Handling $^{22}\text{NaCl}$ by the Blood-Brain Barrier and Kidney
Its Relevance to Salt-Induced Hypertension in Dahl Rats

Shlomoh Simchon, William Manger, Eugene Golanov, Jacob Kamen, George Sommer, Chris H. Marshall

Abstract—We previously reported that inappropriate renal vasoconstriction in Dahl salt-sensitive (DS) rats fed high NaCl diets may cause sodium retention. The present study examined the distribution and elimination of $^{22}\text{Na}$ in DS and Dahl salt-resistant (DR) rats, and we determined whether an abnormality in renal function might also cause sodium retention in DS rats. Following an intravenous bolus of 4 $\mu$Ci $^{22}\text{NaCl}$ in prehypertensive DS and DR rats with similar blood pressures on low (0.23%) or high (8% for 4 days) NaCl diets, urinary clearance of $^{22}\text{Na}$ in 1 hour was about 4 times less in DS than DR rats, and renal retention of $^{22}\text{Na}$ was up to 8 times greater in DS than DR rats ($P<0.01$), suggesting that a renal functional defect may contribute to salt retention in DS rats; however, its uptake in tail artery, heart, lungs, liver, and spleen was similar in DS and DR rats. Uptake in brain was up to 5 times greater in DS than DR rats ($P<0.01$). Cerebrospinal fluid $^{22}\text{Na}$ radioactivity (in counts per minute) revealed that the blood-brain barrier is 5 to 8 times more permeable to sodium in DS than DR rats ($P<0.01$). Cerebrospinal fluid volume and brain water content increased significantly ($P<0.01$) in DS but not DR rats on an 8% NaCl diet. Intracerebroventricular bolus injection of 0.06 mL of 4.5 mol/L NaCl acutely and transiently induced the same degree of hypertension in DR and DS rats, whereas similar volume injections of isotonic saline, 4.5 mol/L Na-acetate, or 4.5 mol/L NaBr did not produce hypertension in either strain. We conclude that functional abnormalities in DS rat kidneys may cause retention of NaCl and that an increased blood-brain barrier permeability to NaCl may enhance its access to sites in the brain that are then activated and induce hypertension. (Hypertension. 1999;33[part II]:517-523.)

Key Words: brain ■ cerebrospinal fluid ■ hemodynamics ■ ventricles, cerebral ■ kidney ■ rats, Dahl

The mechanism of NaCl-induced hypertension in Dahl salt-sensitive (DS) rats and in salt-sensitive humans is unclear. Yet considerable evidence suggests that the renal abnormality that causes blood pressure (BP) elevation in acquired and inherited hypertension may be associated with a diminished sodium excretion.1,2 The cause of renal hemodynamic and/or functional abnormalities in DS rats that might play a role in the development of salt-sensitive hypertension is unknown; however, a number of biochemical derangements that might impair renal function have been implicated, including deficiency in the generation of cyclic GMP,3 20-hydroxyeicosatetraenoic acid,4 kallikrein,5 prostaglandin E2,6 or dopamine.7 It has also been postulated that hypertension develops in DS rats because of a decrease in nitric oxide production.8,9

DS rats appear to have a genetic functional derangement in the kidney that causes salt retention10 rather than a deficit of nephrons that Brenner et al11–13 postulated as a cause of hypertension. Although evidence suggests that sodium chloride (NaCl) is retained by kidneys of DS rats,14–17 accumulation of sodium in blood or tissues has not been conclusively demonstrated.

