SUMMARY The relative roles of the sympathetic nervous system and renin-angiotensin system in the development of two-kidney renal hypertension were studied using four groups of rats: Group I = vehicle control; Group II = 6-OH-dopamine (2 weeks prior to renal clipping then weekly throughout the study); Group III = adrenal medullectomy plus vehicle; Group IV = 6-OH-dopamine plus adrenal medullectomy. Six weeks after clipping of a single renal artery, plasma renin activity (PRA) was comparably elevated in all groups. However, mean blood pressure (MBP) of Group II was lower than that of Group I controls (154.7 ± 6.8 vs 197.3 ± 6.6 mm Hg respectively). The MBP of Group III (207.0 ± 5.2 mm Hg) was not different from that of Group I whereas in Group IV (134.2 ± 18.0 mm Hg) it was markedly lower. All groups of rats were given a single dose of captopril (30 mg/kg p.o.) to inhibit the renin-angiotensin system. Despite differences in starting MBP, captopril caused similar reductions (38-50%) of MBP and increases in PRA in all groups. Similar results were obtained in two-kidney renal hypertensive rats with hypertension of 12 weeks' duration. It is concluded that the sympathetic nervous system does not contribute to the elevated PRA in two-kidney renal hypertensive rats but does contribute significantly to the development of hypertension in this model.

KEY WORDS • renin-angiotensin • sympathoadrenal • captopril • renal hypertension • 6-hydroxydopamine • adrenal medullectomy

THE significance of the sympathetic nervous system in the development and/or maintenance of renal hypertension remains controversial. A recurring problem in the interpretation and understanding of previously published findings is the tendency to classify all forms of hypertension involving kidney manipulation into that of "renal hypertension" despite obvious and often dramatic differences in methodology. Thus, many studies have examined the potential role of the sympathetic nervous system in renal hypertension induced by various means including one-kidney one clip,\(^1\) two-kidney one clip,\(^2\) two-kidney two clip,\(^3\) two kidney two wrapped,\(^4\) and aortic ligation between the two renal arteries.\(^5\) It is not surprising that the results are often confusing and seemingly contradictory. Since it has been demonstrated that one- and two-kidney renal hypertension are etiologically different,\(^6\) it would also seem more appropriate to examine the involvement of the sympathetic nervous system separately in either form of hypertension. The present study is concerned with the relative significance of sympathetic nerves and the adrenal medulla in the development of two-kidney one clip renal hypertension in rats.

Earliest evidence for a mechanism other than renal in the maintenance of renal hypertension was provided indirectly by Koletsky et al.\(^7\) They demonstrated that, in rats with aortic ligation between the two renal arteries (a form of two-kidney hypertension), etiological participation of the renin-angiotensin system was limited only to the acute stage (2 weeks) of hypertension despite subsequent maintenance of hypertension in these rats for up to 6 months. Similarly, these authors found\(^8\) that resection of the ischemic kidney was effective in normalizing blood pressure in two-kidney renal hypertension only to the extent that a high renin mechanism was present. No increase in renin activity occurred after nephrectomy even though hypertension persisted. Similarly, studies with angiotensin II (AII) receptor antagonists have shown that two-kidney renal hypertension (either two-kidney one clip or aortic ligation between the renal arteries) is renin-dependent during the first 4 weeks.
whereas some other mechanism is responsible for maintenance of the hypertension beyond that time.18-19 Douglass et al.16 examined the effects of sympathectomy with guanethidine on the development of two-kidney one clip renal hypertension and concluded that the sympathetic nervous system was not necessary. However, this is in distinct contrast to the effectiveness of guanethidine in normalizing blood pressure in established renal hypertension. Also, these authors did not take into account the presence of the adrenal glands and their potential participation in renal hypertension. Thus, the present study was designed to examine the relative roles of the sympathetic nerves and the adrenal gland individually, and in conjunction (sympathoadrenal system), on the development of two-kidney one clip renal hypertension in rats, as well as the role of the renin-angiotensin system in this model.

