Importance of the Renal Nerves in the Pathogenesis of Experimental Hypertension

SHERRY R. WINTERNITZ, M.D., AND SUZANNE OPARIL, M.D.

SUMMARY Anatomical studies have demonstrated sympathetic innervation of the renal arterioles, juxtaglomerular apparatus, and renal tubules. Physiologic studies of the effects of the renal efferent nerves on renin release and renal sodium handling indicate that they play an important role in body fluid homeostasis and cardiovascular regulation. In addition, evidence is accumulating that stimulation of intrarenal mechanoreceptors and chemoreceptors causes an increase in renal afferent nerve activity and that alterations in renal afferent nerve traffic are, in turn, associated with changes in blood pressure and in vasoconstrictor tone in the contralateral kidney. Further, recent studies have demonstrated functionally significant connections between renal afferent nerves and the central nervous system. Interruption of the renal sympathetic nerves has been shown to prevent or attenuate hypertension in a number of animal models, suggesting that the renal nerves have an important role in the pathogenesis of experimental hypertension. In the spontaneously hypertensive rat of the Okamoto strain (SHR) and the DOCA-NaCl rat, the delay in the development of hypertension produced by renal denervation is due in part to increased sodium excretion thought to be secondary to interruption of the renal efferent nerves. In contrast, in one-kidney, one clip and two-kidney, one clip Goldblatt hypertension in the rat and coarctation hypertension in the dog, the depressor effect of renal denervation is unrelated to changes in urinary sodium excretion or plasma renin activity. In these models the attenuation of hypertension following renal denervation appears to be secondary to a decrease in peripheral sympathetic activity. Evidence in the one-kidney model suggests that interruption of the renal afferent nerves lower blood pressure via an effect on central noradrenergic mechanisms.

KEY WORDS • hypertension • renal sympathetic nerves • renal denervation

THE renal nerves, including both renal efferent sympathetic nerves, which influence renal sodium excretion and renin release, and the renal afferent nerves, which carry signals from mechanoreceptors and chemoreceptors in the kidney, are involved in cardiovascular homeostasis. It has recently been shown that renal denervation lowers blood pressure and/or prevents the development of hypertension in a number of experimental models. In the current review we discuss anatomic and physiologic studies of the renal nerves which form the basis for the concept that renal neural mechanisms are important in the pathogenesis of hypertension.

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Supported by grants from the American Heart Association, Alabama Affiliate, and the National Heart, Lung and Blood Institute (HL 25451). Dr. Winternitz is the recipient of a National Heart, Lung, and Blood Institute Clinical Investigator Award (HL 00707-02).

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Anatomical and Functional Studies of the Renal Efferent Nerves

A number of investigators, including Bradford and Mitchell, have described the innervation of the renal arterioles and have demonstrated that the primary efferent neural control of the renal vasculature is via the sympathetic nervous system. More recently innervation of the juxtaglomerular apparatus has been demonstrated, and Barajas and Barajas and Muller, using both electron microscopic and histochemical techniques, have provided evidence for direct innervation of the renal tubules in monkey and rat kidneys. DiBona has demonstrated similar evidence for tubular innervation in the dog.

The renal nerves have been shown to control sodium excretion both by an effect on arteriolar vasoconstriction, resulting in a change in intrarenal hemodynamics, and by a direct effect on renal tubular sodium reabsorption. Bernard in 1859 was the first to demonstrate that unilateral sectioning of the splanchnic nerve in the anesthetized dog resulted in increased urine volume from the affected kidney. A number of subsequent studies have shown that renal denervation in the anesthetized animal results in diuresis and
natriuresis. Bello-Reuss et al. studied the effects of acute unilateral denervation on renal sodium handling in the anesthetized rat. In that study, denervation resulted in an increase in urine volume and urinary sodium excretion in the absence of alterations in renal blood flow or glomerular filtration rate. Based on these observations the authors concluded that the diuresis and natriuresis seen following denervation resulted from a decrease in sodium and water reabsorption in the proximal tubule. Additional support for this conclusion comes from the studies of DiBona. demonstrating that renal sympathetic nerve stimulation results in enhanced tubular reabsorption of sodium in the dog.

An additional mechanism whereby the renal nerves may influence cardiovascular homeostasis is by influencing the activity of the renin angiotensin system. In 1965, Vander found that stimulation of the renal nerves resulted in an increase in renin release from the kidney. Other investigators have demonstrated that reflex stimulation of the sympathetic nervous system via a number of mechanisms enhances renin secretion. In addition, Thanes and DiBona have shown that the renal nerves modulate the release of renin in response to non-neural mechanisms such as suprarenal aortic constriction.