The DS rat is an excellent animal model for study of the mechanism of salt-induced hypertension. The moderate hypertension that develops after several months of a 1% NaCl diet may be the most appropriate model to study, because the hypertension results from increased total peripheral resistance (TPR) without detectable blood volume expansion,16,18 as occurs in most humans with essential hypertension. An 8% NaCl diet causes rapid onset of severe hypertension initially because of blood volume expansion and elevated cardiac output (CO); however, CO rapidly returns to normal and hypertension is maintained by increased TPR, even though hypervolemia persists.16,18

Our major objective was to determine how $^{22}\text{NaCl}$ is handled in prehypertensive DS and Dahl salt-resistant (DR) rats on a low NaCl (0.23%) diet before hemodynamic or structural abnormalities related to hypertension have occurred. Furthermore, we studied the effects of hypertension induced by 8% NaCl on the handling of $^{22}\text{NaCl}$ by the blood-brain barrier (BBB). We also investigated the BP effects of acute injection of hypertonic NaCl or other sodium compounds into cerebrospinal fluid (CSF) of DS and DR rats to determine any difference in BP response between prehypertensive DS and DR rats on a low NaCl diet.

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Materials and Methods

Animals and Procedures

Ninety-five DR and 95 DS male rats (Brookhaven National Laboratory strain obtained from Harlan Sprague Dawley Inc, Indianapolis, Ind) were fed a low salt diet (0.23% NaCl) (Harlan Teklad) from weaning. Dietary sodium content was confirmed in our laboratory. All rats were housed in an animal facility with controlled temperature and lighting and were used according to National Institutes of Health and New York University Medical Center guidelines for animal care. Studies were performed on 3 groups of rats on the following diets: group A, 44 DR and 44 DS rats fed a 0.23% NaCl diet until 6 weeks of age; group B, 23 DR and 23 DS rats fed a 0.23% NaCl diet until 6 weeks of age and then fed an 8% NaCl diet for 4 days; and group C, 23 DR and 23 DS rats fed a 0.23% NaCl diet until 6 weeks of age and then fed an 8% NaCl diet for 3 or 4 weeks.

Five DR and 5 DS rats were used for a preliminary study to detect any deterioration of rats during experiments. BPs were measured at regular intervals by the indirect tail-cuff method (Narco Biosystems). Rats from groups A and B were nonmedicative before experimental protocols.

Experimental Protocols

For each rat group, 6 different protocols were conducted.

Protocol 1

In protocol 1, we investigated 22Na uptake in different organs. Fifteen DR and 15 DS rats (5 DR and 5 DS rats from each group) were anesthetized with sodium pentobarbital (35 mg/kg IP, usually adequate for Dahl rats; supplemental doses used as required), and a polyethylene catheter (PE-240) was inserted into an incision into the trachea to ensure stable ventilation. A second catheter (PE-50) was inserted into a femoral artery and connected to a Statham transducer and a polygraph recorder (model 7, Grass Instrument Co) for recording of arterial pressure. A third catheter (PE-50) was inserted into the femoral vein for intravenous injections. A dose of 4 μCi 22NaCl (NEI Research Products) in 0.4 mL saline was injected intravenously, and 0.2-mL blood samples were taken every 15 minutes to determine 22Na radioactivity in counts per minute (cpm) in plasma. Fifty-five minutes after the first injection, 10 μCi 125I-labeled albumin (University of Missouri, Research Reactor Facility) in 0.1 mL saline was injected intravenously. Five minutes after the injection of 22NaCl, rats were decapitated and blood was collected for estimation of 22Na radioactivity. Several tissues (including part of the tail artery, lungs, and liver as well as the entire heart, spleen, kidney, and brain) were removed and weighed and their 22Na radioactivity determined. 22Na and 125I radioactivities were measured in plasma and tissues with a gamma counter (well counter) connected to a multichannel analyzer. The well counter is a scintillation crystal detector formed with a central well into which the samples are inserted and their radioactive emission counted. The major advantage of this arrangement is the increased counting efficiency that results from surrounding the sample with the detector. The well counter was connected to a PCA-P computer-based program with a PCA-P card, which permits collection of radioactive data (Oxford Instruments Inc) and is installed in an IBM-compatible personal computer. The radioactivity of each isotope is corrected for any overlapping of isotopes. Relative uptake, ie, tissue cpm/plasma cpm, of 22NaCl (60 minutes after injection) to relative uptake of 125I (5 minutes after injection) was determined. If the ratio 22Na/125I equals 1, it would indicate that the same volume is occupied by the 2 isotopes and that the 55 minutes difference in time of injection did not affect their distribution. If this ratio is greater than 1, it would indicate the degree of tissue uptake of 22Na. The radioactivity of 22Na in plasma was always determined as the average between the extrapolated counts per minute at zero time and the counts per minute 60 minutes after injection of 22NaCl.

