Surgical Reversal of Two-Kidney One Clip Hypertension During Inhibition of the Renin-Angiotensin System


SUMMARY Conscious rats with two-kidney one clip Goldblatt hypertension had the constricting clip removed during continuous infusion of either dextrose, saralasin, or captopril. Other dextrose-infused animals underwent removal of the ischemic kidney or a sham procedure. Direct arterial blood pressure (BP) was recorded throughout the 15-hour preoperative and subsequent 24-hour postoperative period. Rats were studied in the "early" phase (1-3 weeks duration) or "chronic" phase (> 4 months) of hypertension. Animals subjected to a sham procedure returned to preoperative BP values. The BP of animals unclipped or nephrectomized did not return to previous hypertensive levels. Instead, a biphasic response was seen where BP partially recovered from an operative fall and then slowly declined to normal at 24 hours; this effect occurred in both stages of hypertension. At 24 hours, removal of the ischemic kidney was as effective as removal of the constricting clip in the correction of both early and chronic phase hypertension. Rats infused with saralasin or captopril demonstrated an acute (within 2 hours) and sustained fall in BP, but not to nonhypertensive levels. This fall was significant in all animals (p < 0.01) apart from chronic phase rats infused with saralasin where no significant fall was seen. Although animals infused with saralasin or captopril commenced at a lower preoperative BP, the biphasic pattern of response to unclipping was identical to that of dextrose-infused unclipped rats. Thus, sustained inhibition of the renin-angiotensin system did not modify the correction of hypertension produced by removal of the constricting clip, and the response to surgical correction did not appear to be entirely mediated by changes in the activity of the renin-angiotensin system, particularly in the chronic stage. Equally, the rapidity of correction is not consistent with a role for vascular hypertrophy.

(Hypertension 4: 69-76, 1982)

THREE factors have received most attention in the study of the development and maintenance of Goldblatt two-kidney one clip hypertension in the rat: the renin-angiotensin system, sodium retention, and vessel wall hypertrophy. Plasma renin is raised early in the development of hypertension but after several months renin levels fall and may become normal, and renin-angiotensin blockade with a competitive antagonist produces little or no blood pressure response. However, removal of the clip in both early and chronic phases of hypertension lowers blood pressure (BP) to normal according to some, but not all reports, and plasma renin falls to normal or subnormal levels. On the other hand, removal of the ischemic kidney lowers the BP and plasma renin levels to normal in the early phase but is probably less effective in the chronic phase, despite lowering plasma renin to subnormal levels. Recently, we have shown that the fall in BP following unclipping or nephrectomy is associated with a positive sodium balance in Goldblatt two-kidney one clip hypertension of both short- and long-term duration. This argues against a role for sodium retention in the maintenance of the raised BP or as a cause for the reduction of plasma renin activity in the chronic phase of this model. Furthermore, others have shown that cardiac output and stroke volume increase after unclipping, which suggests that the mechanism of BP reduction operates at the peripheral vascular level as opposed to acting through a reduction in venous return to the heart.

A fall in BP can be demonstrated within 2 hours after unclipping, although the residual effects of anesthesia at this time remain uncertain. Certainly, BP is normal at 24 hours after surgical correction in both early and chronic phases, despite the fact that structural changes in the vessel wall are not reversed until 3 weeks after normalization of BP and do not
appear to be completely reversed in the chronic phase. These results have led to the suggestion that smooth muscle tone is subnormal immediately after unclipping.11

The purpose of the present study was to investigate, with a continuous recording system, the change in BP before and for 24 hours after operation in conscious rats and study the contribution of the renin-angiotensin system. The effect of unclipping under continuous pharmacological blockade of the renin-angiotensin system using saralasin (sarcosine alanine angiotensin II) and captopril (angiotensin I converting enzyme inhibitor) was investigated in differing durations of hypertension.