**Studies on the Importance of the Renal Efferent Nerves in the Pathogenesis of Experimental Hypertension**

Perhaps the earliest demonstration that the renal nerves may be important in the control of systemic arterial pressure came in 1945 when Kotke et al. showed that chronic renal artery-nerve stimulation resulted in sustained hypertension in dogs. Interpretation of these data is somewhat clouded by the autopsy finding in one of the dogs of renal hemorrhage and nearly complete occlusion of the renal arteries by thrombus formation in the region of the electrodes. Thus, renal ischemia may have contributed to the pathogenesis of the observed hypertension. The potential importance of the renal nerves in the pathogenesis of hypertension was further elucidated in the study of Katholi et al., which showed that chronic intrarenal norepinephrine infusion in conscious dogs produces sustained hypertension. Intrarenal norepinephrine infusion resulted in both a positive sodium balance and an increase in plasma renin activity. However, the hypertension did not appear to be renin-dependent, as blockade of the renin-angiotensin system with the angiotensin II antagonist, saralasin, did not result in a significant decrease in blood pressure. Unlike norepinephrine infusion, chronic infusion of norepinephrine into the vena cava did not result in hypertension despite a similar increase in circulating norepinephrine. Based on this observation, the authors suggested that selective increases in renal sympathetic activity are required to maintain the hypertension. This hypothesis is in agreement with that of Guyton, who proposed that a generalized increase in peripheral resistance producing an increase in blood pressure would result in a pressure natriuresis and a return of arterial pressure to normal levels.

Based on the preceding observations, it is reasonable to postulate that in experimental models of hypertension in which there is evidence of increased sympathetic nervous system activity, increased renal sympathetic nerve activity, in particular, may play a role in the pathogenesis of hypertension. In support of this hypothesis, recent studies in our laboratories suggest that in both the spontaneously hypertensive rat of the Okamoto strain (SHR) and the DOCA-NaCl model increased renal efferent nerve activity contributes to the development of hypertension by causing enhanced urinary sodium retention.

A number of studies indicate that the sympathetic nervous system is important in the pathogenesis of hypertension in the SHR. Concentrations of catecholamines and catecholamine synthesizing enzymes in individual brain-stem and hypothalamic nuclei of SHR have been shown to differ from those of control Wistar-Kyoto (WKY) rats, 30-33 and treatment with both central and peripheral sympatholytic agents has been shown to delay and attenuate the development of hypertension in the SHR. In addition, Judy and colleagues, employing direct measurements of pre- and postganglionic sympathetic nerve activity, have shown nerve traffic in SHR to be increased in comparison to age-matched WKY controls. In agreement with their findings, Thoren and Ricksten found efferent renal sympathetic nerve activity of SHR to be twice that of control WKY. Other investigators, however, have failed to confirm these findings. For example, Francisco et al. found no differences in efferent renal nerve activity between SHR and WKY. Touw et al. found that the elevated vascular resistance observed in SHR did not appear to be secondary to an increase in neurally mediated vasoconstrictor tone. The apparent discrepancies between studies have been attributed to methodological differences such as mode of anesthesia, methods of processing nerve signals or different sources of animals, but have not been fully explained.

Several investigators have shown that renal denervation delays the development of hypertension in young SHR. A preliminary report from one laboratory indicates that in SHR repeat denervations at 3-week intervals result in a chronic attenuation of hypertension. In studies performed in our laboratories, 7-week-old (early hypertensive) and 18-week-old (established hypertensive) male SHRs were subjected to bilateral renal denervation. The subsequent course of development of hypertension and of sodium excretion was monitored and compared with sham-operated controls. In 7-week-old animals, renal denervation delayed the onset and slowed the rate of development of hypertension. These alterations were associated with a significantly greater fractional excretion of sodium (percentage of sodium intake excreted) during the first 3 weeks after denervation compared to sham-operated controls (fig. 1). Blood pressure 2 weeks after surgery was 169 ± 3.5 (sham) 150 ± 2.4 mm Hg (denervated).
Corresponding values for mean weekly fractional excretion of sodium (FENa) in the two groups. P values refer to comparisons between groups using Student's t test for unpaired data.

