Role of central nervous system aldosterone synthase and mineralocorticoid receptors in salt-induced hypertension in Dahl salt-sensitive rats

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Huang BS, White RA, Jeng AY, Leenen FHH. Role of central nervous system aldosterone synthase and mineralocorticoid receptors in salt-induced hypertension in Dahl salt-sensitive rats. Am J Physiol Regul Integr Comp Physiol 296: R994–R1000, 2009. First published December 31, 2008; doi:10.1152/ajpregu.90903.2008.—In Dahl salt-sensitive (S) rats, high salt intake increases cerebrospinal fluid (CSF) Na⁺ concentration ([Na⁺]) and blood pressure (BP). Intracerebroventricular (ICV) infusion of a mineralocorticoid receptor (MR) blocker prevents the hypertension. To assess the role of aldosterone locally produced in the brain, we evaluated the effects of chronic central blockade with the aldosterone synthase inhibitor FAD286 and the MR blocker spironolactone on changes in aldosterone and corticosterone content in the brain, hippocampus, and plasma of Dahl S rats. After 4 wk of high salt intake, plasma aldosterone and corticosterone were not changed, but hypothalamic aldosterone increased by ~35% and corticosterone tended to increase in Dahl S rats, whereas both steroids decreased by ~65% in Dahl salt-resistant rats. In Dahl S rats fed the high-salt diet, ICV infusion of FAD286 or spironolactone did not affect the increase in CSF [Na⁺]. ICV infusion of FAD286 prevented the increase in hypothalamic aldosterone and 30 mmHg of the 50–mmHg BP increase induced by high salt intake. ICV infusion of spironolactone fully prevented the salt-induced hypertension. These results suggest that, in Dahl S rats, high salt intake increases aldosterone synthesis in the hypothalamus and aldosterone acts as the main MR agonist activating central pathways contributing to salt-induced hypertension.

METHODS

Male Dahl S and R rats (Charles River, Montreal, PQ, Canada), 5 wk of age, were housed on a 12:12-h light-dark cycle and fed a standard commercial rat chow with regular salt (101 μmol Na⁺/g) and water ad libitum. All experiments were approved by the University of Ottawa Animal Care Committee and conform with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Experimental Protocol

In six groups of Dahl S rats (n = 7/group) under isoflurane inhalation, an L-shaped, 23-gauge stainless steel cannula was placed into the right lateral cerebroventricle. The other end of the cannula...
was connected to an osmotic minipump (model 2002, Alzet) implanted subcutaneously for ICV infusion at a rate of 0.5 μL/h. After surgery, group 1 continued the regular-salt diet and received ICV infusion of vehicle (aCSF). Groups 2–6 were started on a high-salt diet (1.370 μmol Na+/g; Harlan Teklad, Madison, WI) and the following infusions: ICV vehicle (group 2), ICV FAD286 at 10 μg·kg⁻¹·day⁻¹ (group 3), ICV FAD286 at 80 μg·kg⁻¹·day⁻¹ (group 4), subcutaneous (SC) FAD286 at 80 μg·kg⁻¹·day⁻¹ (group 5), and ICV spironolactone in aCSF with 1% alcohol at 10 μg·kg⁻¹·day⁻¹ (group 6) (23). FAD286 is a single (+)-enantiomer. FAD286 hydrogen tartrate (Novartis Institutes for BioMedical Research) was used, because it is soluble in aCSF. Each 1.67 mg of FAD286 hydrogen tartrate provides 1 mg of FAD286 free base, and the amount of the drug in the pumps was adjusted accordingly. After 2 wk, each pump was replaced with a new one filled with the same, freshly prepared solution for an additional 2 wk of infusion. The doses of FAD286 were initially extrapolated from studies using oral FAD286 (8, 25). ICV infusion of FAD286 at a rate of 100 μg·kg⁻¹·day⁻¹ showed marked inhibitory responses in rats with ICV infusions of Na⁺-rich aCSF (21) and in rats after myocardial infarction (22). The ICV dose of spironolactone was adopted from previous studies (9, 23) and is markedly lower than orally active doses (23).

