Dual Hemodynamic Mechanisms for Salt-Induced Hypertension in Dahl Salt-Sensitive Rats

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Cardiac output, blood volume, total peripheral resistance, and renal blood flow were measured in awake salt-sensitive and salt-resistant Dahl rats on normal rat chow (1% NaCl) and on high salt (8% NaCl) diets. Rats were studied after 4, 8, and 46 weeks on a 1% NaCl diet and after 4 and 8 weeks on an 8% NaCl diet. Salt-sensitive rats on 8% NaCl for 4 weeks developed systolic hypertension; by 8 weeks they developed greater systolic and also diastolic hypertension. Salt-resistant rats on 8% NaCl remained normotensive throughout the studies, although renal resistance decreased (p<0.05). At 4 weeks, hypertension in salt-sensitive rats on 8% NaCl was caused by increased blood volume and cardiac output (p<0.05), with normal total peripheral resistance. At 8 weeks, hypertension was due to increased total peripheral resistance (p<0.05); cardiac output was below normal despite persistent elevation of blood volume (p<0.05). Salt-sensitive rats on 1% NaCl for 46 weeks were hypertensive, with elevated total peripheral resistance (p<0.05); cardiac output decreased (p<0.05), whereas blood volume remained unchanged. Salt-resistant rats on 1% NaCl remained normotensive with no changes in hemodynamics. Salt-sensitive rats on 8% NaCl for 4 weeks had an increase in renal vascular resistance but no significant change in nonrenal resistance or total peripheral resistance. The increased total peripheral resistance in salt-sensitive rats on 8% NaCl for 8 weeks and on 1% NaCl for 46 weeks was a reflection of increases of both renal and nonrenal vascular resistance. Salt-induced hypertension in salt-sensitive rats occurs by two mechanisms: on 8% NaCl, hypertension is initiated by increased blood volume and cardiac output but is sustained by increased total peripheral resistance; with prolonged ingestion of a 1% NaCl diet, hypertension results from increased total peripheral resistance without increased blood volume or cardiac output. Salt-sensitive rats on a 1% NaCl diet provide another model, probably more appropriate, to study human salt-sensitive hypertension unaccompanied by blood volume expansion. (Hypertension 1991;17:1063-1071)

Although salt can play a significant role in the genesis of hypertension, the precise mechanism by which it elevates blood pressure is unknown. Experimental evidence indicates an important role for both the kidney and sodium chloride in hypertension. Dahl and coworkers developed two strains of rats: a salt-sensitive strain (DS) that becomes hypertensive with high salt intake, and a salt-resistant strain (DR) that remains normotensive despite high salt intake. The Dahl model has been useful for the study of salt-sensitive hypertension. Salt-induced hypertension in Dahl rats has been attributed to a humoral factor, although participation of neurogenic mechanisms and a deficient renal elimination of salt and water also have been implicated. However, the precise mechanism or mechanisms by which DS rats become hypertensive remain unclear. Because transplantation of kidneys from DR rats into hypertensive DS rats can normalize blood pressure, whereas transplantation of DS kidneys into DR rats can lead to salt-induced hypertension, it appears that an abnormality in kidney function of DS rats is responsible for the salt-induced hypertension. Dahl et al concluded that genetically controlled factors operating primarily through the kidney determine the level of blood pressure. More recently, however, Morgan et al have reported that in addition to the kidney, extrarenal factors also contribute significantly to NaCl-induced hyperten...
sion in DS rats. The discrepancy between these latter results and those of Dahl and coworkers is unclear. Tobian et al., Roman, and Roman and Osborn attributed salt-induced hypertension in DS rats to a reduction in the kidney's natriuretic capacity. Studies by Azar et al. have shown that hypertensive DS rats have fewer glomeruli and fewer "functioning" nephrons than DR rats. Jaffe et al. suggested that hypertension induced by salt in DS rats is mediated through a "Goldblatt effect" and that this is accompanied by development of renal lesions. Pitcock et al. demonstrated that DS rats have fewer renomedullary interstitial cells than DR rats; whether deficiency of a putative antihypertensive lipid elaborated by these cells is implicated in the hypertension of salt-sensitive rats is unknown. Studies by Hirata et al. on the isolated perfused kidney indicated lower renal papillary plasma flow in DS as compared with DR rats; they demonstrated that DS rats had increased natriuretic factor in their atria but that their kidneys were hyporesponsive to this factor. DS rats on a high salt diet have a lower glomerular filtration rate, despite a higher perfusion pressure as compared with DR rats; there is a shift of the arterial pressure–glomerular filtration rate curve so that glomerular filtration rate is less for any given pressure because of exaggerated afferent arteriolar vasconstriction.