Protocol 2

In protocol 2, we investigated the passage of 22Na across the BBB, its uptake by the brain, urinary clearance, and renal retention. We also measured plasma volume with 125I-albumin. Fifteen DR and 15 DS rats (5 DR and 5 DS rats from each group) were anesthetized with sodium pentobarbital (35 mg/kg IP), and catheters were placed as in protocol 1. A fourth catheter (PE-190) was placed transabdominally into the urinary bladder and secured so that all urine was collected. The rat’s head was placed in a stereotactic apparatus, and the dorsal surface of the skull was shaved. After a small scalpel incision, a hole (2 mm diameter), 1 mm caudal and 1.5 mm lateral to the bregma, was made in the skull with a dental drill, and a stainless steel cannula (internal diameter: 1 mm; length, 5.5 mm) was inserted into the left lateral ventricle. Doses of 4 μCi 22NaCl in 0.4 mL saline and 4 μCi 125I-labeled albumin (radioiodinated 125I serum albumin, Mallinkrodt, Inc) in 0.2 mL saline were injected intravenously. Sixty minutes after injection, samples of blood and CSF were taken to determine 22Na and 125I activities (in counts per minute) in plasma and CSF. After rats were decapitated, the brain was removed, weighed, and 22Na and 125I activities determined. 22Na and 125I-albumin activities were measured in plasma, CSF, and tissues as in protocol 1. Passage of 22NaCl across the BBB was calculated from its counts per minute in CSF and plasma and expressed as NaCl cpm in 1 g brain/NaCl cpm in 1 mL plasma. Plasma volume was determined from 125I-labeled albumin and calculated as total 125I cpm injected/125I cpm in 1 mL plasma and expressed per 100 g body wt. Brain uptake was calculated as 22Na cpm in 1 g brain/22Na cpm in 1 mL plasma. Urine was collected for 1 hour, starting 15 minutes after injection of 22NaCl, and 22Na radioactivity in urine was determined. For further study of urinary clearance, plasma counts per minute of 22Na was determined 15, 30, 45, and 60 minutes after 22NaCl injection. Urinary clearance (milliliters plasma per hour) was calculated as total 22Na cpm in urine during 60 minutes collection/22Na cpm in 1 mL plasma. Renal retention was calculated as 22Na cpm in 1 g kidney/22Na cpm in 1 mL plasma.

Protocol 3

In protocol 3, we injected 22NaCl and 125I-labeled albumin into the left cerebral ventricle to measure CSF volume and uptake in brain. Nine DR and 9 DS rats (3 DR and 3 DS rats from each group) were anesthetized with sodium pentobarbital (35 mg/kg IP, usually adequate for Dahl rats; supplemental doses used as required), and a polyethylene catheter (PE-240) was inserted through an incision into the femoral vein for intravenous injections. A dose of 4 μCi 22NaCl (NEI Research Products) in 0.4 mL saline was injected intravenously, and 0.2 mL blood samples were taken every 15 minutes to determine 22Na radioactivity in counts per minute (cpm) in plasma. Fifty-five minutes after the first injection, 10 μCl 125I-labeled albumin (which does not penetrate cells or enter the circulation) in 0.1 mL saline were injected into the cerebral ventricle. Sixty minutes after injection, samples of CSF were taken to determine 22Na and 125I activities (in counts per minute) in CSF. Volume distributions of 22NaCl and 125I-labeled albumin in CSF were calculated from counts per minute in CSF and total counts per minute injected and expressed as total 22Na cpm injected/22Na cpm in 1 mL CSF and total 125I cpm injected/125I cpm in 1 mL CSF, respectively.

