blood pressure rose progressively and at 30 minutes after the injection did not differ significantly from precaptopril baseline values in any group (fig. 1). At this time, however, effective inhibition of converting enzyme could still be demonstrated. Thus, from AI pressor response curves in normal rats we calculated that 7.8 ng would elevate blood pressure by 10 mm Hg, whereas 30 minutes after captopril the dose of AI calculated to produce the same response would be 168.6 ng. Blockade of a similar order could be demonstrated in other groups, so that the pressor response to 25 ng of AI was less than 5 mm Hg in each case.

Angiotensin II Pressor Responses

Rats with DOC-salt hypertension (Group 4) were significantly more responsive to AI1 than normal and renovascular hypertensive animals (Groups 1 through 3, p < 0.05, fig. 2). Early and chronic renovascular hypertensive groups were less responsive than normal rats, but the differences between these groups were not significant by one-way analysis of variance (fig. 2 upper left). However, two-way analysis of variance did show that the depressed responsiveness in renovascular hypertension was significant (p < 0.05), al-

<table>
<thead>
<tr>
<th>Rat</th>
<th>Plasma renin concentration (ng AI/ml/hr)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>134.5 ± 17.2</td>
</tr>
<tr>
<td>Early renovascular hypertension</td>
<td>368.3 ± 53.0</td>
</tr>
<tr>
<td>Chronic renovascular hypertensive</td>
<td>199.2 ± 19.1</td>
</tr>
<tr>
<td>DOC-salt hypertension</td>
<td>3.24 ± 0.3</td>
</tr>
<tr>
<td>Normal nephrectomized</td>
<td>3.1 ± 0.3</td>
</tr>
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Figure 2. Pressor responses to angiotensin II before and after captopril in intact rats (upper and lower left) and bilaterally nephrectomized rats (upper and lower right). Normal • • •; early ◊ ◊ ◊ and chronic □□□; two-kidney one clip renovascular hypertension, and DOC/salt ◊◊◊ hypertensive rats. Each point is the mean of pressor responses in seven animals.

though there was no difference between early and chronic phases. After captopril, pressor response curves of all groups were not significantly different from each other. Thus, captopril enhanced responsiveness to AII significantly in normal and renovascular hypertensive animals ($p < 0.05$) but had no effect on DOC-salt hypertensive rats. Responses in intact animals after captopril were all significantly greater than in comparable bilaterally nephrectomized groups ($p < 0.05$).

After bilateral nephrectomy, the response curves in all groups were the same (fig. 2 upper and lower right, $p > 0.05$). This was due to the fact that responsiveness of nephrectomized DOC-salt hypertensive rats (Group 8) was depressed and that of nephrectomized chronic renovascular hypertensive rats (Group 7) was increased compared to intact animals (Groups 4 and 3 respectively, $p < 0.05$). Captopril did not alter the responsiveness of any group after nephrectomy.

Norepinephrine Pressor Responsiveness

There was no significant difference in pressor response curves between the groups either in intact or in bilaterally nephrectomized rats, either before or after captopril administration. Captopril in intact animals did not significantly alter threshold sensitivity to NE although the slope of the line was increased in all groups (fig. 3 upper and lower left, $p < 0.05$). Captopril-treated intact animals were significantly more responsive than comparable nephrectomized groups (fig. 3 lower left and right, $p < 0.05$). Captopril when administered to nephrectomized animals produced no significant change in responsiveness.

Bilateral nephrectomy reduced responsiveness of all groups to NE but this was only significant in early renovascular and DOC-salt animals (Groups 6 and 8).

Bradykinin Infusions

Pressor responsiveness to both AII and NE were significantly depressed during infusion of bradykinin into nephrectomized normal rats (Group 9, $p < 0.05$, fig. 4).

Saralasin Infusion

Pressor responsiveness to NE was increased during infusions in early renovascular hypertensive rats (Group 10, $p < 0.05$), and the slope of the line was increased ($p < 0.05$, fig. 5).
Plasma Renin Concentration

Compared to PRC in normal rats, PRC in rats with early and, to a lesser extent, chronic renovascular hypertension (Groups 2 and 3, p < 0.05) was significantly elevated, and decreased in rats with DOC-salt hypertension, and in normal rats after bilateral nephrectomy (Groups 4 and 5, p < 0.05).

