Enhanced sympatheexcitatory and pressor responses to central Na\(^+\) in Dahl salt-sensitive vs. -resistant rats

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Enhanced sympatheexcitatory and pressor responses to central Na\(^+\) in Dahl salt-sensitive vs. -resistant rats. Am J Physiol Heart Circ Physiol 281: H1881–H1889, 2001.—An enhanced responsiveness to increases in cerebrospinal fluid (CSF) Na\(^+\) by high salt intake may contribute to salt-sensitive hypertension in Dahl salt-sensitive (S) rats. To test this hypothesis, sympathetic and pressor responses to acute and chronic increases in CSF Na\(^+\) were evaluated. In conscious young (5–6 wk old) and adult (10–11 wk old) Dahl S and salt-resistant (R) rats as well as weight-matched Wistar rats, hemodynamic [blood pressure (BP) and heart rate (HR)] and sympathetic [renal sympathetic nerve activity (RSNA)] responses to 10-min intracerebroventricular infusions of artificial CSF (aCSF) and Na\(^+\)-rich aCSF (containing 0.2–0.45 M Na\(^+\)) were evaluated. Intracerebroventricular Na\(^+\)-rich aCSF increased BP, RSNA, and HR in a dose-related manner. The extent of these increases was significantly larger in Dahl S versus Dahl R or Wistar rats and young versus adult Dahl S rats. In a second set of experiments, young Dahl S and R rats received a chronic intracerebroventricular infusion of aCSF or Na\(^+\)-rich (0.8 M) aCSF (5 \(\mu\)l/h) for 14 days, with the use of osmotic minipumps. On day 14 in conscious rats, CSF was sampled and BP, HR, and RSNA were recorded at rest and in response to air stress, intracerebroventricular \(\alpha\)-adrenoceptor agonist guanabenz, intracerebroventricular ouabain, and intravenous phenylephrine and nitroprusside to estimate baroreflex function. The infusion of Na\(^+\)-rich aCSF versus aCSF increased CSF Na\(^+\) concentration to the same extent but caused severe versus mild hypertension in Dahl S and Dahl R rats, respectively. After central Na\(^+\) loading, hypothalamic “ouabain” significantly increased in Dahl S and only tended to increase in Dahl R rats. Moreover, sympatheexcitatory and pressor responses to intracerebroventricular exogenous ouabain were attenuated by Na\(^+\)-rich aCSF to a greater extent in Dahl S versus Dahl R rats. Responses to air-jet stress or intracerebroventricular guanabenz were enhanced by Na\(^+\)-rich aCSF in both strains, but the extent of enhancement was significantly larger in Dahl S versus Dahl R. Na\(^+\)-rich aCSF impaired arterial baroreflex control of RSNA more markedly in Dahl S versus R rats. These findings indicate that genetic control of mechanisms linking CSF Na\(^+\) with brain “ouabain” is altered in Dahl S rats toward sympathetic hyperactivity and hypertension.

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assess whether intracerebroventricular hypertonic saline elicits greater sympathoexcitatory and pressor responses in conscious Dahl S versus Dahl R rats, and whether the different responses to acute intracerebroventricular Na\(^{+}\) loading are due to increased responsiveness in Dahl S versus Dahl R rats or decreased responsiveness in Dahl R versus other normotensive rats. Moreover, more importantly, it has not yet been assessed whether in Dahl S rats chronic intracerebroventricular infusion of hypertonic saline causes sympathoexcitatory and hypertensive effects, similar as caused by high dietary sodium intake.

The objectives of the present study were therefore: 1) to examine whether acute increases in CSF Na\(^{+}\) increase renal sympathetic nerve activity (RSNA), heart rate (HR), and BP to a larger extent in Dahl S versus Dahl R rats; 2) to compare the sympathoexcitatory and pressor responses to acute central Na\(^{+}\) loading in young versus adult Dahl S and Dahl R rats, because salt sensitivity in Dahl S rats appears to decrease with age (22, 30); and 3) to assess whether chronic intracerebroventricular infusion of Na\(^{+}\)-rich aCSF causes similar increases in CSF Na\(^{+}\) but greater increases in brain “ouabain” and more marked sympathoexcitatory hyperactivity, impairment of arterial baroreflex function, and hypertension in Dahl S versus Dahl R rats.

