Salt-Induced Hypertension in Dahl Salt-Sensitive Rats
Hemodynamics and Renal Responses

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This study was performed with Dahl salt-sensitive (DS) and Dahl salt-resistant (DR) rats to detect differences in cardiovascular hemodynamics and renal responses that might be involved in initiating salt-induced hypertension in DS rats. The effects of 4 weeks of 8% NaCl diet were studied in conscious, male DR and DS rats in which vascular and urinary catheters had been previously implanted. Results were compared with those obtained from control groups of DR and DS rats on 4 weeks of 1% NaCl diet. DR rats on 8% salt diet did not develop hypertension, and cardiac output and blood volume were unchanged; glomerular filtration rate, urinary flow, sodium excretion, and plasma atrial natriuretic factor (ANF) increased. DS rats on 8% salt diet developed hypertension, and cardiac output and blood volume increased; glomerular filtration rate, urinary flow, and sodium excretion did not change, despite an increase in ANF. DS and DR rats on 1% NaCl diet were subjected to ANF infusion. After ANF infusion DR rats had a decreased blood volume and an increased glomerular filtration rate, urinary flow, and sodium excretion; DS rats showed no significant changes in blood volume, glomerular filtration rate, urinary flow, or sodium excretion. ANF caused vasodilation in all regions studied in DR rats; DS rats showed vasodilation in all regions except the kidney. After acute volume expansion, although both DR and DS rats responded by an increase in cardiac output, only DS rats developed prolonged hypertension. This finding suggests an inadequate vasodilatory mechanism in DS rats. In response to acute volume expansion, renal resistance decreased in DR rats but not in DS rats. It is concluded that the primary hemodynamic disturbance in DS rats with salt-induced hypertension is an increase in cardiac output caused by blood volume expansion in the absence of any vasodilation. Comparison of the responses of DS and DR rats to high salt diets, ANF infusion, and acute volume expansion indicates that the salt-induced hypertension in DS rats is initiated by a diminished renal response to ANF. (Hypertension 1989;13:612–621)
in Dahl rats has been attributed to a humoral factor,\textsuperscript{3-8} the mechanism by which DS rats become hypertensive remains to be elucidated. Page\textsuperscript{9} has clearly and concisely reviewed the current knowledge of the role of the kidney and salt metabolism in the production of experimental hypertension. Since transplantation of kidneys from DR rats into hypertensive DS rats can normalize blood pressure and 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\textsuperscript{10} concluded that genetically controlled factors operating primarily through the kidney determine the level of blood pressure. Salt-induced hypertension in DS rats has been attributed to a reduction in the natriuretic capacity of the kidneys.\textsuperscript{11-14} Studies by Azar et al\textsuperscript{15} have shown that hypertensive DS rats have fewer glomeruli and fewer functioning nephrons than DR rats. Jaffe et al\textsuperscript{16} 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\textsuperscript{17} demonstrated that DS rats have fewer renal medullary 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. Data obtained by Hirata et al\textsuperscript{18} from the isolated perfused kidney indicated lower renal papillary plasma flow in DS 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. Others, however, reported that DS rats on a high salt diet have a lower glomerular filtration rate (GFR) despite a higher perfusion pressure as compared with DR rats and that there was a rightward shift of the arterial pressure-GFR curves due to exaggerated afferent arteriolar vasoconstriction in DS rats.\textsuperscript{19,20}

We determined the effects 1) of 4 weeks of high dietary NaCl (8% vs. 1%) on hemodynamics and renal function in DS and DR rats, 2) of atrial natriuretic factor (ANF) infusion, and 3) of acute volume expansion on these parameters in DS and DR rats on 1% NaCl diets. Awake rats were used to avoid any effect of anesthesia on systemic and renal hemodynamics, renal function, and blood volume.

**Materials and Methods**

**Procedures**

Experiments were performed on 37 conscious male DR and DS rats (Brookhaven National Laboratory, Upton, New York) weighing about 250 g. After anesthesia with sodium pentobarbital (35 mg/kg i.p.), a polyethylene catheter (PE-50) was inserted into the abdominal aorta via a femoral artery for blood withdrawal and recording of arterial pressure. A second catheter (PE-50) was advanced into the inferior vena cava (catheter tip located caudal to the renal veins) via a femoral vein for intravenous infusions. A third catheter was advanced transabdominally into the urinary bladder. Catheters were tunneled subcutaneously and exteriorized at the back of the neck and fixed to the skin with sutures. Rats were allowed to recover from this relatively minor surgery for 1 day; they appeared completely awake, normally active, and pain-free at the time of experimentation. During the experiment, the rat was kept unrestrained in a dark box with a small hole through which the three intravascular catheters and the bladder catheter were exteriorized, and cardiovascular pressures were monitored by using Statham transducers and a polygraph recorder (model 7, Grass Instrument Co., Quincy, Massachusetts). Three protocols were used to study the effects of dietary sodium, ANF infusions, and acute volume expansion.

