Effects of high salt intake on brain AT$_1$ receptor densities in Dahl rats

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Wang, Jun Ming, Shereeni J. Veerasingham, Junhui Tan, and Frans H. H. Leenen. Effects of high salt intake on brain AT$_1$ receptor densities in Dahl rats. Am J Physiol Heart Circ Physiol 285: H1949–H1955, 2003. First published July 3, 2003; 10.1152/ajpheart.00744.2002.—To assess effects of dietary salt on brain AT$_1$ receptor densities, 4-wk-old Dahl salt-sensitive (Dahl S) and salt-resistant (Dahl R) rats were fed a regular (101 μmol Na/g) or high (1,370 μmol Na/g)-salt diet for 1, 2, or 4 wk. AT$_1$ receptors were assessed by quantitative in vitro autoradiography. AT$_1$ receptor densities did not differ significantly between strains on the regular salt diet. The high-salt diet for 1 or 2 wk increased AT$_1$ receptor binding by 21–64% in the Dahl S rats in the subfornical organ, median preoptic nucleus, paraventricular nucleus, and suprachiasmatic nucleus. No changes were noted in the Dahl R rats. After 4 wk on a high-salt diet, increases in AT$_1$ receptor binding persisted in Dahl S rats but were now also noted in the paraventricular nucleus, median preoptic nucleus, and suprachiasmatic nucleus of Dahl R rats. At 4 wk on the diet, intracerebroventricular captopril caused clear decreases in blood pressure only in the Dahl S on the high-salt diet but caused largely similar relative increases in brain AT$_1$ receptor densities in Dahl S and R on the high-salt diet versus regular salt diet. These data demonstrate that high salt intake rapidly (within 1 wk) increases AT$_1$ receptor densities in specific brain nuclei in Dahl S and later (by 4 wk) also in Dahl R rats. Because the brain renin-angiotensin system only contributes to salt-induced hypertension in Dahl S rats, further studies are needed to determine which of the salt-induced increases in brain AT$_1$ receptor densities contribute to the hypertension and which to other aspects of body homeostasis.

brain renin-angiotensin system; salt-induced hypertension; angiotensin II; autoradiography

The brain renin-angiotensin system (RAS) plays an important role in sodium homeostasis and central cardiovascular regulation. Components of the brain RAS are distributed in central regions that mediate these functions, including circumventricular organs, such as the organum vasculosum laminae terminalis (OVLT) and the subfornical organ (SFO), which are exposed to blood-borne angiotensins, and many regions within the blood-brain barrier (BBB), such as the paraventricular nucleus (PVN), median preoptic nucleus (MnPO), and suprachiasmatic nucleus (SCh) (1, 3). These areas express a predominance of AT$_1$ receptors that bind ANG II and ANG III with high affinity. Central AT$_1$ receptor stimulation appears to play a critical role in the development of salt-induced hypertension in the Dahl salt-sensitive (Dahl S) rat, a genetic model of salt-sensitive hypertension. Chronic central infusion of CV-11974, a nonpeptide AT$_1$ receptor antagonist, prevents the development of hypertension in Dahl-Iwai salt-sensitive rats on a high-salt diet (23). Our group (8, 9) demonstrated that chronic blockade of central AT$_1$ receptors, by intracerebroventricular infusion of losartan, prevents sympathectomy and exacerbation of hypertension in Dahl S rats as well as spontaneously hypertensive rats (SHR) on a high-salt diet.

The activity of the RAS may be enhanced by the increased release of its active peptides as well as by the upregulation of specific receptors. In Dahl S rats, AT$_1$ receptor mRNA increased threefold in brain homogenates following 6 wk of a high-salt diet (21). A high-salt diet for 2–5 wk caused a marked increase in angiotensin-converting enzyme (ACE) mRNA and activity measured in hypothalamic and pons homogenates in Dahl S rats compared with Dahl salt-resistant (Dahl R) rats (27). These studies (22, 27) do not provide information on where the increases in expression occur in the brain, and AT$_1$ receptor mRNA increases may not accurately reflect changes in the functional receptor. Lesion studies indicate that certain hypothalamic areas mediate the salt-induced hypertension in Dahl S rats. Lesions of the anteroventral third ventricle region (5) or bilateral lesions of the PVN (4, 6) attenuated the salt-induced hypertension in this model. Lesions of the anteromedial hypothalamus, which included the PVN, SCh, and intervening periventricular tissue, prevented the development of hypertension in Dahl S rats on a high-salt diet (2, 4). Because these regions contain components of the brain RAS (7, 14), we hypothesized that in one or more of these areas changes in the activity of the brain RAS, specifically the above-mentioned increases in AT$_1$ receptors mRNA, may contribute to salt-induced hypertension in Dahl S rats. The objective of the present study was therefore to identify changes in AT$_1$ receptor density induced by high di-
etary salt intake within the brain areas involved in sodium homeostasis and cardiovascular regulation in Dahl S versus Dahl R rats.

