Brain “Ouabain” Mediates the Sympathoexcitatory and Hypertensive Effects of High Sodium Intake in Dahl Salt-Sensitive Rats

Bing S. Huang, Frans H.H. Leenen

Abstract To assess whether brain ouabain-like activity (OLA) mediates the hypertensive effects of high sodium intake in Dahl salt-sensitive (Dahl S) rats, the effects of blockade of brain OLA on mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) were evaluated in conscious Dahl salt-resistant (Dahl R) and Dahl S rats on a regular (120 μmol/g) or high sodium (1370 μmol/g) diet from 4 to 7 weeks of age. Dahl S rats given high sodium showed higher basal MAP and augmented responses of MAP and RSNA to air stress and to intracerebroventricular injection of the α2-adrenergic receptor agonist guanabenz as compared with Dahl R rats or Dahl S rats given regular sodium. In contrast, the sympathoexcitatory and pressor responses to intracerebroventricular injection of ouabain (0.3 and 1.0 μg) were markedly attenuated in Dahl S rats given high sodium. Intracerebroventricular preinjection of 0.3 μg ouabain significantly enhanced blood pressure and RSNA responses to air stress and intracerebroventricular guanabenz in Dahl S rats given regular sodium to the levels observed in Dahl S rats given high sodium. Intracerebroventricular digoxin-specific antibody Fab (DAF) fragments (132 μg/8 μL for 5 minutes) did not change basal MAP and RSNA during the first 4 hours after administration in Dahl S rats on a high sodium diet for 3 weeks. However, 18 hours after the injection of DAF fragments, basal MAP and RSNA were significantly decreased, reaching values for Dahl S rats on a regular sodium diet. The magnitude of increases or decreases in MAP and RSNA to air stress or intracerebroventricular guanabenz were significantly attenuated by the DAF fragments in Dahl S rats on a high sodium but not regular sodium diet. Concomitant intracerebroventricular infusion of DAF fragments (200 μg per day) prevented the development of hypertension after a high sodium diet in Dahl S rats and prevented an augmentation in pressor and sympathoexcitatory responses to air stress. After discontinuing the infusion of DAF fragments, resting MAP gradually increased to the high levels found in Dahl S rats given high sodium treated with γ-globulins. These results support the concept that high sodium intake may cause hypertension in Dahl S rats by increasing endogenous brain OLA, thereby enhancing sympathetic outflow and basal blood pressure as well as sympathoexcitatory and pressor responses to stress. (Circ Res. 1994;74:586-595.)

Key Words • renal sympathetic nerve activity • brain ouabain-like activity • osmotic minipump • air stress • brain α2-adrenergic receptor stimulation • digoxin-specific antibody Fab fragments

High sodium intake enhances the development of hypertension in rats genetically predisposed to hypertension.1-5 In spontaneously hypertensive rats (SHR), high sodium intake results in increased activity in sympathoexcitatory pathways and enhanced responses to air stress, for example, as well as in decreased activity in sympathoinhibitory pathways.3-4 The latter decrease is associated with enhanced sympathoinhibitory responses to the α2-adrenergic receptor agonist guanabenz, presumably because of upregulation and/or decreased receptor occupancy of α2-adrenergic receptors in the anterior hypothalamus.3 The sympathoexcitatory and pressor responses to increases in central sodium appear to be mediated by brain ouabain-like activity (OLA); in conscious rats digoxin-specific antibody Fab (DAF) fragments administered intracerebroventricularly block the sympathoexcitatory and pressor responses to intracerebroventricular hypertonic saline, ouabain, and brain extracts containing OLA.5 In both biologic and chemical aspects, this brain OLA appears to be similar to ouabain, isolated from human plasma,6 or a ouabain isomer isolated from bovine hypothalamus.7 SHR show an increased hypothalamus and pituitary OLA.8 High dietary sodium further increases brain OLA in SHR and decreases responses to exogenous ouabain in SHR, consistent with increased occupancy of brain ouabain receptors by brain OLA and decreased availability of the receptors for exogenous ouabain.9

The possible causal role of brain OLA in the neural and hemodynamic effects of high sodium intake in other forms of salt-sensitive hypertension, such as that found in Dahl salt-sensitive (Dahl S) rats, has not yet been examined. Neural mechanisms appear to contribute significantly to the development of hypertension in Dahl S rats. The sympathetic nervous system is essential for the development of hypertension.10 Sympathoexcitatory and pressor responses to electrical stimulation of the ventromedial hypothalamus11 or to environmental stress12 are enhanced in Dahl S rats on a high sodium diet compared with Dahl S rats on a regular sodium diet or Dahl salt-resistant (Dahl R) rats on a high or regular sodium diet. Furthermore, lesions in certain paraventricular regions in the brain13-16 minimize or prevent sodium-induced hypertension in Dahl S rats. High sodium intake increases the Na+ concentration of the
cerebrospinal fluid in Dahl S rats but not in Dahl R rats.\textsuperscript{17} If an increase in brain OLA plays a primary role in the development of hypertension in Dahl S rats on a high sodium diet, one may expect that (1) responses to centrally administered ouabain diminish (ie, there is increased receptor occupancy); (2) exogenous ouabain mimics central effects of high sodium; and (3) blockade of brain OLA prevents/reverses the sodium-induced hypertension. Therefore, in Dahl S and Dahl R rats given regular versus high dietary sodium, we evaluated (1) basal mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA), as well as pressor and sympathoexcitatory responses to intracerebroventricular ouabain, (2) responses to air stress and the intracerebroventricular \( \alpha_{2} \)-adrenergic receptor agonist guanabenz and to what extent exogenous ouabain mimics the effects of high sodium on these responses, and (3) the ability of intracerebroventricular DAF fragments to prevent and reverse sodium-induced sympathoexcitatory hypertension in Dahl S rats.

