Reversal of Two-Kidney One Clip Renovascular Hypertension in the Rat

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SUMMARY Attempted correction of two-kidney, one clip Goldblatt hypertension in the rat was carried out by three techniques: removal of the constricting clip, removal of the ischemic kidney, and converting enzyme blockade by oral captopril. Since duration of hypertension is said to be a critical factor, groups of rats were studied after short term (< 6 weeks from clipping) and chronic (> 4 months) hypertension. Blood pressure, sodium balance, and plasma renin concentration (PRC) were followed before and after these correcting procedures. In a control group of animals, removal of a loose renal artery clip did not influence blood pressure and only caused trivial postoperative retention of sodium. Unclipping, however, normalized blood pressure in both short-term and chronic hypertension. After a major postoperative fall, blood pressure returned to somewhat elevated levels after nephrectomy in animals with chronic (but not short-term) hypertension. Sodium balance became markedly positive with the fall in blood pressure of operated hypertensive animals and was significantly correlated with the fall in blood pressure in these four groups at 7 days (r = 0.43). Captopril also produced a fall in blood pressure at 24 hours, with a positive sodium balance, although the relationship between blood pressure fall and sodium balance did not reach statistical significance (r = 0.30). The PRC was elevated in all hypertensive groups, although individual values overlapped with values from normal rats and non-hypertensive rats with a loose renal artery clip. The PRC fell to normal or subnormal values after either operative procedure and stabilized for at least 2 months independently of whether blood pressure fell or not. It is concluded that neither sodium retention nor renin hypersecretion maintains blood pressure in this model. Also, the rapidity of the blood pressure fall is not consistent with a role for vascular hypertrophy. The greater efficacy of unclipping suggests that the revascularized kidney after this procedure exerts a vasodepressor function independent of sodium excretion or the renin-angiotensin system. (Hypertension 2: 256-265, 1980)

KEY WORDS • Goldblatt hypertension • plasma renin concentration • two-kidney hypertension • sodium balance • nephrectomy • unclipping

The mechanism by which sustained blood pressure elevation follows renal artery constriction is still uncertain. Attention has been focused on three factors: the renin-angiotensin system, sodium retention, and resistance vessel hypertrophy. The presence of a contralateral nonischemic kidney appears to be of critical importance to the pathogenesis of hypertension. When contralateral nephrectomy is performed (one-kidney, one clip hypertension), plasma and renal renin levels are normal or reduced,1 and sodium balance is positive.2 In the two-kidney, one clip model, on the other hand, renin levels are elevated during the early phases of hypertension, although renin may then fall.3-4 In addition, there is little or no blood pressure response to renin-angiotensin blockade with the competitive angiotensin antagonist saralasin, after hypertension has been present for several months.6-8 Sodium balance is usually negative in the early phase of hypertension, presumably as a result of perfusion pressure natriuresis by the nonischemic kidney,9 although importance has been attached to transient sodium retention observed during the development of hypertension.9-10 It has also, however, been postulated on the basis of indirect evidence that sodium retention assumes a role in blood pressure maintenance after several months when the renin-angiotensin system becomes less important.9 In addition, hypertension induces arteriolar hypertrophy that may maintain peripheral resistance,11 even when such factors as renin-angiotensin activation and sodium retention appear not to play a role. Whether these three factors, i.e., renin, sodium balance, and vascular hypertrophy explain the elevation of blood pressure that follows renal artery constriction is open to doubt.12 Most studies have focused on physiological changes occurring during the development or maintenance of renovascular hypertension. In the present work, sodium balance and plasma renin have been studied in rats with two-kidney, one clip hypertension during

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corrective operative procedures — removal of the constricting clip and nephrectomy of the ischemic kidney. Further, the response to surgery has been compared with the response to reduction of blood pressure with the converting enzyme inhibitor captopril (SQ 14,225). In view of the possibility raised in other studies that different mechanisms are important in longstanding hypertension compared with the earlier phases of blood pressure elevation, we have studied rats within a few weeks of renal artery clipping and after several months.

