Laboratory Studies

Inability of Dorsal Spinal Rhizotomy to Prevent Renal Wrap Hypertension in Rats

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SUMMARY Renal hypertension has been shown to be prevented and reversed by renal denervation. It has been postulated that the afferent nerves from the kidney are responsible for mediating the hypertensive stimulus that activates the sympathetic nervous system and increases arterial pressure. This study was designed to directly test the hypothesis that the afferent renal nerves are necessary for the development and maintenance of renal hypertension. In the first experiment, dorsal spinal rhizotomies or sham rhizotomies were performed in rats between T9 and L1, through which afferent renal nerves have been shown to traverse. After the one-kidney, one-wrap procedure, the increase in systolic arterial pressure and water intake was similar in the two groups of rats. To determine whether the removal of afferent renal nerves reversed the hypertensive process, animals with established renal hypertension were subjected to dorsal rhizotomy or the sham-rhizotomy procedure. Again, there was no significant effect on systolic arterial pressure and water intake. Although combined dorsal and ventral rhizotomy and subdiaphragmatic vagotomy did not affect the onset of hypertension, spinal transaction at the level of C8 effectively prevented the rise in arterial pressure. Although efferent neural mechanisms contribute to the hypertensive process, these studies suggest that afferent renal nerves are not directly involved in the development and maintenance of one-kidney, one-wrap renal hypertension. (Hypertension 7: 722-728, 1985)

Key Words • one-kidney, one-wrap renal hypertension • renal afferent nerves • sympathetic nervous system

CONSIDERABLE attention recently has been focused on the importance of the renal nerves in the etiology of experimental hypertension. Sectioning of the renal nerves in the abdomen has been reported to delay the development of hypertension in spontaneously hypertensive rats (SHR),1-3 New Zealand genetically hypertensive rats,4 and aortic baroreceptor denervated animals.5 Successive bilateral renal denervation every 3 weeks has prevented 30 to 40% of the expected progressive elevation of arterial pressure in aging SHR.6 There has been some question as to whether the renal nerves are necessary for renal artery stenosis-induced hypertension. Early studies using dogs suggested that renal denervation has no effect on these forms of renal hypertension.7,8 More recent studies in rats have indicated that renal denervation in two-kidney, one clip and one-kidney, one clip, as well as one-kidney, figure-8 wrap Grollman renal hypertension delays the onset of high blood pressure and reverses the severity of the established hypertension.9-15 In fact, complete and irreversible kidney denervation by renal autotransplantation has been shown to delay for at least 9 weeks the onset of one-kidney, figure-8 wrap Grollman hypertension.14 Finally, it has been observed that deoxycorticosterone (DOCA)-salt hypertension also is delayed by renal denervation.15

The mechanisms by which renal denervation delays the development of experimental hypertension remain unknown. Elevated efferent sympathetic nervous system activity has been implicated in the etiology of DOCA-salt hypertension,16 spontaneous hypertension,17 and renal hypertension.9,18 It has been suggested that increased renal sympathetic tone in DOCA-salt hypertension and the SHR facilitates sodium retention and is necessary for the development of hypertension. On the other hand, the importance of the renal afferent nerves in the etiology of two-kidney and one-kidney renal hypertension has been suggested by Brody20 and recently emphasized by Katholi et al.10
Renal catecholamines have been shown to be absent in one-kidney, figure-8 wrap hypertensive rats. Renal denervation in one-kidney, one-clip rats resulted in a decrease in hypothalamic norepinephrine content, returned plasma norepinephrine to control levels, and caused a reduced depressor response to ganglionic blockade. These observations led to the hypothesis that clipping the renal artery or wrapping the kidney caused an increase in renal afferent nerve signals that triggered an increase in peripheral sympathetic efferent activity and thus resulted in renal hypertension. As renal denervation severed both renal afferent and efferent fibers, no direct experimental evidence has indicated whether the renal hypertensive process involved renal afferent nerves, efferent nerves, or both.

It has been reported that the major projections of renal afferent fibers to the spinal cord are directed ipsilaterally through the ninth thoracic to the first lumbar spinal dorsal roots in rats by using the methods of horseradish peroxidase and the fluorescent dye true blue. Therefore, renal afferent denervation can be approached by selective section of these dorsal spinal roots in rats. In addition, efferent nerves have in some instances been shown to traverse through the ventral roots. For this reason, the present study employed dorsal and combined dorsal and ventral rhizotomy to determine whether renal afferent nerves contribute to the development and maintenance of one-kidney, one-wrap renal hypertension; and whether renal afferent denervation resulted in a functional decrease in the contribution of the sympathetic nervous system to arterial pressure.