Methods

Female white Wistar rats (160–250 g weight) were used throughout. All surgical procedures were performed under ether anesthesia. Two-kidney one clip hypertension was produced by placing a silver clip (0.2 mm internal diameter) on the left renal artery through a loin incision; the right kidney was not touched. Indirect BP measurements were by a light plethysmographic method. Animals with pressures in excess of 150 mm Hg were studied either within 6 weeks of renal artery constriction (early phase) or after 16 weeks (chronic phase).

The carotid artery (P50) and jugular vein (P30 and P10) were cannulated, and the catheters brought out between the scapulae. They were protected by a light flexible metal tube which was attached to the animals by a linen jacket and maintained under minimal tension by a lightly counterbalanced arm. On recovery from anesthesia, the rats were placed in a plastic container (30 x 30 cm) and given free access to food and water. The BP was monitored continuously using a Statham P23 gb transducer connected to a Grass polygraph recorder. Patency of the catheter was maintained by a slow infusion of heparinized 5% dextrose (10 IU/ml) at 0.25 ml/hr.

After BP had stabilized, infusions of either 5% dextrose, saralasin (10 μg/kg/min), or captopril (8.3 μg/kg/min) were given through the venous line at a rate of 0.25 ml/hr. Animals infused with captopril had a bolus dose of 500 μg immediately before the beginning of the infusion. Effective blockade of angiotensin II (AI1) was assessed by the BP response to 50 ng AI1 before and during infusion of saralasin, and of angiotensin converting-enzyme by 50 ng AI before and during infusion of captopril. All agents were administered in glucose solution (50 g/liter). The animals were infused for 15 hours (overnight) and then subjected to a further operation, which consisted of either unclipping, left nephrectomy, or a sham procedure, the same loin incision; operation was invariably completed within 10 minutes. The sham procedure consisted of exposing and cleaning the clip but not removing it. Infusions and direct BP recordings were continued throughout and for a further 24 hours after the second operation.

Animals were randomly allocated to one of the following groups: each group contained eight animals with early and eight with chronic hypertension.

- **Group 1:** 5% dextrose and sham operation
- **Group 2:** 5% dextrose and left nephrectomy
- **Group 3:** 5% dextrose and unclipping
- **Group 4:** saralasin and unclipping
- **Group 5:** captopril and unclipping

After 24 hours the cannulas were removed, and the animals allowed to recover. Indirect BP measurement was carried out and a sample of blood for plasma renin concentration (PRC) taken at 7 and 60 days in animals that survived. The PRC was measured on samples of tail vein blood obtained under light ether anesthesia. The technique and effect of anesthesia in this model have been described previously.

All results were expressed as mean values ± SEM, and paired or unpaired t tests were used to make statistical comparisons. The PRC was transformed into logarithms before such comparisons were made, since PRC is logarithmically and not normally distributed.

Results

The mean direct arterial BP was calculated from the diastolic plus one-third of the pulse pressure.

**Early Phase Hypertension**

The rats were studied at 28 ± 1.3 days after renal artery clipping. All had a significantly raised PRC (table 1), compared to values for normotensive unoperated or loose-clipped controls, which have been described previously.

**Dextrose Infusion**

There was no significant change in BP during the preliminary 15-hour infusion period. All showed the same fall in BP during operation. The BP rose rapidly to the preoperative level within 2 hours after the sham procedure, followed by a small overshoot and then stabilization by 24 hours at a level that was not significantly different from the initial value. When nephrectomy or unclipping was performed, the BP did not return to hypertensive levels at any stage, but there was a postoperative rise followed by a slow fall to normal (fig. 1). There was no significant difference in the pattern of response at any stage with either procedure.