Materials and Methods

Operative Procedures

A loin incision was made and a constricting silver clip (internal diameter 0.2 mm) was placed on the left renal artery under ether anesthesia. Removal of the clip and left nephrectomy were carried out in animals through the same incision.

Metabolic Balance Studies

Individual animals were housed in glass metabolic cages (Jencon's "Metabowl"). Balance procedures were carried out according to a previously described method. Rats were allowed to familiarize themselves with the cages for 5 to 7 days before balances were begun. Balances were then analyzed for at least 4 days before any definitive procedure was carried out. Food (standard rat chow) was presented as a paste made up with a measured volume of deionized water. Uneaten food residue was weighed at the end of each balance period of 24 hours. Feces were collected separately, ashed at 450°C in porcelain crucibles, and then dissolved in 10 to 20 ml N/10 hydrochloric acid; sodium content was measured in the same way after ashing. Urine volume and sodium content were measured daily. Sodium balance was calculated as intake [food provided—food residue] – output [urine volume × sodium concentration] + (fecal sodium)]. Balances were followed for a week after definitive procedures (i.e., unclipping, nephrectomy, captopril administration). Animals were then removed from the cages, the unclipped and the nephrectomized animals were maintained in ordinary rat cages for 2 months, and blood pressure and plasma renin concentration were measured at the end of this period to ensure physiological stability.

Blood Pressure Measurement

Blood pressure measurement was carried out under ether anesthesia by an indirect light plethysmographic method. Hypertensive animals were selected on the basis of blood pressure measurement of 150 mm Hg or more. Approximately two-thirds of clipped animals met this criterion.

Plasma Renin Concentration (PRC Assay)

Tail-vein blood was collected in the midafternoon under ether anesthesia, in a precooled tube moistened with a drop of concentrated solution of potassium ethylene diamine tetraacetate (EDTA): plasma was separated after spinning in a refrigerated centrifuge and frozen at -20°C. A 100 μl sample of plasma was incubated with 400 μl of nephrectomized rat plasma as substrate at pH 6.5 in the presence of enzyme inhibitors, and the generated angiotensin I measured by radioimmunoassay. The method used was similar to that of Sealey et al., except that phenylmethylsulphonyl fluoride was used as the angiotensinase inhibitor. A normal range for rat plasma renin concentration was obtained using blood samples collected in the same way from 10 normotensive, non-operated rats from the same colony.

Experimental Groups

Animals were studied after the development of early hypertension (less than 6 weeks after clipping) and chronic hypertension (more than 4 months after clipping). Each group comprised 8 animals. The groups were:

Group 1. (Early Hypertension, Unclipping)

The constricting clip was removed from the renal artery, blood pressure was checked at 7 days before and immediately before operation, the day following operation, and at 7 and 60 days.

Group 2. (Early Hypertension, Left Nephrectomy)

Animals were studied in an identical fashion to Group 1 before and after removal of the left ischemic kidney.

Group 3. (Early Hypertension, Converting Enzyme Blockade)

Captopril (SQ 14,225) was administered in the food paste so that animals received approximately 2.5 mg over one 24-hour period. Blood pressure was measured after 12 and 24 hours of captopril.

Groups 4, 5, 6.

These groups were treated identically to Groups 1, 2, and 3 respectively except that rats with chronic hypertension were studied.

Group 7. (Control, Loose Clip, Unclipping)

A loose, renal artery clip (internal diameter 0.5 mm) was applied to the renal artery. Animals were then treated identically to Group 1.

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 are expressed as mean values ± SEM.
Results

The interval between operation and beginning metabolic balances was 30.9 ± 1.3 days for Groups 1–3 and 180.0 ± 19.6 days for Groups 4–6.