**Methods**

Male, Sprague-Dawley rats (Charles River) weighing between 75-100 g were used in this study. Sodium intake was approximated at about 5 mEq/kg/day. The rats were randomly divided into four groups and designated and treated as shown in table 1. In addition, a second batch of rats was treated identically as shown in table 1, except that they were maintained for 12 weeks after renal artery clipping instead of 6 weeks. Rats were dosed daily for 2 days on each of 2 consecutive weeks prior to surgery with either 6-OH dopamine as described for sympathectomy by Finch et al.,16 or ascorbic acid vehicle. At time "0", all rats were made two-kidney one clip renal hypertensive (2K-RHR) by anesthetizing them with ether and placing a 0.2 mm silver clip on the left renal artery through a flank incision and leaving the contralateral kidney intact. Rats continued to receive either 6-OH dopamine or vehicle, as described in table 1. Three weeks after renal artery clipping, all rats were anesthetized with ether once again and their aortae were intubated for the measurement of blood pressure and heart rate as described by Weeks and Jones24 and Laffan et al.25 Three weeks after intubation, blood samples from these conscious, freely moving rats were withdrawn through the aortic cannula for determination of plasma renin activity (PRA) as previously described.26 Mean blood pressure (MBP) and heart rate were then determined both before and after the administration of a single dose of captopril (30 mg/kg p.o.) in all groups of rats. Adrenal medullectomy was performed at the same time as renal artery clipping. A small incision was made in both the left and right flank. A small slit was made in the adrenal capsule and the medulla was removed by gently squeezing on the gland with forceps.

The identical procedure described above was carried out on the second batch of rats except that PRA blood samples and blood pressure and heart rate determinations before and after captopril were taken at 12 weeks instead of 6 weeks after renal artery clipping.

Other groups of 2K-RHR treated exactly as described above were prepared at the end of Weeks 6 and 12 respectively for the determination of blood pressure and heart rate. Responses to sympathetic nerve stimulation and pressor agents were obtained in these rats after the pithing procedure described by Gillespie and Muir.21

All results are expressed as the mean ± se.

**Results**

**Blood Pressure and Heart Rate at 6 Weeks**

Six weeks after renal artery clipping, the MBP of Group I rats was markedly elevated in comparison with nonclipped rats (197.3 ± 6.6 vs 118.9 ± 2.1 mm Hg respectively; p < 0.001). In Group II rats sympathectomized with 6-OH dopamine, the MBP was

**Table 1. Experimental Design and Treatment Schedules for Rats in Groups I-IV. Week Number "O" was Designated as the Week of Surgery Involving Renal Artery Clipping.**

<table>
<thead>
<tr>
<th>Week no.</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2; day 1</td>
<td>0.25% ascorbic acid (in normal saline; 2.5 ml/kg i.v.)</td>
<td>6-OH dopamine (50 mg/kg i.v. in 0.25% ascorbic acid 2.5 ml/kg i.v.)</td>
<td>0.25% ascorbic acid, i.v.</td>
<td>6-OH dopamine, 50 mg/kg i.v.</td>
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<tr>
<td>day 2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-1; day 1</td>
<td>0.25% ascorbic acid, i.v.</td>
<td>6-OH dopamine, 100 mg/kg i.v.</td>
<td>0.25% ascorbic acid, i.v.</td>
<td>6-OH dopamine, 100 mg/kg i.v.</td>
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<tr>
<td>day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td></td>
<td></td>
<td>Renal Clipping</td>
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<td>Adrenal medullectomy</td>
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<td>+ 0.25% ascorbic acid, i.p.</td>
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<td></td>
<td>BP &amp; HR Measurements as Well as Captopril and PRA</td>
</tr>
</tbody>
</table>
MECHANISMS OF RENAL HYPERTENSION

significantly lower than Group I rats (fig. 1). In contrast, adrenal medullectomy (Group III) had no significant effect on the development of renal hypertension. In rats that were both adrenal-medullectomized and injected with 6-OH dopamine (Group IV), MBP was significantly lower than in Group I, II, and III rats.

The heart rates of the 6-week rats in Groups I, II, and III were not significantly different from each other although slightly reduced in the 6-OH dopamine-treated rats (Group II). However, the heart rates of the Group IV rats were significantly lower than in either vehicle-treated rats (Group I), or Group II and Group III rats (fig. 1).