Protocol 4

In protocol 4, we injected hypertonic NaCl, Na-acetate, and NaBr solutions into the left cerebral ventricle. Twenty-one DR and 21 DS rats from group A were prepared as in protocol 2, and 60 μL of 4.5 mol/L NaCl, Na-acetate, or NaBr was injected into the left lateral ventricle. BP and heart rate were monitored through a femoral artery as described in protocol 1.

Protocol 5

In protocol 5, we studied systemic, renal, and cerebral hemodynamic changes in 15 DR and 15 DS rats (5 DR and 5 DS rats from each group) using a transducer and polygraph as indicated in protocol 1. CO (milliliters per minute) and blood flow distribution (milliliters per minute per 100 g tissue) were determined by a microsphere method previously validated in our laboratory by comparison with electromagnetic flowmeter and 133Xe washout techniques. The brain, kidney, and heart and a section of thoracic aorta were immediately removed. 85Sc radioactivity in tissues was determined with a gamma counter (Packard Instrument Co) connected to a multichannel analyzer (Tracer Northern Co). TPR was calculated as the ratio of mean arterial BP (MAPB) to CO. Renal vascular resistance (RVR), coronary vascular resistance, or...
cerebral vascular resistance (CVR) was calculated as the ratio of MABP to renal, coronary, or cerebral blood flow, respectively.

Protocol 6
In protocol 6, we studied kidney and brain water content in 15 DR and 15 DS rats (5 DR and 5 DS rats from each group), calculated from the difference between their wet and dry weights after placement in an oven at 50°C for 24 hours, a period sufficient for drying.

Statistical significance of changes was evaluated by analysis of variance, followed by the Student-Newman-Keuls test for multiple comparisons.

Results
A preliminary study, to detect any deterioration of rats during experiments, revealed that 2 hours after anesthesia induction, intubation, and surgical procedures described in the protocols, arterial blood gases and pH were within normal ranges: PO₂ from 80 to 90 mm Hg, PCO₂ from 38 to 40 mm Hg, and pH from 7.34 to 7.40. Blood gases were determined with a blood gas analyzer.

Protocol 1
MABP in DS rats fed an 8% NaCl diet for 3 weeks increased significantly (P<0.01) and was accompanied by an increase in plasma volume (P<0.01), whereas DR rats on the same diet remained normotensive without any increase in plasma volume (Table 1). After an intravenous bolus of 22NaCl and 26NaCl to 6-week-old prehypertensive DS and DR rats with similar BPs and renal hemodynamics on low (0.23%) or high (8% for 4 days) NaCl diets, as well as to hypertensive, hypervolemic DS rats fed an 8% NaCl diet for 3 weeks, renal tissue 26Na retention, calculated from the relative volume distribution of 22NaCl (60 minutes after injection) to 26NaCl (5 minutes after injection), was 6 to 8 times greater (P<0.01) in DS than DR rats. One hour after an intravenous bolus of 22NaCl, its uptake in tail artery, heart, lungs, liver, and spleen was similar in DS and DR rats, but its uptake in brain was about 3 to 5 times greater (P<0.01) in DS than DR rats (Table 1).