**Materials and Methods**

**General**

Male inbred Dahl S and Dahl R rats were obtained from Harlan Sprague Dawley Inc, Indianapolis, Ind. At 4 weeks of age, rats were placed on either a regular or high sodium diet (rat chow containing 120 or 1370 \( \mu \)mol sodium per gram of food, Harlan Sprague Dawley Inc, Madison, Wis). All rats were housed in a room on a 12-hour light/dark cycle at a controlled temperature of 24\(^\circ\)C and were allowed water ad libitum. Two different experimental protocols using different groups of rats were followed. In the first protocol, 20 Dahl S and 17 Dahl R rats were randomized to receive high versus regular sodium diets from 4 to 7 weeks of age. At 7 weeks of age, responses to ouabain, air stress, and guanabenz were evaluated, followed by the administration of central DAF fragments in all rats. In a separate group of 15 Dahl S rats all on a high sodium diet from 4 to 7 weeks of age, the time course of changes induced by DAF fragments as compared with \( \gamma \)-globulins was studied at 7 weeks of age. In the second protocol, 33 Dahl S rats received a high versus regular sodium intake from 4 to 7 weeks of age with concomitant administration of DAF fragments or \( \gamma \)-globulins (n=7 to 9 per group).

**Experimental Protocol I: Sympathoexcitatory and Hypertensive Effects of Sodium and Their Reversal by Intracerebroventricular DAF Fragments**

Intracerebroventricular cannulas were placed at 6 weeks of age, as described previously.\textsuperscript{9} Briefly, after induction of anesthesia with sodium pentobarbital (65 mg/kg IP), a 23-gauge, 14-mm-long, stainless-steel tubing was implanted and fixed above the left lateral cerebral ventricle as a guide cannula (0.5 mm posterior and 1.4 mm lateral to bregma and 2.8 mm deep from dura). Penicillin G (30 000 IU IM Derapen, Ayerst Laboratories, Montreal, Canada) was injected after surgery. On the day of the actual experiment, the rats were anesthetized with halothane, and catheters were placed in the right femoral artery and jugular vein. After intravenous injection of methohexitol sodium (30 mg/kg Brevital supplemented with 10 mg/kg as needed, Eli Lilly Canada Inc, Toronto), a pair of platinum electrodes (Leico Industries, New York, NY) was placed around the left renal nerve through a flank incision.\textsuperscript{9} The nerve and the electrodes were fixed to each other with silicone rubber (SilGel 604, Wacker, Munich, Germany). The electrodes and catheters were tunneled subcutaneously to the back of neck, and the rats were then allowed to recover from the anesthesia and surgery.

Four to 5 hours after the surgery, each rat was placed in a small cage that permitted movement back and forth. The intra-arterial catheter was connected to a transducer, and blood pressure (BP) and heart rate (HR) were recorded through a polygraph (model 7E, Grass Instrument Co, Quincy, Mass) and a Grass 7P44 tachograph. The electrodes were linked to a Grass P511 bandpass amplifier, and RSNA (spikes per second) was counted by a nerve traffic analyzer (model 706C, University of Iowa Bioengineering, Iowa City). For repeated measurements, the window setting for recording of RSNA remained the same for each rat. At the end of an experiment, the rats were killed, and the background noise of the RSNA recording was measured by directly recording the activity 20 minutes after the animal was killed. The actual RSNA was determined by subtracting noise from total activity.\textsuperscript{18} Data were digitized through a microcomputer. A 26-gauge, L-shaped, stainless-steel needle was used for intracerebroventricular injection. The shorter arm of the needle was inserted into the guide cannula so that its tip protruded 0.8 to 1.0 mm from the tip of the guide cannula into the lateral ventricle. The longer arm was outside the guide cannula and connected to a Hamilton microsyringe (20-\( \mu \)L volume) via a polyethylene catheter (PE-10 fused to PE-50). Fig 1 shows typical tracings of BP, HR, and RSNA responses induced by air stress and intracerebroventricular guanabenz or ouabain in a Dahl S rat on a high sodium diet.

