Chronic activation of brain areas by high-sodium diet in Dahl salt-sensitive rats

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Budzikowski, Adam S., Faranak Vahid-Ansari, and Frans H. H. Leenen. Chronic activation of brain areas by high-sodium diet in Dahl salt-sensitive rats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H2046–H2052, 1998.—To map changes in neuronal activity in the brains of Dahl salt-sensitive (Dahl S) vs. salt-resistant (Dahl R) rats by high-sodium diet, we used immunohistochemical detection of Fra-like proteins as a marker for long-term neuronal activation. Compared with Dahl R rats during regular sodium intake, Dahl S rats showed modestly higher expression of Fra-like immunoreactivity (Fra-LI) in the supraoptic nucleus, anterior hypothalamic area (AHA), central gray, and nucleus of solitary tract (NTS) at 5, 6, and 9 wk of age but clearly elevated Fra-LI in the magnocellular part of the paraventricular nucleus (PVN) at 6 wk of age (not at 5 and 9 wk). In the median preoptic nucleus (MnPO) Fra-LI was lower at 9 wk of age and no differences were observed in the parvocellular PVN and subfornical organ in Dahl S vs. Dahl R rats on regular sodium intake. Compared with Dahl S rats on a regular-sodium diet, Dahl S rats on a high-sodium diet from 4 to 9 wk of age had significantly increased blood pressure and experienced transient activation of magnocellular PVN and MnPO and virtually no changes in the activity of the parvocellular PVN, AHA, and NTS. In contrast, Dahl R rats showed marked activation in the magnocellular PVN after 1 and 2 wk on a high-sodium diet compared with Dahl R rats on a regular-sodium diet. The present study demonstrates that Dahl S rats show differential activation of brain areas participating in regulation of osmotic and cardiovascular homeostasis during development of sodium-sensitive hypertension.

Dahl salt-resistant rats; sodium intake; neuronal activation; immediate early genes

IN DAHL SALT-SENSITIVE (Dahl S) rats high-sodium diet increases hypothalamic "ouabain," decreases sympathetic inhibition, increases sympathoexcitation, desensitizes baroreflex function, and causes hypertension (14, 15, 18). These responses to high-sodium diet can be prevented by intracerebroventricular administration of Fab fragments binding ouabain, brain "ouabain," and related steroids with high affinity (14, 15). In Dahl S rats the development of hypertension on high sodium intake is associated with increases in cerebrospinal fluid (CSF) sodium, suggesting activation of not only peripheral but also central sodium and/or osmoreceptors (21). Lesions of the anteroventral third ventricle region (AV3V) and the paraventricular nucleus (PVN) minimize or prevent development of this form of hypertension (1, 10, 11). However, the actual sequence of events and the pathways involved have not been documented so far.

Immediate early genes like c-fos and jun encode transcriptional factors that are considered to reflect activation of neurons responding to synaptic activation, voltage-gated calcium entry, and alterations in concentrations of second messenger systems (27). Immunohistochemical detection of Fos has been proven to be a good marker of short-term neuronal activation, including neurons involved in cardiovascular homeostasis (2, 7). However, after an initial increase Fos downregulates quickly (25, 31). With continuous stimulation Fos expression is followed by expression of other Fos family proteins, like Fos-B, Fra-1, and Fra-2. These immediate early genes persist longer than Fos and can therefore be used to map long-term neuronal activation (6, 13, 26, 29).

In the present study we used immunohistochemical detection of these proteins, as Fos-like immunoreactivity (Fra-LI), to map activation of central nervous system areas involved in cardiovascular and osmotic homeostasis during development of sodium-sensitive hypertension in Dahl S compared with Dahl salt-resistant (Dahl R) rats on high sodium intake.

METHODS

Male Dahl S and Dahl R rats (4 wk of age; Harlan Sprague Dawley, Madison, WI) were housed two or three per cage in a temperature-controlled environment with a 12:12-h light-dark cycle and with free access to a regular-sodium diet (101 µmol Na/g) and water. After an acclimatization period of 3–4 days, the rats were randomized to either continue on a regular-sodium diet or start a high-sodium diet (1,370 µmol Na/g) purchased from Harlan Sprague Dawley. All animals were treated in accordance with the procedures outlined in the Guide for the Care and Use of Experimental Animals endorsed by the Medical Research Council of Canada.

