Renal Nerves and the Development of Dahl Salt-Sensitive Hypertension

JEFFREY L. OSBORN, RICHARD J. ROMAN, AND JOSEPH D. EWENS

SUMMARY Several experimental forms of hypertension require intact renal innervation for the development or maintenance (or both) of the elevated arterial pressure. We determined the relationships between urinary sodium and water excretion and arterial pressure in Dahl salt-sensitive rats (DS) with innervated (n = 6) and denervated (n = 7) kidneys after switching from a low to a high sodium diet. Arterial pressure significantly increased in both groups within 48 hours after they began to eat an 8% sodium chloride diet. This hypertension increased to 188 ± 9 and 190 ± 7 mm Hg, respectively, in rats with innervated and denervated kidneys after 12 days. Mean arterial pressures were not significantly different between groups on any day. The rise in arterial pressure of DS placed on a high sodium intake was associated with an elevation of urine flow rate and urinary sodium excretion in rats with either innervated or denervated kidneys. Urine flow rates and urinary sodium excretions were greater in denervated than in innervated rats on Days 4 through 7 after beginning the high sodium diet. This diuresis and natriuresis in rats with denervated kidneys were associated with greater water and sodium intakes on Days 4 to 7 of the high sodium diet when compared with rats with innervated kidneys. These results demonstrate that, following exposure to a high sodium intake, DS have increased arterial pressure within 24 hours. The development of this arterial hypertension is not dependent on intact renal innervation. In conscious DS, the renal innervation does participate in the regulation of urinary sodium excretion by promoting renal sodium and water reabsorption. However, DS with either innervated or denervated kidneys maintained sodium balance and rapidly became hypertensive when fed a high salt diet. Thus, elevation of sodium intake in DS results in arterial hypertension independent of measurable renal neurogenic alterations in salt and water balance. (Hypertension 11: 523-528, 1988)

KEY WORDS • renal innervation • hypertension • blood volume • sodium balance

In several experimental models of hypertension, functional innervation of the kidneys has been reported as a requirement for the development of elevated arterial pressure.1-7 In these studies, renal denervation was shown to either abolish2,6,7 or reduce the magnitude3-5 of the hypertension. Alterations in the development of hypertension by renal denervation may result from interruption of renal afferent or renal efferent sympathetic pathways. Stimulation of renal afferent pathways has been reported to be important in the development of hypertension during ipsilateral renal artery stenosis8 or during intrarenal infusion of adenosine.9 Activation of renal efferent nerve fibers either by direct electrical stimulation or by reflex activation of sympathetic outflow increases renal vascular resistance, tubular sodium reabsorption, and renin release.10 These factors could elevate arterial pressure through the vasoconstrictor effects of subsequent increases in circulating angiotensin II or by expansion of the extracellular fluid volume through increased sodium and water reabsorption (or by both mechanisms).

Abnormalities in neural control mechanisms of hypertensive animals have been reported, including prehypertensive Dahl salt-sensitive rats (DS). Impaired baroreceptor reflex control of sympathetic outflow has been postulated as a potential mechanism underlying the development of this salt-sensitive form of arterial hypertension. This mechanism has been proposed based on studies by Gordon and Mark11 demonstrating that prehypertensive DS have altered baroreceptor reflex control of heart rate and total peripheral resistance. The precise nature of this abnormality was localized in part to reduced aortic depressor nerve responses of DS to increased arterial pressure. Thus, DS may be unable to appropriately decrease sympa-
thetic outflow in response to stimulation of baroreceptor reflex mechanisms by blood or extracellular fluid volume expansion.

The DS rapidly become hypertensive when placed on a high sodium diet. An inability to excrete sodium chloride in a normal fashion has been suggested as the initiating factor in the development of elevated arterial pressure in this strain of rats. It is now well established that very modest increases in renal sympathetic nerve traffic can result in increased renin release and increased renal tubular sodium reabsorption. Taken together, the findings of these studies have led to the hypothesis that, when DS are placed on a high sodium intake, small increases in body fluid volumes do not result in appropriately decreased renal sympathetic outflow. In the presence of a high sodium intake without reduced renal nerve activity, the DS would be unable to efficiently excrete the increased sodium chloride load, therefore leading to elevation of extracellular fluid volume, cardiac output, and arterial pressure. The present studies were conducted to test this hypothesis and to determine the changes in sodium and water balance and arterial pressure in DS with innervated and denervated kidneys after being placed on a high sodium chloride diet.