Figure 1. Top Panel: Systolic blood pressures of renal denervated and sham-operated spontaneously hypertensive rats (SHR). The arrow indicates time of operation. Bottom Panel: Corresponding values for mean weekly fractional excretion of sodium (FENa) in the two groups. P values refer to comparisons between groups using Student's t test for unpaired data.

(p < 0.001), corresponding to fractional sodium excretions of 65% ± 1.3% (sham) vs 80% ± 2.3% (denervated) (p < 0.001). By the fifth week after surgery, at which time the renal norepinephrine content of denervated animals had increased, suggesting reinnervation, blood pressures in the two groups converged (sham, 199 ± 6.5 mm Hg vs denervated, 180 ± 3.5 mm Hg, NS) and there was no difference in sodium excretion (sham 77% ± 2.5% vs denervated 79% ± 2.3%). In addition, measurements of renal norepinephrine content of animals sacrificed at 1, 3, and 6 weeks following denervation demonstrated a highly significant positive correlation between absolute change in blood pressure (blood pressure at sacrifice — control blood pressure) and renal norepinephrine content. Plasma and kidney renin activity of denervated animals did not differ significantly from that of sham-operated controls. In 18-week-old rats, renal denervation did not alter blood pressure or urinary sodium excretion. These results indicate that the renal sympathetic nerves contribute to the development of hypertension in the SHR by causing increased urinary sodium retention.

In a similar study by Katholi et al., renal denervation was carried out in uninephrectomized 5-week-old Sprague-Dawley rats 1 week before beginning DOCA-NaCl treatment. Consistent with findings in the SHR, renal denervation resulted in a delay in the development of hypertension in DOCA-NaCl treated animals. In addition, during the first 2 weeks of DOCA-NaCl treatment, urinary sodium excretion of denervated animals was significantly greater than that of sham-operated controls.

In summary, these studies indicate that, in a genetic model, the SHR, and in a sodium-dependent model, the DOCA-NaCl rat, the renal efferent nerves may contribute importantly to the development of hypertension via an effect on urinary sodium excretion. Two observations deserve mention in connection with these studies. First, it should be noted that these studies do not exclude the possibility that the blood pressure-lowering effect of renal denervation in these models may be due, in part, to interruption of the renal afferent nerves. This is important in view of data from a number of laboratories suggesting that the renal afferent nerves play a role in the regulation of arterial pressure. Second, experiments recently completed in our laboratory indicate that high sodium intake in the SHR may affect both peripheral and central noradrenergic activity. In those studies, the feeding of high sodium diets to young SHR resulted in an exacerbation of hypertension accompanied by evidence of increased plasma norepinephrine levels, an exaggerated depressor response to ganglionic blockade with hexamethonium, and an increase in the norepinephrine content of the anterior and dorsomedial hypothalamic nuclei. These data are compatible with the hypothesis that alterations in sodium intake modulate the severity of hypertension in SHR by altering the level of activity of noradrenergic systems.

Studies of the Importance of the Renal Afferent Nerves in Cardiovascular Regulation

Several lines of evidence suggest that the renal afferent nerves are involved in cardiovascular regulation. Both mechanoreceptors and chemoreceptors have been demonstrated in the kidney of several species, including rat, cat, and dog. Further, a variety of stimuli have been shown to result in alterations in afferent renal nerve activity (fig. 2). Ueda et al., studying anesthetized dogs, showed that increases in intrarenal pressure produced by compression of the kidney, renal vein occlusion, or elevation of perfusion pressure increased the action potentials of the afferent renal nerves. Niijima observed a similar increase in afferent renal nerve activity in the rabbit following an increase in arterial perfusion pressure. More recently, in extensive studies Recordati et al. showed that stim-
ulation of renal chemoreceptors results in increases in renal afferent nerve activity. These investigators demonstrated two populations of chemoreceptors in the rat kidney, both of which are sensitive to renal ischemia and one of which senses changes in the ionic composition of the pelvic urine.