We previously reported that hypertension that developed in DS rats in response to a high salt diet (8% NaCl) for 4 weeks resulted, at least partly, from an inability of the kidney to vasodilate and to increase sodium and water excretion; an expanded blood volume (BV) and an increased cardiac output (CO) were responsible for the hypertension. According to Guyton et al., in the presence of an increased CO and augmented tissue blood flow, total peripheral resistance (TPR) increases significantly with "long-term autoregulation" (i.e., vasoconstrictor regulation of tissue perfusion), and CO returns to normal; elevated TPR then becomes responsible for the hypertension.

In the current study we report the effects of 4 and 8 weeks of high dietary NaCl (8%) and 4, 8, and 46 weeks of normal rat chow containing 1% NaCl on cardiovascular and renal hemodynamics in DS and DR rats. These experiments were designed to compare hemodynamic mechanisms initiating and maintaining hypertension on 8% NaCl with those responsible for the development of hypertension on
a 1% NaCl diet. Awake rats were used to avoid any effect of anesthesia on cardiovascular and renal hemodynamics and BV.

**Methods**

**Procedures**

Experiments are reported on 31 awake male DR and 35 DS rats (Brookhaven National Laboratory, Upton, N.Y., and Harlan Sprague Dawley, Inc., Indianapolis, Ind.) that had been fed normal Purina rat chow (1% NaCl) from weaning. Rats were subjected to one of the following protocols.

**Protocol A, high salt diet.** In this protocol, 13 DR and 15 DS rats were fed a normal rat chow (1% NaCl) diet from birth until 6 weeks of age. Diets then were changed to a high salt diet containing 8% NaCl. Rats were studied after either 4 or 8 weeks on an 8% NaCl diet, when animals were 10 or 14 weeks old and weighed approximately 250 and 360 g, respectively. Tap water was provided ad libitum.

**Protocol B, normal salt diet.** In this protocol, 18 DR and 20 DS rats were fed a normal rat chow (1% NaCl) diet from birth until they were 10, 14, and 46 weeks old. Rats weighed approximately 500 g at 46 weeks. Tap water was provided ad libitum.

Before surgical procedures, blood pressures and heart rates were monitored weekly in conscious rats by the tail-cuff method (Nacro Instruments, Houston, Tex.) and recorded on a polygraph recorder (model 7, Grass Instrument Co., Quincy, Mass.).

**Surgical Procedure**

After anesthesia with sodium pentobarbital (35 mg/kg i.p.), a PE 50 polyethylene catheter was inserted into the abdominal aorta via a femoral artery for blood withdrawal and recording of arterial pressure (volume removed during blood sampling was replaced by the infusion of an equal volume of donor blood). A second PE 50 catheter was advanced into the inferior vena cava via a femoral vein for intravenous infusions. A third catheter was advanced via the carotid artery into the left ventricle for injection of microspheres; the position of the catheter tip was confirmed by pressure tracing. Catheters were tunneled subcutaneously and exteriorized at the back of the neck and fixed to the skin with sutures. Rats were allowed to recover for 24 hours. They appeared normally active and pain free at the time of experimentation. Determination of plasma corticosterone 24, 48, and 72 hours after similar surgical procedures