**Methods**

Male Sprague-Dawley rats weighing 290 to 330 g were individually housed in wire cages in a room with constant temperature (approximately 22 °C) and a 12-hour light, 12-hour dark cycle. All animals were maintained on rat chow and tap water ad libitum.

**Spinal Rhizotomy**

Dorsal spinal rhizotomy was performed while the rats were under sodium pentobarbital anesthesia (50 mg/kg i.p.). The vertebrae attached to the 13 ribs were used for anatomical orientation, and a midline, dorsal longitudinal incision was made over the spine. The paraspinal musculature was scraped from vertebral laminae, and muscles attached to the left transverse processes from T9 to L1 were sectioned. Then, the left dorsal roots from T9 to L1, inclusive, were removed with small bone scissors. Under a dissecting microscope, the dura was opened directly over the left transverse processes from T9 to L1 were sectioned. The left vertebral laminae from T9 to L1, inclusive, were removed with small bone scissors. Under a dissecting microscope, the dura was opened directly over the left dorsal root entry zone from T9 to L1 and the left dorsal roots were cut near their zone of entry by angled microscissors. Overlying muscles and connective tissue were separated, and the skin was closed with 4-0 silk. The sham-operated rats underwent the same operation, except that the left dorsal roots from T9 to L1 were not sectioned. The dorsal and ventral rhizotomized rats received the same operation, but ventral roots from T9 to L1 were also severed. All spinal rhizotomized and sham-operated rats were allowed at least 10 days to recover from the operation.

**Spinal Transection**

Rats for spinal transection were anesthetized with chloral hydrate (1 mg/kg). An incision was made from the base of the skull to the level of T4. The prominent second thoracic spinous process of the second thoracic vertebrae was used for anatomical orientation. The major strap muscles were separated with forceps, and the paraspinal musculature was scraped from the vertebral laminae from C6 to T1. The vertebral laminae at C7 was removed with small bone scissors, and the dura at C8 was exposed and removed. The spinal cord at C8 was transected with small scissors.

**Arterial and Venous Catheterization**

Each animal was prepared with femoral artery and vein catheters for the direct measurement of arterial pressure and drug injection. While the rats were anesthetized with methoxyflurane, a heparin-filled Tygon catheter with a Teflon tip was inserted into the lower abdominal aorta through the left femoral artery. Polyethylene tubing (PE-50) was placed in the left femoral vein. All catheters were exteriorized in the dorsal mid-cervical region of the neck. One day later, arterial pressure was monitored in the conscious rats with an Ailtech (MS20) pressure transducer (Ailtech Associates, Inc., Deerfield, IL, USA) with a Beckman Dynograph Recorder (Beckman Instruments, Inc., Fullerton, CA, USA). Heart rate was obtained from the arterial pressure pulse with a cardiotachometer. A 30-minute to 60-minute baseline mean arterial pressure and heart rate were obtained for each rat. To assess the contribution of the sympathetic nervous system to arterial pressure in one-kidney Grollman hypertension, hexamethonium bromide (25 mg/kg i.v.) and atropine sulfate (0.4 mg/kg i.v.) were sequentially administered in each rat. Arterial pressure and heart rate were recorded for 10 minutes after each injection.

**Verification of Renal Afferent Denervation**

The spinal cords of all animals that underwent dorsal rhizotomy, combined dorsal and ventral rhizotomy, and spinal section were visually inspected. Only the animals in which complete rhizotomy between T9 and L1 or spinal section was observed were included in the data.

To further assess renal afferent denervation, a midline laparotomy was performed with the rats under sodium pentobarbital anesthesia. A miniature flow probe was placed on the superior mesenteric artery to record the blood flow with a pulsed Doppler flowmeter. Arterial pressure was monitored through the femoral artery. The left renal nerves were dissected from surrounding tissue and severed distally. The central end of the renal nerves was placed on a bipolar stimulating electrode and stimulated with a frequency of 16 Hz at 8 V. In sham-operated rats (n = 3), the responses to electrical stimulation of the central end.
were characterized by increased vascular resistance (+11.0 ± 1.8%) of the superior mesenteric artery. The response to stimulation of the left renal nerves in dorsal rhizotomized rats (n = 4) was not observed (−0.5 ± 2.3%).