METHODS

Male Dahl R and S rats (Harlan Sprague Dawley, Madison, WI; age 3–4 wk), were housed in a temperature-controlled environment at 24°C on a 12:12-h light-dark cycle, fed regular rat chow, and allowed tap water ad libitum for at least 3 days before entering the study. All experimental procedures were carried out in accordance with the guidelines of the Canadian Institutes of Health Research outlined in the “Guide for the Care and Use of Experimental Animals”. All chemicals were purchased from Sigma (Oakville, Ontario, Canada) except where otherwise noted.

Dietary treatments. At 4 wk of age, rats were randomly assigned to receive either a regular salt (101 mmol Na/g) or a high-salt (1,370 mmol Na/g, Teklad; Madison, WI) diet for 1, 2, or 4 wk (5–6 rats/group at each time point, for a total of 66 Dahl S and 66 Dahl R rats). At these ages, Dahl S rats are in the developmental phase of salt-induced hypertension and exhibit attendant increases in hypothalamic and pons ACE activity (27). To exclude confounding influences of surgical stress on brain AT<sub>1</sub> receptor densities, blood pressures and AT<sub>1</sub> receptor binding were measured in separate, parallel in time, groups of rats.

Blood pressure and heart rate measurement. At the end of the dietary period, rats were anesthetized by halothane-oxygen inhalation, and a polyethylene catheter was inserted into the left carotid artery, tunneled subcutaneously, and exteriorized at the nape. After an overnight recovery period, resting mean arterial pressure (MAP) and heart rate (HR) were recorded using a data acquisition program (Dataquest LabPro, Data Science International; St. Paul, MN), which allowed online analysis of the pulsatile blood pressure signal from the arterial catheter (averages of 10-s periods, sampling rate 500 Hz). After an accommodation period of 30 min, resting MAP and HR were recorded for 30 min in conscious, freely moving animals. Mean values over these 30 min were used for statistical analysis.

Intracerebroventricular injection of captopril and blood pressure and HR measurement. Captopril was administered into the lateral ventricle to eliminate endogenous ANG II and thereby eliminate possible differences in receptor density caused by ANG II-induced endocytosis. An additional group of 20 rats (10 Dahl S and 10 Dahl R) had an intracerebroventricular cannula stereotaxically implanted (13) after 3 wk on a regular or high-salt diet. One week later, i.e., after 4 wk of the diet, conscious rats were all injected intracerebroventricularly with captopril (100 μg in 2 μl of artificial cerebral spinal fluid, repeated after 1 h). Blood pressure and HR were recorded for 2 h (see above), and then the rats were decapitated for autoradiography.