As additional control groups, two groups of Dahl R rats (n = 7/group) of the same age as the Dahl S rats were provided with a regular or high-salt diet. After 4 wk, BP and HR were assessed, and brain tissue and blood were collected for aldosterone and corticosterone assays.

At the end of the 4 wk of treatment, under isoflurane anesthesia, a catheter was placed into the left carotid artery, sealed, and externalized at the back of the neck. A carotid, rather than femoral, catheter was used to allow rapid collection of free-running blood for biochemical assays. On the following morning, at ~18 h after the surgery, catheters were connected to a pressure transducer linked to a polygraph (model 7E, Grass Instrument, Quincy, MA) and a tachograph (model 7P44, Grass). Real-time digital data were obtained using a personal computer equipped with a Grass data acquisition and analysis program (Polyview 2.0). Rats were allowed to rest in their original cages for 30 min, and thereafter resting MAP and HR were recorded for 20 min in conscious, unrestrained animals. Average values over the 20 min were used for statistical analysis. After the BP measurement, 3 ml of blood were collected via the catheter into ice-chilled tubes for electrolyte, aldosterone, and corticosterone measurements. Subsequently, under isoflurane anesthesia, each rat was placed in a stereotaxic frame, and 100–200 μl of CSF were collected at ~5 μl/s from the cisterna magna (19). Plasma and CSF electrolytes were determined using an ion-selective electrode (model 917, Hitachi). The rats were then decapitated, and the brains were removed and stored at ~80°C. The whole hypothalamus, hippocampus, and brain stem were dissected according to Glowinski and Iversen (10).

Plasma and brain aldosterone were measured by RIA as described previously (18). Briefly, plasma was applied to preconditioned C18 cartridges, the cartridges were washed with 12% methanol, and aldosterone was eluted with 80% methanol. The eluates were dried in a vacuum concentrator and then redissolved in PBS containing 0.5% BSA for the RIA. Aldosterone antiserum (catalog no. 07-108216, ICN Pharmaceuticals; 1:90,000 dilution) and 2,25I-labeled aldosterone (catalog no. 07-108226, ICN Pharmaceuticals) were added, and the tubes were incubated for 16–24 h at 4°C. After separation with dextran-coated charcoal, the supernatant was counted using a Canberra-Packard AutoGamma counter. For the assay of hypothalamic aldosterone, the tissue was first homogenized in 100% methanol. After centrifugation, the supernatant was dried in a vacuum concentrator. The residues were redissolved in 0.1% trifluoroacetic acid and centrifuged, and the supernatants were applied to preconditioned cartridges and assayed as described for plasma. For the standard curve, the lowest detectable value was 0.5 pg per tube. Since the average weight of hypothalamic tissue extracted was 80–90 mg and the amount added to each RIA tube was ~14 μg, the sensitivity for hypothalamic aldosterone was 0.5/14 = 0.035 pg/mg. Observed levels of hypothalamic aldosterone (~0.1 pg/mg) are well above this level. The intra-assay variation was 7%, and all samples from the experiment were done in one assay. The recovery, i.e., spiking with known concentrations of aldosterone, was ≥88%. Cross-reactivities were 0.03 and 0.14% for corticosterone and deoxycorticosterone (DOC), respectively. Parallelism of the assay was demonstrated. Plasma and tissue corticosterone were determined with RIA using a corticosterone 125I RIA kit (catalog no. 07-120103, MP Biomedicals).