**Experiment 1**
To determine the effect of dorsal rhizotomy on the onset and maintenance of one-kidney, one-wrap renal hypertension, six dorsal rhizotomized rats and six sham-rhizotomized animals were studied for 4 weeks. During the first week, body weight and systolic arterial pressure (by the nonheating, photoelectric cell method) were measured three times for average control values. Daily water intake measurements were taken to determine baseline fluid intake. At the end of the week, all the animals were subjected to one-kidney, figure-8 wrap procedure described by Grollman. Systolic arterial pressure and body weight, measured 3 times each week, and daily determinations of water intake were continued over the next 4 weeks.

At the end of the study, each animal was catheterized for a direct measurement of arterial pressure and heart rate. In addition, the functional sympathetic component contributing to blood pressure was assessed by administering atropine sulfate (0.4 mg/kg i.v.) and hexamethonium bromide (25 mg/kg i.v.).

**Experiment 2**
Renal afferent denervation was performed in animals with established one-kidney, one-wrap renal hypertension to determine its importance in the maintenance of elevated arterial pressure. After measurement of control systolic arterial pressure, body weight, and water intake, all animals were subjected to the one-kidney, one-wrap hypertensive procedure. Three weeks later, when systolic arterial pressure had reached a plateau, four animals underwent dorsal rhizotomy and four animals underwent sham rhizotomy. Systolic pressure, body weight, and water intake measurements were continued for 2 weeks after operation. The conscious animals were then catheterized for the determination of resting arterial pressure and heart rate. The functional sympathetic nervous system contribution to arterial pressure was assessed with the use of ganglionic blockade.

**Experiment 3**
To determine the role of afferent nerves passing through the ventral spinal roots and the sympathetic nervous system in the early phase of one-kidney, one-wrap renal hypertension, four groups of animals were studied during the first week of renal hypertension. The first group of rats (n = 9) was sham-rhizotomized; the second group of animals (n = 9) received dorsal rhizotomy, the third group of rats (n = 6) underwent both ventral and dorsal rhizotomy, and the final group of animals underwent spinal transection at C8. One week postwrap, all animals were catheterized and direct arterial pressure and heart rate measurements were made while the animals were conscious.

**Experiment 4**
To eliminate the possibility of afferent nerves from the kidney traversing the vagus to activate the hypertensive process, subdiaphragmatic vagotomy (n = 5) or sham vagotomy (n = 5) was performed. After an initial weight loss the animals resumed normal eating behavior, and body weight returned to preoperative levels. Both groups of rats were then subjected to the renal wrap procedure. Systolic arterial pressure was followed for 14 days. At the end of that period, the animals were prepared with femoral artery and vein catheters for direct recording of blood pressure while conscious.

**Statistics**
Comparisons of the two groups of animals in Experiments 1, 2, and 4 were made by two-way analysis of variance for repeated measures. Significant changes within each group were determined by one-way analysis of variance for repeated measures and multiple range test using the mean square error from the overall two-way analysis of variance. Differences between groups were detected with a modified t test. Differences in resting direct arterial pressures between two groups were analyzed by Student’s t test. Arterial pressure differences in Experiment 3 were analyzed by one-way analysis of variance. Significance was taken as p < 0.05.

**Results**

**Experiment 1**
The effects of dorsal rhizotomy on the development and maintenance of one-kidney, one-wrap renal hypertension are shown in Figure 1. The renal wrap procedure resulted in a progressive, significant rise in systolic arterial pressure in both the dorsal rhizotomized and sham-rhizotomized animals (p < 0.05). Systolic arterial pressure reached a plateau of 187 ± 7 mm Hg in the rhizotomized animals and 179 ± 7 mm Hg in the sham-rhizotomized animals by Day 11. At no time during the 4-week course of hypertension were there any differences in systolic arterial pressure between the two groups of rats. Changes in water intake and body weight were also similar in the two groups of animals through the course of hypertension (see Figure 1).