Quantitative in vitro ANG II receptor autoradiography. Procedures for quantitative assessment of ANG II binding in vitro receptor autoradiography were performed according to a standard protocol (21, 26). Briefly, rats were decapitated, and the brains were removed rapidly, frozen in 2-methylbutane at −40°C, and stored at −20°C. All tissues were processed within a 1-wk period. Serial 20-μm-thick sections were cut on a cryostat, thaw mounted onto Superfrost Plus microscope slides (VWR; West Chester, PA), and dehydrated overnight in a desiccator at 4°C. Sections were preincubated in a buffer (10 mM sodium phosphate, 120 mM NaCl, and 5 mM disodium EDTA, pH 7.4) containing 0.2% bovine serum albumin for 15 min at 20°C. Sections were then incubated in the same buffer containing 0.2% bovine serum albumin, 0.5 mg/ml bacitracin, and 125I-labeled Sar<sup>1</sup>Ile<sup>8</sup>-ANG II (0.3 μCi/ml, Washington State University Peptide Radioiodination Service Centre; Pullman, WA) for 1 h. AT<sub>1</sub> receptor binding was determined by including PD-123319 (10<sup>−5</sup> M), an AT<sub>2</sub> receptor antagonist, to exclude AT<sub>2</sub> receptor binding. Nonspecific binding was determined in the presence of 1 μM ANG II. After four successive 1-min washes in ice-cold buffer to remove nonspecifically bound ligand, sections were air-dried and then exposed to Kodak X-OMAT 270 RA automatic processor (Rochester, NY), and relative optical density within outlined the brain areas, heart, and kidneys was quantified by computerized densitometry following the calibration to relative optical densities of the 125I standards using a computer-assisted image analysis system, AIS/C (Imaging Research; St. Catharines, Ontario, Canada). Specific binding density was determined by subtraction of nonspecific binding (2–5%) from total binding (expressed as fmol/mg wet weight of tissue). Animals were coded such that experimental groups were not known during densitometry.

The rat brain nuclei localization in sections was defined according to the rat brain atlas of Paxinos and Watson (17). The PVN, MnPO, and SC were chosen to represent nuclei with significant numbers of AT<sub>1</sub> receptors inside the BBB, and the OVLT and SFO were chosen as nuclei outside the BBB playing a major role in sodium homeostasis and cardiovascular regulation. Densities of six to eight sections for a given nucleus were averaged to provide the AT<sub>1</sub> receptor density for this nucleus. In the 4-wk studies, the heart and kidneys were included to represent peripheral tissues that express an intrinsic RAS. For the heart, five sections in the mid one-third perpendicular to the long axis of the heart were obtained, and densities for each section were measured and then averaged for the left ventricle (LV) and right ventricle (RV). For the kidneys, 10 sagittal sections through the renal hilus were obtained, and densities for each section were measured and then averaged for the renal cortex and medulla separately.

Statistical analysis. Data are presented as means ± SE. All comparisons between groups were determined by a two-way analysis of variance followed by the Student-Newman-Keuls test where applicable. The level of significance was set at P < 0.05. For AT<sub>1</sub> receptor densities, absolute values were compared within strains on high salt versus regular salt intake and between strains on the same diet.

RESULTS

Resting hemodynamics. Resting MAP tended to be higher in Dahl S versus R rats on a regular salt diet from 4 to 8 wk of age (Table 1). MAP of Dahl S rats progressively increased on the high-salt diet with rats becoming severely hypertensive after 4 wk of the diet (Table 1). Resting HR did not change significantly between groups during the first 2 wk on the diet but increased significantly in Dahl S rats after 4 wk on the high-salt diet (Table 1). Body weight was similar in Dahl R and Dahl S rats on the regular salt diet. Dahl S rats on the high-salt diet gained significantly less weight than Dahl R rats on the same diet or either strain on regular salt diet (Table 1).
**Table 1. Resting MAP, HR, and body weight of Dahl S and R rats on regular or high-salt diets from 4 to 5, 4 to 6, and 4 to 8 wk of age**

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<td>MAP, mmHg</td>
<td>94 ± 2</td>
<td>104 ± 3</td>
<td>98 ± 3</td>
<td>118 ± 4†</td>
<td>103 ± 4</td>
<td>101 ± 2</td>
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<td>HR, beats/min</td>
<td>391 ± 11</td>
<td>405 ± 6</td>
<td>403 ± 10</td>
<td>445 ± 15‡</td>
<td>405 ± 11</td>
<td>408 ± 11</td>
</tr>
<tr>
<td>Weight, g</td>
<td>135 ± 14</td>
<td>131 ± 13</td>
<td>130 ± 12</td>
<td>129 ± 10</td>
<td>199 ± 15</td>
<td>183 ± 17</td>
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Values are means ± SE; n = 6 rats/group. MAP, mean arterial pressure; HR, heart rate; Dahl R, Dahl salt-resistant rats; Dahl S, Dahl salt-sensitive rats; regular salt diet, 101 μmol Na/g; high-salt diet, 1,370 μmol Na/g. *P < 0.05 vs. same strain on regular diet; †P < 0.05 vs. Dahl R rats on same salt diet.