**Figure 2.** Effect of 4 and 8 weeks of high salt diet (8% NaCl) on blood volume and systemic hemodynamics (cardiac output [C.O.] and total peripheral resistance) of awake Dahl salt-resistant (R-Dahl) and salt-sensitive (S-Dahl) rats. Total peripheral resistance is represented as mm Hg/ml/sec/100 g. Vertical lines indicate standard deviation. *Statistical difference from control (p<0.05). MABP, mean arterial blood pressure.
in additional DS and DR rats revealed no evidence of stress from these procedures. Our experience also has indicated that anesthesia with pentobarbital followed by minor surgical procedures in the rat causes no remarkable alteration of plasma catecholamines. Furthermore, systolic blood pressure and heart rate determined by the tail-cuff method in conscious rats just before anesthesia and cannulation were similar to those obtained 24 hours after cannulation. The rats lost only about 3% of their body weight and were eating and drinking moderately well. During the experiment, each rat was kept unrestrained in a dark box with a small hole through which the catheters were exteriorized. Cardiovascular pressures were monitored using Statham transducers and a polygraph recorder (model 7, Grass Instrument Co.).

Methods

Hemodynamic parameters. CO and renal blood flow (RBF) were determined by a microsphere method, using 15.0±1.0-μm diameter microspheres (New England Nuclear Corp., Boston) labeled with 46Sc injected into the left ventricle within 10–15 seconds. A reference blood sample was withdrawn from the abdominal aorta at a rate of 0.8 ml/min for 2 minutes (total of 1.6 ml blood). At the end of the experiment, rats were killed by injecting a saturated KCl solution into the left ventricle, and various organs (kidneys, heart, and samples of skin and skeletal muscle) were immediately removed. Activity of 46Sc in various tissues was determined with a gamma counter (Packard 5130, Auto-Gamma System, Packard Instrument Co., Inc., Downers Grove, Ill.) connected to a multichannel analyzer (Tracor Northern, Inc., Middleton, Wis.). Resistance to flow was calculated as the ratio of mean arterial pressure to regional blood flow per weight. Renal resistance was calculated as the ratio of mean arterial pressure to RBF per gram kidney, and nonrenal vascular resistance was calculated as the ratio of mean arterial pressure to CO–RBF per gram body weight. TPR was calculated as the ratio of mean arterial pressure to CO.

Blood volume. Red cell volume was determined using red blood cells labeled with 51Cr (sodium chromate 51Cr, E.R. Squibb & Sons, Inc., Brunswick, N.J.) by the indicator dilution technique. Plasma volume was determined by a standard technique, using 125I-labeled albumin (radioiodinated 125I serum albumin, Mallinckrodt, Inc., St. Louis).
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Results are presented as mean±SD. Statistical comparisons of variables between groups were performed by analysis of variance followed by the Newman-Keuls range test.

Blood urea nitrogen and creatinine were determined in DS and DR rats after 10 weeks on an 8% NaCl diet and in DS and DR rats after 46 weeks on a 1% NaCl diet.

Results

The effects of an 8% NaCl diet on arterial blood pressure are shown in Figure 1. DR rats remained normotensive on both the 1% and 8% NaCl diets for 4 and 8 weeks, as did DS rats on a 1% NaCl diet. DS rats on 8% NaCl developed only moderate systolic hypertension by 4 weeks, but at 8 weeks, they developed more marked systolic and moderate diastolic hypertension (Figure 1).

The effects of an 8% NaCl diet on BV, CO, and TPR are shown in Figure 2. At 4 and 8 weeks, DR rats on 8% NaCl had no statistically significant changes in TPR, CO, and BV. The 8% salt diet resulted in a significant expansion of BV (p<0.05) at 4 weeks (as previously reported18) and 8 weeks in DS rats. At 4 weeks, DS rats on 8% NaCl had a marked increase in CO (p<0.05), whereas TPR was not significantly changed. After 8 weeks, however, CO decreased to below control, and TPR became markedly elevated (p<0.05).