Although the functional significance of increased renal afferent nerve activity remains uncertain, the findings of several investigators support the view that the renal afferent nerves may influence cardiovascular homeostasis. Electrical stimulation of the renal afferent nerves has been shown to produce a change in systemic arterial pressure, although there is disagreement among investigators as to whether this response is pressor or depressor. The apparent discrepancies in findings among laboratories have been attributed to differences in anesthesia. Stimulation of the renal afferents of one kidney has been shown to produce a reflex increase in efferent nerve activity and arteriolar vasoconstriction in the opposite kidney. The finding that this response is abolished by spinal cord sectioning suggests that this reno-renal reflex is mediated via supraspinal neurons. A number of studies have demonstrated connections between the renal afferent nerves and the central nervous system. Fernandez et al. observed that renal denervation in the rat resulted in an increase in the norepinephrine content of whole hypothalamus without a significant change in blood pressure, suggesting that the renal nerves may modulate hypothalamic noradrenergic activity. In addition, Brody and colleagues found that stimulation of the renal afferent nerves in the rat resulted in alterations in resistance in hindlimb, mesenteric and renal vascular beds, and that this response was abolished by lesioning the periventricular tissue surrounding that anteroventral third ventricle, the AV3V region, indicating that there is a feedback loop between the renal afferents and the anterior hypothalamus.

More direct evidence is provided by the studies of Calaresu and Cirriello, who measured the electrical activity of spontaneously firing single units in the medulla and hypothalamus during stimulation of the afferent renal nerves of anesthetized cats. These investigators found responsive units in a number of areas, including the lateral tegmental field, the area of the paramedian reticular nucleus and the dorsal vagal complex of the medulla, the lateral hypothalamic area, the lateral preoptic area, and the paraventricular nucleus of the hypothalamus. Many of the regions where responsive units were detected are thought to be involved in body fluid regulation and cardiovascular homeostasis. The additional finding of this study, that most of the areas responsive to afferent renal nerve stimulation also responded to carotid sinus nerve stimulation, provides further evidence for a role of the renal afferent nerves in cardiovascular regulation.

The Role of the Renal Afferent Nerves in the Pathogenesis of Experimental Hypertension

Recently completed studies of the effects of renal denervation in the one-kidney, one clip Goldblatt model of experimental hypertension (1K1C) suggest that the renal afferent nerves play a role in the pathogenesis of this form of experimental hypertension. Plasma norepinephrine, used as an index of peripheral sympathetic activity, has been found to be elevated in 1K1C. In addition, Eide et al. found increased tyrosine hydroxylase activity and norepinephrine content in the hypothalamus of 1K1C 2 weeks after renal artery clipping, suggesting that central noradrenergic activity is altered in this model. Central sympathectomy interventions, including treatment with intracisternal 6-hydroxydopamine or lesioning of the posterior hypothalamus, have been shown to prevent or attenuate the hypertension in 1K1C.

To study the importance of the renal sympathetic nerves in the maintenance of hypertension in 1K1C, Katholi et al. examined the effects of renal denervation or sham operation performed 2 weeks after clipping the left renal artery in uninephrectomized rats. Systolic blood pressure increased from 125 ± 3 mm Hg to a stable level of 185 ± 7 mm Hg (p < 0.001) at 2 weeks after clipping. Renal denervation (n = 13) resulted in a decrease in blood pressure (137 ± 7 mm Hg; p < 0.01) with no change (186 ± 8 mm Hg; NS) seen after sham operation (n = 14). There was no difference in mean daily water intake (sham 32.7 ± 0.7 vs denervated 32.5 ± 0.8 ml), mean daily sodium intake (sham 2.16 ± 0.02 vs denervated 2.13 ± 0.02 mEq), or mean fractional urinary sodium excretion (sham 89 ± 5 vs denervated 88 ± 5%) during the 2 weeks postoperation. Plasma renin activity (sham 6.32 ± 1.9 vs denervated 6.31 ± 1.8 ng/ml/hr) and creatinine clearance (sham 1.30 ± 0.10 vs denervated 1.15 ± 0.24 ml/min) were not significantly different at sacrifice (2 weeks postoperation). Six of the renal denervated rats were followed for 11 weeks after surgery. Blood pressure rose again to hypertensive levels (187 ± 8 mm Hg) by 5 weeks after renal denervation. Repeat renal denervation resulted in a significant de-
crease in blood pressure ($p < 0.001$) to 142 ± 8 mm Hg. The data indicate that the renal nerves are necessary for the maintenance of IKIC in the rat and that this effect is not mediated by renin or alterations of sodium intake or excretion, water intake, or renal function, suggesting that the effects of renal denervation in IKIC are not due to an interruption of renal efferent nerves.