**AT₁ receptor density in the central nervous system.** In all groups, high densities of ¹²⁵I-labeled Sar¹Ile⁸-ANG II binding to AT₁ receptors were readily detected in the OVLT, SFO, PVN, MnPO, and SCh (Table 2 and Fig. 1). Table 2 shows the absolute AT₁ receptor densities in the different nuclei of Dahl S and R rats on the high or regular salt intake. Figure 2 shows the results on a high-salt diet expressed as a percentage of the regular salt diet. On the regular salt diet, Dahl S and R rats exhibited fairly similar densities in all nuclei studied. In Dahl R rats, the high salt intake for 1 or 2 wk did not change AT₁ receptor binding in all brain areas investigated. However, in Dahl R rats on the high-salt diet for 4 wk, AT₁ receptor densities were increased (22–30%) in the PVN, MnPO, and SCh (all inside the BBB) but not in the OVLT or SFO (outside the BBB, Table 2 and Figs. 1 and 2). Dahl S rats exhibited a different pattern of changes in binding. Significant increases in AT₁ receptor binding in the PVN, MnPO, and SCh were noted in Dahl S rats within 1 wk on the high-salt diet and persisted after 2 and 4 wk, compared with Dahl S rats on the regular salt intake (Table 2 and Figs. 1 and 2). After 1 and 2 wk on the high salt intake, most of these increases were also significant compared with Dahl R rats on the high salt intake (Table 2). After 4 wk, significantly higher densities persisted in the PVN, SCh, and MnPO of Dahl S versus Dahl R rats on the high-salt diet (Table 2). Dahl S rats also showed significant increases in AT₁ receptor binding in the SFO, particularly after 1 wk on the high-salt diet. In both Dahl R and S rats, high salt intake did not change AT₁ receptor densities in the OVLT (Table 2 and Fig. 2).

**AT₁ receptor binding in peripheral organs.** On the regular salt intake, the heart of both strains showed low AT₁ receptor densities (Table 3). The high-salt diet for 4 wk did not change AT₁ receptor binding in either the LV or RV (Table 3). On the regular salt intake, AT₁ receptor densities were significantly higher in the renal cortex of Dahl R versus Dahl S rats but similar in the medulla (Table 3). High salt intake decreased renal AT₁ receptor binding similarly in Dahl S and Dahl R rats by 17–22% in the medulla and a ~30% in the cortex (Table 3).

**AT₁ receptor binding after intracerebroventricular injection of captopril.** Intracerebroventricular injection of captopril decreased heart rate (by ~20 beats/min) and resting MAP (by 30 mmHg, P < 0.05) in the Dahl S rats on a high-salt diet for 4 wk (Fig. 3A). In Dahl S rats on the regular salt diet or Dahl R rats on either the high- or regular salt diet, resting MAP showed minor (5–10 mmHg, not significant) decreases after captopril (Fig. 3A).

In both Dahl S and R rats on the high versus regular salt intake for 4 wk, after intracerebroventricular treatment with captopril, relative differences in AT₁ receptor densities in the brain nuclei studied were substantially larger (Fig. 3B) compared with the modest differences without captopril (Fig. 2). The extent of these differences was similar in both strains in the SFO, PVN, MnPO, and SCh. In the OVLT, after captopril, a high salt-induced increase in AT₁ receptor

**Table 2. Effects of high-salt diet on AT₁ receptor densities in brain areas of Dahl S and R rats on regular salt or high-salt diets from 4 to 5, 4 to 6, or 4 to 8 wk of age**

<table>
<thead>
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<th>Dahl R</th>
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<td></td>
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<tr>
<td>OVLT</td>
<td>1,089 ± 72</td>
<td>1,146 ± 92</td>
<td>1,245 ± 24</td>
<td>1,282 ± 46</td>
<td>946 ± 46</td>
<td>1,016 ± 69</td>
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<tr>
<td>SFO</td>
<td>1,068 ± 77</td>
<td>1,254 ± 36</td>
<td>1,095 ± 66</td>
<td>1,792 ± 99†</td>
<td>409 ± 64</td>
<td>1,218 ± 107</td>
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<tr>
<td>PVN</td>
<td>599 ± 8</td>
<td>535 ± 43</td>
<td>899 ± 66</td>
<td>752 ± 43‡</td>
<td>651 ± 21</td>
<td>736 ± 29</td>
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<tr>
<td>MnPO</td>
<td>446 ± 81</td>
<td>478 ± 98</td>
<td>481 ± 36</td>
<td>665 ± 57‡</td>
<td>470 ± 34</td>
<td>491 ± 31</td>
</tr>
<tr>
<td>SCh</td>
<td>565 ± 89</td>
<td>568 ± 78</td>
<td>604 ± 23</td>
<td>816 ± 35†</td>
<td>508 ± 34</td>
<td>545 ± 31</td>
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</table>