**Study Protocols**

After a 30-minute stabilization period, basal MAP, HR, and RSNA were recorded. The following tests were performed with intervals of at least 30 min: Responses to Central Ouabain Two doses (0.3 and 1.0 \( \mu \)g/1 to 2 \( \mu \)L) of ouabain (Sigma) in saline were injected intracerebroventricularly at a 15-minute interval. To assess possible peripheral effects of ouabain, 5 minutes after BP, HR, and RSNA had returned to basal levels, 1.0 \( \mu \)g ouabain was injected intravenously.
Responses to Air Stress and Central α₁-Adrenergic Receptor Stimulation

A standardized air stress was provided for 30 seconds, followed by a 10-minute rest. The air stream (1 to 1.5 PSL) was directed to the face of the rat through a plastic tubing located 3 to 4 cm in front of the animal. Subsequently, guanabenz (Sigma Chemical Co, St Louis, Mo) dissolved in saline was injected intracerebroventricularly at increasing doses (7.5, 25, and 75 µg per 1 to 3 µL) at 5- to 10-minute intervals, when responses to the previous dose had subsided. Only responses to the highest dose will be presented.

Effects of Exogenous Ouabain on the Responses to Air Stress and Intracerebroventricular Guanabenz

Ouabain (0.3 µg/1 µL) was injected intracerebroventricularly. When responses reached their plateau, a 30-second air stress was provided. After the responses to the air stress had subsided, 75 µg/3 µL guanabenz was injected intracerebroventricularly.

To assess the reproducibility of the responses to air stress and guanabenz, in a separate group of Dahl S rats (n=8) on a regular sodium diet from 4 to 7 weeks of age, these responses were tested twice according to the above protocols, but saline was given without ouabain.

Effects of Intracerebroventricular DAF Fragments on Basal MAP, HR, and RSNA and Responses to Air Stress and Intracerebroventricular Guanabenz

After a rest of at least 30 minutes, in all Dahl S and Dahl R rats used for the above protocols, DAF fragments (132 µg/8 µL, Digibind, Burroughs Wellcome, Inc, Montreal, Quebec, Canada) dissolved in saline were injected intracerebroventricularly for 5 minutes, and BP, HR, and RSNA were recorded for 30 minutes after the injection. This dose of DAF fragments was chosen to provide for excess binding capacity of endogenous OLA, taking into account its ability to bind ouabain or OLA and the amounts of OLA present centrally. The rats were then disconnected from the polygraph and amplifier, and the catheters were sealed with a stylet after having been filled with heparinized saline (200 U/mL). The rats were returned to their regular cages, with access to the original diets. Each rat was returned to the small cage at 18 hours after the injection of DAF fragments, and BP, HR, and RSNA were recorded after a 30-minute rest, followed by the measurement of responses to air stress and 75 µg ICV guanabenz.

Time Course of Changes Caused by Intracerebroventricular DAF Fragments

After the resting values as well as responses to air stress and intracerebroventricular guanabenz (75 µg/3 µL) as described above had been recorded, Dahl S rats on a high sodium diet received 132 µg/8 µL DAF fragments (n=7) or, as a control for DAF fragments, 132 µg/8 µL γ-globulins (n=8) dissolved in saline, both injected intracerebroventricularly. At 4, 14, 18, and 24 hours after the injection, basal BP, HR, and RSNA (the latter only at 4 and 18 hours) were recorded after a 30-minute rest. In between these measurements, rats were returned to their regular cages. At 18 hours after DAF or γ-globulin injection, responses to air stress and intracerebroventricular guanabenz (75 µg/3 µL) were repeated.

Experimental Protocol II: Prevention of Sympathoexcitatory and Hypertensive Effects of High Sodium by Intracerebroventricular DAF Fragments

At 4 weeks of age, Dahl S rats were placed on either a high or regular sodium diet. At 5 weeks of age, a 23-gauge, stainless-steel, right-angled cannula was implanted into the left lateral ventricle and fixed to the skull of anesthetized rats. This age for the surgery was selected because at 5 weeks the BP is still normal (MAP, 99±4 versus 98±2 mm Hg for high versus regular sodium diet) and at 4 weeks the rats are too small in size and still growing rapidly, which would likely result in dislocation of the intracerebroventricular canulas. The cannula was connected to an osmotic minipump (model 2002, Alza Corp, Palo Alto, Calif) via 50/60-PE tubing for chronic intracerebroventricular infusion of DAF fragments or γ-globulins (200 µg/12 µL per day for both). The osmotic pumps were implanted subcutaneously on the back of the rats. The rats were then returned to their own housing, and original diets were resumed. At 7 weeks of age, the rats were anesthetized with halothane, the right femoral artery was catheterized, and renal nerve electrodes were placed. The experiment started at least 4 hours after recovery from anesthesia. After 30 minutes of rest, air stress was provided twice, with a 10-minute interval (the average of the responses to the two stresses was used for statistical analysis). Subsequently, the rats were anesthetized with halothane, the minipump was removed, the rats were returned to their cages, and original diets were continued. BP and HR were measured at 18, 24, 42, and 48 hours, and RSNA was measured at 24 hours after the removal of the pump. Rats rested at least 30 minutes before each measurement, and BP, HR, and RSNA were monitored for 10 minutes.