METHODS

Male Dahl S and Dahl R rats (Harlan Sprague Dawley; Indianapolis, IN) and Wistar rats (Charles River Breeding Laboratories; Montreal, Canada) were housed on a 12:12-h light-dark cycle and were allowed a 5-day acclimatization period on normal rat chow and tap water. All procedures in the present study were carried out according to the guidelines of the University of Ottawa Animal Care Committee for the use and care of laboratory animals.

Acute Central Sodium Loading

Five- to 6- (young) and 10- to 11 (adult)-wk-old Dahl S and R and Wistar rats were used. With the rat under halothane anesthesia, a 23-gauge, stainless steel guide cannula was implanted and fixed to the skull (coordinates: 0.4 mm posterior and 1.4 mm lateral to the bregma and the tip at 2.8 mm ventral to the dura) for intracerebroventricular infusion of aCSF or Na\(^{+}\)-rich aCSF into the right lateral ventricle (15). Early in the morning, about 1 wk after brain surgery, with the rats under halothane anesthesia, polyethylene (PE) catheters were inserted into the right femoral artery and vein. With additional methohexital sodium anesthesia given to the rat (30 mg/kg iv, supplemented with 10 mg/kg as needed, Brevital, Eli Lilly Canada; Toronto, Canada), through a flank incision, a pair of silver electrodes (A-M System; Everett, WA) was placed around and fixed to the left renal nerve with silicone rubber (SiltGe1 604, Wacker; Munich, Germany) for measurement of RSNA as described previously (15). The catheters and electrodes were tunneled subcutaneously and externalized on the back of the neck.

At least 4 h after the rat recovered from the anesthesia, the intra-arterial catheter was connected to a pressure transducer, and BP and HR were recorded through a polygraph (model 7E, Grass Instrument; Quincy, MA) and Grass 7P44 tachograph. The electrodes were linked to a Grass P511 band-pass amplifier. The amplified and filtered RSNA signals were channeled to a rectifying voltage integrator (Grass model 7P10) and recorded through the polygraph. The integrated voltage signals (in mV) of RSNA, together with BP and HR, were also fed into an online computer equipped with a Grass data acquisition and analysis program (Polyview 2.0) for display, storage, and later analysis of both analog and digital data. At the end of each experiment, noise for RSNA was measured 20 min after the rat had been killed by an intravenous overdose of pentobarbital sodium, and the actual activity was determined by subtracting noise from the total activity (15).

After a 30-min rest, baseline mean arterial pressure (MAP), HR, and RSNA were recorded in resting, unrestrained animals. Subsequently, the following four solutions were intracerebroventricularly infused at 3.8 μl/min for 10 min at 30-min intervals: aCSF, and Na\(^{+}\)-rich aCSF containing 0.2, 0.3, and 0.45 M Na\(^{+}\). For intracerebroventricular infusion, a 30-gauge stainless steel was inserted into the guide cannula so that its tip protruded 0.8–1.0 mm from the tip of the guide cannula and was located inside the lateral ventricle. A 500-μl volume Hamilton microsyringe was used for intracerebroventricular infusion, mounted on a Sage 355 infusion pump. Five minutes before each of the four intracerebroventricular infusions, the vasopressin V1 receptor antagonist d-(CH\(_2\))\(_5\)-Tyr-(Me)arginine vasopressin (30 μg/kg in 0.1 ml saline; Sigma Chemical) was injected intravenously to exclude the vasoconstrictor and sympathoinhibitory responses to increased endogenous vasopressin by the hypertonic saline (2, 15). The vasopressin antagonist caused transient insignificant decreases in BP. The resting BP, RSNA, and HR did not change significantly over the 120–150 min of the experimental period.

Chronic Central Sodium Loading

Five- to six-week-old Dahl S and Dahl R were used. With the rat under halothane anesthesia, an L-shaped, 23-gauge stainless steel cannula (Harvard Apparatus; Holliston, MA) was implanted and fixed to the skull (0.5 mm posterior and 1.4 mm lateral to the bregma and 3.8 mm deep from the dura) (17). The shorter arm of the L-cannula was inserted into the ventricle, and the longer arm was connected with PE tubing to an osmotic minipump (model 2ML2, ALZA; Palo Alto, CA), which was filled with aCSF (0.145 M Na\(^{+}\), the same as in CSF) or Na\(^{+}\)-rich aCSF (0.8 M Na\(^{+}\)), which were pumped into the right lateral ventricle at 120 μl/day for 14–15 days. Considering that the normal secretion rate of CSF in rats is 120–320 μl/h (3), this rate of intracerebroventricular infusion appears to increase CSF volume of the rats by 2–4%, and increase the amount of Na\(^{+}\) in CSF by −2–4% for aCSF infusion and by 11–22% for Na\(^{+}\)-rich aCSF infusion.