Resting mean arterial pressure obtained from indwelling arterial catheters was also similar in both groups of animals. The mean arterial pressure of the sham-rhizotomized animals was 141 ± 4 mm Hg, while the mean arterial pressure of the dorsal rhizotomized animals was 148 ± 7 mm Hg. Baseline heart rate values were 387 ± 11 beats/minute and 407 ± 22 beats/minute in the sham-rhizotomized and dorsal rhizotomized rats respectively. As shown in Figure 2, total ganglionic blockade produced an equivalent decrease in mean arterial pressure in both groups of rats. Although the sympathetic nervous system contribution to arterial pressure was similar in the two groups of rats, the increased arterial pressure may have been maintained in the dorsal rhizotomized animals by an-
other pressor system. As the vasopressin antagonist has been shown to lower arterial pressure in renal wrap hypertensive rats, the vascular antagonist d(CH)2Tyr(Me)AVP was administered after ganglionic blockade. Equivalent decreases in arterial pressure were observed in the two groups of rats (−19.8 ± 2.0 mm Hg in sham-operated rats and −22.0 ± 5.1 mm Hg in renal wrapped rats).

**Experiment 2**

Figure 3 shows the effect of renal hypertension and sham rhizotomy or dorsal rhizotomy on systolic arterial pressure, water intake, and body weight. After the induction of renal hypertension, systolic arterial pressure in the two groups of rats showed a progressive rise that approached a plateau of 204 mm Hg in both groups of animals. Subsequent to sham rhizotomy or dorsal rhizotomy, the arterial pressure of both groups of rats fell during the first 2 days after operation. Arterial pressure then increased in both groups of rats. There were no differences in systolic arterial pressure between the two groups of animals at any time after the rhizotomy procedure. In addition, there were no differences in water intake or body weight between the two groups of rats throughout the study.

Direct measurement of arterial pressure and heart rate showed that there was no significant difference in baseline mean arterial pressure in the two groups of animals (159 ± 8 mm Hg in the dorsal rhizotomized

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**Figure 1.** The effect of previous dorsal rhizotomy and sham rhizotomy is shown for the time course of systolic arterial pressure, water intake, and body weight following the one-kidney, one-wrap procedure. Significant increases in arterial pressure and water intake occurred in both groups of rats when compared with the pre-wrap control period (p < 0.05). There were no differences between the dorsal rhizotomized and sham-rhizotomized rats.

**Figure 2.** The resting mean arterial pressure (MAP) and the change in mean arterial pressure produced by ganglionic blockade with hexamethonium bromide and atropine sulfate are shown for sham-rhizotomized and dorsal rhizotomized rats. There was no significant difference in the resting blood pressure or the ganglionic blockade response in the two groups of rats.

**Figure 3.** Changes in systolic arterial pressure, water intake, and body weight resulting from the one-kidney, one-wrap procedure are shown before and after dorsal rhizotomy or sham rhizotomy. Arterial pressure and water intake remained significantly elevated following the rhizotomy procedure (p < 0.05).
group and 169 ± 13 mm Hg in the sham-rhizotomized animals). Heart rates were also not different between the two groups of rats (394 ± 18 beats/min in rhizotomized animals and 420 ± 15 beats/min in the sham-operated group). Total ganglionic blockade (Figure 4) produced a significant decrease in mean arterial pressure in both groups of animals (49 ± 10 mm Hg in the rhizotomized rats and 52 ± 4 mm Hg in the sham-rhizotomized animals, p < 0.05), but there was no significant difference in the decrease in mean arterial pressure between these two groups of animals.

Experiment 3

The effect of dorsal rhizotomy, dorsal and ventral rhizotomy, and spinal cord transection at C8 is shown in Figure 5. The sham-rhizotomized, renal wrapped animals had a resting mean arterial pressure of 139 ± 4 mm Hg. The mean arterial pressures for the dorsal rhizotomized animals and dorsal and ventral rhizotomized rats were 141 ± 7 mm Hg and 159 ± 9 mm Hg respectively. Animals with spinal cord transection at C8 had a resting mean arterial pressure of 117 ± 3 mm Hg.

Experiment 4

As shown in Figure 6, there were no differences in systolic arterial pressure before one-kidney, figure-8 renal wrap in vagotomized or sham-vagotomized rats. Following the renal wrap procedure, arterial pressure consistently increased in both groups of animals during the 2-week period. Direct measurements of arterial pressure revealed no differences between the vagotomized (152.6 ± 10.8 mm Hg) and the sham-vagoto-
mized (153.6 ± 10.3 mm Hg) rats; however, the vagotomized rats experienced a blunted increase in water intake over the 2-week period compared with the sham-operated rats. In addition, there was a significant weight loss in the vagotomized rats during the post-wrap period (p < 0.05).