Results are means ± SE of bound ¹²⁵I-labeled ANG II (fmol/mg tissue wet wt); n = 5–6 rats/group. OVLT, organum vasculosum laminae terminalis; SFO, subformical organ; PVN, paraventricular nucleus; MnPO, median preoptic nucleus; SCh, suprachiasmatic nucleus. *P < 0.05 vs. same strain on regular salt; †P < 0.05 vs. Dahl R rats on same salt diet.

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binding became apparent, and this increase was significantly larger in Dahl S versus Dahl R rats (Fig. 3B).

DISCUSSION

The present study demonstrates that a high dietary salt intake for 1–2 wk increases AT\textsubscript{1} receptor binding in the brain regions involved in sodium homeostasis, sympathetic activity, and blood pressure regulation in Dahl S rats but not Dahl R rats. After 4 wk on a high-salt diet, these increases in AT\textsubscript{1} receptor binding persist in Dahl S rats, whereas Dahl R rats now also show (more modest) increases of AT\textsubscript{1} receptor binding in the brain areas inside the BBB but not outside the BBB. After 4 wk on the high-salt diet, inhibition of ANG II formation in the brain by intracerebroventricular injection of the ACE inhibitor captopril results in more substantial differences in AT\textsubscript{1} receptor binding on high versus regular salt intake, similarly in the two strains. In contrast to the central nervous system, AT\textsubscript{1} receptor binding in the heart was not affected by high salt and decreased in the renal medulla and cortex of both strains.

The regulation of AT\textsubscript{1} receptors in brain areas involved in sodium homeostasis and cardiovascular regulation may be one of the critical components in regulating the activity of the brain RAS in response to salt. In SHR, high sodium intake for 4 wk increased AT\textsubscript{1} receptor binding in the hypothalamus, thalamus, and striatum (15) but resulted in a slight fall in normotensive Wistar-Kyoto rats. In Dahl rats, 6 wk of a high-salt diet caused a threefold increase in AT\textsubscript{1} receptor mRNA in whole brain homogenates of Dahl S rats but only a slight (−30%) rise in the normotensive controls, Dahl R rats (22). Quantification of the AT\textsubscript{1} receptor mRNA reflects AT\textsubscript{1} receptor gene expression but may not represent the actual changes in the membrane-bound functional receptors. The rate of protein translation and the speed of internalization may also play important roles in the AT\textsubscript{1} receptor density on the cytoplasm membrane. 1\textsuperscript{25}I-labeled Sar\textsuperscript{1}Ile\textsuperscript{8}-ANG II binding to AT\textsubscript{1} receptors reflects binding at the cell membrane and therefore only AT\textsubscript{1} receptor density on the cell membrane. In the present study, we demonstrate increases in 1\textsuperscript{25}I-labeled Sar\textsuperscript{1}Ile\textsuperscript{8}-ANG II binding to AT\textsubscript{1} receptors in the PVN, MnPO, SCh, and SFO after 1 and 2 wk of a high-salt diet in Dahl S rats but not in their normotensive controls, Dahl R rats. After 4 wk of diets, high salt-induced increases in AT\textsubscript{1} receptor binding persisted in these brain regions of Dahl S rats and were now also detected, although to a lesser extent, in the PVN, MnPO, and SCh of Dahl R rats.

