Role of the Renal Nerves in One-Kidney, One Clip Hypertension in Rats

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SUMMARY The effects of renal denervation on the onset and maintenance of one-kidney, one clip Goldblatt (1K1C) hypertension were determined. Renal denervation was performed at the time of 1K1C surgery, and was repeated at 3-week intervals to prevent renal nerve regeneration. Denervation delayed the onset of 1K1C hypertension by about 5 weeks, but the final hypertensive state was unaltered. Mean arterial pressure (MAP) averaged 196 ± 11.4 mm Hg in six rats at 9 weeks after 1K1C surgery and 194 ± 11.3 mm Hg in eight renal-denervated rats at this time. The delay in the development of 1K1C hypertension following renal denervation could not be explained by interference with renin release. This delay in the development of hypertension could be prevented, however, in renal-denervated 1K1C rats by substituting saline for the drinking water. Two weeks after 1K1C surgery and a high sodium diet, MAP averaged 164 ± 6.4 mm Hg in eight rats with intact renal nerves and 173 ± 4.8 mm Hg in nine renal-denervated rats. Intact renal nerves are not necessary for the development or maintenance of 1K1C hypertension. Renal denervation delays development of 1K1C hypertension, possibly by delaying the ability of these rats to retain sodium.

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KEY WORDS • renal denervation • renal norepinephrine • renin • salt loading

STUDIES of several models of renal hypertension in rats have indicated that an intact sympathetic nervous system or intact renal innervation is necessary for the maintenance of this form of hypertension. Dorr and Brody1 reported that immunosympathectomy of one-kidney, one clip Goldblatt (1K1C) rats, produced by treatment with antinerve growth factor, had no effect on the development of the acute phase of renal hypertension. However, the chronic phase of hypertension was markedly attenuated. These findings were later substantiated in a similar experiment by Ayitey-Smith and Varma.2 It has also been suggested that ablation of centers in the central nervous system that may be involved in arterial pressure regulation may interfere with the development or maintenance of renal hypertension by alteration of normal renal nerve reflex pathways.3-5

In a recent study, Katholi et al.6 performed renal denervations on 1K1C hypertensive rats 2 weeks after renal artery clipping. The renal denervation procedure resulted in an almost complete reversal of the hypertension for about 3 weeks, but arterial pressure increased back to the initial hypertensive level 5 weeks after denervation. This reestablishment of hypertension 5 weeks after denervation coincides with the time course of functional renal nerve regeneration.6-9 Repeat renal denervations were performed 7 weeks after the initial renal denervation. This second renal denervation procedure again resulted in a decrease of arterial pressure in the 1K1C hypertensive rats to almost normotensive levels and was maintained for approximately 3 to 4 weeks before blood pressure returned to the previous hypertensive level.6 These authors concluded that renal nerves play an important role in the maintenance of 1K1C hypertension.

The present experiments were undertaken to analyze further the importance of the renal nerves in 1K1C hypertension in rats. In a previous experiment we demonstrated that renal denervation repeated at 3-week intervals would produce a sustained decrease of renal norepinephrine content and a maintained functional renal denervation.9 We used this technique of repetitive renal denervations to study the role of renal nerves in both the development and maintenance of 1K1C hypertension.
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Methods
Renal Denervation of One-Kidney, One Clip Rats
The first series of experiments was performed on 26 male Sprague-Dawley rats (Blue Spruce Farms, Altamont, New York) whose average body weight was 267 ± 3.1 g (mean ± se). The rats were anesthetized with pentobarbital sodium (approximately 50 mg/kg) and via a midline abdominal incision were subjected to 1K1C surgery. This consisted of right nephrectomy and constriction of the left renal artery close to its origin from the aorta by a silver clip with an internal diameter of 0.25 mm. This was the full extent of the initial surgical preparation of 14 rats, and they served as the sham renal-denervated 1K1C group (1K1C-sham rats).