Another two groups of Dahl S rats were anesthetized with isoflurane, and a telemetry probe (model TA11PA-C40, DSI) was placed into the abdominal cavity and secured to the ventral abdominal wall, with the catheter inserted into the abdominal aorta. The telemetry signal was obtained using a data acquisition system, which calculated and stored the mean values of resting BP and HR over a 3-s interval for a 1-min period each hour. Continuous recordings were started 3 days after the probe implantation and lasted 21 days. After 2 days of control recordings, the animals were anesthetized with isoflurane, and a stainless steel cannula was implanted into the left lateral cerebral ventricle and connected to an osmotic minipump (model 2004), which was filled with aCSF (n = 6) or FAD286 (100 μg·kg⁻¹·day⁻¹) dissolved in aCSF (n = 5). The rats were returned to their original cages. At 2 days after ICV cannulation, the high-salt diet was started in all rats for 17–18 days.

Statistical Analysis

Values are means ± SE. Differences between groups were evaluated by one-way ANOVA or one-way repeated-measures ANOVA (telemetry data). When the F values were significant for main effect, Duncan’s test was performed for multiple comparisons. Statistical significance was defined as P < 0.05.

RESULTS

Aldosterone and Corticosterone in Brain and Plasma

In Dahl R rats, the high-salt diet caused significant decreases in aldosterone (Fig. 1) and corticosterone (Table 1) in the hypothalamus and hippocampus. In contrast, in Dahl S rats, high salt intake significantly increased aldosterone in the hypothalamus by ~35% (P < 0.05 vs. regular salt intake; Fig. 1) but had no effect on aldosterone content in the hippocampus. High salt intake caused a modest [P = not significant (NS)] increase in hypothalamic corticosterone and no change in corticosterone content in the hippocampus (Table 1). Levels of both steroids in the brain stem did not change after high salt intake (data not shown). High salt intake had no effect on plasma aldosterone and corticosterone (Fig. 2).

Effects of Central Blockades of Aldosterone Synthase and MR

Aldosterone and corticosterone in brain and plasma of Dahl S rats. ICV infusion of FAD286 at both doses prevented the salt-induced increase in hypothalamic aldosterone content, and levels remained similar to those of rats fed the regular-salt diet. SC infusion of FAD286 at 80 μg·kg⁻¹·day⁻¹ tended (P = 0.08) to attenuate the increase in hypothalamic aldosterone (Fig. 1). In Dahl S rats infused with ICV spironolactone, hypothalamic aldosterone content also remained similar to that in rats fed the regular-salt diet. None of the treatments affected aldosterone content in the hippocampus (Fig. 1) or corticosterone content in either brain area (Table 1).
Plasma aldosterone and corticosterone levels were not significantly altered by ICV or SC FAD286. In contrast, ICV spironolactone lowered plasma aldosterone from ~450 to ~200 pg/ml and corticosterone from ~200 to ~100 ng/ml (Fig. 2).

CSF [Na+] in Dahl S rats treated with vehicle, high salt intake caused a significant increase in CSF [Na+] by ~5 mmol/l. This increase persisted during ICV FAD286 or spironolactone (Table 2). CSF K+ concentration, plasma [Na+] and K+ concentration, and hematocrit were similar in the six groups of rats (Table 2). There were no significant differences in weight gain among groups fed the high-salt diet (Table 2), and all treatment groups developed normally.

Resting BP and HR. In Dahl S rats infused with ICV vehicle, 24-h mean arterial pressure (MAP) increased significantly on day 2 of high salt intake and reached a plateau of ~130 mmHg on days 4–5 (+20 mmHg from control; Fig. 3). After 4 wk of high salt intake, MAP further increased to ~170 mmHg (Fig. 4). In rats treated with ICV FAD286 at 100 μg·kg⁻¹·day⁻¹, MAP rose significantly by day 3 of high salt intake and remained at ~120 mmHg for the remaining 2 wk (Fig. 3). The MAP increase was ~50% less than in rats infused with vehicle. After 4 wk of high salt intake, BP increased to ~150 mmHg in rats treated with ICV FAD286 at 10 μg·kg⁻¹·day⁻¹ and to ~140 mmHg in rats treated with ICV FAD286 at 80 μg·kg⁻¹·day⁻¹. These MAP values were significantly lower than in rats fed the high-salt diet and treated with vehicle but still higher than in rats fed the regular-salt diet (~125 mmHg; Fig. 4). In rats fed the high-salt diet and treated with SC FAD286 at 80 μg·kg⁻¹·day⁻¹, MAP increased to ~155 mmHg, which is somewhat less (P = NS) than in rats fed the high-salt diet and treated with ICV vehicle. In contrast, the high-salt-induced rise