Strehlow et al. (22) also found a slight increase of mRNA expression (−30%) in the brain of Dahl R rats after 6 wk on a high-salt diet. This finding is consistent with another study showing that, in normotensive rats, 3 wk of high salt intake caused a similar range (35%) of

![Fig. 1. Autoradiographs showing 1\textsuperscript{25}I-labeled Sar\textsuperscript{1}Ile\textsuperscript{8}-ANG II AT\textsubscript{1} receptor binding density within representative brain nuclei of a Dahl salt-sensitive (Dahl S) and Dahl salt-resistant (Dahl R) rat on a regular (101 μmol Na/g) or high-salt (1,370 μmol Na/g) diet from 4 to 6 wk of age. Binding density is represented by computer-generated pseudocolor gradient beside the graphs. OVLT, organum vasculosum laminae terminalis; SFO, subfornical organ; MnPO, median preoptic nucleus; SCh, suprachiasmatic nucleus; PVN, paraventricular nucleus; SH, Dahl S rat on high-salt diet; SR, Dahl S rat on regular salt diet; RH, Dahl R rat on high-salt diet; RR, Dahl R rat on regular salt diet.]
upregulation of AT$_1$$_a$ (but not AT$_1$$_b$) receptor mRNA in decorticated brain homogenates (19). In the present study, we noticed an increase in AT$_1$ receptor binding in the PVN, MnPO, and SCh, but not the OVLT and SFO of Dahl R rats, after more a chronic (4 wk) high-salt diet. These results indicate that in normotensive Dahl R rats, a chronic high-salt diet also results in an increase of AT$_1$ receptor binding, but this increase occurs later and to a less extent compared with the Dahl S rats. The increase of AT$_1$ binding only in nuclei inside the BBB in Dahl R rats on a high-salt diet may reflect a subtype difference (10, 14) and/or different functions in these brain areas versus areas outside the BBB (i.e., OVLT and SFO). However, after captopril, these two areas also show higher AT$_1$ receptor densities in Dahl R on a high-salt diet versus regular salt diet (see below).

AT$_1$ receptors in the brain appear to be upregulated in response to its ligand as chronic intracerebroventricular infusion of ANG II in normotensive rats increases AT$_1$ receptor mRNA and protein in whole brain homogenates (18). In the whole hypothalamus, ANG II levels are decreased in Dahl S versus R rats on a regular salt intake and remain decreased on a high salt intake (27). However, such tissue levels do not exclude increased ANG II release in specific nuclei and thereby an increase in AT$_1$ receptor occupancy in vivo. The ANG II-AT$_1$ receptor complex may be internalized very fast (~30 min) through receptor-mediated endocytosis (24, 25). It is therefore possible that the receptor density measured by in vitro autoradiography underestimates

Table 3. AT$_1$ receptor binding densities in the heart and kidney of Dahl S and R rats on regular or high-salt diets from 4 to 8 wk of age

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<tr>
<td>Heart</td>
<td></td>
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</tr>
<tr>
<td>LV</td>
<td>55 ± 6</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>RV</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Medulla</td>
<td>2,537 ± 103</td>
<td>2,108 ± 48*</td>
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<tr>
<td>Cortex</td>
<td>1,822 ± 42</td>
<td>1,289 ± 33*</td>
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Results are means ± SE of bound $^{125}$I-labeled ANG II (in fmol/mg tissue wt; n = 5 rats/group). LV, left ventricle; RV, right ventricle. *P < 0.05 vs. same strain on regular salt; †P < 0.05 vs. R rats on same diet.
the actual increase in number of receptors because of increased internalization subsequent to enhanced angiotensin synthesis in specific brain regions. To assess this possibility, brain ANG II synthesis was blocked by intracerebroventricular administration of the ACE blocker captopril in Dahl S and R rats on high versus regular salt intake for 4 wk, when on a high-salt diet both strains show increased brain AT1 receptor densities. After 2-h inhibition of the brain RAS, the amount of the membrane-bound receptor may increase relative to the extent of receptor turnover. Indeed, after 2 h of intracerebroventricularly administered captopril, the amount of membrane-bound receptor as assessed by in vitro 125I-labeled Sar\(^2\)Ile\(^8\)-ANG II differed more markedly in all brain regions in both strains on a high-salt diet relative to rats on a regular salt diet. Thus after the chronic (4 wk) high salt intake, the receptor turnover appears to be enhanced by the high salt intake in both Dahl S and R rats, and the actual increase in AT1 receptors appears to be substantially more than that found without captopril (Fig. 3B vs. Fig. 2). Moreover, in two areas (OVLT and SFO) of Dahl R rats, differences are apparent only after captopril, suggesting that in these areas the increase in receptor turnover prevents an actual increase in membrane-bound recep-

tors (as measured without captopril). The percent differences after captopril varied between regions, possibly reflecting varying increases in ANG II. Between strains, the percent differences on the high- versus regular salt diet after captopril were fairly similar. However, absolute densities are higher in Dahl S versus Dahl R rats (Table 2), and therefore the actual activity of the RAS in these nuclei is likely higher in the Dahl S versus Dahl R rats.