Protocol 2
Studies of 22Na excretion and its urinary clearance in rats fed 0.23% or 8% NaCl diets (after 4 days or 3 weeks) revealed a progressive and markedly greater renal retention of 22Na and markedly less urinary clearance of 22Na in DS than DR rats (P<0.01), whereas urinary clearance of 22Na increased markedly (P<0.01) in DR rats, without any renal retention (Figure 1). Increased permeability of the BBB to 22NaCl caused 5 to 8 times greater (P<0.01) accumulation of 22Na in the CSF and about 3 to 5 times greater uptake in the brain of normotensive DS than DR rats on 0.23% and 8% (4 days) NaCl diets (Figure 1). When the 8% NaCl diet was extended for 4 weeks, severe hypertension developed in DS rats and 22Na accumulation in CSF doubled when compared with normotensive DS rats; however, uptake in brain did not increase significantly more but remained greater (P<0.01) in DS than DR rats. Figure 2 reveals the rate of decrease in plasma 26Na radioactivity in normotensive DR and DS rats fed 0.23% NaCl or 8% NaCl for 4 days. In DR rats fed 0.23% NaCl, plasma 22Na radioactivity decreased at a rate of 166 (cpm/g plasma)/min, determined from the slope of the graph; whereas in DR rats fed an 8% NaCl diet, 22Na decreased at a greater rate of 244 (cpm/g plasma)/min (P<0.01). In DS rats fed 0.23% NaCl, 22Na decreased at a rate of only 52 (cpm/g plasma)/min and at only a slightly but not significantly greater rate of 83 (cpm/g plasma)/min after an 8% NaCl diet, indicating sodium retention in plasma in DS compared with DR rats.

Protocol 3
Table 2 shows that the volume of CSF determined by dilution of 22NaCl and 125I-albumin was similar in normotensive DS and DR rats on 0.23% NaCl. After an 8% NaCl diet for 4

<table>
<thead>
<tr>
<th>TABLE 1. MABP, Plasma Volume, and Relative Uptake of 22Na to 24Na in Various Organs in DR and DS Rats on Low and High NaCl Diets (Protocol 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.23% NaCl Diet</strong></td>
</tr>
<tr>
<td><strong>MABP, mm Hg</strong></td>
</tr>
<tr>
<td>98.5±3.8</td>
</tr>
<tr>
<td><strong>PV, mL/100 g</strong></td>
</tr>
<tr>
<td>8.5±0.6</td>
</tr>
<tr>
<td><strong>Relative Ratios of 22Na in 60 min/24Na in 5 min</strong></td>
</tr>
<tr>
<td>Red blood cells</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Brain</td>
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<tr>
<td>Kidney</td>
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<td>Liver</td>
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<tr>
<td>Tail artery</td>
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<tr>
<td>Lung</td>
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<tr>
<td>Spleen</td>
</tr>
</tbody>
</table>

PV indicates plasma volume determination from protocol 2 expressed as milliliters per 100 g body wt.

Relative ratios = CPM 22Na in g tissue / CPM 22Na in mL plasma

*P<0.01, DS vs DR rats.
days, MABP remained normotensive, but CSF volume doubled in DS rats and did not change in DR rats (Table 2). When 8% NaCl was continued for 4 weeks, severe hypertension (182 ± 3 mm Hg) developed and CSF increased further (P<0.01) to slightly more than 5 mL (Table 2). The volumes of CSF determined by 22NaCl were similar to those determined by 125I-albumin, suggesting no significant brain uptake of 22NaCl within 1 hour after injection into CSF.

Protocol 4
Acute intracerebroventricular (ICV) injection of a 0.06-mL bolus of 4.5 mol/L NaCl caused significant (P<0.001) similar elevations of BP in DS and DR rats; however, injection of 0.06 mL isotonic saline or 4.5 mol/L Na-acetate or NaBr caused no change in BP. Figure 3 shows that the rate and magnitude of BP increase after a bolus injection of 4.5 mol/L NaCl was similar in DS and DR rats; however, heart rate increased more slowly in DS than DR rats.

Protocol 5
After a 0.23% NaCl diet, BP, CO, and TPR were similar in DS and DR rats (Figure 4). After an 8% NaCl diet for 4 days, although BP in DS and DR rats remained similar and normotensive, CO increased significantly and TPR decreased significantly (P<0.01) in DS rats, whereas CO and TPR remained unchanged in DR rats. RVR and CVR (Figure 4) were similar in DS and DR rats on 0.23% NaCl diets. After an 8% NaCl diet for 4 days, RVR and CVR decreased significantly (P<0.01) in DR rats but remained unchanged in DS rats. When rats ingested an 8% NaCl diet for 4 weeks, RVR and CVR decreased further (P<0.01) in DR rats, whereas they increased significantly (P<0.01) in DS rats.