**Saralasin Infusion**

This caused a maximal fall in BP in all animals by 2 hours (156 ± 9.7 mm Hg), with no further change after 15 hours (159 ± 6.2 mm Hg). Both values were significantly lower than the preinfusion value (p < 0.01, table 1). Despite the lower preoperative value, a fall in BP similar to that of the dextrose group was observed at operation; again there was a temporary postoperative rise followed by a slow fall to normal at
TABLE 1. Early Hypertension: Initial Plasma Renin Concentration (PRC) and Mean Arterial Blood Pressure Preinfusion, Preoperation, and 24 Hours Postoperation

<table>
<thead>
<tr>
<th>Group (n = 8)</th>
<th>PRC (ng AI ml⁻¹ hr⁻¹)</th>
<th>Direct mean blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preinfusion</td>
</tr>
<tr>
<td>Sham operation</td>
<td>690 ± 187</td>
<td>176 ± 8.8</td>
</tr>
<tr>
<td>Left nephrectomy</td>
<td>317 ± 79</td>
<td>189 ± 6.8</td>
</tr>
<tr>
<td>Unclip 5% dextrose</td>
<td>681 ± 157</td>
<td>187 ± 5.4</td>
</tr>
<tr>
<td>Unclip saralasin</td>
<td>485 ± 79</td>
<td>206 ± 5.9</td>
</tr>
<tr>
<td>Unclip captopril</td>
<td>381 ± 71</td>
<td>190 ± 4.2</td>
</tr>
</tbody>
</table>

Results are mean values ± SEM.
* p < 0.05, compared with preinfusion blood pressure.
† p < 0.01.

24 hours (fig. 2). The pressor response to a 50 ng bolus injection of AII before the infusion was 39 ± 3.4 mm Hg with only a 2 ± 0.9 mm Hg response during the infusion.

Captopril Infusion

The maximum fall in BP occurred at 1 hour (158 ± 11.4 mm Hg), and there was no further change after 15 hours (163 ± 7.6 mm Hg); this level was significantly lower than initial values (p < 0.05, table 1). The pattern of response to operation was similar to other unclipped groups; the BP at 24 hours was normal. The pressor response to a 50 ng bolus injection of AII before infusion was 34 ± 6.8 mm Hg and 3 ± 1.6 mm Hg during infusion.

Chronic Phase Hypertension

These animals were studied 134 ± 3 days after renal artery clipping. Direct BPs before infusion were similar to those of rats with early hypertension although the initial PRCs were not significantly different from normal (p > 0.1). This is in keeping with other reports although in a previous study significantly raised renin levels in chronic hypertensive rats were seen.* The group that underwent nephrectomy

![Figure 1. Blood pressure response in early two-kidney one clip hypertension after unclipping, left nephrectomy, or sham operation.](http://hyper.ahajournals.org/)
had significantly higher PRCs than animals unclipped under dextrose or saralasin infusions, but not compared to those infused with captopril (table 2).

Dextrose Infusion

There was no change in BP over the infusion period, and the fall in BP during operation was similar in all three groups. However, 15 minutes postoperatively the BP of nephrectomized animals was significantly lower than that of sham-operated animals ($p < 0.05$). The unclipped animals were not different from the sham-operated ones. The pattern of rise in BP postoperatively was similar in all groups, although the sham and nephrectomy groups rose to higher levels. By 3 hours the sham-operated animals had attained their preoperative BP levels, no overshoot being seen. The nephrectomy group did not quite achieve preoperative values and the BP fell gradually, although at 6 hours it was still not significantly different from that of sham-operated animals. By 12 hours, however, the BP was significantly lower in the nephrectomy group. In contrast, although the BP of the unclipped group was not significantly different from that of the nephrectomy group, it did not show the same postoperative rise, and at 6 hours was significantly lower than in the sham group. After 24 hours, the BP of the sham-operated animals had returned to