Protocol 1. At the end of 14 days of infusion, with the rat under halothane anesthesia, the right femoral artery was catheterized with PE-10 fused to PE-50 tubing for measurement of the BP. The rat was then placed in a stereotaxic frame, and a 2-cm long, 22-gauge stainless steel cannula was placed into the cisterna magna and fixed on the skull with cement and screws, as described previously (17). The upper end of the cannula was connected with a short segment of PE tubing sealed with a styloc.

At least 4 h after the rat recovered from the anesthesia while in the original cage, the intra-arterial catheter was connected to a pressure transducer and a Grass 7P44 tachograph. After a 30-min rest, resting BP and HR were recorded, and 1 ml of blood was withdrawn from the arterial line followed by infusion of 1 ml physiological saline to replace the
Acute Central Sodium Loading

The rat was then placed in the experimental cage and basal BP, HR, and RSNA were recorded at least 4 h after the rat recovered from anesthesia and 30 min after the connection of catheters and electrodes. A standardized mental stress was then provided twice at a 15-min interval by blowing the face of the rat with a jet of air (1–1.5 psi) for 30 s (16). The average of the peak changes in MAP, RSNA, and HR in response to the two applications of stress was used. After a 15-min rest, phenylephrine (5–50 μg/min) was infused intravenously to achieve a ramp increase in MAP with a maximum of 50 mmHg over 1–2 min. After the responses had subsided, the animal was allowed to rest for 40 min, and ouabain at 0.5 μg/2 μl aCSF was then injected intracerebroventricularly. There were no significant changes in resting BP, RSNA, and HR observed over the experimental period.

"Ouabain" Assay

"Ouabain" was extracted by mixing each plasma sample with 1 vol of water containing 0.1% trifluoroacetic acid. Tissues were homogenized in 10 vol methanol-2 M ascorbic acid. The homogenate was centrifuged, and the supernatant was dried and reconstituted with water containing 0.1% trifluoroacetic acid. Plasma and tissue extracts were passed over prewashed 200 mg C-18 disposable Sep-Pak Vac cartridges. "Ouabain" was extracted by mixing each plasma sample containing 2% bovine serum albumin) to each well at 37°C for 18 h. The substrate reaction was terminated by the addition of 1 vol of rinse solution [10 mmol/l, 3,3'-5,5'-tetramethlybenzidine base (TMB) substrate solution]. The enzyme immunoassay microplates were coated with ovalbumin-ouabain by adding to each well. Plates were incubated at 37°C for 2 h with continuous shaking followed by four rinses of 250 μl of rinse solution per well. Anti-ouabain antibodies remaining bound to the ovalbumin-ouabain were reacted with 200 μl per well of a 1:1,000 dilution of goat anti-rabbit IgG-peroxidase by incubating the plates for an additional 1 h at 37°C. Unbound anti-rabbit IgG-peroxidase conjugate was washed away by rinsing. The presence of peroxidase enzyme remaining in each well was determined by the addition of 200 μl per well of 3,3',5,5'-tetramethylbenzidine base (TMB) substrate solution. The substrate reaction was terminated by the addition of 50 μl of 2 M H₂SO₄. The absorbency of each well was measured at 450 nm using an ELISA Reader (Bio-Rad Microplate Reader model 3550, Bio-Rad; Hercules, CA).

The concentration of "ouabain" in each sample was calculated from the absorbency according to the ouabain standard curve.

Data Analysis

Responses of RSNA were expressed as percent changes from the baseline levels. Arterial baroreflex function was analyzed as a logistic model (17), i.e., changes in RSNA (ΔRSNA) or HR (ΔHR) in response to increases and decreases in MAP were analyzed together using the logistic equation: ΔRSNA = P₁ + P₂/[1 + e^(P₃(MAP - P₄))], where P₁ is the lower ΔRSNA plateau that represents the minimal decrease in RSNA, P₂ is the ΔRSNA range, P₃ is a curvature coefficient, and P₄ is ED₅₀ for MAP, i.e., MAP at half the ΔRSNA range. The average gain (G), or slope of the curve between the two inflection points of the curve, is given by G = -P₂ × P₃/4.56. Two-way ANOVA was performed for comparisons between groups. When F ratios were significant, a Duncan multirange test followed to locate the significant differences.