There was no apparent change or difference in coronary vascular resistance between DR (13.51 ± 1.7 [mm Hg/mL]/s per 100 g heart) and DS (11.51 ± 3.5 [mm Hg/mL]/s per 100 g heart) rats.

**TABLE 2. MABP and CSF Volume Determined 1 Hour After Injecting 22NaCl or 125I-Albumin Into Lateral Cerebral Ventricle of Brookhaven DS and DR Rats Fed Low or High NaCl Diets (Protocol 3)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>MABP, mm Hg</th>
<th>Vol 22NaCl, mL</th>
<th>Vol 125I-albumin, mL</th>
<th>22NaCl/125I‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.23% NaCl</td>
<td>101 ± 3</td>
<td>2.12 ± 0.04</td>
<td>1.89 ± 0.25</td>
<td>1.12 ± 0.01</td>
</tr>
<tr>
<td>8% NaCl, 4 days</td>
<td>96 ± 1</td>
<td>2.14 ± 0.05</td>
<td>2.25 ± 0.15</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>8% NaCl, 4 weeks</td>
<td>108 ± 3</td>
<td>2.13 ± 0.07</td>
<td>1.91 ± 0.18</td>
<td>1.11 ± 0.01</td>
</tr>
</tbody>
</table>

Results are mean ± SD. Volume CSF (mL) calculated as total 22Na radioactivity (cpm) injected/22Na radioactivity (cpm) in 1 mL CSF and total 125I radioactivity (cpm) injected/125I radioactivity (cpm) in 1 mL CSF, respectively.

**Figure 1.** Urinary 22Na clearance and renal retention (top panels) and 22Na in CSF and brain (bottom panels) 1 hour after IV injection of 22NaCl in DS and DR rats on low and high NaCl diets (Protocol 2). Urinary 22Na clearance = 22Na in urine collected in 60 minutes/22Na in mL plasma; renal 22Na retention = 22Na in g kidney/22Na in mL plasma; CSF 22Na uptake = 22Na in g CSF/22Na in mL plasma; brain 22Na uptake = 22Na in g brain/22Na in mL plasma. Data are mean ± SD.

**Figure 2.** 22Na radioactivity (CPM)/g plasma at various time intervals after intravenous injection of 22NaCl in DS and DR rats (protocol 2). Data are mean ± SD.

**Figure 3.** Urinary 22Na clearance and renal retention (top panels) and 22Na in CSF and brain (bottom panels) 1 hour after ICV injection of 22NaCl in DS and DR rats (Protocol 4). Urinary 22Na clearance = 22Na in urine collected in 60 minutes/22Na in mL plasma; renal 22Na retention = 22Na in g kidney/22Na in mL plasma; CSF 22Na uptake = 22Na in g CSF/22Na in mL plasma; brain 22Na uptake = 22Na in g brain/22Na in mL plasma. Data are mean ± SD.

**Figure 4.** Urinary 22Na clearance and renal retention (top panels) and 22Na in CSF and brain (bottom panels) 1 hour after ICV injection of 22NaCl in DS and DR rats (Protocol 5). Urinary 22Na clearance = 22Na in urine collected in 60 minutes/22Na in mL plasma; renal 22Na retention = 22Na in g kidney/22Na in mL plasma; CSF 22Na uptake = 22Na in g CSF/22Na in mL plasma; brain 22Na uptake = 22Na in g brain/22Na in mL plasma. Data are mean ± SD.

* DS from DR rats; ** from rats on 0.23% NaCl; ‡ from rats on 8% NaCl 4 days (P<0.01)
Protocol 6
Dry and wet weights of brain and kidneys after a 0.23% NaCl diet and 8% NaCl diet for 4 days were similar in DS and DR rats; however, brain and kidney water contents increased in DS rats on an 8% NaCl diet for 4 weeks ($P<0.01$). In DR rats water content of the brain did not change significantly (Figure 5).