<table>
<thead>
<tr>
<th>Group (n = 8)</th>
<th>PRC (ng AI mL$^{-1}$ hr$^{-1}$)</th>
<th>Direct mean blood pressure (mm Hg) Preinfusion</th>
<th>Preop</th>
<th>24 hrs Postop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>125 ± 22</td>
<td>171 ± 5.6</td>
<td>177 ± 6.3</td>
<td>171 ± 6.5</td>
</tr>
<tr>
<td>Left nephrectomy</td>
<td>212 ± 55</td>
<td>169 ± 6.0</td>
<td>176 ± 6.5</td>
<td>111 ± 9.0*</td>
</tr>
<tr>
<td>Unclip 5% dextrose</td>
<td>96 ± 9.2</td>
<td>173 ± 5.8</td>
<td>172 ± 4.3</td>
<td>116 ± 5.8*</td>
</tr>
<tr>
<td>Unclip saralasin</td>
<td>75 ± 17</td>
<td>174 ± 4.8</td>
<td>156 ± 7.1</td>
<td>115 ± 8.0*</td>
</tr>
<tr>
<td>Unclip captopril</td>
<td>152 ± 90.1</td>
<td>186 ± 5.7</td>
<td>153 ± 8.7*</td>
<td>111 ± 3.8*</td>
</tr>
</tbody>
</table>

*$p < 0.01$, compared to preinfusion blood pressure.
MEAN BLOOD PRESSURE (mm Hg)

**Figure 3.** Blood pressure response in chronic two-kidney one clip hypertension after unclipping, left nephrectomy, or sham operation.

the initial level, whereas it was normal in the nephrectomy and unclipped animals (fig. 3).

**Saralasin Infusion**

The BP fell within 2 hours to its nadir (156 ± 3.5 mm Hg) and remained stable until operation 15 hours later (157 ± 8.7 mm Hg), but neither value was significantly different from the preinfusion value (p > 0.05, table 2). Perioperative fall and postoperative rise was similar to those of the dextrose-infused unclipped animals (fig. 4). At 24 hours the BP was normal, being significantly lower than either preinfusion or preoperative values (p < 0.01, table 2). The pressor response to a 50 ng bolus injection of AII was 39 ± 4.6 mm Hg before and 1.9 ± 1.9 mm Hg during infusion of saralasin.

**Captopril Infusion**

A significant fall in BP had occurred within 1 hour of infusion (p < 0.01), and the BP remained unchanged after that, until operation. There was no difference subsequently between this group and other unclipped animals (fig. 4). At 24 hours, the BP was normal and significantly lower than the preoperative values (p < 0.01, table 2). The pressor response to a 50 ng bolus injection of AII was 32.1 ± 1.5 mm Hg before and 5.3 ± 3.1 mm Hg during infusion of captopril.

**Comparison of Initial PRC with Fall in Blood Pressure following Pharmacological Blockade**

The fall in BP in early and chronic groups with saralasin was significantly correlated with preinfusion PRC both at 2 hours (r = 0.49) and at 15 hours (r = 0.63). There was no significant correlation between the BP fall at 2 hours (r = −0.04) or at 15 hours (r = 0.07) and PRC in the captopril-infused animals.

**Long-Term Response to Surgery**

In early phase hypertension, the BP showed a highly significant fall in both unclipped and nephrectomized animals (p < 0.01); the initial and 7-day values were similar in both groups (table 3). Nephrectomized animals were still normotensive at 60 days. PRC also fell in the nephrectomized group to a level significantly lower at 7 days than that of the unclipped group (p < 0.05). At 60 days, the nephrectomized group showed an insignificant rise in PRC (p > 0.1).

In chronic phase hypertension, the initial, 7-day, and 60-day postoperative BPs were similar in both unclipped and nephrectomized animals, showing significant falls compared with preoperative values (table 3). PRC values, however, were significantly lower in nephrectomized animals at 7 days but not at 60 days when compared with unclipped animals.
FIGURE 4. Blood pressure response in chronic two-kidney one clip hypertension after unclipping during dextrose, saralasin, or captopril infusion.