As part of the initial surgical preparation, the remaining 12 rats (1K1C-denervated group) underwent renal denervation in addition to the 1K1C surgery described above. The renal nerves to the left kidney were removed by stripping the left renal artery and vein of all obvious nerve and connective tissue, and then painting these blood vessels with 10% phenol in ethanol. A fine brush was used to apply the phenol to the renal vessels to avoid damaging surrounding tissues. During this surgical procedure, the renal denervation was completed prior to placement of the clip on the left renal artery. Successive renal denervations were performed by a similar technique at 3-week intervals in these rats to prevent renal nerve regeneration. Sham surgery, consisting of a midline abdominal incision and visualization of the left renal artery and vein, was also repeated at 3-week intervals in the 14 1K1C-sham rats.

Blood Pressure Determinations
Tail-cuff blood pressure was determined in the conscious rats prior to the initial surgical procedures (Week 0) and then weekly thereafter with an electro-sphygmomanometer (Narco Biosystems, Houston, Texas). The rats were placed in a small animal study unit with a temperature-controlled baseplate (39° C) for approximately 10 minutes prior to the first pressure determination. Each tail-cuff pressure reported is the average of three successive measurements; the tail-cuff pressure was then determined weekly for 8 weeks following the original 1K1C surgery.

Mean arterial pressure (MAP) was measured in the conscious, unrestrained rats during the final week (Week 9) of the experiment (3 weeks after the third renal denervation or sham renal denervation). A polyethylene catheter (PE 90) with a stretched tip was inserted into the lower abdominal aorta via the left femoral artery. The catheter was tunneled subcutaneously to the nape of the neck and filled with sodium heparin (1000 U/ml). The MAP was determined 1 to 2 days after catheter implantation by using Statham low-volume displacement pressure transducers (Statham Laboratories, Hato Rey, Puerto Rico) with a Grass model 7D polygraph (Grass Instruments Company, Quincy, Massachusetts). The mean pressures reported are the average pressures found during approximately the final half of a 1-hour recording period, during which the rats rested on a bed of wood shavings in a Plexiglass cage.

Following MAP determination, each rat was sacrificed, and the left kidney was removed for analysis of renal norepinephrine (NE) content. For this analysis, the kidney was minced and placed in ice-cold 0.1 N perchloric acid. The kidney was then homogenized, the homogenate was centrifuged in a refrigerated centrifuge at 20,000 g for 20 minutes, and the supernatant was stored at −85° C. Liquid chromatography with electrochemical detection (Bioanalytical Systems, West Lafayette, Indiana) was used to determine the renal NE content by the technique of Moyer and Jiang.

Plasma Renin Activity
In a second series of experiments, the effect of renal denervation on the normal transient elevation of plasma renin activity (PRA) during development of 1K1C hypertension was determined. The PRA was measured in seven control rats (both kidneys intact, no clip), in 1K1C-sham rats on Day 2 (n = 9) and Day 7 (n = 8) after 1K1C surgery, and in 1K1C-denervated rats on Day 2 (n = 10) and Day 7 (n = 7) after 1K1C surgery and renal denervation. Arterial catheters were implanted 2 days prior to blood sampling for PRA determination. On the day of PRA measurement, MAP was determined in the conscious rat. The MAP measurement was followed by rapid removal of a blood sample via the arterial catheter, and PRA was measured by radioimmunoassay (Angiotensin I [125I] RIA Kit, New England Nuclear, Boston, Massachusetts).

Renal Denervation of One-Kidney, One Clip Rats with High Sodium Intake
In a third series of experiments, the effect of increased sodium intake on the development of 1K1C hypertension was determined in Sprague-Dawley rats with or without intact renal nerves. Twenty rats underwent 1K1C surgery, and 10 of these rats also underwent simultaneous renal denervation. Drinking water for these rats was replaced with 0.9% saline before the rats recovered from anesthesia. These 20 rats make up two final groups: 10 1K1C-sham-high Na rats and 10 1K1C-denervated-high Na rats.