Discussion

In the present experiments responsiveness to All was increased in intact DOC-salt hypertensive rats when compared to normal rats. By contrast, responsiveness of rats with either early or chronic two-kidney one clip renovascular hypertension was significantly reduced (fig. 2). This finding is consistent with other reports of a modest reduction in All responsiveness in this model of renovascular hypertension. Both captopril pretreatment and bilateral nephrectomy eliminated the differences in responsiveness between the groups, bringing the dose response curves into close apposition. There were differences in the effects of these two procedures, however. Thus, captopril increased responsiveness of both normal rats and rats with renovascular hypertension, while responsiveness of animals with DOC-salt hypertension was unaltered. On the other hand, the increase in reactivity produced by bilateral nephrectomy was significantly less than that produced by captopril, and nephrectomy significantly reduced the responsiveness of rats with DOC-salt hypertension. Captopril had no effect upon pressor responsiveness to All in nephrectomized rats. These data strongly suggest that the effect of captopril on pressor responsiveness to All is mediated through a reduction in endogenous All. The most likely explanation for the effects of captopril is that the drug acts by decreasing the occupancy of receptors by endogenous All. This interpretation is supported by the fact that responsiveness of intact animals to All was correlated with PRC, being maximal in rats with DOC-salt hypertension where PRC was low, and minimal in early renovascular hypertension where PRC was high (fig. 2 upper and lower left). It should be noted, however, that PRC levels were measured the day before the study under ether anesthesia. While these values (table 1) do not reflect absolute PRC during the study, they do reflect the relationship between PRC in the experimental groups.
Theoretically, other actions of captopril could play a role. Bradykinin potentiation is unlikely since infusion of this agent reduced responsiveness to All (fig. 4). Similarly, alteration in cell membrane sodium transport is unlikely in short-term experiments, and no change in responsiveness occurred after captopril in bilaterally nephrectomized animals (fig. 2 upper and lower right).

Bilateral nephrectomy will also decrease All receptor occupancy but there is an additional factor in that nephrectomy was associated with a global reduction in responsiveness (fig. 2). This appears to be nonspecific as NE responsiveness was also lower in nephrectomized compared with captopril-treated groups (fig. 3). It is reasonable to conclude that this effect is a consequence of renal failure. Such an effect may account for the lowering of blood pressure produced by nephrectomy in rats with DOC-salt as well as chronic renovascular hypertension (fig. 1).

Apart from the influence of bilateral nephrectomy, the pattern of responsiveness to NE was quite distinct. There was no difference among any experimental group of intact or nephrectomized rats (fig. 3). Captopril did have an unpredicted effect, however, in that the slope of the NE dose response curves was significantly increased in all groups after captopril administration so that responsiveness to NE was enhanced for higher doses (fig. 3). This effect appeared to be mediated through blockade of the renin-angiotensin system, as saralasin infusion had an identical effect upon NE responsiveness (fig. 5). While a similar effect of acute administration of captopril on NE responsiveness has been described in normal intact rats, these observations are in contrast to isolated tissue responses. Malik and Naslietti found that the vasoconstrictor response of isolated rat mesenteric artery to NE was potentiated by renin substrate, and this potentiation was prevented by converting enzyme inhibitor. They concluded that local vascular renin or renin-like enzymes generated All, which potentiated NE and sympathetic responsiveness by augmenting release of NE, inhibiting its uptake and increasing reactivity to it. These results are not necessarily discordant with our observations, which were made in vivo in animals with an intact nerve supply to the cardiovascular system and in which basal NE concentrations in the neuroeffector cleft must have been much higher. Thus, if blockade of the renin-angiotensin system reduced NE concentration at this site sensitivity to exogenous NE would be increased, just as supersensitivity to NE is produced by denervation.

In our studies, renovascular hypertension was investigated at two stages: during the first week or two of blood pressure elevation before hypertrophy had become fully established and after at least 4 months of hypertension. The differences in responsiveness that have been demonstrated in isolated tissue beds could not be detected in either untreated hypertensive rats or in rats either given captopril or bilaterally nephrectomized so that the influence of prior receptor occupancy upon All responsiveness had been reduced or eliminated. In the conditions of these experiments, we
were unable to demonstrate a role for vascular hypertrophy in determining vascular responsiveness. The differences between the responsiveness of animals and isolated tissues that are emphasized by the present study reflect the greater complexity of the intact circulation where changes in pressure in turn reflect both changes in cardiac output and peripheral resistance. In addition to changes in concentrations of circulating vasoactive hormones encountered in the intact animal, the presence of the autonomic nervous system with preservation of baroreceptor reflexes clearly influences the pattern of response to pressor agents. “Resetting” of such reflexes is well established in experimental hypertension, and altered central modulation of these reflexes could theoretically play a role in the differences in responsiveness between experimental groups observed here. Abolition of these differences by captopril, however, argues against such an explanation. Similarly, the changes in basal blood pressure produced by captopril were small at the time dose response curves were carried out and cannot therefore account for the differences in the responsiveness demonstrated.

The initial fall in blood pressure in response to a bolus dose of captopril was not sustained: the mechanism of partial recovery is uncertain. “Escape” from blockade is unlikely as the pressor effects of exogenous AI could still be demonstrated to be blocked throughout the studies. The failure to produce a major sustained fall in blood pressure in rats with renovascular hypertension despite elevated PRCs is consistent with other reports that a prolonged infusion of captopril is necessary for such an effect to be demonstrated. The current studies provide no explanation for the discrepancy between the acute effect upon circulating AI1 and the slower effect upon blood pressure.

In conclusion, therefore, the present studies emphasize the importance of receptor occupancy in the hyperresponsiveness to AI1 demonstrated in DOC-salt hypertension in the intact anesthetized rat. Where this effect was eliminated by converting enzyme inhibition or nephrectomy, AI1 responsiveness was identical in normal animals, in animals with DOC-salt hypertension, and in animals with either early or chronic renovascular hypertension. This indicates that other factors do not play a significant role within the conditions of our experiments. An additional enhancing effect of converting enzyme inhibition on NE responsiveness at higher doses remains to be elucidated.

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