Data Analysis

RSNA responses to DAF fragments or γ-globulins, air stress, ouabain, or guanabenz were expressed as percent changes from resting levels. Peak responses to air stress, guanabenz, or ouabain were calculated for statistical evaluation. All data analyses were performed using sas statistics system software (SAS Institute Inc, Cary, NC). Since values of all variables including RSNA (expressed as percent of resting values) were normally distributed, a two-way (strain and diet, or diet and treatment) ANOVA for repeated measurements was performed for all the data. When F ratios were significant, a Duncan multirange test was followed. Statistical significance was defined as P<.05.

Results

Effects of High Dietary Sodium

Resting MAP and HR

After 3 weeks of high versus regular sodium intake, the resting MAP at the start of the experimental protocols was significantly higher in Dahl S rats on a high sodium diet (130 to 140 mm Hg) compared with Dahl S rats on a regular sodium diet and Dahl R rats on either sodium diet (≈100 mm Hg). Resting values showed only minor changes throughout the experimental protocol. There were no significant differences in resting HR among the four groups of rats (data not shown). Whereas all groups had similar body weights at the beginning of the study, at the end of the 3-week dietary period, significantly less weight gain was evident in Dahl S and Dahl R rats on a high versus regular sodium diet (92±5 versus 146±5 g and 86±4 versus 123±4 g).

Responses to Ouabain

MAP, HR, and RSNA (Fig 2) started to increase at 1 minute after the ouabain injection, reached their plateau within 5 minutes, and returned to the resting levels 15 minutes after the injection. Ouabain (0.3 µg) did not induce any behavioral changes. After 1.0 µg ouabain, rats moved back and forth, causing some fluctuation in MAP, HR, and RSNA. The peak increases in MAP, HR, and RSNA in Dahl S rats on a high sodium diet were markedly less than those in Dahl S rats on a
regular sodium diet and Dahl R rats on either sodium diet. There were no significant differences in peak responses among Dahl S rats on a regular sodium diet and Dahl R rats on either sodium diet. Ouabain (1 μg) administered intravenously did not elicit any responses (data not shown).

**Responses to Air Stress**

Air stress elicited an instant increase in MAP, HR, and RSNA (Fig 3). The extent of these increases was significantly larger in Dahl S rats on a high sodium diet compared with Dahl S rats on a regular sodium diet and Dahl R rats on either sodium diet. The responses to air stress were not different in Dahl R rats on a high versus regular sodium diet.

When air stress was repeated 5 minutes after 0.3 μg ICV ouabain, the magnitude of increases in MAP, HR, and RSNA were significantly enhanced in Dahl S rats on a regular sodium diet compared with the increases induced by air stress alone. As a result, the differences in the increases in MAP, HR, and RSNA in response to air stress became insignificant for Dahl S rats on a high versus regular sodium diet. There were no significant differences between the responses to air stress before and after intracerebroventricular ouabain preinjection in Dahl S rats on a high sodium diet or in Dahl R rats.

**Responses to Guanabenz**

Intracerebroventricular guanabenz (Table 1) decreased MAP, HR, and RSNA within 2 minutes of injection. Decreases lasted ~5 minutes and were dose dependent (not shown). The magnitude of decreases was significantly larger in Dahl S rats on a high sodium diet compared with Dahl S rats on a regular sodium diet and Dahl R rats on either sodium diet.

In Dahl S rats on a regular sodium diet, after intracerebroventricular preinjection of 0.3 μg ouabain, the decreases in MAP, HR, and RSNA in response to 75 μg ICV guanabenz were significantly augmented compared with those elicited without ouabain pretreatment. Ouabain pretreatment had no effects on the responses to 75 μg ICV guanabenz in Dahl S rats on a high sodium diet and in Dahl R rats on either diet. Thus, after ouabain treatment the differences in peak responses to intrace-
Table 1. Effects of Intracerebroventricular Guanabenz (75 μg) on Mean Arterial Pressure, Heart Rate, and Renal Sympathetic Nerve Activity Before and After Ouabain Preinjection in Dahl S and R Rats on a High or Regular Sodium Diet From 4 to 7 Weeks of Age

<table>
<thead>
<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>RSNA, % resting</th>
<th>HR, bpm</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<tr>
<td>Dahl S rats</td>
<td></td>
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<td></td>
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<tr>
<td>H-Na (n=10)</td>
<td>-31±2*</td>
<td>-29±2†</td>
<td>-51±3†</td>
</tr>
<tr>
<td>R-Na (n=10)</td>
<td>-12±2</td>
<td>-30±2††</td>
<td>-29±2</td>
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<tr>
<td>Dahl R rats</td>
<td></td>
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<tr>
<td>H-Na (n=8)</td>
<td>-13±2</td>
<td>-12±1</td>
<td>-26±2</td>
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<tr>
<td>R-Na (n=9)</td>
<td>14±2</td>
<td>-11±2</td>
<td>-30±2</td>
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</table>

MAP indicates mean arterial pressure; RSNA, renal sympathetic nerve activity; HR, heart rate; bpm, beats per minute; before, before ouabain; after, after ouabain; H-Na, high sodium diet; and R-Na, regular sodium diet. Values are mean±SEM.