The tail-cuff pressure was determined in these rats prior to surgery and then every 2 to 4 days for the next 2 weeks. Arterial catheters were implanted 14 days after 1K1C surgery, and MAP was determined in the conscious rats on Day 15. Renal NE content was measured as before.

Statistical Analysis
Tail-cuff pressures of the renal-denervated rats were compared with those of the sham-denervated rats by performing an analysis of variance (ANOVA) for repeated measures with program BMDP2V of the

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BMDP series,11 followed by use of the Bonferroni method for making simultaneous multiple comparisons.12 Two-group comparisons where there were no repeat measurements were performed using standard Student's t tests. All values are means ± se.

Results
Effects of Renal Denervation on the Development and Maintenance of One-Kidney, One Clip Hypertension

The 26 rats used in this series of experiments had an average initial tail-cuff pressure of 115 ± 1.4 mm Hg. The tail-cuff blood pressures prior to surgery (Week 0) and during the next 8 weeks following 1K1C surgery are shown in Figure 1. The average tail-cuff pressure of the 1K1C-sham group increased gradually to a plateau of approximately 175 mm Hg. However, the tail-cuff pressure of the 1K1C-denervated group remained at the control level for about 4 weeks before it began to rise. There was a significant difference (p < 0.05) in the tail-cuff pressures of the 1K1C-sham and 1K1C-denervated groups at Weeks 2 through 5 after the initial surgical preparation. Even though the renal denervation procedure was repeated at Weeks 3 and 6, the tail-cuff pressure of the 1K1C-denervated group rose to the same plateau level as that observed in the 1K1C-sham group. There were no significant differences in tail-cuff pressures between the two groups during Weeks 6 through 8.

MAP measurements made in the conscious rats at Week 9 also indicated no significant difference between the two groups: MAP averaged 196 ± 11.4 mm Hg in the 1K1C-sham group (n = 6) and 194 ± 11.3 mm Hg in the 1K1C-denervated group (n = 8). Theoretically, tail-cuff pressure is a measure of systolic pressure, but in this study it can be seen that the tail-cuff technique gave values less than or equal to MAP. This finding is not unusual and is a result of the fact that the full aortic pressure may not be transmitted to the tail and that the pressure measured by the indirect technique depends on the location of the cuff on the tail and cuff width.13-15

The delay in development of hypertension in the 1K1C-denervated group cannot be explained by effects of the repetitive renal denervation procedures on growth of the rats. The body weights of the two groups of rats increased progressively over the 9 weeks of this study. Average body weight at Week 9 was 407 ± 13.3 g in the 1K1C-sham rats and 434 ± 14.7 g in the 1K1C-denervated group (no significant difference). Also, the eventual increase in blood pressure of the 1K1C-denervated group is not the result of ineffective repeat renal denervations. The average renal NE content in the 1K1C-denervated rats (n = 10) at Week 9 (3 weeks after the third renal denervation) was significantly decreased (p < 0.001) to a level of 8.5 ± 2.1 ng NE/g kidney weight compared with an average of 51.7 ± 9.9 ng NE/g kidney weight in the 1K1C-sham rats (n = 6).

Role of Changes in Plasma Renin Activity on the Delay in Development of One-Kidney, One Clip Hypertension

Changes in PRA following 1K1C surgery were determined in 1K1C-sham and 1K1C-denervated rats. As shown in Table 1, PRA in unoperated control rats averaged 4.33 ± 1.31 ng angiotensin I/ml-hr. Two days after 1K1C surgery, the PRA was significantly increased in both the 1K1C-sham and 1K1C-denervated rats compared with control rats. By Day 7, however, PRA had returned to control levels in both groups. There were no significant differences in PRA between the 1K1C-sham rats and 1K1C-denervated rats at either Day 2 or Day 7 following 1K1C surgery. Also shown in Table 1, the MAP in both groups of rats increased approximately 30 mm Hg by Day 2 after 1K1C surgery. Over the following 5 days there was an additional 17 mm Hg increase in the average MAP of the 1K1C-sham rats. However, the average MAP of the 1K1C-denervated group declined 12 mm Hg during this time period.