Effects of Chronic High-Sodium Diet on Blood Pressure

After 2 or 5 wk on either regular- or high-sodium diet (n = 5 rats/group), Dahl S or Dahl R rats were anesthetized using halothane in oxygen (1.5%) and a polyethylene catheter (CPE 50, Clay Adams, Becton-Dickinson, Sparks, MD) filled with heparinized saline (100 U/ml) placed in the left carotid artery. Twenty-four hours later, the rats were placed in partitioned cages and the catheters were connected to a pressure transducer (Abbott Labs, Chicago, IL). The arterial blood pressure was recorded in conscious unrestrained rats after a 30-min acclimatization period using an MP100WSW data-acquisition system (Biopac System, Goleta, CA).

Effects of Chronic High-Sodium Diet on Immediate Early Gene Expression

Dahl S or Dahl R rats were fed either diet for 1, 2, or 5 wk (n = 5 rats/group at each time point). Animals were then deeply anesthetized by intraperitoneal injection of pentobarbital sodium (100 mg/kg; Somnotol MTC Pharmaceuticals, Cambridge, ON, Canada) and perfused transcardially with 200 ml of 0.9% saline followed by 150 ml of 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde (PFA) at room temperature. Brains were then removed and postfixed in 4% PFA for 24–48 h at 4°C. Coronal sections were cut using a...
A vibratome from the forebrain starting at the vertical limb of the diagonal band of Broca ending at the arcuate nucleus and from the medulla starting at the level of the obex to the parabrachial nucleus according to the atlas of Paxinos and Watson (23).

Fra-LI was detected using an affinity-purified rabbit polyclonal antibody (c-fos K-25, Santa Cruz Biotechnology, Santa Cruz, CA) recognizing amino acids 128–152 in the NH₂-terminal region of Fos. This antibody recognizes Fos, Fos-B, Fra-1, and Fra-2 proteins (8). To detect the expression of Fos-like immunoreactivity, we used affinity-purified sheep antibody CRB OA-11–823 (Cambridge Research Biochemicals, Cambridge, UK) recognizing amino acids 2–16 in the NH₂-terminal region of Fos. Immunohistochemistry was performed using a standard procedure (29). Briefly, sections were washed in 0.01 M PBS containing 0.3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. Sections were then rinsed three times in PBS and incubated in PBS containing 0.3% Triton X-100, 0.02% sodium azide, and primary antibody using either c-fos K-25 (1:2,000) or CRB OA-11–823 (1:500) for 48 h. Next, the sections were washed three times with PBS and incubated with either biotin-labeled donkey anti-rabbit or donkey anti-sheep secondary antisera (Jackson Laboratories, West Grove, PA; 1:200) for 16 h. The sections were washed three times with PBS and incubated for 3 h with PBS containing 0.3% Triton X-100 and streptavidin-horseradish peroxidase (Amersham Canada, Oakville, ON, Canada; 1:100). After three washes in PBS, the sections were rinsed in 0.1 M acetate buffer, pH 6.0. The reaction was visualized using a glucose oxidase-diaminobenzidine-nickel method. The reaction was terminated by washing in acetate buffer. Subsequently the sections were mounted on chrom-alum-coated slides, dried, dehydrated through a graded series of alcohols and two changes of xylene, and coverslipped for microscopic observation.

Positive immunoreactive neurons were quantified in the different brain areas using an image-analysis system equipped with Image 1.47 software (29). Digitization of sampled areas (600 × 800 µm) was performed at ×100 magnification using a charge-coupled device camera linked to a microscope. The area of interest was then outlined and thresholding was performed on the digitized image to allow for exclusion of small positive profiles such as fragments of nuclei (<8 µm), glial cells, and weakly stained neurons from the final analysis. To ensure consistency between measurements, we chose the threshold value on the basis of the background staining. In every rat, for each area a total of two measurements of Fra-LI-positive nuclei was done, each performed on a separate section. Averages from these measurements per area per rat were used for statistical analysis.

Statistical Analysis

All data are presented as means ± SE. Factorial ANOVA was performed on the cell count for each region with strain, diet, and time as factors, followed by the Newman-Keuls test where applicable (30). Individual differences between groups of rats were separated using a modified t-test (30).