### Table 1. Corticosterone content in hypothalamus and hippocampus of Dahl R and Dahl S rats fed high- or regular-salt diet for 4 wk and treated with vehicle, FAD286, or spironolactone

<table>
<thead>
<tr>
<th></th>
<th>Corticosterone Content, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>Dahl R</td>
<td></td>
</tr>
<tr>
<td>RNa</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>HNa</td>
<td>23 ± 6*</td>
</tr>
<tr>
<td>Dahl S</td>
<td></td>
</tr>
<tr>
<td>RNa + ICV Veh</td>
<td>111 ± 14</td>
</tr>
<tr>
<td>HNa + ICV Veh</td>
<td>136 ± 12</td>
</tr>
<tr>
<td>HNa + ICV FAD10</td>
<td>125 ± 22</td>
</tr>
<tr>
<td>HNa + ICV FAD80</td>
<td>130 ± 20</td>
</tr>
<tr>
<td>HNa + SC FAD80</td>
<td>123 ± 22</td>
</tr>
<tr>
<td>HNa + ICV Spir</td>
<td>92 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Dahl R and Dahl S, Dahl salt-resistant and salt-sensitive rats, respectively; RNa and HNa, regular- and high-salt diet, respectively; ICV, intracerebroventricular; Veh, vehicle (artificial cerebrospinal fluid); FAD10 and FAD80, FAD286 at 10 and 80 μg·kg⁻¹·day⁻¹; SC, subcutaneous; Spir, spironolactone. *P < 0.05 vs. Dahl R, RNa.
in MAP was prevented completely by treatment with ICV spironolactone (Fig. 4).

In both groups of rats, 24-h HR increased on days 1 and 2 after ICV cannulation (Fig. 3). After the commencement of high salt intake, HR remained at higher levels than HR measured before ICV cannulation. HR was significantly lower (~25 beats/min) in FAD286-treated rats from cannulation. In contrast, in rats infused with high salt intake, HR remained at higher levels than HR measured before ICV cannulation. In rats treated with ICV FAD286 at 10 min in rats treated with ICV vehicle compared with rats fed the regular-salt diet (Fig. 4). In rats treated with ICV FAD286 at 80 μg·kg⁻¹·day⁻¹ or ICV spironolactone, HR remained at the level of rats fed the regular-salt diet.

**DISCUSSION**

The present study provides direct evidence for a biologically relevant role of aldosterone produced locally in the CNS in the regulation of cardiovascular homeostasis in Dahl rats fed a high-salt diet: 1) high salt intake causes a decrease of hypothalamic aldosterone and corticosterone in Dahl R rats but an increase in Dahl S rats; 2) in Dahl S rats, treatment with ICV FAD286 prevents the salt-induced increase in hypothalamic aldosterone and prevents a major part of the increases in BP and HR, whereas MR blockade fully prevents the hyperten-