Blood Pressure and Heart Rate at 12 Weeks

In different groups of two-kidney one-clip rats observed at 12 weeks after renal artery clipping, the results were qualitatively similar to those described for 6-week 2K-RHR. Blood pressure of 12-week Group I rats was significantly higher than the corresponding 6-week rats ($p < 0.05$; fig. 1). Rats treated with 6-OH dopamine (Group II) had lower MBP than vehicle-treated rats, whereas blood pressure of adrenal-medullectomized rats (Group III) was not different from control. In rats treated with 6-OH dopamine and also having bilateral adrenal medullectomy (Group IV), blood pressure was dramatically lower than any other corresponding group (fig. 1).

As in the 6-week rats, the heart rates of Groups I, II, and III were not different from each other whereas the heart rate of Group IV rats was significantly lower than that of the vehicle-treated rats as well as the Group II and III rats (fig. 1).

Pressor Responses in Pithed 12-Week Two-Kidney Renal-Hypertensive Rats (2K-RHR)

Pressor responses to electrical stimulation of the entire sympathetic outflow (10 Hz, 80 V, 20 sec), norepinephrine (1 μg/kg), and tyramine (0.5 mg/kg) were obtained in pithed 12-week 2K-RHR of Groups I-IV. The results are summarized in figure 2.

Pressor responses to electrical stimulation normally observed in vehicle-treated rats (Group I) were actually reversed to depressor responses in 6-OH dopamine-treated rats (Group II). The pressor response to norepinephrine was significantly enhanced whereas that to tyramine was significantly reduced in Group II rats in comparison to controls (fig. 2).

In adrenal-medullectomized rats (Group III), pressor responses to electrical stimulation, norepinephrine, and tyramine were not significantly different from control values (fig. 2).

In adrenal-medullectomized rats additionally receiving 6-OH dopamine (Group IV), the pressor response to nerve stimulation was totally blocked and that to tyramine significantly reduced, whereas the response to norepinephrine was significantly enhanced.

Similar results were obtained in representative 6-week 2K-RHR (not shown).

Effect of Captopril on Blood Pressure and Heart Rate in 6-Week Two-Kidney Renal-Hypertensive Rats (2K-RHR)

A single oral dose of captopril (30 mg/kg) in vehicle-treated 6-week 2K-RHR caused reductions of MBP to normal levels for the first 12 hours, a significant reduction in MBP persisting for over 18 hours (fig. 3 A).

In rats receiving 6-OH dopamine, captopril caused similar reductions in MBP as in vehicle controls but the responses were not maintained for as long a period of time (fig. 3 B). Because the starting MBPs of these animals were lower than in the vehicle controls, absolute levels of pressure achieved after captopril were lower than in vehicle-treated animals (fig. 3 B).

The effects of captopril in adrenal-medullectomized rats were qualitatively and quantitatively similar to those observed in vehicle-treated rats (fig. 3 C).

In adrenal-medullectomized rats additionally receiving 6-OH dopamine, captopril caused a significant reduction in MBP, although smaller in
Figure 2. Summary of the blood pressure responses ($\Delta MBP$) to stimulation of the entire sympathetic outflow (Panel A), norepinephrine (Panel B) and tyramine (Panel C) in pithed 12-week RHR. Values are the mean ± SE of at least six rats.

Effect of Captopril on 12-week Two-Kidney Renal-Hypertensive Rats (2K-RHR)

The results obtained with captopril in these rats were qualitatively similar to those observed in 6-week 2K-RHR. Captopril (30 mg/kg p.o.) caused a significant and prolonged reduction of MBP in vehicletreated rats (fig. 5 A). However, the change in MBP was less than that observed in 6-week RHR (c.f. fig. 3A), and MBP was still substantially elevated after captopril. In 6-OH dopamine-treated rats, captopril caused a marked reduction in MBP, which was of shorter duration than in vehicle controls (fig. 5 B).

Similarly, captopril normalized MBP in adrenal-medullectomized rats despite severe hypertension before drug (fig. 5 C).