Subsequent studies were undertaken to determine whether the depressor effects of renal denervation in this model were dependent on interruption of renal afferent nerves. In initial experiments IKIC rats were subjected to renal denervation or sham operation 2 weeks after clipping. Uninephrectomized age- and sex-matched rats were used as controls. Renal denervation resulted in a significant decrease in blood pressure from 200 ± 7 to 150 ± 6 mm Hg. After operation, plasma norepinephrine and mean blood pressure before and after ganglionic blockade were determined in conscious unrestrained animals. In agreement with the findings of other investigators, Katholi et al. found that plasma norepinephrine was significantly higher in hypertensive sham-operated rats than in control uninephrectomized animals (422 ± 42 pg/ml, sham vs 282 ± 25 pg/ml, control, $p < 0.01$). Renal denervation resulted in a decrease in plasma norepinephrine to normal levels (273 ± 22 pg/ml). Ganglionic blockade in sham-operated animals resulted in a significantly greater decrease in blood pressure than occurred in renal-denervated or control animals. These data indicate that the fall in blood pressure following renal denervation in IKIC is, in part, secondary to a decrease in peripheral sympathetic activity.

To examine the hypothesis that interruption of the renal afferent nerves results in a decrease in central and thereby peripheral sympathetic activity, experiments were then undertaken to study the effects of renal denervation on central norepinephrine stores. Renal denervation of sham-operated animals resulted in a significantly greater decrease in blood pressure than occurred in renal-denervated or control animals. These data indicate that the fall in blood pressure following renal denervation in IKIC is, in part, secondary to a decrease in peripheral sympathetic activity.

<table>
<thead>
<tr>
<th>Table 1. The Effects of Renal Denervation on Hypothalamic Norepinephrine and Dopamine Content in the One-Kidney, One Clip Goldblatt Hypertensive Rat</th>
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<tr>
<td><strong>Pre-Op</strong></td>
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<td>BP mm Hg</td>
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<td>Sham (n = 9)</td>
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<tr>
<td>Denervated (n = 12)</td>
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<td>Control (n = 11)</td>
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*p < 0.001, †p < 0.01 (differences from control), unpaired Student's t test.

Changes were observed in other brain regions studied. Dopamine content was not different between groups in any region. These data, combined with the finding of a decrease in peripheral sympathetic activity following renal denervation, suggest that the renal afferent nerves may play a role in established IKIC by modulating central sympathetic nervous system activity.

Results of studies of the effects of renal denervation on two-kidney, one clip Goldblatt hypertension in the rat (2KIC) and coarctation hypertension in the dog provide additional evidence that the renal afferent nerves are involved in the pathogenesis of experimental hypertension. Similar to the findings observed in 1KIC, Katholi et al. found that denervation of the clipped kidney of 2KIC performed 6 weeks after renal artery clipping resulted in a significant attenuation of hypertension. Plasma norepinephrine levels and the depressor response to ganglionic blockade in hypertensive 2KIC were increased in comparison to those of normotensive unclipped controls. Renal denervation resulted in a return of plasma norepinephrine levels and the depressor response to ganglionic blockade to control levels. As in the 1KIC, these results indicate that the attenuation of hypertension following renal denervation in 2KIC results from a decrease in peripheral sympathetic activity.

Whitlow and Katholi found that bilateral renal denervation resulted in a decrease in blood pressure in dogs with chronic coarctation hypertension. There was no change in sodium excretion or plasma renin activity following denervation, suggesting that the effects of denervation were not due to interruption of the renal efferent nerves. As was the case in 1KIC and 2KIC, there was evidence of decreased peripheral sympathetic activity following renal denervation.

Taken together, the aforementioned studies indicate that in a number of experimental models the renal afferent nerves contribute to the maintenance of hypertension and that the depressor effect of renal denervation in these models is, at least in part, secondary to a decrease in sympathetic nervous system activity.

**Conclusions**

There is evidence that both the renal efferent sympathetic nerves and the renal afferent nerves are involved in cardiovascular homeostasis. Recent studies of the effects of renal denervation in a number of experimental models indicate that renal neural mechanisms
are important in the pathogenesis of hypertension. The mechanisms involved appear to differ in different models. In some models such as the SHR the renal nerves appear to influence the development of hypertension via an effect on urinary sodium excretion, while in others such as the one-kidney, one clip Goldblatt rat, evidence suggests that the renal afferents modulate the activity of the sympathetic nervous system.

Acknowledgments

The authors thank Dr. Richard Katholi for his many contributions to the work reviewed here and Karen Riley for her support, perseverance, and assistance in preparation of the manuscript.

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Importance of the renal nerves in the pathogenesis of experimental hypertension.
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Hypertension. 1982;4:III108
doi: 10.1161/01.HYP.4.5_Pt_2.III108

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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