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**Table 2. Body weight gain, CSF and plasma electrolytes, and hematocrit in Dahl S rats fed regular- or high-salt diet for 4 wk and treated with vehicle, FAD286, or spironolactone**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body Wt Gain, g</th>
<th>Concen in CSF, mM</th>
<th>Concen in Plasma, mM</th>
<th>Hct, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>[Na⁺]</td>
<td>[K⁺]</td>
<td>[Na⁺]</td>
</tr>
<tr>
<td>RNa + ICV Veh</td>
<td>7</td>
<td>103±10*</td>
<td>148±1*</td>
<td>3.3±0.2</td>
<td>146±2</td>
</tr>
<tr>
<td>HNa + ICV Veh</td>
<td>7</td>
<td>81±9</td>
<td>154±2</td>
<td>3.4±0.2</td>
<td>147±2</td>
</tr>
<tr>
<td>HNa + ICV FAD10</td>
<td>7</td>
<td>79±10</td>
<td>154±1</td>
<td>3.2±0.1</td>
<td>146±2</td>
</tr>
<tr>
<td>HNa + ICV FAD80</td>
<td>7</td>
<td>77±4</td>
<td>153±2</td>
<td>3.2±0.1</td>
<td>147±2</td>
</tr>
<tr>
<td>HNa + SC FAD80</td>
<td>5</td>
<td>80±6</td>
<td>153±2</td>
<td>3.3±0.2</td>
<td>148±2</td>
</tr>
<tr>
<td>HNa + ICV Spir</td>
<td>7</td>
<td>83±4</td>
<td>155±2</td>
<td>3.3±0.2</td>
<td>146±2</td>
</tr>
</tbody>
</table>

Values are means ± SE. [Na⁺], Na⁺ and K⁺ concentration; Hct, hematocrit. *P < 0.05 vs. others.

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**Fig. 4.** Resting MAP and HR in Dahl R rats fed regular- or high-salt diet for 4 wk and Dahl S rats fed regular- or high-salt diet for 4 wk and treated with ICV infusion of aCSF, FAD286 at 10 or 80 μg·kg⁻¹·day⁻¹, or spironolactone at 10 μg·kg⁻¹·day⁻¹ or SC infusion of FAD286 at 80 μg·kg⁻¹·day⁻¹. Values are means ± SE (for n, see Table 2). *P < 0.05 vs. other Dahl S, except SC FAD. *P < 0.05 vs. Dahl S, regular salt or high salt + ICV FAD at 80 μg·kg⁻¹·day⁻¹. bP < 0.05 vs. Dahl S, regular salt.
Enzymes involved in steroid biosynthesis are present in the CNS (24), and aldosterone can be synthesized in the rat brain (12). In rats, cytochrome P-450 11β-hydroxylase (CYP11B1) converts DOC to corticosterone, and cytochrome P-450 aldosterone synthase (CYP11B2) catalyzes the steps between DOC and aldosterone (28). The brain is a major extra-adrenal site of aldosterone synthesis (29). Transcript levels are found in the hypothalamus and brain stem (31). CYP11B1 and CYP11B2 expression in specific brain nuclei has not been studied. Variations in gene expression of CYP11B2 are generally associated with parallel changes in enzyme levels (15).

High salt intake increased aldosterone content in the hypothalamus significantly by ∼35% at 4 wk, whereas aldosterone levels in the hippocampus, brain stem, and plasma did not change. This 35% increase represents the increase in the whole hypothalamus and may underestimate increases in specific nuclei/areas, such as the paraventricular or supraoptic nuclei, where aldosterone may putatively be produced or exert its role in activating CNS pathways. Further studies are needed to assess where in the hypothalamus aldosterone actually increases. Inasmuch as a chronic increase in CSF [Na+] also increases hypothalamic aldosterone in Wistar rats (18, 21), it appears that, in Dahl S rats, high salt intake increases CSF [Na+] (19) and, thereby, hypothalamic aldosterone. How the increase in CSF [Na+] activates aldosterone synthesis is an intriguing question that requires further study. There are two main regulatory steps of aldosterone synthesis: 1) the early rate-limiting step in which cholesterol is converted to pregnenolone and 2) the final steps in which DOC is transformed to aldosterone (1). Since hypothalamic corticosterone also tended to increase after high salt intake, it is possible that high salt intake increases local production of aldosterone at the early and final steps of biosynthesis. In contrast to Dahl S rats, high salt intake markedly decreased hypothalamic aldosterone and corticosterone in Dahl R rats without affecting the plasma levels. This suggests that, as a salt-resistant strain, Dahl R rats fed a high-salt diet may possess inhibitory mechanisms to decrease aldosterone and corticosterone synthesis in the CNS. Since high salt intake does not increase CSF [Na+] in Dahl R rats (19), one may speculate that these inhibitory mechanisms could be activated by transient increases in plasma [Na+] (7), which stimulate Na+ sensors, such as Na+, (17). The latter are expressed in neurons in circumventricular organs, such as the organum vasculosum laminae terminalis and subfornical organ (16), which lack the blood-brain barrier.