In adrenal-medullectomized rats additionally receiving 6-OH dopamine, captopril caused a significant reduction in MBP below normotensive levels, which was of relatively short duration (fig. 5 B).

As shown in figure 4B, the reduction in MBP caused by captopril expressed as a percentage of the control MBP for each group was least in vehicle-treated controls and greatest in the variously treated groups, with no significant differences among the latter.

Plasma Renin Activity (PRA)

The PRAs were significantly and comparably elevated in all groups of 6-week 2K-RHR in comparison with normotensive Sprague-Dawley control rats (fig. 6). Captopril (30 mg/kg p.o.) significantly increased PRAs in all groups from their respective control values. Despite substantial differences in PRA after captopril among the various groups, none of the values was significantly different from each other because of the wide scatter of values.

In 12-week 2K-RHR, PRAs in vehicle-treated rats were slightly but not significantly higher than in normotensive controls (fig. 7). In contrast, PRAs from Group II-IV were all significantly higher. Captopril significantly increased PRAs in all groups from their respective controls (fig. 7). There were no significant differences among PRA values in Groups I-IV treated with captopril.

Discussion

There appear to be at least three components involved in the maintenance of two-kidney renal hypertension beyond 6 weeks' duration: 1) the renin-angiotensin system, 2) the sympathoadrenal system...
and 3) salt and/or volume retention. The involvement of salt and/or volume in renal hypertension has been described previously and will not be considered further in this report.

**Renin-Angiotensin System**

Many studies with ANG II antagonists have suggested that the renin-angiotensin system plays a primary initiating role in the development of two-kidney renal hypertension (less than 4 weeks) but not in its maintenance. Thus, ANG II antagonists cause significant reductions in blood pressure in 2K-RHR during the early, but not the late, stage of hypertension. These studies are in good agreement with those of Koletsky et al., which showed that a renal pressor substance in the renal vein disappeared some 2 weeks after the initiation of two-kidney renal hypertension whereas hypertension was still maintained. However, recent studies with captopril, an orally active inhibitor of angiotensin converting enzyme, have shown that this agent is very effective in reducing blood pressure of both acute and chronic 2K-RHR.

These results strongly implicate the renin-angiotensin system as being important in maintaining chronic renal hypertension despite the return of the PRA to control levels. With regard to ANG II antagonists in chronic two-kidney renal hypertension, Rieger et al. have reported that, while a single injection of the ANG II antagonist saralasin produced only
small and variable reductions in 2K-RHR, prolonged infusion was able to normalize pressure. Therefore, the reported lack of efficacy cited by others (see above for references) in chronic 2K-RHR might very well be due to a methodological problem rather than a lack of involvement for the renin-angiotensin system. Furthermore, PRA values may not be a good reflection of the importance of the renin-angiotensin system in chronic two-kidney renal hypertension since the vascular renin-angiotensin system may take on greater significance during this time.21, 22, 26

Further evidence for renin-angiotensin being important during the chronic phase of two-kidney renal hypertension was obtained in the present study. Captopril was able to normalize blood pressure in vehicle-treated 6-week 2K-RHR as previously shown in other studies.22 However, despite significantly higher MBP in 12-week 2K-RHR, captopril was not as effective in decreasing MBP as in 6-week 2K-RHR, suggesting that some additional mechanism became operative in these rats, probably the sympathoadrenal system (see below). In rats that were either sympathectomized with 6-OH dopamine, adrenal-medullectomized, or had a combination of both procedures, captopril was still capable of significantly decreasing MBP further. In fact, the percentage reduction was the same for all treated groups, suggesting that the renin-angiotensin involvement was unchanged by inactivation of the sympathoadrenal system in 2K-RHR. This is further supported by the lack of effect of sympathectomy and/or adrenal medullectomy on PRAs and the observation that MBP was reduced to hypotensive levels in these 2K-RHR whereas the drug was ineffective in normotensive rats with a normal renin-angiotensin system.25, 26

Thus, a renin-angiotensin component is established in the maintenance of two-kidney renal hypertension. Furthermore, it is apparently independent of the sympathoadrenal system which contributes its own component.