Discussion

Recent studies have demonstrated that peripheral denervation of a kidney by autotransplantation (Haywood JR, Patel NP, Corry RD, unpublished observations, 1984) or renal nerve section combined with phenol9"13 protects an animal against the severity of renal hypertension. The mechanism through which denervation of the clipped or wrapped kidney prevents the onset of high blood pressure is unclear. One line of reasoning suggested that as no excessive sodium loss occurred and no decrease in renin-angiotensin system activity could be detected in renal denervated rats, efferent neural mechanisms probably were not contributing to the hypertensive process. This concept was reinforced by the observation of Fink and Brody,21 who demonstrated a reduced renal catecholamine content in the renal wrapped kidney, which indicated the absence of a functional sympathetic tone to the kidney. For these reasons, investigators suggested that afferent nerves from the kidney provided the triggering mechanism for the rise in arterial pressure in renal hypertension.9, 20

Activation of mechanoreceptors or stimulation of chemoreceptors as a result of impaired renal blood flow or altered chemical composition of the urine has been shown to increase afferent renal nerve activity.30, 31 Electrical stimulation of the afferent renal nerves has been shown to result in an increase in renal and mesenteric vascular resistance that is mediated by an activation of the sympathetic nervous system.14 Thus, it was postulated that renal wrapping or reduced renal blood flow activated the afferent nerves from the kidney and caused an elevation in arterial pressure.20 This hypothesis is consistent with observations demonstrating an enhanced functional contribution of the sympathetic nervous system in one-kidney renal hypertension.19

Studies using anterograde tracing methods have shown that the majority of afferent nerves from the left kidney enter the spinal cord at the level of T9 to L1.22-25 These observations were confirmed physiologically in this study when removal of these dorsal roots effectively abolished the mesenteric vasoconstriction resulting from electrical afferent nerve stimulation.

The present experiments directly tested the contribution of afferent nerves from the kidney in one-kidney renal hypertension. Left dorsal roots between T9 and L1 were sectioned to selectively remove afferent input to the central nervous system from the kidney. This procedure failed to alter the onset of the rise in arterial pressure or to reverse established hypertension in the one-kidney Grollman wrapped rats. The changes in water intake and body weight that normally accompany the rise in arterial pressure also were unaffected by dorsal rhizotomy. The inability of the dorsal rhizotomy to affect the course of hypertension suggests that the renal afferent nerves have little direct influence on the hypertensive process.

Other possible pathways through which renal afferent nerves could enter the central nervous system also were tested by selective denervation. It has been shown that some afferent fibers may traverse the ventral roots of the spinal cord.19 To determine the possible involvement of these fibers in the hypertensive process, combined dorsal and ventral rhizotomies were performed. This procedure also failed to influence the onset of hypertension. As afferent nerves from the kidney may pass through the vagus,32 the effect of subdiaphragmatic vagotomy was also tested. Although differences in water intake and body weight were observed, denervation of the vagus was ineffective in altering the rise in arterial pressure during the 2-week period.

Some remaining afferent fibers passing through T8 and L2 may be sustaining the hypertension; however, denervation of the majority of the fibers would be expected to cause a greater reduction in arterial pressure in animals with established hypertension. An exaggerated hypertensive response was not observed after dorsal rhizotomy.

Section of the spinal cord at C8 was effective in preventing the rise in arterial pressure in one-kidney, one-wrap renal hypertension. This finding is consistent with the increased functional contribution of the sympathetic nervous system that has been observed in the early and chronic stages of this model of hypertension.19 In addition, the dorsal rhizotomy procedure did not affect the sympathetic nervous system contribution as ganglionic blockade responses were similar in dorsal rhizotomized and sham-operated rats. This observation suggests that the mechanism of renal hypertension is not altered by dorsal rhizotomy.

In summary, the removal of renal afferent nerves did not prevent, reverse, or affect the mechanism mediating one-kidney, one-wrap renal hypertension. Although nerves from the kidney may be entering the central nervous system through dorsal roots other than T9 to L1, the results of this study strongly suggest that afferent renal nerves alone are not responsible for the development of this kind of hypertension. It is possible, however, that these nerves interact with other central nervous system influences, either neural or humoral, to modulate the hypertensive process.

Acknowledgments

The authors thank Ms. Janet Wall for her excellent secretarial assistance.

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Hypertension. 1985;7:722-728
doi: 10.1161/01.HYP.7.5.722

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
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