Blood Pressure Changes

Removal of the constricting clip from animals with early and chronic hypertension produced an acute fall in blood pressure to normal at 24 hours; blood pressure then remained normal after that up to 60 days (fig. 1). Removal of the ischemic kidney also produced a fall to normal in all animals, but then blood pressure showed a significant rise in the animals with chronic hypertension (p < 0.05, fig. 2). At the end of 7 days, blood pressure was significantly higher in this group than in unclipped rats (p < 0.05). Five of these animals had blood pressures above normal at this stage, and blood pressures remained at approximately these levels for the next 2 months. Left nephrectomy of rats with the early phase of hypertension restored blood pressure to normal in all rats at 24 hours, although in one animal, blood pressure rose to 180 mm at 7 days, and this was still elevated (150 mm Hg) after 2 months. Captopril produced a significant fall in blood pressure in rats with the early and chronic phase of hypertension (p < 0.05 fig. 3). There was a nonsignificant fall in blood pressure following removal of a loose, renal artery clip from control animals from 92.7 ± 3.3 to 83.7 ± 3.2 mm Hg at 24 hours and 86.3 ± 3.8 mm Hg at 7 days.

Metabolic Balance

Cumulative sodium balance showed no consistent change in any of the groups of hypertensive rats before definitive procedures were carried out (figs. 1–3). Unclipping or nephrectomy of rats in the early or chronic phase of hypertension (Groups 1, 2, 4, and 5) was associated with a substantial positive cumulative sodium balance developing over the first 2 or 3 postoperative days. There was no significant difference in cumulative sodium balance at the end of the experimental period 7 days postoperatively between early and chronically hypertensive rats or between animals subjected to declipping and animals subjected to nephrectomy (p > 0.1). However, chronically hypertensive rats that underwent left nephrectomy (Group 5) could be divided into two subgroups. In 3 rats, blood pressure fell and remained within normal limits (< 120 mm Hg); in these, sodium balance was +3.08 ± 1.55 mmol. In five rats, the blood pressure was above the upper limit of normal at the end of the balance period; in these, sodium balance was substantially less positive (1.54 ± 0.55 mmol), although for these small numbers, the difference was not statistically significant (p < 0.3 > 0.2). The change in

Figure 1. Effect of removal of constricting clip on cumulative sodium balance and systolic blood pressure (B.P.).
cumulative sodium balance was significantly correlated with the fall in blood pressure in the four groups of operated hypertensive rats \( (r = 0.43; p < 0.05; \text{fig. 4}) \). The fall in blood pressure produced by captopril induced a modest positive sodium balance in rats with early hypertension (Group 3), although this only reached maximal values 2 days after the drug was stopped. There was only a trivial degree of sodium retention in chronically hypertensive rats (Group 6, fig. 3). Four rats in Group 3 showed little or no change in blood pressure with captopril. Sodium balance at the end of the 24-hour period of administration was \(-0.52 \pm 0.68\) mmol in these rats compared with \(+1.11 \pm 0.46\) mmol in the four rats in which blood pressure fell by a greater amount \((p < 0.1 > 0.05)\). Only two rats in Group 6 failed to show a fall in blood pressure with captopril, and sodium balance was \(+0.27 \pm 0.54\) mmol compared with a balance of \(+0.7 \pm 0.36\) mmol in six rats in which blood pressure did fall \((p < 0.6 > 0.5)\). For the two groups of captopril-treated rats, the correlation between blood pressure fall and sodium balance was 0.30; this was not statistically significant \((\text{fig. 5}; p < 0.3 > 0.2)\). After removal of the clip from loose-clipped animals, cumulative sodium balance became positive, reaching a maximum of \(+0.94 \pm 0.3\) mmol by the third day.
Figure 4. Relationship between cumulative sodium balance and fall in systolic blood pressure (B.P.) in the four groups of animals in which operative correction of hypertension was attempted.