*P<.05 vs other groups; †P<.05 vs Dahl R rats; and ‡P<.05 vs rats in the same group before ouabain.

Intracerebroventricular guanabenz in Dahl S rats on a high versus regular sodium diet became insignificant.

Reproducibility of the Responses

In all groups, the second intracerebroventricular injection of 0.3 μg ouabain caused increases in BP, HR, and RSNA similar to those elicited by the first injection of 0.3 μg ouabain (for Dahl S rats on a regular sodium diet: MAP, 14±2 versus 12±3 mm Hg; HR, 24±3 versus 26±2 beats per minute; RSNA, 24±3% versus 26±2% resting; data for other groups not shown). To exclude the possibility that more prolonged recovery from anesthesia and surgery rather than ouabain was responsible for enhanced responses to the second air stress and guanabenz in Dahl S rats on a regular sodium diet, responses to air stress and 75 μg ICV guanabenz were assessed twice over the same time span (1.5 to 2 hours) before and after the administration of intracerebroventricular saline. The responses were found to be similar for air stress (MAP, 8±1 versus 9±1 mm Hg; HR, 20±2 versus 19±2 beats per minute; and RSNA, 24±3% versus 25±3% basal; P>.05 for all) and guanabenz (MAP, -9±2 versus -12±2 mm Hg; HR, -22±3 versus -21±2 beats per minute; RSNA, -25±2% versus -23±3% basal; P>.05 for all).

Reversal of Sympathoexcitatory and Hypertensive Effects of High Sodium Intake by Intracerebroventricular DAF Fragments

Effect of DAF Fragments on Resting MAP, HR, and RSNA

As shown in Table 2, at 18 hours after intracerebroventricular injection of 132 μg DAF fragments, BP of Dahl S rats on a high sodium diet had decreased significantly and had reached the level of BP in Dahl S rats on a regular sodium diet or Dahl R rats on either diet. In Dahl S rats on a high sodium diet, RSNA also decreased significantly after the administration of DAF fragments and was significantly lower than that in the other groups of rats when expressed as a percentage of the level before the injection. In Dahl S rats on a regular sodium diet or Dahl R rats on either sodium diet, resting BP and RSNA were not affected by the DAF fragments. In all groups of rats, no changes in HR were observed after intracerebroventricular injection of DAF fragments (data not shown).

Fig 4 shows the time course of the decrease in BP by DAF fragments in Dahl S rats on a high sodium diet. At 4 hours after the injection of DAF fragments or γ-globulins, no changes in BP were observed. However, 14 hours after the injection of intracerebroventricular DAF fragments, resting MAP had significantly decreased, and by 18 and 24 hours after the injection, BP had decreased to the level of BP in Dahl S rats on a regular sodium diet (Fig 4 and Table 2). In contrast, after intracerebroventricular injection of γ-globulins, BP (Fig 4) remained elevated and unchanged up to 24 hours. As with the BP, intracerebroventricular DAF fragments did not change the resting RSNA after 4 hours (96±3% versus before injection) but caused a significant decrease after 18 hours in Dahl S rats on a high sodium diet (Table 2).

Responses to Air Stress and Intracerebroventricular Guanabenz After DAF Fragments

In Dahl S rats on a regular sodium diet, air stress administered 18 hours after intracerebroventricular injection of 132 μg DAF fragments elicited increases in BP, HR, and RSNA similar to those induced by air stress before treatment with DAF fragments (Fig 5). Similarly, responses to air stress in Dahl R rats on either diet were not affected by the DAF fragments (data not shown). In Dahl S rats on a high sodium diet, the responses were not different before versus after 132 μg γ-globulin injection (Fig 5). In contrast, in Dahl S rats on a high sodium diet, the responses to air stress after the injection of DAF fragments were significantly attenuated compared with those induced before the injection of intracerebroventricular DAF fragments and compared with those induced after the injection of γ-globulins (Fig 5). After intracerebroventricular injection of DAF fragments, there were no longer significant differences in MAP, HR, and RSNA responses to air stress between Dahl S rats on a high sodium diet and the other groups of rats.