Functional studies (8, 23) have clearly established that the brain RAS, through AT1 receptor activation, plays an essential role in the sympathoexcitation and development of hypertension in Dahl S rats on high-salt diet. The larger blood pressure response to intracerebroventricular captopril in the Dahl S rats on high salt intake is consistent with these previous studies. The early development of hypertension in Dahl S rats on a high-salt diet is indeed accompanied by a clear increase in AT1 receptor densities in brain areas, which are involved in the regulation of sympathetic tone and cardiovascular function. Rapid upregulation by high salt of AT1 receptor densities in specific brain regions of Dahl S rats may per se not be sufficient to increase the sympathetic tone and blood pressure (12) but will likely enhance responses to increased release of angiotensins (11). On the other hand, after more chronic high salt intake, AT1 receptor densities also increased (although to a less extent) in these brain regions of Dahl R rats. Autoradiography is not sensitive enough to assess the actual cellular distribution of the AT1 receptors in a given nucleus. Further studies are needed to assess whether AT1 receptors playing a role in central regulation of sodium and water homeostasis are similarly increased by the high salt intake in the two strains, but AT1 receptors involved in regulation of sympathetic activity, and thereby blood pressure, only increased in Dahl S rats. In situ hybridization and other functional approaches may provide further insight in this regard.

In contrast to salt-induced increases of AT1 receptor mRNA (22) and AT1 receptor binding in specific brain regions (the present study) in Dahl S and Dahl R rats, AT1 receptor mRNA (22) and AT1 receptor binding decreased in the renal medulla and cortex in both Dahl S and Dahl R rats. The intrinsic tissue RASs therefore appear to be regulated in a distinct and organ-specific manner in response to dietary salt (16, 20, 22).

Limitations of study. We did not study the possible functional implications of the changes in brain AT1 receptors in Dahl S and R rats by high salt intake. The discussion on this aspect should be considered “speculative.” Second, in the captopril experiment we did not include the four control (i.e., no captopril) groups and therefore do not know the extent per se of changes induced by captopril within each of the four groups (i.e., Dahl S and R rats on high vs. regular salt intake). However, the primary objective of this particular experiment was to assess whether relative differences in AT1 receptor binding between rats on a high-diet salt versus regular salt diet would become larger after inhibition of endogenous ANG II formation.

Fig. 3. Effects of intracerebroventricular (icv) infusion of captopril (100 μg/2 μl of artificial cerebral spinal fluid at time = 0 and 60 min) on mean arterial pressure (MAP) (A) and AT1 receptor binding densities in brain nuclei (B) of Dahl S and Dahl R rats on a regular (101 μmol Na/g, R) or high-salt (1,370 μmol Na/g, H) diet from 4 to 8 wk of age. A: results are the changes vs. baseline. Values are means ± SD from 5 rats in each group. B: results are presented as percentage of densities on high salt vs. regular salt (set as 100%) in the same strain. ∗P < 0.05 vs. same strain on regular salt; ∗P < 0.05, S vs. R rats.
In conclusion, salt-induced hypertension in Dahl S rats is accompanied by increased densities of AT1 receptors in brain areas both inside and outside of the BBB. Such salt-induced increases in AT1 receptors in these brain areas may reflect increased local release of angiotensins and may contribute to the development of salt-induced sympathetic hyperactivity and hypertension. However, more chronic high salt intake also increases AT1 receptor densities in the same brain areas in Dahl R rats. It is possible that some of the AT1 receptor populations assessed are part of the central pathways involved in sodium and water homeostasis in both strains and other populations are part of central pathways involved in regulation of sympathetic activity, only activated in the Dahl S rats.

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DISCLOSURES

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REFERENCES