Materials and Methods

Experiments were conducted in 10-week-old female DS (Brookhaven Laboratories, Upton, NY, USA) weighing 200 to 250 g. Rats were housed in metabolism cages in an animal care facility at the Medical College of Wisconsin approved by the American Association for Laboratory Animal Care. The temperature and humidity of the room were controlled, and the rats were maintained on a 12-hour light and 12-hour dark cycle. Before study, all animals were kept on a low sodium diet and were allowed water ad libitum. At least 5 days before study, each animal was subjected to unilateral nephrectomy (right kidney) and either renal denervation (n = 7) or sham operation (n = 6). All surgical procedures were conducted with strict adherence to the National Institutes of Health guidelines. All protocols had received prior approval by the Animal Care Committee of the Medical College of Wisconsin. Rats were anesthetized with ketamine (100 mg/kg) and acepromazine (1.8 mg/kg) i.m. Both kidneys were exposed by a retroperitoneal flank incision. In one group, renal denervation was accomplished by stripping all visible renal nerves along the renal artery and vein from the aorta to the hilus of the kidney. The connective tissue also was stripped away from the hilus to each pole of the kidney. Both the renal artery and renal vein then were painted with a 10% phenol solution solubilized in 95% ethanol. Sham operation was accomplished by exposing the kidney and gently manipulating the area surrounding the renal artery and vein, with care being taken to avoid disruption of the renal innervation. The flank incisions then were closed.

At the time of this surgical procedure, a chronic indwelling arterial cannula was inserted into a femoral artery. This cannula consisted of a 4-cm segment of heparin-complex-treated polyvinyl tubing (inside diameter, 0.50 mm; outside diameter, 0.80 mm; Dural Plastics, Dural, N.W.T. Australia) connected to Tygon microbore tubing (inside diameter, 0.020 in.; outside diameter, 0.060 in.; Norton Plastics, Akron, OH, USA) by a stainless steel pin (22 gauge; 0.7 cm). Near the junction of the pin and the microbore tubing, the tubing was curved to an angle of approximately 160 degrees by applying heat. The polyvinyl tubing was inserted into the femoral artery to the pin and secured into position. The catheter (Tygon microbore) then was tunneled subcutaneously to the back of the neck and exteriorized near the scapular region. All catheters were filled with an anticoagulant solution of heparin (1000 U/ml) containing fibrinolysin (2.0 mg/ml). All catheters were flushed on alternating days and refilled with fresh anticoagulant solution to maintain patency. This technique provides indwelling arterial pressures in nearly all animals for up to 6 weeks with a success rate of 90 to 95%. The femoral incision then was closed, and the animals were allowed 5 days to recover. All rats were treated postoperatively with 40,000 units of penicillin G-streptomycin to prevent infection.

All rats then were placed in metabolic cages for the determination of 24-hour sodium and water balances and were maintained on a low sodium chow (0.3% NaCl; Dyets, Bethlehem, PA, USA) during the recovery period. Control measurements of sodium and water balance were made for 24 hours on the day before the high sodium diet (8% NaCl chow; catalog no. 100073, Dyets) was begun. Daily sodium and water balances in both groups of rats then were determined for 7 consecutive days and on the 12th day after beginning the high sodium diet. Arterial pressure was determined by direct measurement from the indwelling arterial catheter on the control day and on Days 1, 3, 5, 7, 10, and 12 of the high sodium intake. Plasma sodium concentration also was measured in both groups of rats from arterial blood samples (100 μl) collected on Days 1, 3, and 7 after elevation of sodium intake.