Sympathoadrenal System

The present study clearly demonstrates a prominent role for the sympathoadrenal system in the maintenance of two-kidney renal hypertension. After destruction of the sympathetic nervous system with 6-OH dopamine, the magnitude of hypertension was significantly though moderately reduced 6 and 12 weeks after renal artery clipping. These results are similar to those of Fernandez et al.7 who also found an attenuation by 6-OH dopamine of the hypertension caused by aortic ligation between the renal arteries. In 2K-RHR with established hypertension of 3 and 9 weeks, a single intravenous injection of 6-OH dopamine caused marked reductions in blood pressure.22 In other related studies, Guazzi et al.26 and Bellini et al.27 also provided indirect evidence that neurogenic activity was also involved in cat and rat two-kidney renal hypertension of greater than 6 weeks' duration. Moreover, we have reported that guanethidine was capable of further decreasing blood pressure to normal in chronic (10-month) 2K-RHR receiving maximally effective doses of captopril and hydrochlorothiazide.28 Although Grewal and Kaul29 found that 6-OH dopamine prevented the development of two-kidney two clip renal hypertension in 80% of weanling rats, they could show no significant inhibition when adult rats were used.

A major difference between our study and others
was our protracted use of 6-OH dopamine to prevent the regeneration of sympathetic neurons observed after only short-term treatment with 6-OH dopamine. Furthermore, we have demonstrated functional sympathectomy in this study whereas others have not. Douglass et al. were unable to attenuate the development of 2K-RHR with prior sympathectomy with guanethidine. The reason for the discrepancy in our studies is unknown but might very well be related to methodological differences. Although the tyramine pressor response was not totally abolished in this study by 6-OH dopamine treatment, functional sympathectomy was apparently completed since the response to nerve stimulation was abolished. The partial tyramine response was probably due to release of catecholamines from chromaffin tissue, which 6-OH dopamine does not deplete. In any case, our study reveals an influence of sympathectomy on the development of renal hypertension; if the sympathectomy was not total, we may have underestimated the role of the sympathetic nervous system, but the influence is nevertheless demonstrated.

Unlike 6-OH dopamine treatment, adrenal medullectomy was without effect on the development of 2K-RHR, an observation previously made for DOCA-salt and one-kidney one clip renal hypertension. Similarly, in established two-kidney renal hypertension of 9 weeks' duration, bilateral adrenalectomy had no significant effect on blood pressure.

It is interesting to note that, despite the lack of the effect of bilateral adrenal medullectomy alone on two-kidney renal hypertension, this procedure was dramatically effective in reducing blood pressure and heart rate when combined with 6-OH dopamine treat-
ment in both 6- and 12-week 2K-RHR. Similar results had been observed in established DOCA-salt and chronic renal hypertension in rats. Thus, the adrenal medulla appears to play an important role in compensating for impaired sympathetic neural function. This is shown indirectly by the twofold increase in adrenal tyrosine hydroxylase after peripheral sympathectomy, and directly by the maintenance of plasma norepinephrine and a marked increase in plasma epinephrine levels in 6-OH dopamine-treated rats.

Thus, that the sympathoadrenal system is involved in the development and maintenance of two-kidney renal hypertension appears to be well established. However, the precise origin of the mechanisms by which this system interacts with the renal pressor system is unclear. A central origin of increased sympathetic discharge appears likely since central 6-OH dopamine prevents but does not alter established two-kidney renal hypertension. An interaction of peripherally formed AII with central sympathetic structures may very well be the initiating factor. In addition, AII has been found to enhance responses to sympathetic stimulation by facilitating release, and exogenous norepinephrine by enhancing end organ responses.

FIGURE 6. Plasma renin activity (ng AI/ml/hr) of conscious normotensive Sprague-Dawley rats (control) and 6-week RHR. N = before captopril (No drug); C = after captopril (30 mg/kg p.o.) Horizontal bar indicates the mean values. Group numbers correspond to the same treatments as in figure 1 and Methods. See text for details.

FIGURE 7. Plasma renin activity of normotensive (control) and 12-week RHR. See figure 6 for details.
responsiveness and inhibiting adrenergic neuronal up-take. Further studies are necessary to determine which of these mechanisms are of importance in the development of renal hypertension.

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