**Protocol 1: Effect of dietary sodium.** Starting at 4 weeks of age, rats ($n=7$ and $n=8$ for DR and DS rats, respectively) were fed rat chow (Ralston Purina Co., Indianapolis, Indiana) containing, on a dry-weight basis, either 1% NaCl (normal salt diet) or...
Protocol 2: Effect of atrial natriuretic factor infusion. Synthetic rat ANF (28 amino acids, Sigma Chemical Co., St. Louis, Missouri) was infused intravenously (70 nmol/kg/min for 30 minutes) into 8-week-old rats (n=5 for DR and for DS rats) that had been maintained since weaning on 1% NaCl diet. Cardiac output, blood flow distribution, plasma ANF levels, red blood cell and plasma volumes, glomerular filtration rate, and sodium concentration were determined.

Protocol 3: Effect of volume expansion. Acute blood volume expansion was performed on 8-week-old normotensive rats (n=6 for DR and for DS rats) that had been maintained after weaning on 1% NaCl diets. Donor blood (obtained from animals of the same species and maintained on identical diets) was infused intravenously (40±6 ml/kg body wt) for 5 minutes to approximately double blood volume. Cardiac output, blood flow distribution, and plasma ANF concentration were measured.

Methods

Hemodynamic parameters. Cardiac output and blood flow distribution were determined by a microsphere method, which had been validated in our laboratory by comparison with electromagnetic flow meter and xenon-133 washout technique. Latex microspheres (15.0±1.0 μm in diameter, New England Nuclear Corp., Boston, Massachusetts) labeled with scandium-46 and suspended in 10% dextran solution (mol wt 78,000) were used. A known amount of microsphere suspension (about 50,000 spheres/kg body wt) was injected into the left ventricle within 10-15 seconds, and the catheter was immediately flushed with 0.7 ml 0.9% NaCl. Reference blood sample was withdrawn from the abdominal aorta at a rate of 0.8 ml/min. At the end of the experiment, rats were killed by injection of a saturated KCl solution into the left ventricle, and tissues were immediately removed. Activity of scandium-46 in various tissues was determined with a gamma counter (Packard 5130, Auto-Gamma System, Packard Instrument Co., Downers Grove, Illinois) connected to a multichannel analyzer (Tracor Northern Co., Middleton, Wisconsin). Resistance to flow in various organs was calculated as the ratio of mean arterial pressure to regional blood flow.

Plasma atrial natriuretic factor levels. Blood samples (2.5 ml each) from the femoral artery were placed in precooled tubes containing EDTA for determination of plasma concentration of ANF. Volume removed during blood sampling was simultaneously replaced by infusion of donor blood. ANF was extracted from plasma with prewashed C18 Sep-Pak cartridges (Millipore Waters, Milford, Connecticut) and measured by radioimmunoassay by means of rat antibodies (ANP kit, Peninsula Lab., Inc., Belmont, California).

Blood volume. Red blood cell volume was determined with red blood cells labeled with chromium-
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**Figure 2.** Bar graphs showing effect of 4 weeks of high salt diet (8% NaCl) on plasma atrial natriuretic factor (ANP) concentration and blood volume of awake salt-resistant (R) and salt-sensitive (S) Dahl rats. Shaded bars are rats on 8% salt diet; clear bars are rats on 1% salt diet. *Significant difference (p<0.05) between 1% and 8% NaCl diets.

ANP (pg/ml)

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Blood Volume (ml)

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**Figure 3.** Bar graphs showing effect of 4 weeks of high salt diet (8% NaCl) on renal hemodynamics and glomerular filtration rate (GFR) of awake salt-resistant (R) and salt-sensitive (S) Dahl rats. Shaded bars are rats on 8% salt diet; clear bars are rats on 1% salt diet. *Significant difference (p<0.05) between 1% and 8% NaCl diets. RBF, renal blood flow.