Figure 5. Relationship between cumulative sodium balance and fall in systolic blood pressure (B.P.) in rats treated with captopril.
and then falling to $0.21 \pm 0.48$ mmol at the end of the balance. Weight showed no consistent change in the preoperative period (fig. 6). At the end of 7 days, the hypertensive animals showed a minor weight gain (<5% body weight). The loose-clipped controls showed no change.

Urine volumes fell in parallel with the fall in sodium excretion in the unclipped and nephrectomized groups and in the early hypertensive animals treated with captopril, although the fall in blood pressure produced in chronically hypertensive rats by captopril was not associated with any change in urine volume. Urine volume at 7 days was not altered by removal of a loose, renal artery clip (table 1).

**Plasma Renin Concentration**

(Figs. 7 and 8; table 2). Rats in the early and chronic phase of hypertension showed a significantly elevated PRC compared with the 10 normal, nonoperated rats ($p < 0.01$). There was no significant difference between PRC in rats with early and chronic hypertension ($p < 0.4 > 0.3$). Rats with a loose, renal artery clip showed a nonsignificant elevation of PRC ($p < 0.2 > 0.1$). The PRC fell significantly in all the hypertensive animals that underwent either unclipping or nephrectomy ($p < 0.01$, fig. 8). The PRC values at the end of the balance period were significantly less than normal in the nephrectomized early and
TABLE 2. Plasma Renin Concentrations in Experimental Groups and Normal Controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Preoperative</th>
<th>Postoperative</th>
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<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>60 days</td>
</tr>
<tr>
<td>Early</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclipping</td>
<td>801.6±315.9</td>
<td>111.0*±25.5</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>1296.0±1040.3</td>
<td>27.4*±11.5</td>
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<tr>
<td>Captopril</td>
<td>1132.2±485.7</td>
<td>74.8*±22.6</td>
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<tr>
<td></td>
<td>991.5±210.8</td>
<td>57.5*±16.8</td>
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<tr>
<td>Chronic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclipping</td>
<td>1793.6±1122.3</td>
<td>49.4*±23.6</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>1322.2±542.2</td>
<td>78.4*±22.6</td>
</tr>
<tr>
<td></td>
<td>1353.9±210.8</td>
<td>57.5*±16.8</td>
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<tr>
<td>Controls</td>
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<tr>
<td>Loose clip</td>
<td>228.5±46.9</td>
<td>110.8±18.2</td>
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<tr>
<td></td>
<td>135.5±16.8</td>
<td>57.5*±16.8</td>
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<td>Data are mean values ± SEM. For each group, n = 8, except the normal rat group where n = 10. *Significantly different from preoperative values for the same group.</td>
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Figure 8. Postoperative plasma renin concentrations (P.R.C.) at the end of metabolic balance.

Discussion

In this study, a fall in blood pressure was produced in two-kidney, one clip Goldblatt hypertension by removal of the renal artery constriction, unilateral nephrectomy of the ischemic kidney, and converting enzyme blockade. The results provide evidence of relevance to our understanding of three factors that may play a role in the pathogenesis and maintenance of hypertension — sodium and water balance, the renin-angiotensin system, and vascular hypertrophy induced by the increased load upon the resistance vessels in hypertension.