RESULTS

**Sodium-Dependent Hypertension in Dahl S vs. Dahl R Rats**

High-sodium diet did not change the mean arterial pressure (MAP) in Dahl R rats after 2 or 5 wk (Table 1). In contrast, significant changes were observed in Dahl S rats after both 2 and 5 wk on high-sodium diet. After 2 wk on high sodium, the MAP had increased to 124 ± 3 mmHg, whereas a marked increase (to 199 ± 7 mmHg) occurred in Dahl S rats after 5 wk on high sodium (Table 1).

**Table 1. Blood pressure and body weight in Dahl S vs. Dahl R rats after 2 or 5 wk on either regular- or high-sodium diet**

<table>
<thead>
<tr>
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<th>Body Wt, g</th>
<th>MAP, mmHg</th>
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<tr>
<td></td>
<td>2 wk</td>
<td>5 wk</td>
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<tr>
<td>Dahl R rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular sodium</td>
<td>179 ± 3</td>
<td>270 ± 7</td>
</tr>
<tr>
<td>High sodium</td>
<td>164 ± 5</td>
<td>249 ± 5</td>
</tr>
<tr>
<td>Dahl S rats</td>
<td></td>
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</tr>
<tr>
<td>Regular sodium</td>
<td>199 ± 5</td>
<td>308 ± 10</td>
</tr>
<tr>
<td>High sodium</td>
<td>191 ± 3</td>
<td>267 ± 7</td>
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Values are means ± SE; n = 5 rats/group. MAP, mean arterial pressure; Dahl S and Dahl R rats, Dahl salt-sensitive and salt-resistant rats, respectively. *P < 0.01 vs. rats on regular-sodium diet at same time point within strain.

**Effects of Chronic High-Sodium Diet on Fos Immunoreactivity**

The whole brain was scanned for the presence of Fos-like-positive immunoreactive neurons after Dahl R and Dahl S rats were fed either regular- or high-sodium diet for 1, 2, or 5 wk. Fos immunoreactive neurons were undetectable in all examined regions in both Dahl R and Dahl S rats at all time points.

**Effects of Chronic High-Sodium Diet on Fra-LI**

In contrast to Fos-like immunoreactivity, Fra-LI-positive neurons were detected in osmosensitive nuclei of the lamina terminalis, including the median preoptic nucleus (MnPO) (ventral and dorsal part) and the subfornical organ (SFO). Fra-LI neurons were also observed in the supraoptic nucleus (SON) and in areas regulating cardiovascular function, including the PVN, anterior hypothalamic area (AHA), nucleus of solitary tract (NTS), and central gray (CG). No immunoreactivity was observed in supraschiasmatic, arcuate, and parabrachial nuclei or the rostral ventrolateral medulla. Figure 1 presents the pattern of Fra-LI-positive neurons in the PVN of Dahl R and Dahl S rats on a high-sodium diet for 2 wk.

**Fra-LI Neurons in Osmosensitive Nuclei**

SFO. On regular sodium intake, Fra-LI in the SFO was barely detectable in Dahl R rats at all time points. In Dahl S rats, some Fra-LI-positive neurons were present in the SFO at 2 wk on a regular-sodium diet (Fig. 2B). On a high-sodium diet, both Dahl R and Dahl S rats showed significant and similar expression of Fra-LI after 2 and 5 wk. These increases were slightly higher after 2 wk in Dahl S rats than in Dahl R rats (Fig. 2B). These immunoreactive neurons were found in the core but not at the periphery of the SFO.

MnPO. In the MnPO, Fra-LI was clearly present in both Dahl R and Dahl S rats on a regular-sodium diet at all time points, but it decreased with maturation and at
9 wk of age was less in Dahl S than in Dahl R rats (Fig. 2A). In Dahl S rats high-sodium diet significantly increased the expression of Fra-LI (1 and 2 wk), which was followed by a decrease in activity, whereas it had no effects in Dahl R rats (Fig. 2A).

SON. In the SON, the number of Fra-LI-positive neurons was significantly higher in Dahl S than in Dahl R rats after both 2 and 5 wk on a regular-sodium diet (Fig. 3B). High-sodium diet markedly increased expression of Fra-LI in Dahl S rats after 2 wk on a regular-sodium diet (Fig. 3A). High-sodium diet did not change Fra-LI expression in either of the strains (Fig. 3B). In the magnocellular part of the PVN, Fra-LI expression was also low, except for clear expression in Dahl S rats after 2 wk on a regular-sodium diet (Fig. 3A). High-sodium diet significantly increased expression of Fra-LI in Dahl S and Dahl R rats in the magnocellular portion of the PVN. However, in Dahl S rats this increase was only noted after 1 wk and in Dahl R rats after both 1 and 2 wk, but no longer after 5 wk (Fig. 3A).