Discussion
The present study suggests that both a renal functional defect and an inappropriate renal vasoconstriction, which occurs when DS rats are placed on a high NaCl diet, are responsible for salt retention. Other experimental studies provide evidence suggesting that the kidney retains NaCl in DS rats.$^{14-16}$

It appears that the BBB is considerably more permeable to $^{22}$NaCl in DS than in DR rats, resulting in a significantly greater increase in CSF and brain $^{22}$Na in DS than in DR rats. Furthermore, CSF $^{22}$Na was found to be significantly more elevated in DS rats on an 8% NaCl diet for 4 days than on 0.23% NaCl even though DS rats remained normotensive. It is uncertain whether increased CO in DS rats on this high salt diet for 4 days enhanced passage of $^{22}$NaCl across the BBB. The fact that CSF volume significantly expanded after 8% NaCl suggests that accumulation of NaCl in CSF was accompanied by fluid retention in DS rats. The latter finding is also consistent with the occurrence of increased fluid in the brain of these DS rats (Figure 5). Increased renal sodium retention was also accompanied by fluid retention in kidneys of DS rats on 8% NaCl for 4 weeks.

Nakamura and Cowley$^{22}$ demonstrated that hypertensive DS rats fed a high salt diet exhibited a greater CSF concentration of sodium than DR rats; however, since the increase in CSF sodium followed the onset of hypertension, they concluded that this increase in sodium did not initiate NaCl-induced hypertension but might perpetuate it. After a high NaCl diet, plasma and CSF volume expansion might obscure an increase in total sodium content if only sodium concentration is determined. We observed that after an 8% NaCl diet, hypertension in DS rats was initiated by blood volume expansion and increased CO but was perpetuated by increased TPR,$^{16}$ whereas prolonged ingestion of 1% NaCl caused hypertension by increasing TPR.$^{18}$ Conceivably, increased access of NaCl to the brain of DS rats may be responsible for the increased TPR.

ICV administration of hypertonic NaCl solutions produces an increase in arterial BP.$^{23-26}$ Furthermore, lesions in the anteroventral third ventricle abolish the acute hypertensive response of ICV NaCl and also prevent or attenuate development of salt-induced hypertension in rats on high NaCl diets.$^{27,28}$ It is noteworthy that vasopressin injections blocked about half the antihypertensive effect of anteroventral third ventricle lesions in DS rats fed an 8% NaCl diet.$^{28}$ Access of increased concentrations of NaCl to the brain may occur in DS rats ingesting excess NaCl and might activate the sympathetic nervous system or some other pressor system and increase TPR and BP. Leenen et al$^{29}$ suggested that ouabain-like activity in the brain may mediate a sympathoexcitatory and hypertensive response to excess NaCl consumption in DS rats. Acute ICV injection of 1.5 mol/L NaCl in anesthetized dogs caused hypertension and tachycardia that coincided with
elevation of plasma catecholamines; however, part of the pressor effect may be due to nonneurogenic activation, eg, secretion of vasopressin.\textsuperscript{25} Finally, in addition to effects on the brain, it is conceivable that chronic ingestion of excess NaCl by DS rats may alter vascular smooth muscle and in some way increase vasoconstriction and TPR.