Effects of an 8% NaCl diet on renal and nonrenal resistance are shown in Figure 3. An 8% NaCl diet for 4 and 8 weeks caused a progressive increase in renal resistance of DS rats but a progressive decrease in renal resistance of DR rats (p<0.05). At 8 weeks on an 8% salt diet, the resistance of other organs (nonrenal) also increased (p<0.05) in DS but not in DR rats.

The effects of a 1% NaCl diet for 10, 14, and 46 weeks on arterial blood pressure are shown in Figure 4. After 46 weeks on a 1% salt diet, DS rats had a mild increase in systolic, diastolic, and mean arterial blood pressures (p<0.05). Blood pressure of DR rats remained unchanged.

The effects of a 1% salt diet on BV, CO, and TPR are shown in Figure 5. DS rats on this diet for 46 weeks had a decrease in CO (p<0.05), whereas TPR increased (p<0.05); BV remained unchanged. No significant change occurred in DR rats.

The effects of a 1% salt diet on renal and nonrenal resistance are shown in Figure 6. Forty-six weeks on
this diet resulted in an increase in both renal and nonrenal resistance in DS rats (p<0.05) but no change in DR rats. Blood urea nitrogen and serum creatinine did not change remarkably in DS or DR rats after 10 weeks on an 8% NaCl diet or after 46 weeks on a 1% NaCl diet. There was no evidence of renal insufficiency or deterioration of health in any animals studied.

Discussion

The objective of this study was to investigate the mechanism of salt-induced hypertension in conscious DS rats. The results indicate that initiation of hypertension induced by 8% dietary salt for 4 weeks in DS rats was due to an increased CO accompanied by an expansion of BV. Progression of hypertension after 8 weeks on this high salt diet was associated with an increase in TPR, whereas CO decreased to below normal. After 4 weeks on an 8% NaCl diet, no significant change in TPR occurred in DS rats, despite a significant increase in renal resistance (Figure 3). Whether the increase in TPR after 8 weeks on an 8% NaCl diet was an autoregulatory response or a reflection of some neurohumoral or intrinsic vascular mechanism is unclear. Na⁺-K⁺ pump activity is increased in the tail artery isolated from DS rats. This could be secondary to a genetic increase in sodium permeability in vascular smooth muscle, which increases sodium influx and depolarizes the vascular membrane. DR rats did not become hypertensive after 4 and 8 weeks on an 8% NaCl diet, but they manifested a selective decrease in renal vascular resistance per gram kidney weight (Figure 3), with no significant changes in nonrenal resistance per gram body weight. Our results are in agreement with those of Fink et al, who reported that in the autoperfused kidney, there was a reduced renal vascular resistance in DR rats on high salt intake but not in DS rats; however, in this preparation, DS kidneys failed to vasodilate during high salt intake, whereas we demonstrated renal vasoconstriction in DS rats after 4 weeks on 8% NaCl.

Hypertension in DS rats accompanied by failure of renal vasodilatation in response to salt loading is similar to one type of salt-sensitive human hypertension, designated as “non-modulating” by Hollenberg and Williams. These authors defined two types of human essential hypertensives: 1) “modulators,” hypertensive patients whose RBF increases appropriately in response to an increase in salt intake (as
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![Diagram](image)

**FIGURE 6.** Effect of 10, 14, and 46 weeks of 1% NaCl diet on renal and nonrenal resistance of Dahl salt-resistant (R-Dahl) and salt-sensitive (S-Dahl) rats. Renal resistance is calculated as mean arterial blood pressure (MABP)/(renal blood flow [RBF]/kidney wt in grams). Nonrenal resistance is calculated as MABP/(cardiac output [C.O. - RBF]/(body wt – kidney wt)). Vertical lines indicate standard deviation. *Statistical difference from control (p<0.05).