ICV infusion of FAD286 to inhibit CYP11B2 in the CNS started before high salt intake prevented the salt-induced increase in hypothalamic aldosterone. ICV FAD286 did not affect the mild increase in hypothalamic corticosterone and had no effect on plasma aldosterone. In Wistar rats, ICV FAD286 prevents the increase in hypothalamic aldosterone, but not corticosterone, caused by ICV Na+–rich aCSF (21). Together, these findings indicate that ICV FAD286, indeed, causes specific blockade of the aldosterone synthase enzyme in the CNS. Similar to our previous studies in Wistar rats (21, 22), ICV FAD286 did not decrease hypothalamic aldosterone to levels lower than in rats with regular salt intake. These findings may indicate the presence of two pools of aldosterone in the hypothalamus, one locally produced in the brain and one reflecting uptake from the circulation. Presumably, ICV FAD286 only blocks biosynthesis of aldosterone locally in the brain without affecting aldosterone uptake from the circulation. SC FAD286 at 80 μg·kg–1·day–1 did not affect plasma aldosterone levels but attenuated (P = NS) the increase in hypothalamic aldosterone. This finding suggests that, at this dose, FAD286 does not affect adrenal aldosterone synthesis but does somewhat inhibit aldosterone synthesis in the CNS.

Surprisingly, ICV spironolactone not only prevented the increase in hypothalamic aldosterone, but it also markedly decreased plasma aldosterone and corticosterone levels in Dahl S rats fed the high-salt diet. Central blockade of angiotensin (AT1) receptors prevents stress-induced release of corticotrophin-releasing factor (26). Blockade of brain MR by ICV spironolactone may decrease downstream AT1 receptor activation (20) and, therefore, decrease corticotrophin-releasing factor and ACTH and adrenal aldosterone and corticosterone release. The lower plasma aldosterone may then lead to less uptake of aldosterone into the hypothalamus, resulting in lower hypothalamic content. Alternatively, independent of the MR, as one of its nonspecific actions, spironolactone may directly inhibit enzymes involved in steroid biosynthesis (32), thereby preventing increases in hypothalamic aldosterone by high salt intake.

The present study confirms the findings of Gomez-Sanchez et al. (13) that ICV infusion of an MR antagonist prevents the hypertension caused by high salt intake in Dahl S rats. These studies indicate that, in the brain, MR activation plays an essential role in the pathways leading to the salt-sensitive hypertension. ICV infusion of the 3β-HSD inhibitor trilostane also fully prevents salt-induced hypertension in Dahl S rats (14), indicating that steroids produced locally in the CNS are responsible for the MR activation. Our findings with FAD286 suggest that the MR activation is, to a large extent, the result of binding by aldosterone produced locally in the hypothalamus. ICV FAD286 blocked the increase in hypothalamic aldosterone but, in contrast to an MR blocker, only partially (50–70%) prevented the early and later stages of the hypertension. It is possible that higher doses of FAD286 may be needed to achieve more complete blockade of aldosterone production and release in specific nuclei. Alternatively, corticosterone and/or changes in 11β-HSD-2 activity may contribute as well. Inhibition of 11β-HSD-2 increases sympathetic activity, which can be blocked by an MR blocker, suggesting that corticosterone in the CNS may act as an agonist for MR (34).