In Dahl S rats on a high sodium diet, 18 hours after intracerebroventricular injection of 132 μg DAF fragments, the magnitude of decreases in MAP, HR, and RSNA in response to 75 μg ICV guanabenz was signif-
TABLE 2. Resting Mean Arterial Pressure and Renal Sympathetic Nerve Activity Before and 18 Hours After the Administration of Intracerebroventricular Digoxin-Specific Antibody Fab Fragments of γ-Globulins in Dahl S and R Rats on High or Regular Sodium Intake From 4 to 7 Weeks of Age

<table>
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<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>RSNA, % before injection</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<td>First experiment</td>
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<td>DAF fragments</td>
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<tr>
<td>Dahl S rats</td>
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<tr>
<td>H-Na (n=10)</td>
<td>136±4*</td>
<td>104±3†</td>
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<tr>
<td>R-Na (n=10)</td>
<td>105±6</td>
<td>100±3</td>
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<tr>
<td>Dahl R rats</td>
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<tr>
<td>H-Na (n=8)</td>
<td>103±5</td>
<td>97±4</td>
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<td>R-Na (n=9)</td>
<td>99±6</td>
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<tr>
<td>Dahl S rats</td>
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<tr>
<td>H-Na (n=7)</td>
<td>135±4</td>
<td>106±6††</td>
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<td>γ-Globulins</td>
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<td></td>
</tr>
<tr>
<td>Dahl S rats</td>
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</tr>
<tr>
<td>H-Na (n=8)</td>
<td>137±5</td>
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MAP indicates mean arterial pressure; RSNA, renal sympathetic nerve activity; before, before injection of digoxin-specific antibody Fab (DAF) fragments or γ-globulins; after, after injection of DAF fragments or γ-globulins; H-Na, high sodium diet; and R-Na, regular sodium diet. Values are mean±SEM.

*P<.05 vs other groups of rats before or after injection of DAF fragments; †P<.05 vs rats in the same group before injection of DAF fragments; and ††P<.05 vs after γ-globulin injection.

Significantly attenuated compared with the decreases induced by the same dose of guanabenz without DAF fragment pretreatment as well as compared with the decreases after γ-globulin injection (Fig 6). Pretreatment with DAF fragments did not change the responses to guanabenz in Dahl S rats on a regular sodium diet, nor did pretreatment with γ-globulins in Dahl S rats on a high sodium diet. DAF fragments abolished the enhancement of the peak decreases in MAP, HR, and RSNA in response to intracerebroventricular guanabenz present in Dahl S rats on a high sodium diet (Fig 6).

Prevention of Sympathoexcitatory and Hypertensive Effects of High Sodium Intake by Intracerebroventricular DAF Fragments

When the rats were 7 weeks of age, basal MAP had significantly increased in Dahl S rats on a high sodium diet with the administration of intracerebroventricular γ-globulins compared with other groups. In contrast, in Dahl S rats on a high sodium diet that were treated with intracerebroventricular DAF fragments, MAP had not increased at all and was similar to MAP in Dahl S rats on a regular sodium diet that were subjected to either treatment (Fig 7). HRs were similar in all groups of rats (data not shown).

Increases in MAP, HR, and RSNA caused by air stress were significantly larger in Dahl S rats on a high sodium diet that were treated with intracerebroventricular γ-globulins compared with the other groups (Fig 8). In contrast, in Dahl S rats on a high sodium diet that were treated with DAF fragments, responses to air stress were not augmented and were similar to those found in rats on a regular sodium diet. DAF fragments did not change the responses in Dahl S rats on a regular sodium diet.

The resting MAP values after the discontinuation of intracerebroventricular DAF or γ-globulin infusion are shown in Fig 7. There were no significant changes in MAP over the 48 hours after stopping the infusions in Dahl S rats on a high sodium diet that were treated previously with γ-globulins or in Dahl S rats on a regular sodium diet that were subjected to either treatment. In contrast, in Dahl S rats on a high sodium diet that were treated previously with DAF fragments, MAP gradually increased after the removal of the pump. At 48 hours after the discontinuation of the DAF fragments, MAP reached the level found in rats on a high sodium diet that were treated with γ-globulins.

In Dahl S rats on a high sodium diet that were treated with intracerebroventricular γ-globulins or in Dahl S rats on a regular sodium diet subjected to either treatment, RSNA had decreased to ~80% of the original activity 24 hours after the removal of the pumps. In contrast, in Dahl S rats on a high sodium diet that were treated previously with DAF fragments, RSNA was slightly increased 24 hours after the removal of the pumps and was significantly higher than RSNA values found in other groups, when RSNA was expressed as the percentage of pre-removal activity for each rat: 103±4% versus 78±3%, 83±4%, or 80±3%, for RSNA.
in rats on a high sodium diet that were treated with DAF fragments versus rats on a high sodium diet that were treated with γ-globulins, rats on a regular sodium diet that were treated with DAF fragments, or rats on a regular sodium diet that were treated with γ-globulins, respectively.