occurs in normotensive subjects), and 2) “non-modulators,” salt-sensitive hypertensives in whom RBF does not increase in response to increased salt intake. The inability of DS rat kidneys to increase blood flow in response to an increase in salt intake suggests that DS rats may be a model of nonmodulator human hypertension. The genetic defect responsible for the development of hypertension in DS rats on a high salt diet remains to be determined. It appears that this defect involves the renal vasculature and the response of the kidney to atrial natriuretic peptide in the presence of increased secretory mechanism for atrial natriuretic peptide. Whether the defect is confined to the kidney remains to be determined. Evidence from an increased Na+-K+ pump activity in the tail artery of DS rats in the presence of increased secretory mechanism for atrial natriuretic peptide suggests that an abnormality in vessels other than the kidney may exist. Ganguli et al compared hemodynamics in anesthetized female DS and DR rats on 0.3% or 8% NaCl diets for 3 or 7 days. After 3 days of 8% NaCl, CO was increased in both DR and DS rats; TPR was decreased in DR rats but increased in DS rats. Blood pressure remained unchanged in DR rats but increased in DS rats. After 7 days of 8% NaCl, CO was not increased in DS or DR rats. TPR remained relatively constant in DR rats on either 0.3% or 8% NaCl for 7 days but was elevated in DS rats on 8% NaCl for 7 days. The reasons for these differences from our results are not clear but may, in part, be due to differences in experimental conditions or animals (anesthesia, differences in duration of high salt ingestion, sex differences of rats, etc.). Pfeffer et al reported hemodynamic studies on Dahl female rats under ether anesthesia receiving various amounts of dietary NaCl for 9 weeks. They demonstrated that graded pressure elevation in DS rats was produced by corresponding increases in TPR, as CO did not vary. Greene et al reported that increasing the concentration of sodium in a liquid diet from 0 to 20 meq/day for 96 hours in conscious DS rats resulted in fluid retention and an increased CO, which triggered a rise in blood pressure. DR rats on the same protocol exhibited similar increases in both fluid retention and CO but no rise in blood pressure. They concluded that a major deficit in the cardiovascular response to volume expansion in DS rats is responsible for the hypertension. Their studies also suggested that it was the retention of fluid and not the sodium per se that triggered hypertension. Others have demonstrated an impaired baroreflex in prehy-
pertensive DS rats.\textsuperscript{35,36} Although our results indicated a tendency for BV and CO to increase in DR rats on an 8% NaCl diet, these increases were not statistically significant. Our measurements were performed 4 and 8 weeks after increasing dietary NaCl from 1% to 8%. Whether differences between our protocol and that of Greene et al\textsuperscript{34} account for the difference in results in DR rats is unclear. DS rats on a 1% NaCl diet developed mild systolic and diastolic hypertension by 46 weeks of age. Hypertension resulted from an increase in TPR, and both renal and nonrenal resistance increased. CO was significantly decreased ($p<0.05$) in these DS rats, but BV remained unchanged.

Our results demonstrated a dual mechanism for salt-induced hypertension in DS rats. One mechanism involved an increased BV and CO after an 8% NaCl diet was ingested for 4 weeks; after 8 weeks, an increased TPR sustained the hypertension. The second mechanism involved an increased TPR after a 1% NaCl diet was ingested for 46 weeks; CO decreased and BV remained unchanged. The cause of the increased TPR and hypertension in DS rats after prolonged ingestion of 1% NaCl remains to be determined. Abnormal renal hemodynamics play an important role in initiating hypertension in DS rats on a high salt diet. It remains to be determined if renal hemodynamics also play a role in rats on more moderate NaCl intake. The development of hypertension in DS rats on normal rat chow appears to be a more appropriate model for studying salt-sensitive human essential hypertension.

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