AHA. Few Fra-LI-positive neurons were detectable in the AHA of Dahl R rats on a regular-sodium diet. Compared with Dahl R rats on a regular-sodium diet, a modest but significant increase in Fra-LI was detected in Dahl S rats at all time points. High-sodium diet did not change this pattern (Fig. 2D).

NTS. In both Dahl S and Dahl R rats, Fra-LI-positive neurons were detected in the caudal part of the NTS. In Dahl R and Dahl S rats on a regular-sodium diet, few Fra-LI-positive neurons were observed after both 1 and 2 wk and none after 5 wk in the caudal NTS (Fig. 3D). In Dahl R rats on high sodium intake, modest but significant increases in Fra-LI were observed at all time points. In Dahl S rats, high-sodium diet significantly increased Fra-LI at 5 wk with no significant changes after 1 and 2 wk (Fig. 3D).

CG. In the CG, few scattered positive neurons were detected in Dahl R rats on a regular-sodium diet after 1, 2, and 5 wk of follow-up. On regular-sodium diet, Fra-LI expression was more pronounced in Dahl S rats than in Dahl R rats at all time points (Fig. 3C). In Dahl R rats, high-sodium diet significantly increased Fra-LI after 2 and 5 wk. In Dahl S rats, high-sodium diet only increased Fra-LI at 5 wk, and no significant changes were observed after 1 and 2 wk (Fig. 3C).

**DISCUSSION**

Only limited information is available regarding differences in brain functions between Dahl S and Dahl R rats (1, 10, 11, 14, 15, 18). In the present study we utilized immunohistochemical detection of Fra-LI to identify differences in Dahl S and Dahl R rats on regular sodium intake and in response to a high-sodium diet in a variety of neuronal populations. The study reveals major differences between Dahl R and Dahl S rats on a regular-sodium diet in the activity of magnocellular neurons in the PVN and to a lesser extent in the SON, AHA, and CG and shows that development of sodium-sensitive hypertension is accompanied by a differential change in Fra-LI expression mostly in brain areas involved in osmoregulatory responses. With this stimulus and in this time frame the detected Fra-LI is not influenced by the presence of Fos.
Fra-LI in Dahl S and Dahl R Rats on Regular Sodium Intake

On a regular-sodium diet, Dahl S rats show higher activity in the SON, AHA, and CG. On the other hand, activity of the magnocellular PVN fluctuates from being higher at 6 wk of age and lower at 9 wk of age. Dahl S rats show increased pituitary arginine vasopressin (AVP) content (24) but no difference in plasma AVP compared with Dahl R rats (19). This pattern of Fra-LI may therefore reflect an initial increase in the synthesis of AVP, but without increased release leading to an accumulation of the peptide in neurons followed by feedback inhibition of further synthesis.

The slightly higher activity in AHA neurons of Dahl S rats may not be related to sympathoinhibitory neurons in this area because responses to intracerebroventricular injection of the α2-agonist guanabenz, which can be used as a marker for the functional activity of this area (22), do not differ between the two strains on regular sodium intake (16). The functional relevances of minor differences in the Fra-LI in the MnPO, SFO, and NTS remain to be established and are possibly related causally or are a consequence of the small increase in MAP observed in Dahl S rats on regular sodium intake (Table 1).

Fra-LI in Dahl S and Dahl R Rats on High Sodium Intake

Transient activation of the magnocellular PVN, SON, and MnPO and virtually no changes in the activity of the parvocellular PVN, AHA, and NTS accompany the development of hypertension in Dahl S rats.

Osmoregulatory brain areas. Both Dahl R and Dahl S rats showed modest activation of the SFO by a high-sodium diet. Nakamura and Cowley (21) reported that an increase in CSF sodium, which could be an osmotic stimulus activating SFO, only occurs in sodium-sensitive rats. However, the pattern of immunoreactivity (activation of mostly the core but not the periphery of the SFO) is consistent with the activation of this structure by a humoral factor circulating in the blood but not the CSF (2, 20, 28) and suggests that changes in CSF sodium were of lesser importance in the activation of this area.