RESULTS

Acute Sodium Loading

On regular sodium intake, resting MAP was significantly higher in adult Dahl S versus other groups of rats. Resting HR was significantly higher in young Dahl S versus Dahl R and tended to be higher in adult Dahl S versus R (Table 1).

In both adult and young rats, intracerebroventricular aCSF did not change resting parameters, whereas intracerebroventricular Na⁺-rich aCSF increased MAP, RSNA, and HR in a dose-related manner (Fig. 1).

Table 1. Body weight and resting MAP and HR in young and adult Dahl S, Dahl R, and Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>12</td>
<td>100 ± 6</td>
<td>432 ± 27</td>
<td>224 ± 11</td>
</tr>
<tr>
<td>Adult</td>
<td>6</td>
<td>103 ± 3</td>
<td>433 ± 16</td>
<td>341 ± 7</td>
</tr>
<tr>
<td>Dahl R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>10</td>
<td>104 ± 3</td>
<td>418 ± 12</td>
<td>226 ± 6</td>
</tr>
<tr>
<td>Adult</td>
<td>8</td>
<td>106 ± 6</td>
<td>427 ± 17</td>
<td>328 ± 5</td>
</tr>
<tr>
<td>Dahl S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>10</td>
<td>108 ± 3</td>
<td>468 ± 11*</td>
<td>231 ± 7</td>
</tr>
<tr>
<td>Adult</td>
<td>8</td>
<td>120 ± 5*</td>
<td>469 ± 15</td>
<td>337 ± 4</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = number of rats. MAP, mean arterial pressure; HR, heart rate; Dahl R, Dahl salt-resistant rats; Dahl S, Dahl salt-sensitive rats. *P < 0.05 vs. others; †P < 0.05 vs. young Dahl R.

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The parallel increases in MAP, RSNA, and HR started 1–2 min following the start of the infusion; reached their plateau in another 1–2 min; and returned to resting levels within 2 min following termination of the infusion. Responses to the Na⁺ loading at all three rates were significantly larger in young Dahl S versus young Dahl R or Wistar rats. In adult Dahl S rats, only responses to 0.3 and 0.45 M Na⁺ were significantly larger versus adult Dahl R or Wistar rats.

MAP, RSNA, and HR responses to 0.3 or 0.45 M Na⁺ (Figs. 1 and 2) were significantly smaller in adult versus young Dahl S rats. In contrast, responses only tended to be smaller in adult versus young Dahl R or Wistar rats (Fig. 2).

Chronic Sodium Loading

After 2 wk of treatment, the gain of body weight, K⁺ concentration in both plasma and CSF, Na⁺ concentration in plasma, and resting HR were similar in the four groups of rats. Na⁺ concentration in CSF and resting BP were significantly increased in Dahl S and Dahl R rats with Na⁺-rich aCSF versus aCSF. However, whereas Na⁺ concentration in the CSF similarly increased by about 10 mM in Dahl S and Dahl R rats after intracerebroventricular Na⁺-rich aCSF, the extent of increases in resting MAP was significantly larger in Dahl S versus Dahl R rats (Table 2). Compared with the controls, central Na⁺ loading signifi-

Fig. 1. Mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) responses to intracerebroventricular (icv) infusion of artificial cerebrospinal fluid (aCSF) (0.145 M Na⁺) or aCSF containing 0.2, 0.3 or 0.45 M Na⁺ in young (A) and adult (B) Dahl salt-sensitive (S), Dahl salt-resistant (R), and Wistar rats. bpm, Beats per minute. Data are means ± SE. For n values, see Table 1. *P < 0.05 vs. Dahl R and Wistar rats at corresponding rate of Na⁺ infusion. **P < 0.05 vs. Dahl R at corresponding rate of Na⁺ infusion.

Fig. 2. MAP, RSNA, and HR responses to icv infusion of aCSF containing 0.45 M Na⁺ in young versus adult Dahl S (DS), Dahl R (DR), and Wistar rats. Data are means ± SE. For n values, see Table 1. *P < 0.05 young vs. adult rats within strain. †P < 0.05 vs. age-matched DR or Wistar rats.
cantly increased hypothalamus “ouabain” in Dahl S, but only tended to increase it in Dahl R rats (Fig. 3). Na⁺-rich aCSF tended (not significantly) to increase the “ouabain” content in the brain cortex in both Dahl S (23.9 ± 5.5 vs. 15.4 ± 2.5 ng/g tissue) and Dahl R rats (26.0 ± 4.8 vs. 15.9 ± 6.4 ng/g tissue), but had no effects on plasma “ouabain”(1.5 ± 0.3 vs. 1.3 ± 0.4 ng/ml for Dahl S, and 0.7 ± 0.0 vs. 0.8 ± 0.0 ng/ml for Dahl R rats). Exogenous ouabain intracerebroventricular increased MAP, HR, and RSNA. These sympathoexcitatory and pressor responses were attenuated in both Dahl S and R rats by Na⁺-rich aCSF, but the extent of attenuation was significantly greater in Dahl S versus R rats (Fig. 4).