TABLE 3. Indirect Blood Pressure (BP) and Plasma Renin Concentration (PRC) Before and After Unclipping or Nephrectomy

<table>
<thead>
<tr>
<th>Hypertension phase</th>
<th>Initial BP (mm Hg)</th>
<th>Initial PRC (ng AI ml⁻¹hr⁻¹)</th>
<th>7 days postop BP (mm Hg)</th>
<th>7 days postop PRC (ng AI ml⁻¹hr⁻¹)</th>
<th>60 days postop BP (mm Hg)</th>
<th>60 days postop PRC (ng AI ml⁻¹hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Unclip</td>
<td>174 ± 2.4</td>
<td>421 ± 62</td>
<td>106 ± 4.7; 109 ± 15;</td>
<td>106 ± 4.7; 109 ± 15;</td>
<td>109 ± 6.2; 68 ± 14;</td>
<td>109 ± 6.2; 68 ± 14;</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>164 ± 5.1</td>
<td>270 ± 73</td>
<td>111 ± 6.2; 35 ± 11;</td>
<td>111 ± 6.2; 35 ± 11;</td>
<td>118 ± 7.9; 38 ± 8;</td>
<td>118 ± 7.9; 38 ± 8;</td>
</tr>
<tr>
<td>Chronic Unclip</td>
<td>166 ± 5.0</td>
<td>125 ± 45</td>
<td>111 ± 5.6; 65 ± 10;</td>
<td>111 ± 5.6; 65 ± 10;</td>
<td>116 ± 6.8; 15 ± 5;</td>
<td>116 ± 6.8; 15 ± 5;</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>169 ± 15</td>
<td>187 ± 42</td>
<td>95 ± 5.6; 30 ± 10;</td>
<td>95 ± 5.6; 30 ± 10;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05; comparison of unclip and nephrectomy groups.
†p < 0.05; comparison of PRC and BP to initial PRC and BP.
‡p < 0.01; comparison of PRC and BP to initial PRC and BP.

Discussion

In the present study, infusion of either saralasin or captopril failed to normalize the BP in Goldblatt two-kidney one clip rats, but despite this, corrective surgery normalized the BP within a few hours.

Both saralasin and captopril lowered the BP during the early phase of Goldblatt hypertension but not to normal levels, and after several months the response to saralasin was smaller and did not achieve significance. This difference between early and late phases of...
hypothesis that chronic hypertension is associated with a sustained increase in plasma renin activity. Our investigation showed a significant correlation between plasma renin concentration and BP response to saralasin.

In our studies, the maximum fall in BP produced by either competitive antagonists of All or converting enzyme inhibition occurred within 1 hour or so of infusion. The BP remained stable over the next 14 hours, in contrast to observations of Reigger et al. of a progressive BP fall to normal levels over 12 hours of infusion with both saralasin and captopril. They interpreted this to indicate that BP was maintained by a slowly developing hypersensitivity to angiotensin II. In this and previous work, we found no evidence of such a slow reversal of hypertension. Although the BP did fall following unclipping or nephrectomy during more prolonged inhibition of the renin-angiotensin system in the present experiments, this fall was similar to that observed in animals undergoing infusion with dextrose alone. The delayed normalization of BP cannot therefore be attributed to a late effect of the antagonist or inhibitor. More prolonged administration of captopril orally has, however, resulted in a fall in BP over several days; our present studies do not throw light upon the nature of this more long-term response. It is of interest to observe that the response to captopril, unlike the response to saralasin, was not related to preinfusion plasma renin either in the rats with early or chronic hypertension. In addition, captopril produced a significant fall in BP in the chronic phase of hypertension when plasma renin activity was normal. This qualitative difference in responsiveness to captopril as compared with saralasin suggests that captopril is lowering the BP at least in part by a different mechanism from renin-angiotensin blockade. One possibility lies in bradykinin potentiation through inhibition of the enzyme kininase II.