Discusson

The present study provides four significant new findings: (1) In Dahl S rats, high sodium causes hypertension associated with blunted responses of BP, HR, and RSNA to central ouabain. (2) Sodium-induced hypertension in Dahl S rats is associated with enhanced BP, HR, and RSNA responses to air stress and to central α2-adrenergic receptor stimulation, and in Dahl S rats on regular sodium, pretreatment with intracerebroventricular ouabain mimics these effects of high dietary sodium. (3) Intracerebroventricular DAF fragments significantly decrease resting BP and RSNA as well as reverse enhanced responses to air stress or central α2-adrenergic receptor stimulation in Dahl S rats on a high sodium intake. (4) Intracerebroventricular DAF fragments concomitant with a high sodium diet prevent sodium-induced hypertension and sympathoexcitation in Dahl S rats.

Dietary Sodium and Central Effects of Ouabain

In Dahl S rats, high sodium intake caused hypertension and markedly attenuated the sympathoexcitatory and pressor responses to central ouabain. In SHR, high sodium similarly attenuates responses to central ouabain.8 Since high sodium increases brain content of OLA in both SHR and Dahl S rats (F.H.H. Leenen, unpublished data), the attenuated responses to ouabain may reflect an increased receptor occupancy by endogenous OLA and decreased availability of receptors for exogenous ouabain. Such an increase appears to occur in centers involved in sympathetic control only in SHR and Dahl S rats, since high sodium decreases sympathoexcitatory responses to exogenous ouabain only in hypertensive rats and not in WKY9 and Dahl R rats.

In Dahl S rats, high sodium intake enhanced sympathoexcitatory and pressor responses to air stress, consistent with findings by Koepke et al.12 In Dahl S rats, high sodium intake also enhanced sympathoinhibitory and depressor responses to centrally administered guanabenz, consistent with upregulation and/or decreased receptor occupancy of α2-adrenergic receptors in the anterior hypothalamus as a consequence of less sympathoinhibition.20 Thus, in both SHR27,28 and Dahl S rats, high sodium intake may cause both withdrawal of sympathoinhibition and enhancement of sympathoexcitatory responses. Moreover, in both Dahl S rats and SHR on a regular sodium intake, central injection of ouabain enhanced the responses to air stress and guanabenz, similar to the extent caused by high sodium intake. This enhancement by exogenous ouabain was not found in Dahl R or WKY9 rats. Thus, both strains of rats genetically predisposed to hypertension respond to high sodium intake with enhanced sympathetic and BP responses to air stress and guanabenz, and this pattern of changes can be reproduced in rats on a regular sodium intake by pretreatment with ouabain. However, considering the extensive genetic polymorphism described between Dahl S and R rats as well as between SHR and WKY rats, the above-described differences in response to central ouabain may or may not be related to the pathogenesis of sodium-induced hypertension.

Central Effects of DAF Fragments

In vitro, DAF fragments not only bind digoxin but also bind ouabain and human OLA22 as well as rat brain OLA (F.H.H. Leenen, unpublished data) with high affinity. DAF fragments diffuse rapidly into the interstitial space and bind free glycosides.23 Because the affinity of glycosides for DAF fragments is higher than for Na+K+-ATPase, the glycosides further dissociate from the cell membrane and will not reassociate with Na+K+-ATPase.23 We previously reported6 that DAF fragments block the sympathoexcitatory and pressor effects of centrally administered ouabain, brain OLA, and hypertonic saline in normotensive rats, suggesting that sympathoexcitatory and pressor effects of increased brain sodium are mediated by brain OLA. The present study shows that in Dahl S rats on a high sodium diet DAF fragments (but not γ-globulins) reverse the enhanced responses to air stress and guanabenz, decrease basal RSNA, and return the increased resting MAP down to normal levels. Moreover, intracerebroventricular administration of DAF fragments concomitant with high sodium intake prevents the development of sodium-induced hypertension and augmentation of sympathoexcitatory and pressor responses to air stress in Dahl S rats. DAF fragments were ineffective in Dahl S rats on a regular sodium diet and in Dahl R rats. These findings indicate that the responses to the DAF fragments are specific for the Dahl S rats on a high sodium diet.

The hemodynamic effects of DAF fragments in hypertensive rats have, so far, been evaluated in only one study:24 in SHR on a high sodium diet intravenous DAF fragments had no effects on basal MAP, but BP was followed for only 30 minutes. No study has assessed the effects of intracerebroventricularly administered DAF...
fragments on the prevention or reversal of hypertension. In Dahl S rats on a high sodium diet, up to 4 hours after the intracerebroventricular administration of DAF fragments, no changes in basal BP and RSNA were noted. However, after 14 hours, the BP had significantly decreased, and 18 hours after intracerebroventricular administration of the DAF fragments, the sympathoexcitatory and pressor effects of high dietary sodium in Dahl S rats had been reversed. This time delay is similar to a recent observation by Balzan et al\textsuperscript{22} showing that simultaneous administration of DAF fragments and ouabain prevented the binding of ouabain to human erythrocyte receptors, but the reversal by DAF fragments of an established binding of ouabain to the receptors required 12 hours.