Air-jet stress elicited rapid increases in MAP, RSNA, and HR. These responses were enhanced in both Dahl S and Dahl R rats with Na⁺-rich aCSF versus aCSF. The extent of enhancement was clearly larger ($P < 0.05$) in Dahl S versus R rats (Fig. 5).

Intracerebroventricular guanabenz caused dose-related decreases in MAP, HR, and RSNA. These responses were enhanced in both Dahl S and Dahl R rats with intracerebroventricular Na⁺-rich aCSF, but the extent of enhancement was significantly larger in Dahl S versus Dahl R rats (Fig. 6).

Changes in MAP by intravenous phenylephrine or nitroprusside elicited reflex changes in RSNA and HR in opposite directions. As shown in Fig. 7 and Table 3, arterial baroreflex control of RSNA was impaired in both Dahl S and R rats by intracerebroventricular Na⁺-rich aCSF. The impairment was more marked in Dahl S versus R rats. Reflex control of HR showed a minor (not significant) desensitization in both Dahl S and R rats by intracerebroventricular Na⁺-rich aCSF (maximal slopes for Dahl S: $-2.0 ± 0.2$ vs. $-1.8 ± 0.1$ beats·min⁻¹·mmHg⁻¹; for Dahl R: $-2.1 ± 0.3$ vs. $-1.9 ± 0.3$ beats·min⁻¹·mmHg⁻¹).

**DISCUSSION**

The present study provides several new findings. First, in Wistar and Dahl rats, acute intracerebroventricular infusion of Na⁺-rich aCSF increases BP, RSNA, and HR in a Na⁺ dose-related manner, and the extent of the increases is significantly larger in Dahl S versus Dahl R rats after icv infusion of aCSF or Na⁺-rich aCSF for 2 wk.
rats versus that of age-matched Dahl R or Wistar rats. Second, the extent of enhancement is significantly less in adult versus young Dahl S. Third, chronic intracerebroventricular infusion of Na\(^+\)-rich aCSF increases Na\(^+\) concentration in the CSF to the same extent in Dahl S and R rats but increases hypothalamic "ouabain" only significantly in Dahl S rats and attenuates sympathoexcitatory and pressor responses to intracerebroventricular exogenous ouabain to a greater extent in Dahl S versus R rats. Fourth, chronic intracerebroventricular Na\(^+\) loading causes more marked sympathetic hyperactivity and hypertension associated with more impairment of arterial baroreflex control of RSNA in Dahl S rats.

We hypothesized that high salt intake activates central sympathoexcitatory mechanisms by increasing CSF Na\(^+\) concentration (17). Increased CSF Na\(^+\) may depolarize relevant excitatory neurons in the brain through amiloride-sensitive Na\(^+\) channels (24) leading to sympathoexcitation. This hypothesis is supported by our findings that in normotensive rats increases in CSF Na\(^+\) by acute (2, 15) or chronic (17) intracerebroventricular administration of hypertonic saline cause similar sympathetic hyperactivity and hypertension as seen in Dahl S on high salt (16), and blockade of brain "ouabain" or brain RAS prevents these responses (15, 17). Increases in CSF Cl\(^-\) or osmolarity of CSF do not cause such sympathoexcitation and hypertension (2). Differences between salt-sensitive and salt-resistant strains may relate to different increases in CSF Na\(^+\) on high salt intake and/or different neural responsiveness to CSF Na\(^+\). Nakamura and Cowley (21) demonstrated that high salt intake persistently increases CSF Na\(^+\) in Dahl S but not Dahl R rats. In Dahl S versus R rats on regular salt intake, the blood-brain barrier is more permeable to Na\(^+\), and Na\(^+\) uptake in brain is up to

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**Fig. 5.** Peak MAP, RSNA, and HR responses to air-jet stress in DS versus DR after icv infusion of aCSF or Na\(^+\)-rich aCSF containing 0.8 M Na\(^+\) for 2 wk. Data are means ± SE (n = 6 rats for all groups) *P < 0.05 vs. others. †P < 0.05 vs. DR with aCSF.