RBF (ml/min/100gm)

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Renal Resistance (mmHg-sec/ml)

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GFR (ml/min)

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**Results**

Effects of an 8% NaCl diet for 4 weeks (protocol 1) on systemic hemodynamics of DR and DS rats are shown in Figure 1. In comparison with DR rats on 1% salt diet, DR rats on 8% salt diet had a small but significant decrease in total peripheral resistance (p<0.05); the lower value of mean arterial pressure and the slightly higher value of cardiac output were not statistically significant. DS rats on 8% salt diet had an increase in mean arterial blood pressure; cardiac output increased from 121±4 to 205±30 ml/min (p<0.05), and total peripheral resistance remained relatively constant. The increase in cardiac output in DS rats resulted from an increase
Effects of ANP infusion on systemic hemodynamics of DR and DS rats on 1% NaCl diet (protocol 2) are shown in Figure 5. ANP infusion increased cardiac output from 118±9 to 162±6 ml/min (p<0.05) and decreased total peripheral resistance (p<0.05) of DR and DS rats, whereas mean arterial pressure remained relatively constant; hence, a vasodilatory response occurred in both DR and DS rats during ANP infusion. Hematocrit increased (p<0.05) in DR rats subjected to ANP infusion (Table 2) but not in DS rats. This increased hematocrit is a result of decreased (p<0.05) plasma volume in DR rats (Table 2). In DR rats ANP infusion caused a decrease (p<0.05) in blood volume (Figure 6), whereas in DS rats blood volume did not change; plasma levels of ANF increased in both strains (p<0.05). ANF infusion in DR rats also caused marked increases in renal blood flow and GFR, which were accompanied by a decrease in renal resistance (p<0.05) (Figure 7), but these responses were not found in DS rats. Urinary flow and sodium excretion also increased in DR (p<0.05) but not in DS rats (Figure 8). The control values of flow resistance are summarized in Table 3. ANF caused vasodilation (decreased resistance) in all
EFFECTS OF ANP INFUSION IN RATS ON 1% NaCl DIET

FIGURE 6. Bar graphs showing effect of 70 nmollkg/min atrial natriuretic factor (ANP) infusion for 30 minutes on plasma ANP concentration and blood volume of awake salt-resistant (R) and salt-sensitive (S) Dahl rats on 1% NaCl diet. Shaded bars are rats receiving ANP infusion; clear bars are control rats receiving saline infusion. *Significant difference (p<0.05) between control and ANP infusion.

Discussion
The objective of this study was to investigate the initiating mechanism of salt-induced hypertension in conscious DS rats. The results indicate that initiation of hypertension induced by high salt intake for 4 weeks in DS rats is due to an increased cardiac output resulting from an expansion of blood volume.

Ganguli et al.31 compared hemodynamics in anesthetized female DS and DR rats on 0.3% or 8% NaCl diets for 3 days or 7 days. After 3 days on 8% NaCl diet, cardiac output was increased in both DR (+18%) and DS (+10%) rats; peripheral resistance was decreased (-14%) in DR rats but increased (+10%) in DS rats. Blood pressure remained unchanged in DR rats but increased (+20%) in DS rats. After 7 days on 8% NaCl diet, cardiac output was not increased in DS or DR rats. Peripheral resistance remained relatively constant in DR rats on either 0.3% or 8% NaCl diet for 7 days but was elevated (+12%) in DS rats on 8% NaCl diet for 7 days.

We found that conscious male DS rats on 8% NaCl diet for 4 weeks had a significant increase in blood volume, which agrees with the findings of Pamnani et al.32 and Overbeck et al.33 Since no significant change in total peripheral resistance occurred, the increase in blood volume and the accompanying increase in cardiac output were responsible for initiating the hypertension. DR rats on 8% NaCl diet displayed a significant decrease in total peripheral resistance with insignificant changes.
in cardiac output and blood pressure. The reasons for these differences from the results of Ganguli et al\textsuperscript{31} are not clear but may in part be due to differences in experimental conditions or animals (anesthesia, differences in duration of high salt ingestion, or sex differences of the rats). Extension of our observations revealed that at 8 weeks, cardiac output had returned to normal, whereas peripheral resistance became greatly increased and blood volume remained elevated in DS rats on 8\% NaCl diet.\textsuperscript{32}