Table 1. Effects of Renal Denervation of One-Kidney, One Clip (1K1C) Rats on the Development of Hypertension and Ability to Release Renin

<table>
<thead>
<tr>
<th>Rat group</th>
<th>PRA (ng ANG I/ml-hr)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>4.33 ± 1.31</td>
<td>113 ± 2.0</td>
</tr>
<tr>
<td>1K1C-sham Day 2 (n = 9)</td>
<td>10.6 ± 3.39*</td>
<td>142 ± 5.9*</td>
</tr>
<tr>
<td>1K1C-sham Day 7 (n = 8)</td>
<td>3.31 ± 0.59*</td>
<td>159 ± 4.5*</td>
</tr>
<tr>
<td>1K1C-denervated Day 2 (n = 10)</td>
<td>36.8 ± 14.2*</td>
<td>143 ± 5.6*</td>
</tr>
<tr>
<td>1K1C-denervated Day 7 (n = 7)</td>
<td>2.87 ± 0.98*</td>
<td>131 ± 2.4*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. ANG I = angiotensin I. MAP = mean arterial pressure.
*p < 0.05 compared with control.
Effect of High Sodium Intake on the Development of One-Kidney, One Clip Hypertension

The effects of a high Na intake on the development of 1K1C hypertension were studied in 10 sham renal-denervated rats (1K1C-sham-high Na group) and in 10 renal-denervated rats (1K1C-denervated-high Na group). The only difference between these groups and the rats in the previous experiments is that, following the surgical preparation, 0.9% saline was substituted for the drinking water of these rats.

The tail-cuff pressures during the first 2 weeks after surgery are shown in Figure 2. The control tail-cuff pressures on these rats prior to surgery (Day 0) averaged 114 ± 2.5 mm Hg in the 1K1C-sham-high Na group and 110 ± 1.4 mm Hg in the 1K1C-denervated-high Na group. Following the 1K1C surgery and high sodium intake, the blood pressure of the renal-denervated rats increased over the same time course as that in the rats with intact renal nerves. The MAP measurements in the conscious rats 15 days after the 1K1C surgery also indicated no significant difference between the two groups. At this time the MAP averaged 164 ± 6.4 mm Hg in the 1K1C-sham-high Na rats and 173 ± 4.8 mm Hg in the 1K1C-denervated-high Na rats.

The effectiveness of the renal denervation was verified by renal NE measurements on Day 15 after sham denervation or renal denervation. Renal NE content of the 1K1C-denervated-high Na rats averaged 6.52 ± 1.11 ng NE/g kidney weight, which was significantly less (p < 0.05) than that of the 1K1C-sham-high Na group (48.5 ± 17.0 ng NE/g kidney weight). In addition, daily saline intake measurements in these two groups indicated no differences between the groups. The saline intake gradually increased from approximately 40 to 90 ml/day in both groups during the 2 weeks of high sodium intake. Average daily saline intake was 78 ± 3.1 ml/day in the 1K1C-sham-high Na rats and 78 ± 3.4 ml/day in the 1K1C-denervated-high Na rats.

FIGURE 2. Effects of renal denervation on development of one-kidney, one clip (1K1C) hypertension in rats having high sodium intake.

Discussion

The sympathetic nervous system may play an important role in the development and/or maintenance of many forms of hypertension, including renal hypertension. One mechanism by which overactivity of the sympathetic nervous system might be responsible for a chronic increase in arterial pressure could be increased renal nerve activity. Katholi et al. determined that renal denervation of 1K1C hypertensive rats would reverse the hypertension, but that the decrease in pressure after renal denervation could not be explained by reduced ability to secrete renin or by decreased sodium and water retention. In addition, it has been demonstrated that there is impaired neurogenic control of the renal vasculature in renal hypertensive rats. This evidence would argue against a role of the renal efferent nerves in 1K1C hypertension.