**Fig. 6.** Peak decreases in MAP, RSNA, and HR in response to guanabenz at 30 (A) and 60 (B) μg icv in DS versus DR rats after icv infusion of aCSF or Na\(^+\)-rich aCSF containing 0.8 M Na\(^+\) for 2 wk. Data are means ± SE (n = 6 rats for all groups). *P < 0.05 vs. others. †P < 0.05 vs. DR with aCSF.

**Fig. 7.** Arterial baroreflex control of RSNA analyzed as a logistic model in DS versus DR after icv infusion of aCSF or Na\(^+\)-rich aCSF containing 0.8 M Na\(^+\) for 2 wk. Each point is mean ± SE of changes in RSNA (% resting) relative to MAP changed by intravenous phenylephrine and nitroprusside (n = 6 rats for all groups).
five times greater after intravenous NaCl injection (25). Similarly, CSF Na⁺ concentration is significantly higher in humans with salt-sensitive hypertension versus normal subjects (12). These studies suggest that in Dahl S rats or salt-sensitive humans a genetic abnormality of the choroid plexus altering ion transport contributes to the increased CSF Na⁺ in response to high salt intake. However, in Nakamura and Cowley’s study (21), BP increased 1–2 days before significant increases in CSF Na⁺ in Dahl S rats, suggesting that the increases in CSF Na⁺ may only contribute to the maintenance of salt-induced hypertension. On the other hand, sampling of CSF was only done over 24-h periods (21), and this approach may not detect increases in CSF Na⁺ during the first few days on high salt intake, because rats consume food mainly at night and increases in CSF Na⁺ during the night may initially be masked by normal Na⁺ concentration in the CSF produced during the daytime.

Alternatively, or in addition, increases in CSF Na⁺ may evoke greater sympathoexcitation and hypertension in Dahl S versus Dahl R rats. Indeed, the pressor response to intracerebroventricular injection of a single dose of hypertonic saline was three times larger in anesthetized Dahl S versus Dahl R rats (11). In contrast, Simchon et al. (25) reported recently that intracerebroventricular bolus injection of hypertonic saline containing 4.5 M Na⁺ caused large pressor responses, but to a similar extent in Dahl S versus Dahl R rats. Neither study assessed sympathetic nerve activity. Both studies were performed in rats under general anesthesia and did not use a vasopressin antagonist. General anesthesia may markedly affect the sympathetic and pressor responses (5). An increase in plasma vasopressin (2) also contributes to the pressor responses to intracerebroventricular hypertonic saline, even with very low infusion rates (14). Moreover, in the study by Simchon et al. (25), a large volume (60 μl) of hypertonic saline with a high Na⁺ concentration (4.5 M Na⁺) was used for the intracerebroventricular bolus injection. In rats, a sudden increase of CSF volume by 60 μl likely increases intracranial pressure and thereby BP. In the present study, we demonstrated that in conscious rats increases in CSF Na⁺ by aCSF, containing 0.2–0.45 M Na⁺ infused intracerebroventricularly at 3.8 μl/min for 10 min after the use of vasopressin antagonist, increase not only BP but also RSNA and HR, in a Na⁺ dose-related manner. Moreover, the magnitudes of these responses were significantly larger in Dahl S versus Dahl R and Wistar rats but were similar in Dahl R versus Wistar rats.

Responses to acute increases in CSF Na⁺ clearly decrease in adult versus young Dahl S rats and only to a minor extent in adult versus young Dahl R or Wistar rats. Although the extent of hypertension by high salt intake has been shown to decrease with age in Dahl rats (22, 30), to our knowledge, no study has so far assessed responsiveness to central sodium loading in young versus adult rats. It is not clear why the responsiveness to CSF Na⁺ decreases with maturation in Dahl S rats. The activity of 11β-hydroxysteroid dehydrogenase-1 (11β-HSD1), which confers the specificity for the mineralocorticoid receptors, is significantly lower in the liver of 6- but not 10-wk-old Dahl S versus age-matched Dahl R rats (6). The 11β-HSD1 mRNA in the mesenteric artery of 8-wk-old Dahl S is also lower compared with Dahl R rats (26). Because mineralocorticoids may affect activity of Na⁺ channels in the brain (20), it is possible that maturation affects expression or activity of 11β-HSD1 in the brain and thereby responses to intracerebroventricular sodium. In addition, maturation may affect responses of brain “ouabain” to central sodium, as well as change the profile of brain α-subunits of Na⁺-K⁺-ATPase. Higher resting BP and/or sympathetic tone in adult versus young rats may also contribute to the decreased responsiveness to CSF Na⁺. It remains to be determined whether this attenuation of the enhanced responses to CSF Na⁺ in Dahl S rats explains the smaller hypertensive response to high salt intake in older Dahl S rats.