On the morning of each day of arterial pressure measurement, the indwelling arterial catheter was connected to a pressure transducer (Statham P23id, Oxford, CA, USA) and arterial pressure was determined for at least 30 minutes using a direct writing oscillograph (Grass polygraph, Quincy, MA, USA). Daily water intake was determined using a calibrated water bottle, and urine flow rate was measured in a graduated cylinder from the directly collected urine. Sodium intake was quantitated daily by the gravimetric determination of food intake. Total sodium excretion was determined by measuring both urinary and fecal sodium losses. Feces were collected daily and solubilized in 20% nitric acid. Plasma, urinary, and fecal sodium concentrations then were determined by flame photometry. Sodium excretion was calculated as the product of urine flow rate and urinary sodium concentration.

At the conclusion of the study, the rats were anes-
thetized with sodium pentobarbital (50 mg/kg i.p.) and the kidneys were removed and frozen for determination of tissue catecholamine concentration. Tissue norepinephrine was determined by radiochemical assay techniques (CAT-a-Kit; Upjohn, Kalamazoo, MI, USA).

All data were calculated as the means ± SEM. Differences between rats with innervated and denervated kidneys were determined by a one-way analysis of variance. Mean differences were determined by the Sheffe's procedure. The 0.05 level of probability was used as the criterion of significance.

**Results**

The mean arterial pressures of DS with innervated and denervated kidneys are shown in Figure 1 before and after receiving a high sodium intake. Average arterial pressures on a low sodium intake (control) were 118 ± 4 and 114 ± 5 mm Hg for rats with innervated and denervated kidneys, respectively. Blood pressure increased significantly within 24 hours in rats with innervated kidneys to 129 ± 4 mm Hg (p < 0.05) and to 136 ± 3 mm Hg (p < 0.05) by 48 hours in rats with denervated kidneys. Arterial pressure continued to increase in both groups of rats to a peak of 188 ± 9 (innervated group) and 190 ± 7 mm Hg (denervated group) after 12 days of a high sodium chloride diet (see Figure 1). Mean arterial pressures were not significantly different between rats with innervated and denervated kidneys on any day in which measurements were obtained.

Body weights of all rats were measured throughout the study. In the control period, rats with innervated kidneys averaged 204 ± 6 g and those with denervated kidneys averaged 208 ± 4 g. The average body weights increased equally throughout the study to 223 ± 7 g (innervated) and 227 ± 4 g (denervated) by Day 12. The body weights of each group were not different from each other on any day of the study. At the conclusion of the experiment, kidney tissue norepinephrine concentrations of rats with innervated kidneys was 80.6 ± 16.9 pg/mg and that of rats with denervated kidneys was 2.4 ± 0.6 pg/mg (p < 0.05). These results document the completeness of renal denervation in these DS over the time course of this study.

The elevation of arterial pressure after rats were placed on an 8% sodium chloride diet (see Figure 1) was associated with a concomitant increase in urinary sodium excretion. Urinary sodium excretion increased in rats with innervated and denervated kidneys to a maximum of 21.2 ± 1.6 and 21.5 ± 1.4 mEq/day, respectively by Day 12 of the high sodium chloride diet (Figure 2). This rise in urinary sodium excretion, however, was blunted in rats with innervated kidneys, as shown by significantly lower urinary sodium excretions in these animals on Days 4, 6, and 7 of the high salt diet. The lower urinary sodium excretions of DS with innervated kidneys on Days 4 to 7 of a high sodium intake were associated with reduced sodium intakes over this same period when compared with DS with denervated kidneys (Figure 3). These sodium intake values were significantly different from each other on Days 4 and 7 after elevating sodium intake (see Figure 3). These similar increases in sodium excretion and sodium intakes resulted in no significant changes in 24-hour sodium balances in DS with innervated and denervated kidneys. Thus, DS with innervated kidneys transiently excreted less sodium than DS with denervated kidneys 4 to 7 days after being placed on an 8% sodium chloride diet. The DS with innervated kidneys also had lower sodium intakes over this same period when compared with DS with denervated kidneys, resulting in no significant alterations in sodium balance.