The AV3V plays a major role in the development of hypertension in Dahl S rats (17). Our study demon-
strates virtually no changes in Fra-LI in the MnPO (major part of AV3V) in Dahl R rats but activation followed by decreased activity in Dahl S rats. This pattern of changes in activation of the MnPO is consistent with a study showing that the AV3V is necessary for development of hypertension only in the initial phase and that lesions of this area in Dahl S rats with established hypertension fail to reverse the hypertension (17). In addition, in spontaneously hypertensive rats (SHR), high sodium intake causes a similar activation of the MnPO (4), and “ouabain” release in this nucleus plays a critical role during development of sodium-sensitive hypertension in SHR (3).

In Dahl R rats, introduction of a high-sodium diet resulted in an initial, intense (up to 5-fold) activation of neurons in the magnocellular PVN followed by a minor decrease in activity after 5 wk of diet. In contrast, in Dahl S rats activation of these neurons is shorter lasting (only after 1 wk of diet) and is lower or at the control level for the rest of the observation period. Greene et al. (12) reported that in both strains introduction of a high-sodium diet was followed by an equal volume expansion and increase in cardiac output and that the reason for the blood pressure increase in Dahl S rats lies in a deficit in the adaptation of cardiovascular regulatory systems to accommodate that volume increase. Dahl R rats may have persistently higher Fra-LI expression in the PVN because the stimuli (either volume or blood pressure increase) that could decrease activity of magnocellular neurons remain small. In the later phase, other mechanisms coping with increased sodium intake and volume expansion probably take over, and activation of the PVN becomes unnecessary to maintain osmolality of extracellular fluid within the physiological range. Interestingly, plasma AVP remains elevated in later stages of hypertension in Dahl S rats on high sodium (19) despite decreasing levels of Fra-LI in magnocellular neurons.

The absence of an increase in Fra-LI in the parvocellular PVN by high sodium suggests that this area does not play an important role in the development of sodium-sensitive hypertension. On the other hand, an increase in the activity of this area could be expected, considering the role the parvocellular division of the

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**Fig. 3.** Number of Fra-LI-positive neurons per section in the paraventricular nucleus of the hypothalamus (magnocellular (A) and parvocellular division (B); −2.12 mm from the bregma), the central gray (C; −7.64 mm from the bregma), and the rostral part of the nucleus of solitary tract (D; −11.6 mm from the bregma) of Dahl R and Dahl S rats on regular-sodium and high-sodium diets. Data are presented as means ± SE; n = 5 rats/group. *P < 0.05 vs. age-matched control on Reg Na within strain; *P < 0.05 vs. Dahl R rats on similar diet at same time point.
PVN plays in regulation of sympathetic activity and that bilateral electrolytic lesions of the entire PVN blunts the development of hypertension (1, 11). This division of the PVN also may be a major source of neurons releasing "ouabain" (32, 33), which mediates increased sympathetic activity in this model of hypertension (14, 16). Because these lines of evidence suggest involvement of the paravascular PVN in the development of hypertension, it is conceivable that in this particular situation immediate early genes other than those of the Fos family are activated or activation occurs without any increase in immediate early genes.

Other brain areas. The activity of AHA was not affected in both strains by a high-sodium diet. This observation is consistent with a study by Chen et al. (5) that demonstrated no differences in the norepinephrine stores and turnover rates in the AHA in Dahl rats on high sodium. On the other hand, a functional test for the activity of this area (MAP and heart rate response to intracerebroventricular guanabenz) indicates that there is a decrease in the activity of AHA in Dahl S rats on a high-sodium diet (16).

In contrast to Dahl S rats, in Dahl R rats the activity of the caudal NTS increased. Because that part of the NTS receives input from cardiovascular afferents, this observation may indicate increased input from peripheral receptors in Dahl R rats. This observation is consistent with sensitization of the arterial baroreflex by high-sodium diet in Dahl R rats (9, 15). Similarly, lack of changes in Fra-LI in Dahl S rats may result from a decrease in gain and resetting of the baroreflex by high sodium in Dahl S rats (9, 15).

In conclusion, on regular sodium intake, in comparison to Dahl R rats, Dahl S rats have higher Fra-LI in the magnocellular PVN, SON, AHA, and CG. In Dahl S rats the development of hypertension on high-sodium diet is associated with transient activation of the magnocellular PVN, SON, and MnPO and no change in the activity of the paravascular PVN, AHA, and NTS. In Dahl R rats, prolonged activation of the magnocellular PVN and small increases in activity in the NTS may reflect brain activity related to prevention of an increase in blood pressure by high dietary sodium.

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