Our finding that acute ICV injection of 4.5 mol/L NaCl caused a rapid similar rise in BP of DS and DR rats suggests that there is no difference between the sensitivity of the brain of DS and DR rats to stimulation by NaCl. Ikeda et al.\textsuperscript{26} reported that an acute ICV bolus injection of 2 μL of 0.3 mol/L NaCl induced a slight but significant transient elevation of BP that was slightly greater in DS than DR rats. We found no change in BP in DS or DR rats when we repeated the bolus injection used by Ikeda et al.\textsuperscript{26} Explanation for these differences in results is unclear. Our findings indicate that the brain responds specifically to stimulation by NaCl and not to other sodium salts. It is noteworthy and perhaps relevant that only increased dietary NaCl causes hypertension; addition of other sodium compounds to the diet does not elevate BP.\textsuperscript{30,31} The rapid increase in BP after acute ICV injection of 4.5 mol/L NaCl suggests that the sympathetic nervous system may have been activated to cause similar hypertension in DS and DR rats. The reason for a less rapid increase of heart rate in DS rats remains unclear. Miyajima and Buțug\textsuperscript{32,33} suggested that chronic infusion of hypertonic sodium into the third ventricle of Sprague-Dawley rats reduces hypothalamic inhibition of sympathetic vasomotor tone and impairs the baroreflex, which then elevates BP.

Increased \(^{22}\text{Na}\) uptake by the brain of DS rats appeared to result from increased permeability of the BBB to sodium, whereas renal \(^{22}\text{Na}\) retention resulted from impaired excretion of \(^{22}\text{Na}\). Uptake was similar in all other tissues studied in DS and DR rats. It seems unlikely that an abnormality of NaCl uptake in the arteries or the various organs studied is involved in the development of salt sensitivity of DS rats ingesting excessive amounts of NaCl. However, our studies could not determine whether there was any difference between an intracellular and extracellular location of \(^{22}\text{Na}\) in DS and DR rats.

Of particular interest was the finding that despite a decrease in TPR, resistance in renal and cerebral arteries of DS rats did not decrease when they ingested an 8% NaCl diet for 4 days. In contrast, RVR and CVR decreased significantly in DR rats on the same diet. After 4 weeks of 8% NaCl, RVR and CVR increased significantly in DS rats, whereas both resistances decreased significantly in DR rats. These results suggest that an abnormality in the vascular response to excess NaCl ingestion may be confined to renal and cerebral vessels of DS rats.

In summary, intravenous administration of \(^{22}\text{Na}\) to normotensive DS and DR rats on a 0.23% NaCl diet revealed similar \(^{22}\text{Na}\) uptake in the tail artery, aorta, heart, lungs, liver, and spleen. Renal retention of \(^{22}\text{Na}\) occurred in DS but not DR rats, and urinary clearance of \(^{22}\text{Na}\) was 4 times less in normotensive DS than DR rats, indicating impaired renal function, possibly due to increased tubular reabsorption of \(^{22}\text{Na}\) in DS rats, in agreement with Roman and Kaldunski\textsuperscript{15} and Sterzel et al.\textsuperscript{17} Increased permeability of the BBB caused significantly greater accumulation of \(^{22}\text{Na}\) in CSF and brain of DS than DR rats. Ingestion of 8% dietary NaCl for 4 days did not alter the normotensive BP, but CSF volume doubled in DS rats without changing brain water content. After 4 weeks on 8% NaCl, DS rats were markedly hypertensive and CSF volume increased further and was accompanied by an increased brain water content. Acute ICV administration of 4.5 mol/L NaCl induced a similar rapid, transitory hypertension and a tachycardia in both DS and DR rats, whereas other sodium compounds did not elevate BP or heart rate. An 8% NaCl diet for 4 days caused an inappropriate response of renal and cerebral arteries in DS rats. Unlike the decrease in vascular resistance occurring in DR rats, CVR and RVR did not decrease in DS rats. When rats ingested 8% NaCl for 4 weeks, CVR and RVR decreased further in DR rats, whereas these resistances increased significantly in DS rats.

In conclusion, it is hypothesized that a functional genetic abnormality of the kidneys causes impairment of NaCl excretion and inappropriate renal vasoconstriction in DS rats ingesting excess NaCl. Impairment of glomerular filtration rate and/or tubular function may also account for decreased NaCl excretion. This then causes retention of NaCl, which accumulates in CSF of DS rats because of an increased permeability of the BBB to NaCl. Increased access of NaCl to the brain may then activate the sympathetic nervous system and/or other pressor systems, which may cause and perpetuate hypertension.

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References

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