The water intakes and urine flow rates of DS with innervated and denervated kidneys placed on a high sodium diet are shown in Figures 4 and 5, respectively. In the first 24 hours after elevation of sodium intake,
FIGURE 3. Changes in sodium intake of DS with innervated (●; control) and denervated (○; DNX) kidneys before (C) and for 12 days after receiving a high sodium intake (8% NaCl chow). Before Day 1 all rats were maintained on a low sodium chow (0.3% NaCl). Asterisk indicates significant difference (p < 0.05) between groups.

Water intake of rats with innervated kidneys increased from 16.7 ± 1.0 to 46.8 ± 2.8 ml/day (p < 0.05) and that of rats with denervated kidneys increased from 23.4 ± 2.0 to 44.0 ± 2.0 ml/day (p < 0.05). Water intake continued to increase in both groups of rats to 88.8 ± 5.2 and 90.0 ± 6.4 ml/day by Day 12 in rats with innervated and denervated kidneys, respectively. These increases in water intake, however, were blunted in rats with innervated kidneys when compared with that of rats with denervated kidneys on Days 4 to 7 after increasing sodium intake (p < 0.05; see Figure 4).

Urine flow rates increased concomitantly with water intakes after increasing sodium intake of DS with innervated and denervated kidneys (see Figure 5). Control urine flow rates averaged 9.5 ± 0.9 and 14.6 ± 1.5 ml/day in rats with innervated and denervated kidneys, respectively. The urine flow rates of these rats with innervated and denervated kidneys increased within 24 hours to 29.3 ± 5.4 and 23.6 ± 4.1 ml/day, respectively, and to a peak of 83.5 ± 4.5 and 82.7 ± 6.1 ml/day, respectively, by Day 12 of the high sodium intake (see Figure 5). As was observed with water intake, urine flow rates of rats with innervated kidneys were significantly reduced compared with rats with denervated kidneys on Days 4 to 7 of the high sodium diet. Each of these changes in urine flow rate after increasing sodium intake were associated with equivalent increases in water intake (see Figure 5).

Discussion

Several investigators have suggested that elevated renal sympathetic efferent nerve activity may importantly influence the control of arterial pressure and potentially lead to the pathogenesis of hypertension.1-7 In these previous studies, bilateral renal denervation was shown to significantly alter the development of several forms of experimental hypertension. It has been suggested that some form of renal dysfunction underlies the development of elevated arterial pressure in the DS when these animals are placed on a high salt diet.13-15 Most studies have indicated that an inability of DS to excrete sodium when challenged with a high sodium intake may lead to a greater expansion of extracellular fluid volume, sodium retention, and ultimately, to the elevation of arterial pressure.14,15

A major factor that potentially could impair renal excretory ability and increase extracellular fluid volume and, consequently, arterial pressure in DS fed a high salt diet is elevated renal sympathetic nerve activity. Recent evidence indicates that efferent renal sympathetic nerve traffic may be an important controller of urinary sodium excretion16,17 and, ultimately, overall...
sodium balance. Gordon and Mark have reported that DS exhibit an altered baroreceptor reflex sensitivity to changes in arterial pressure when compared with Dahl salt-resistant rats. Taken together, these results suggest that elevated renal sympathetic outflow in DS, possibly from impaired baroreceptor reflex mechanisms, could lead to excess renal fluid and electrolyte retention and elevated arterial pressure.

The present studies were conducted to evaluate the development of arterial hypertension and the regulation of salt and water balance in conscious DS with innervated and denervated kidneys when these animals were placed on a high sodium intake. Arterial pressure increased similarly in DS with innervated and denervated kidneys (see Figure 1), and the development of this arterial hypertension peaked in both groups of rats within 10 days after exposure to the high sodium diet. Thus, unlike other genetic forms of experimental hypertension, the presence of intact renal innervation is not required for the development of salt-sensitive hypertension in the DS.