Hypertension that developed in DS rats on an 8\% NaCl diet may be a direct result of the expansion in blood volume; however, Ferrari et al\textsuperscript{35} demonstrated an impairment in cardiopulmonary baroreceptor reflex modulation of the sympathetic nervous system (during volume expansion) that may predispose to development of hypertension in DS rats during a high sodium diet. Our results indicate that acute volume expansion caused hypertension in DS rats but not in DR rats on 1\% NaCl diet and that compensatory vasodilation, which prevented hypertension in DR rats, is defective in DS rats. An absence of compensatory adjustment in cardiac baroreceptor reflex function in DS rats\textsuperscript{36} might also contribute to this defective response to acute volume expansion. Whether a circulating hormonal factor plays a role in the genesis of salt-induced hypertension (as suggested by parabiotic studies between DS and DR rats) is uncertain. Evidence suggests that an ouabain-like substance, which has been implicated as a causative factor in some forms of experimental volume-expanded hypertension, is not present in salt-induced hypertension in DS rats; there was no impairment of the Na\textsuperscript{+</sub>}-K\textsuperscript{+} pump activity.\textsuperscript{32,33} Bioassay, using tail artery ruthenium-86 uptake technique, revealed no evidence of ouabain-like substance in plasma of volume-expanded DS rats on high NaCl diet (F. Haddy, oral communication). Abel and coworkers\textsuperscript{37} found no difference in the contractile sensitivity to norepinephrine or serotonin in the caudal artery of DS and DR rats whether or not they were fed 8\% NACl diets; furthermore, using electron-probe analysis, they reported no difference in intracellular potassium, sodium, and chloride content between DR and DS rats. They concluded that arterial muscle membrane mechanisms are unaltered in genetically hypertensive DS rats.

We found a striking difference between DS and DR rats in the response of the kidney; DS rats did not show the decrease in renal vascular resistance observed in DR rats on high salt intake or during acute volume expansion. In contrast, renal resistance was inappropriately elevated in DS rats on high salt-diet. Furthermore, DS rats did not show the increases in GFR, urinary flow, and sodium excretion in response to high salt diet, which were observed in DR rats. Thus, the kidneys of DR rats respond to volume expansion by vasodilation, whereas this renal vasodilation is absent in DS rats. Ingestion of 8\% NaCl diet caused plasma ANF concentrations to increase significantly in DS and DR rats. This finding suggests that the secretory mechanism of ANF in the heart is functioning normally in both strains; this is in agreement with the observations of Tanaka and Inagami.\textsuperscript{38} Our
findings that the kidneys of DS rats have a reduced response to synthetic ANF with respect to renal blood flow, GFR, diuresis, and natriuresis are in agreement with the results reported by Hirata et al\textsuperscript{18} in which crude atrial extracts from DS rats also showed reduced response. In our experiments, ANF caused vasodilation in all regions studied in DR rats, but in DS rats the kidney was the one organ that did not vasodilate. These results indicate that abnormal renal function in DS rats, including a reduced responsiveness to humoral factors such as ANF, may play an important role in initiating salt-induced hypertension. Although DS rats might also exhibit decreased renal vascular or renal functional responses to other agents, this possibility has not been tested; however, acute volume expansion suggested an impaired capacity of the kidneys of normotensive DS rats to vasodilate in contrast to a compensatory vasodilation in DR rats. Sterzel et al\textsuperscript{39} were unable to demonstrate differences between DS and DR rats in response to ANF; however, they administered ANF by intravenous bolus rather than by continuous infusion. Whether these differences in experimental design account for this disagreement with our results as well as those of Hirata et al\textsuperscript{18} is unclear.

In addition to a defective hemodynamic response to humoral factors such as ANF, studies by Appel and Dunn\textsuperscript{40} on ANF receptors and the response of cyclic guanosine monophosphate (GMP) to ANF provide evidence that there may be a tubular defect in the kidney of DS rats; this response may partially be due to a deficient activity of guanulate cyclase in the papillary collecting duct, resulting in an inadequate production of cyclic GMP. Hinko et al\textsuperscript{41} reported that the ANF receptor is likely to be different in DS and DR strains since the rate of dissociation of ANF from its renal receptor was decreased by high NaCl intake in DR rats, whereas no change occurred in DS rats.

Our results support the findings of others\textsuperscript{12,42} that hypertension that develops in response to a high salt diet results, at least partly, from an inability of
the kidney to vasodilate and to increase sodium and water excretion. Our findings and those of Hirata et al. indicate that a diminished renal response to ANF in DS rats may be responsible for this impaired sodium and water excretion. Since DS rats can liberate ANF appropriately after volume expansion, it remains to be determined if there is an abnormality in the number of function of ANF renal receptors.

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**KEY WORDS** • atrial natriuretic factor • cardiac output • glomerular filtration rate • renal blood flow

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