Results obtained with chronic CSF Na⁺ loading are consistent with the acute data, showing that similar chronic increases in CSF Na⁺ caused more severe hypertension associated with enhanced sympathoexcitatory and pressor responses to air-jet stress, enhanced sympahtoinhibitory and depressor responses to intracerebroventricular α₂-adrenoceptor agonist guanabenz, and more severe impairment of arterial baroreflex control of RSNA in Dahl S versus Dahl R rats. Responses to air stress and intracerebroventricular guanabenz are used to assess activity of central sympahtoexcitatory and sympathoinhibitory pathways (18, 27). Enhanced inhibitory responses to exogenous α₂-adrenoceptor agonist indicate an upregulation or decreased occupancy of central α₂-adrenoeceptors in hypothalamic sympathoinhibitory pathways (27), consistent with a decreased activity in central sympathoinhibitory pathways and thereby increased sympathetic outflow. The present study shows that in Dahl S versus Dahl R rats, chronic CSF Na⁺ loading causes more marked sympathetic hyperactivity and hypertension.

### Table 3. Estimated parameters of arterial baroreflex control of RSNA in Dahl S and R rats after intracerebroventricular infusion of aCSF or Na⁺-rich aCSF for 2 wk

<table>
<thead>
<tr>
<th></th>
<th>Dahl R</th>
<th>Dahl S</th>
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<tbody>
<tr>
<td></td>
<td>aCSF</td>
<td>0.8 M Na⁺</td>
</tr>
<tr>
<td>Upper plateau, % basal</td>
<td>85 ± 2</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Lower plateau, % basal</td>
<td>-97 ± 2</td>
<td>-92 ± 3</td>
</tr>
<tr>
<td>Max slope, %/mmHg</td>
<td>-3.8 ± 0.2</td>
<td>-3.1 ± 0.3</td>
</tr>
<tr>
<td>Avg slope, %/mmHg</td>
<td>-3.4 ± 0.3</td>
<td>-2.5 ± 0.1†</td>
</tr>
<tr>
<td>ED_{50}, mmHg</td>
<td>103 ± 4</td>
<td>120 ± 5†</td>
</tr>
</tbody>
</table>

Data are means ± SE; for n values, see Table 2. Max, maximal; Avg, average; ED_{50}, MAP at half the JRSNA range. †P < 0.05 vs. others; ‡P < 0.05 vs. Dahl R with aCSF; †P < 0.05 vs. Dahl S and R with aCSF.
associated with a marked increase in “ouabain” in the hypothalamus of Dahl S rats. Moreover, sympathoexcitatory and pressor responses to acute intracerebroventricular injection of exogenous ouabain were attenuated only to a minor extent in Dahl R rats by chronic intracerebroventricular infusion of Na+-aCSF and markedly in Dahl S rats. These results are consistent with a more marked increase in occupancy of brain “ouabain” receptors by increased endogenous “ouabain,” and therefore decreased availability to exogenous ouabain. Taken together, the present as well as previous studies support the concept that elevated CSF Na⁺ concentration increases brain “ouabain,” and the latter causes sympathetic hyperactivity and hypertension (15–17).

How an increase in CSF Na⁺ activates brain “ouabain” and central pathways leading to sympathoexcitation is not clear yet. Recent studies show that brain amiloride-sensitive Na⁺ channels may relate to sympathoexcitation (24). Through these channels, increased CSF Na⁺ may depolarize excitatory neurons in autonomic centers and thereby increase sympathetic outflow (23). It was suggested that rats with salt-sensitive hypertension exhibit a higher activity of amiloride-sensitive Na⁺ channels in the brain (23). The neuropeptide Phe-Met-Arg-Phe-NH₂ (FMRF amide) activates Na⁺ channels in the brain and makes Wistar rats salt sensitive (24). Brain mineralocorticoid receptors also appear to play an important role in the salt-sensitive hypertension in Dahl S rats (8). Because mineralocorticoids such as aldosterone increase the activity of amiloride-sensitive Na⁺ channels (20), one may speculate that a genetic alteration in mineralocorticoid metabolism and high activity of amiloride-sensitive Na⁺ channels in the brain contribute to increased responsiveness to CSF Na⁺ in Dahl S versus Dahl R rats. The relationships among CSF Na⁺, brain “ouabain,” and brain Na⁺ channels have not yet been studied. It is possible that CSF Na⁺ may increase brain “ouabain” through activated brain Na⁺ channels or, alternatively, CSF Na⁺ may increase brain “ouabain” first and the latter activates brain Na⁺ channels leading to sympathoexcitation.