Katholi et al. postulated that a reflex mechanism involving increased afferent renal nerve activity following renal artery clipping could cause an increase in sympathetic nerve activity and, therefore, hypertension. Renal denervation might then reverse 1K1C hypertension by interfering with afferent renal nerve mechanisms. Brody et al. have also proposed that afferent renal nerves may be important in several forms of hypertension, including renal hypertension. They have suggested that neural connections between the kidney and the forebrain could be involved in the pathogenesis of renal hypertension and that anteroven-tral third ventricle (AV3V) lesions may reverse renal hypertension by interfering with this system.

The results of the present study indicate that chronic renal denervation of rats maintained on a normal sodium diet results in a 5- to 6-week delay in development of 1K1C hypertension, but has no effect on the final hypertensive state. Therefore, neither afferent nor efferent renal nerves are necessary for development or maintenance of renal hypertension. Since it has been demonstrated that established 1K1C hypertension can be reversed by renal denervation, we would predict that chronic renal denervation (denervation repeated at 3-week intervals) of rats with established renal hypertension would cause a transient decrease in arterial pressure. However, the arterial pressure would gradually rise back to the original hypertensive level whether renal nerves are allowed to regenerate or not. In addition, the prevention or reversal of renal hypertension by AV3V lesions might be expected to be transient.

Measurement of changes in PRA in the early stages of development of 1K1C hypertension indicated that renal denervation does not interfere with the ability of these rats to release renin after renal artery clipping (Table 1). The renin data indicate a tendency for the PRA to undergo a greater transient increase in the 1K1C-denervated rats than in the 1K1C-sham rats, but there were no statistically significant differences between groups. In addition, the studies of the ability of the rats to release renin provided evidence for an early transient increase in blood pressure immediately after 1K1C surgery, even in renal-denervated rats. This early pressure increase was probably missed in the long-
term study since tail-cuff pressure was only monitored weekly.

A reasonable hypothesis for the delay in development of hypertension after renal denervation might be that denervation could interfere with sodium and fluid retention after clipping the renal artery. However, Katholi et al. found no differences in fluid or sodium balances in rats with established 1K1C hypertension that were sham-operated (hypertension maintained) or renal-denervated (hypertension reversed). These results suggest that accumulation of sodium and water are not involved in the development of 1K1C hypertension. On the other hand, studies in the dog have indicated that development of 1K1C hypertension is associated with sodium and fluid retention, and reversal of this hypertension following unclipping of the renal artery is associated with negative balances of both sodium and water. This effect of unclipping the renal artery was determined in conscious dogs that were instrumented with externally adjustable renal artery occluders. It is likely that measurement of small changes in sodium or water homeostasis over a prolonged period during the development of 1K1C hypertension in the rat would be technically difficult due to the small quantities involved. Also, changes in sodium or fluid balance after renal denervation might be obscured by the effects of anesthesia and surgery.

The effects of placing renal-denervated 1K1C rats on a high sodium intake were studied in an effort to clarify the mechanism of the delay in the development of renal hypertension following ablation of the renal nerves. If renal denervation results in a tendency toward increased renal sodium and water excretion, then development of hypertension might be expected to be delayed. To circumvent the potential technical difficulties associated with determination of sodium and fluid balances in the rat, the 1K1C rats were placed on a high sodium diet during development of hypertension. It was postulated that in the presence of this high sodium intake any interference with the ability of the rats to retain salt and water due to renal denervation would be minimized. The results of that study indicated that 1K1C hypertension develops equally rapidly in rats maintained on a high sodium intake whether the renal nerves are present or not.

In conclusion, the renal nerves are not necessary for the development or maintenance of 1K1C hypertension. Renal denervation can delay the onset of 1K1C hypertension, possibly by delaying the ability of these rats to retain sodium and water. Placing the rats on a high sodium intake will prevent even this delay in the development of renal hypertension in renal-denervated rats.

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