High salt intake, in general, decreases plasma ANG II and aldosterone levels (12, 31). In Dahl S rats fed a high-salt diet, plasma aldosterone was significantly decreased after 3 wk (4) but was not changed after 4 wk (5). In Dahl S rats fed a high-salt diet from 6 to 16 wk of age, plasma renin activity was initially suppressed but then rose markedly (29). In the present study, Dahl S rats fed a high-salt diet from 6 to 10 wk of age developed severe hypertension, and plasma aldosterone was similar to that in rats with regular salt intake. It appears that high salt intake only initially suppresses the circulatory renin-angiotensin-aldosterone system in Dahl S rats but then activates it, presumably because of hypertension-induced renal damage. On the other hand, plasma aldosterone levels were
also similar in Dahl R rats fed high- and regular-salt diets. It is possible that, in conscious rats, persistent stress from the arterial cannulation and the stress during blood sampling may lead to an activation of the circulatory renin-angiotensin-aldosterone system and influence plasma aldosterone levels, masking a decrease in aldosterone by salt intake.

MR and epithelial Na⁺ channels are coexpressed in various brain areas, including the choroid plexus (2), a major site of CSF production. Increased epithelial Na⁺ channel activity on the basolateral side and Na⁺-/K⁺-ATPase activity on the CSF side of the choroidal epithelium may enhance Na⁺ transport into the CSF (2, 6, 27). We previously showed that, in Wistar rats fed a high-salt diet, ICV infusion of ouabain, to inhibit Na⁺-/K⁺-ATPase activity in the brain, decreased CSF [Na⁺], whereas in Dahl S rats fed a high-salt diet, ICV infusion of antibody Fab fragments, to bind ouabain-like compounds and, thereby, uninhibit Na⁺-/K⁺-ATPase, further increased CSF [Na⁺] (19). In the present study, blockade of MR or aldosterone synthase in the brain had no effects on the increase in CSF [Na⁺]. It appears that, in Dahl S rats, increased activity of Na⁺-transport mechanisms in the choroid plexus contributes to increased Na⁺ transport from blood into CSF through MR-independent mechanisms.

**Limitation of the Study**

Drugs such as FAD286 or trilostane have not been extensively studied and may have actions other than inhibition of steroidogenesis. However, inasmuch as ICV infusion of aldosterone causes sympathetic hyperactivity and hypertension in rats, ICV infusion of FAD286 or trilostane lowers aldosterone levels in the CNS and largely prevents the salt-induced hypertension in Dahl S rats, and ICV infusion of an MR blocker also prevents the hypertension, it appears less likely that an off-target effect of FAD286 and trilostane would be their primary mechanism of action in the brain.

In the present study, hypothalamic aldosterone levels were modestly higher in Dahl S rats fed the regular-salt diet than in whole brain of Wistar rats (12) and in hypothalamus of Sprague-Dawley rats (33). This may be related to between-laboratory variations in methodology or maybe reflect differences in aldosterone levels in hypothalamus vs. whole brain as well as strain differences.

**Conclusion and Perspectives**

The present study demonstrates that, in Dahl S rats, high salt intake, presumably by increasing CSF [Na⁺], activates a steroidogenic pathway in the hypothalamus, leading to enhanced aldosterone production, and thereby, contributes to the salt-induced hypertension. High-salt-induced MR activation in the brain appears to contribute to neuronal activation, but not to modulation of Na⁺ entry into the CSF in Dahl S rats. These findings highlight a differential role for aldosterone and MR in these two CNS mechanisms contributing to salt-sensitive hypertension.

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**GRANTS**

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**DISCLOSURES**

A. Y. Jeng is an employee of Novartis Institutes for BioMedical Research.

**REFERENCES**