Two stages of hypertension have been studied, here termed "early" and "chronic." We have purposely refrained from the use of the term "acute," which could be confused with the rise in blood pressure sometimes seen in the immediate postclipping period. The "early" group underwent unclipping or nephrectomy in the fifth postoperative week. In our hands, blood pressure in this model begins to rise, on average, 10–14 days after clipping (exceptionally after 21 days), so that hypertension was of approximately 2 weeks' duration at the time of the second operation; by contrast, "chronic" hypertension had been present for several months. It is important to study both stages of hypertension, since there is evidence from inhibitor studies that the renin-angiotensin system is more important in early than in chronic hypertension (as defined here). Further, Gavras et al. have suggested that "volume factors" play a major role in hypertension of duration equal to our "chronic" model. We cannot comment upon hypertension of shorter duration than our "early" group, although our previous balance studies showed gradual development of a negative sodium balance proportionate to the degree of blood pressure elevation. It seems unlikely, therefore, that studies at an earlier stage of the...
The blood pressure fall produced by unclipping restored blood pressure to normal in both early and chronic hypertension; this fall was sustained indefinitely. After an immediate, substantial postoperative fall, blood pressure returned to hypertensive levels in the nephrectomized animals with chronic hypertension (fig. 2), although it remained substantially below initial values. Nephrectomy restored the blood pressure of most rats with short-term hypertension to normal. Captopril produced a less marked fall in blood pressure in both groups. The blood pressure response to unclipping is consistent with other reports, although Floyer observed persistent hypertension after removal of the clip in rats with hypertension of 8–46 weeks' duration. The response to removal of the ischemic kidney in this model is consistent with the previous findings of restoration of blood pressure to normal in animals with short-term hypertension and failure to normalize blood pressure in animals with hypertension of longer duration. On the other hand, Gross observed normalization of blood pressure at this stage. In other studies, captopril produced a similar blood pressure fall to that observed here in rats with short-term two-kidney, one clip Goldblatt hypertension, although more prolonged administration produces a further decline in blood pressure, which may be reduced to normal levels after several days. The fact that blood pressure was not normalized in our animals, therefore, cannot be used as evidence that mechanisms other than the renin-angiotensin system were responsible for blood pressure maintenance, as longer periods of administration or higher doses might have produced a further fall in blood pressure. The objective of the present experiments was to examine the effect of a 24-hour inhibition of converting enzyme upon sodium balance.

The lowering of blood pressure in all six groups was associated with sodium retention (figs. 1–3), whereas removal of the loose, renal artery clip was associated with trivial sodium retention only. While the state of sodium balance was clearly dependent on many factors, it was significantly correlated with a change in blood pressure in the surgically corrected groups (fig. 4). The slope of the line relating blood pressure and sodium balance was identical in the captopril group (fig. 5), although the numbers are smaller and did not reach statistical significance. The net gain of sodium in these animals tended to be less, however. This may reflect the shorter period of balance (24 hours used for computing the correlation), or alternatively, captopril may have an additional effect upon sodium excretion. One possibility is that the bradykinin potentiating action of captopril plays a role in the net effect of this compound on sodium balance. It is interesting to note that sodium balance continued to become positive even after captopril was withdrawn and after blood pressure had returned to hypertensive levels (fig. 3). This suggests that the action of captopril on sodium excretion and the compensatory homeostatic responses so stimulated may be much more complex than the response to surgical correction of blood pressure. Previous studies in this model showed a fall in sodium excretion in the 8 hours after declipping, which was attributed to lack of postoperative food intake. Ten Berg and co-workers found a trend towards sodium retention that did not reach statistical significance following declipping of the renal artery in rats with two-kidney, one clip Goldblatt hypertension. Dietz and co-workers found no relationship between change in blood pressure and change in urinary sodium excretion over the 6 hours after declipping. Our results refute the suggestion that the blood pressure fall induced by any of these procedures is mediated by increased sodium excretion. The degree of sodium retention induced by surgical correction of hypertension demonstrated here is approximately equal to the negative sodium balance that we have previously observed during the development of hypertension in this model, suggesting that the sodium retention represented correction of sodium depletion produced by pressure natriuresis through the nonischemic kidney. The fall in urine volume (table 1) is attributable to relief of pressure diuresis.

The responsiveness of blood pressure to angiotensin II blockade is lost in the chronic phase of hypertension in this model, and it has been suggested that sodium retention assumes a primary role. In the present studies, however, the degree of sodium retention that followed blood pressure correction was just as great in the chronic as in the early phases, and there was no evidence for different handling of sodium in these two stages. Our results do not support the view that blood pressure in either the earlier or the chronic phase of two-kidney Goldblatt hypertension is mediated by sodium retention.