The inability of renal denervation to prevent the development of hypertension in DS on a high sodium intake probably did not result from incomplete renal denervation. The renal tissue norepinephrine concentration of rats with denervated kidneys averaged only 2.6 ± 0.6 pg/mg tissue. This abolition of tissue norepinephrine indicates that the surgical stripping of all visible renal nerves and application of phenol to the renal pedicle effectively eliminated the renal innervation. In addition, these data document that there was no significant reinnervation of DS following surgical denervation during the time course of the study. Post-denervation supersensitivity to circulating catecholamines may have been responsible for the inability of renal denervation to alter the development of hypertension in these rats. This possibility seems unlikely, however, since these animals did exhibit denervation diuresis and natriuresis when compared with rats with innervated kidneys (see Figures 5 and 2, respectively).

Thus, the development of arterial hypertension in DS with denervated kidneys during high sodium intake likely resulted from mechanisms unrelated to intact renal innervation.

Both the DS with innervated kidneys and DS with denervated kidneys rapidly increased urinary sodium excretion when sodium intake was elevated by exposure to a high salt diet (see Figure 2). This natriuretic response to increased sodium intake was similar for 3 days. Both rats with innervated and denervated kidneys, however, maintained sodium and water balance, confirming our previous observation that DS rapidly become hypertensive without retaining detectable quantities of sodium and water.

The renal nerves of the DS do appear to participate in the normal regulation of urinary sodium excretion, however, following exposure to a high salt diet. The rate of increase in urinary sodium excretion of DS with innervated kidneys was significantly less than that of DS with denervated kidneys on Days 4 to 7 after increasing sodium intake (see Figure 2), indicating that renal sympathetic outflow did exhibit an important antinatriuretic influence in these neurally intact animals. Sodium balance, however, remained constant after these rats were placed on a high salt diet because during this same period of denervation-induced natriuresis (i.e., Days 4–7) sodium intakes of rats with denervated kidneys were elevated (see Figure 3). Similar differences in urine flow rate and water intake were observed between DS with innervated and denervated kidneys 4 to 7 days after exposure to a high salt diet: DS with denervated kidneys had greater urine flow rates and water intakes than did DS with innervated kidneys during this period. These differences in sodium excretion and urine flow rate responses to the high sodium diet may have been a result of a more effective pressure natriuresis and diuresis resulting from the markedly elevated arterial pressure. Renal denervation has been shown to shift the pressure natriuresis–diuresis relationship toward lower arterial pressures in normotensive rats, presumably because under these conditions the natriuretic and diuretic responses to elevated arterial pressure are unopposed by the antinatriuretic influence of efferent renal sympathetic nerve activity. Thus, in DS with denervated kidneys that rapidly becoming hypertensive, the rise in urinary sodium excretion was exaggerated. The present studies, however, do not account for the possibility that DS with denervated kidneys increased their sodium intakes, which in turn elicited an elevated sodium excretion. Although the present experimental design does not directly test this hypothesis, this possibility seems unlikely since both DS with innervated and DS with denervated kidneys exhibited similar food and therefore sodium intakes before exposure to the high salt diet.

Bilateral surgical denervation of DS kidneys effectively eliminates both efferent and afferent neural pathways. Previous studies have suggested that sympathetic outflow may participate in the development of hypertension in rats following reduced renal perfusion pressure and in dogs during intrarenal infusion of adenosine or norepinephrine. The present data indicate that, at least in the DS, elevation of sodium intake by an 8% sodium chloride diet does not activate renal afferent neural pathways leading to elevation of arterial pressure.

In conclusion, the results of this study confirm our previous observation that the DS strain of rats increases arterial pressure within 24 hours after being placed on a high sodium intake. The development of arterial hypertension in DS fed a high sodium diet occurs independently of intact renal innervation. This elevation of arterial pressure is associated with an immediate and marked natriuresis and diuresis, and the rats remain in sodium and water balance. Renal sympathetic outflow, however, reduces these natriuretic and diuretic responses, resulting in appropriate reductions in sodium and water intake by DS with innervated kidneys. Thus, the rapid development of systemic arterial hypertension in DS occurs as a possible mechanism to maintain normal fluid and electrolyte balance.
independently of activation of renal sympathetic efferent and afferent neural pathways.

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References

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