The present as well previous studies indicate that several differences between Dahl S and Dahl R rats may contribute to their different sodium sensitivity. First, as shown in the present study, responsiveness to CSF Na⁺ is significantly larger in Dahl S versus Dahl R rats. Therefore, even similar increases in CSF Na⁺ by high salt intake will lead to larger increases in brain “ouabain” and sympathetic hyperactivity. Second, high salt intake may actually increase CSF Na⁺ concentration more in Dahl S versus Dahl R rats (21), which will contribute to greater increases in brain “ouabain” in Dahl S versus Dahl R rats on high salt (19). Third, whereas high salt intake impairs baroreflex control of RSNA in Dahl S rats likely through central mechanisms (16), it sensitizes arterial baroreflex function in Dahl R rats (16) possibly by peripheral mechanisms (4). Impairment of baroreflex function worsens, and sensitization of baroreflex inhibits sympathetic hyperactivity. All these differences can contribute to the sodium sensitivity in terms of sympathetic hyperactivity and hypertension in Dahl S versus Dahl R rats. Although the sodium sensitivity was similar in Dahl R versus Wistar rats in the acute study, chronic sodium loading was not performed in Wistar rats in the present study. In a previous study (17), we showed that in Wistar rats, a similar degree of chronic central sodium loading caused similar patterns of increases in CSF Na⁺, brain “ouabain” content, and resting BP, and impairment of baroreflex function as observed in Dahl R rats in the present study. The pattern of responses to high salt intake is also similar in Dahl R and Wistar rats (16, 13). Thus it seems unlikely that the difference in sodium sensitivity between Dahl S and Dahl R rats is due to decreased responsiveness in Dahl R versus normotensive Wistar rats.

The pathophysiological changes induced by high salt intake and chronic CSF Na⁺ loading are identical in Dahl S rats, but not in Dahl R rats. Whereas all relevant changes were observed in Dahl S rats by either high salt intake or chronic CSF Na⁺ loading, in Dahl R rats effects of chronic CSF Na⁺ loading and high salt intake differ. In the present study, chronic CSF Na⁺ loading increased CSF Na⁺ by 9–10 mM in both Dahl S and R rats, whereas high salt intake significantly increased CSF Na⁺ only in Dahl S but not in Dahl R rats (21). Moreover, in Dahl R rats, high salt intake sensitizes baroreflex function likely by peripheral mechanisms (4, 16), whereas chronic CSF Na⁺ loading does not affect plasma sodium concentration and does not sensitize baroreflex function but desensitizes it presumably by central mechanisms. Thus it appears that in Dahl R rats high salt does not increase CSF Na⁺ sufficiently to increase brain “ouabain” high enough to overcome the effects of baroreflex sensitization and therefore does not lead to sympathetic hyperactivity or hypertension.

A possible limitation of the study was that acute experiments were performed at least 4 h after the recovery from the anesthesia. Effects of the surgery leading to high resting sympathetic drive and possible residual effects of the short-acting anesthetic may still influence the results. In freely moving rats, the RSNA signal deteriorates over 1–2 days. Moreover, fluid and food intake are often less than optimal the first one to two nights after surgery, which may also influence the baseline values. Thus the timing of these assessments is a compromise between these factors.

In summary, the present study demonstrates that acute intracerebroventricular infusion of Na⁺-rich aCSF causes significantly larger sympathoexcitatory and pressor responses in Dahl S rats versusagematched Dahl R or Wistar rats. Chronic intracerebroventricular infusion of Na⁺-rich aCSF increases CSF Na⁺ concentration to the same extent in Dahl S and R rats, but this infusion causes clear increases in brain “ouabain” associated with more marked sympathetic hyperactivity and hypertension and more impairment of arterial baroreflex control of RSNA in Dahl S rats. These results suggest that enhanced neuronal respon-
siveness to CSF Na\(^+\) is one of the mechanisms contributing to salt-sensitive hypertension in Dahl S rats. Genetic control of mechanisms linking CSF Na\(^+\) with brain “ouabain” appears to be altered in Dahl S rats toward sympathetic hyperactivity and hypertension.

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