Intracerebroventricular DAF fragments concomitant with high sodium intake prevented hypertension in Dahl S rats. After discontinuing the intracerebroventricular DAF fragments, the basal MAP showed a small increase at 24 hours and reached the level found in Dahl S rats on a high sodium diet that were treated with intracerebroventricular γ-globulins at 48 hours. Therefore, it appears that at least 18 to 24 hours is required to clear the DAF fragments out of the central nervous system and to build up a level of free brain OLA high enough to induce the sympathoexcitatory and pressor effects of high sodium. Since Dahl S rats did not show pressor effects after 1 week on a high sodium diet (see \textquotedblleft Materials and Methods\textquotedblright), the rapid occurrence of the pressor effects of high sodium after discontinuing the DAF fragments may possibly relate to an upregulation of brain ouabain receptors and/or a high rate of brain OLA synthesis after 3 weeks on a high sodium diet and perhaps further activation of brain OLA in response to chronic DAF fragments.

Central Ouabain-like Activity: Mediator of the Pressor Effects of Sodium in Dahl S rats?

The precise mechanisms through which high sodium intake causes hypertension in Dahl S rats have not yet

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**FIG 5.** Bar graphs showing increases in mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) in response to air stress before (control) and after intracerebroventricular injection of 132 μg digoxin-specific antibody Fab (DAF) fragments or 132 μg γ-globulins at 7 weeks of age in Dahl S rats on a high sodium diet (H-Na) or a regular sodium diet (R-Na) from 4 to 7 weeks of age. Values are mean±SEM (n=8 to 10). *P<.05 vs corresponding values for R-Na. **P<.05 vs H-Na control value (before DAF) and vs values after γ-globulin injection.

**FIG 6.** Bar graphs showing decreases in mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) in response to intracerebroventricular injection of 75 μg ouabain before (control) and after intracerebroventricular injection of 132 μg digitoxin-specific antibody Fab (DAF) fragments or 132 μg γ-globulins at 7 weeks of age in Dahl S rats on a high sodium diet (H-Na) or a regular sodium diet (R-Na) from 4 to 7 weeks of age. Values are mean±SEM (n=7 to 10). *P<.05 vs corresponding value for R-Na. **P<.05 vs H-Na control value (before DAF) and vs values after γ-globulin injection.
been elucidated. The present study suggests that the signal of high dietary sodium may be transmitted via increasing brain OLA. High sodium intake increases the sodium concentration of the cerebrospinal fluid and the content of OLA in brain areas such as the hypothalamus and pons in Dahl S rats but not, or only to a minor extent, in Dahl R rats (F.H.H. Leenen, unpublished observation). Moreover, high sodium intake appears to decrease the availability of brain receptors for exogenous ouabain only in Dahl S rats and not in Dahl R rats. Since intracerebroventricular DAF fragments prevent as well as reverse the chronic sympathoexcitatory and pressor effects of high sodium intake in Dahl S rats, we propose that in Dahl S rats high sodium intake increases cerebrospinal fluid sodium concentration and thereby brain OLA and that the latter causes sympathoexcitatory and pressor responses. However, the high sodium-induced increase in brain OLA alone appears to be not enough to cause chronic sympathoexcitatory and pressor responses. In WKY control rats, high sodium intake also increases the content of OLA in the hypothalamus, but MAP does not increase. Moreover, intracerebroventricular ouabain mimics the sympathetic effects of high dietary sodium only in Dahl S rats and SHR but not in Dahl R and WKY rats. These findings may point to a difference in Dahl S versus Dahl R rats and in SHR versus WKY rats in the binding affinity of brain OLA to Na, K-ATPase or the amount of the enzyme in the central regions mediating the effects of dietary sodium on sympathetic and BP regulation.

**Summary and Conclusion**

The development of sodium-induced hypertension in Dahl S rats is associated with decreased sympathoexcitatory and pressor responses to exogenous ouabain, consistent with increased receptor occupancy by endogenous OLA. In Dahl S rats on a normal sodium diet but not in Dahl R rats, central ouabain mimics the sympathoexcitatory and pressor effects expressed in Dahl S rats on a high sodium diet. Centrally administered DAF fragments decrease the BP and reverse the augmented sympathoexcitation induced by high dietary sodium in Dahl S rats. Moreover, intracerebroventricular administration of DAF fragments concomitant with a high sodium diet prevents sodium-induced hypertension and augmentation of pressor and sympathoexcitatory responses to stress. Together, these findings suggest that brain OLA plays a primary role in mediating the central effects of high sodium intake in Dahl S rats, which is genetically linked with the development of hypertension in Dahl S rats on a high sodium diet, and is not secondary to the development of hypertension. Since the pattern of changes caused by high sodium intake is similar in SHR, it is possible that sodium-sensitive hypertension in general is mediated by central ouabain-like activity. Whether this is unique for models genetically predisposed to hypertension or extends to sodium-sensitive models with high sympathetic activity, such as Grollman hypertension and deoxycorticosterone acetate-salt hypertension has yet to be explored.

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Brain "ouabain" mediates the sympathoexcitatory and hypertensive effects of high sodium intake in Dahl salt-sensitive rats.

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