All groups showed a major fall in BP during the surgical procedure. However, since this response was also seen in the sham-operated group, it is reasonable to conclude that it is a nonspecific response to anesthesia and surgery. The BP recovered between 2 and 4 hours after surgery in the sham group. However, animals that underwent corrective surgery showed only a partial recovery of BP, which then declined to normal over the next 6 to 12 hours. It appears that either unclipping or removal of the ischemic kidney activates a slow fall in BP, which takes a total of between 5 and 16 hours. This fall is partially obscured in the early postoperative period by the nonspecific effect of operation. The interaction between these two vasodepressor processes accounts for the remarkable biphasic decline in BP documented here.

Both nephrectomy and unclipping reduced BP to normal at 24 hours, where it remained for at least 60 days following operation. This finding confirms our previous observation, although the nephrectomized animals showed a partial recovery of hypertension in the present study, BP remained normal in nephrectomized animals as in other groups, although only six animals survived. The fall in renin to normal or even subnormal levels following unclipping or nephrectomy has previously been documented by us. In the present study the fall was somewhat greater in the nephrectomized animals, which exhibited a significantly subnormal renin at the end of the study. This may reflect a greater degree of sodium retention, greater loss of renin-secreting tissue, or changes in the remaining kidney induced by longstanding hypertension or possibly a combination of all three.

In the chronic phase of hypertension in the present study, nephrectomy was associated with a temporary recovery in BP to a level not significantly different from that observed in the sham-operated group, while unclipped animals remained at a significantly lower BP level (fig. 3). This transient difference in behavior is of interest but difficult to explain in the context of this study. Our studies do not support the concept that vascular hypertrophy maintains BP in the chronic phase of hypertension. The BP fell over a period of hours, while structural changes have been shown to take several weeks to reverse. In addition, the BP was no higher after corrective surgery in the chronic than in the early phase of hypertension. Although the reversal of hypertension in the early model is associated with a fall in peripheral resistance, this fall cannot readily be attributed to reversal of structural changes.

Since corrective surgery is associated with a major fall in plasma renin activity it is theoretically possible that the renin-angiotensin system plays a role in maintaining the BP in this model. It could also be argued that, as infusion of saralasin lowers the BP significantly in the early phase of hypertension, albeit not to normal levels, full relief of hypertension is dependent on reduced renin secretion in addition to another effect which is dependent on surgical reversal. This is less obviously so in the chronic phase where saralasin has a smaller effect. The present studies suggest that other mechanisms play a major role in both phases. Thus, the response pattern of a partial recovery of BP in the immediate postoperative period followed by a slow decline in BP was identical whether captopril, saralasin, or dextrose was being infused. The only difference was that the inhibitor-infused animals underwent surgery at a slightly lower BP level.

Since blockade of the pressor effects of exogenous A1 or AII could be demonstrated, the only mechanism by which the renin-angiotensin system could maintain the BP in this situation would be one in which the system was isolated from the effects of inhibitors for the duration of the experiment. While we have elsewhere produced evidence that vaso-active AII is generated locally within the resistance vessel wall by renin derived from the kidneys, our experiments indicate that such a site is readily accessible to the inhibitors. Thus, the most likely conclusion from our experiment is that changes in renin-angiotensin activity do not account for the fall in BP when hypertension of this type is reversed, particularly in the chronic phase.

Since neither changes in sodium balance nor reversal of vascular hypertrophy appear to be of prime importance in this situation, the relatively slow decline in
BP that we observed must be attributed to another mechanism. The nature of that mechanism remains obscure. Vasopressor agents other than renin might be removed or vasodepressor material might be released as a result of corrective surgery. Various non-renin pressor peptides have been extracted, i.e., nephrinotensin, corticotensin, and renopressin, and appear to be different from renin, AI or All. There is no general agreement on the role of such systems. Other evidence suggests that renal medullary interstitial cells synthesize vasodepressor compounds that have the requisite properties necessary to produce the slow BP changes. The physiology of the renal system needs to be studied further to resolve these uncertainties.

Acknowledgments
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G I Russell, R F Bing, H Thurston and J D Swales

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