Plasma renin concentration (PRC) was elevated in the majority of hypertensive animals, although values overlapped with those observed in normal and loose-clipped rats. While elevated plasma renin has been observed at 5, 6, and 10 weeks in this model, other groups have observed normal renin levels in hypertension after a few weeks or months. Renin samples were obtained under light ether anesthesia, which stimulates renin secretion. Thus, PRC was two to three times as high as values we have reported in the early phases of two-kidney, one clip hypertension, when samples were obtained by immediate cardiac puncture of decapitated animals. The relative magnitude of PRC in hypertensive and normal rats is preserved, however, and since restraint is as powerful a stimulus as anesthesia, we chose the latter method; nevertheless, other groups using the same method of sampling detected low PRC values in animals with chronic hypertension. Unclipping or nephrectomy dramatically reduced PRC to normal or subnormal levels in all hypertensive animals, and PRC remained at these levels for at least 2 months. It seems unlikely that renin secretion was suppressed to subnormal values by sodium overload. One interesting possibility is that hypertension caused, in the nonischemic kidney, structural changes of the...
type that have been postulated in low renin essential hypertension in man, in this case, suppression of renin secretion by the previously ischemic kidney in the unclipped animals might be attributable to increased renal perfusion pressure. It is difficult to attribute the fall in blood pressure to correction of hyperreninemia. The failure of even prolonged infusions (up to 12 hours) of angiotensin antagonists to lower blood pressure in the chronic phase of this model supports evidence from other species that with the passage of time, renin no longer plays a role in the pathogenesis of renovascular hypertension. In the present studies, renin fell equally in nephrectomized and unclipped animals with chronic hypertension; despite this, the nephrectomized animals remained moderately hypertensive. It is possible, therefore, that high renin, in this phase at least, is a consequence of hypertension perhaps resulting from negative sodium balance. If so, the persistent hypertension after nephrectomy cannot have been sufficient to activate this mechanism.

The present work does not suggest that resistance vessel hypertrophy produced as a result of prolonged hypertension is the only factor in blood pressure maintenance in this model. The blood pressure fell within 24 hours of surgical correction of renal ischemia. By contrast, vascular hypertrophy takes at least several weeks to reverse following relief of renal artery constriction in rats with chronic Goldblatt hypertension. While vascular hypertrophy may therefore be an important factor in determining sensitivity to vasoactive agents in hypertensive animals, it seems likely that blood pressure is maintained by another mechanism that is reversed rapidly by surgical correction of renal ischemia.

Neither sodium retention, renin hypersecretion, nor vascular hypertrophy appear to be responsible for sustained hypertension in the two-kidney one clip Goldblatt model, therefore, although the present studies do not exclude an initiating role for either renin or the sodium ion. The greater efficacy of unclipping compared with nephrectomy in lowering blood pressure in chronic hypertension is probably not a species-specific phenomenon. Thus, in man, revascularization procedures are reported as producing a greater lowering of blood pressure than unilateral nephrectomy in the treatment of renovascular hypertension. The reasons for this discrepancy are unclear, but it seems possible that the revascularized kidney releases vasodepressor material into the circulation. During the development of renovascular hypertension, bioassay studies indicate that the nonischemic kidney releases prostaglandin-like material in sufficient quantities to moderate peripheral vasoconstriction; it is reasonable to assume that relief of ischemia causes a similar response in the previously ischemic kidney. This seems more likely than the alternate hypothesis that the secretion of pressor material other than renin is inhibited by unclipping; if this were the case, nephrectomy should be at least as effective as unclipping rather than the reverse.
REVERSAL OF RENOVASCULAR HYPERTENSION/Thurston et al.


Reversal of two-kidney one clip renovascular hypertension in the rat.
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