Activation of brain renin-angiotensin-aldosterone system by central sodium in Wistar rats

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Huang, Bing S., Warren J. Cheung, Hao Wang, Junhui Tan, Roselyn A. White, and Frans H. H. Leenen. Activation of brain renin-angiotensin-aldosterone system by central sodium in Wistar rats. Am J Physiol Heart Circ Physiol 291: H1109–H1117, 2006. First published April 7, 2006; doi:10.1152/ajpheart.00024.2006.—Functional studies indicate that the sympathoexcitatory and pressor responses to an increase in cerebrospinal fluid (CSF) [Na⁺] by central infusion of Na⁺-rich artificial cerebrospinal fluid (aCSF) in Wistar rats are mediated in the brain by mineralocorticoid receptor (MR) activation, ouabain-like compounds (OLC), and AT₁-receptor stimulation. In the present study, we examined whether increasing CSF [Na⁺] by intracerebroventricular infusion of Na⁺-rich aCSF activates MR and thereby increases OLC and components of the renin-angiotensin system in the brain. Male Wistar rats received via osmotic minipump an intracerebroventricular infusion of aCSF or Na⁺-rich aCSF, in some groups combined with intracerebroventricular infusion of spironolactone (100 ng/h), antibody Fab fragments (to bind OLC), or as control. After 2 wk of infusion, resting blood pressure and heart rate were recorded, OLC and aldosterone content in the hypothalamus were assessed by a specific ELISA or radioimmunoassay, radioligand binding densities in various brain nuclei were measured by autoradiography using [125I]labeled 351 A and [125I]labeled ANG II. When compared with intracerebroventricular aCSF, intracerebroventricular Na⁺-rich aCSF increased CSF [Na⁺] by ~5 mmol/l, mean arterial pressure by ~20 mmHg, heart rate by ~65 beats/min, and hypothalamic content of OLC by 50% and of aldosterone by 33%. Intracerebroventricular spironolactone did not affect CSF [Na⁺] but blocked the Na⁺-rich aCSF-induced increases in blood pressure and heart rate and OLC content. Intracerebroventricular Na⁺-rich aCSF increased ACE and AT₁-receptor-binding densities in several brain nuclei, and Fab fragments blocked these increases. These data indicate that in Wistar rats, a chronic increase in CSF [Na⁺] may increase hypothalamic aldosterone and activate CNS pathways involving MR, and OLC, leading to increases in AT₁-receptor and ACE densities in brain areas involved in cardiovascular regulation and hypertension.

the brain mineralocorticoid receptor; ouabain; angiotensin-converting enzyme; AT₁-receptor

NEURAL MECHANISMS play a major role in the development of salt-induced hypertension in Dahl salt-sensitive (S) rats (3). In Dahl S rats but not salt-resistant (R) rats, high salt intake increases cerebrospinal fluid (CSF) [Na⁺] (20), associated with increases in hypothalamic ouabain-like compounds (OLC) (41) and in angiotensin-converting enzyme (ACE) activity (46) and AT₁-receptors (44). Functional studies indicate that high salt-induced sympathetic hyperactivity and hypertension can be prevented by CNS blockade of aldosterone synthesis (15), mineralocorticoid receptors (MR) (14, 32), epithelial sodium channels (ENaC) (42), OLC, or of AT₁-receptors (19). We proposed (23) that in Dahl S rats on high salt, genetically determined enhancement in the activity of MR-ENaC activates central pathways involving OLC and AT₁-receptors resulting in sympathetic hyperactivity and hypertension.

Recent studies (1, 11) showed that MR and ENaC are present in the choroid plexus and ventricular ependyma, as well as in neurons involved in cardiovascular regulation in the hypothalamus. MR and ENaC in these regions may contribute to the regulation of Na⁺ entry from the blood into the CSF and brain interstitium, as well as of neuronal responses to increases in interstitial [Na⁺]. Indeed, intracerebroventricular infusion of aldosterone at low rates increases brain OLC and blood pressure (BP) (12, 23), particularly when combined with small increases in CSF [Na⁺] (23). Blockade of brain OLC prevents intracerebroventricular aldosterone-induced sympathetic hyperactivity and hypertension (40). Increasing CSF [Na⁺] by intracerebroventricular infusion of Na⁺-rich artificial CSF (aCSF) elicits sympathoexcitatory and pressor responses in a number of rat strains (21, 22, 25, 38). In Wistar rats, sympathoexcitatory and pressor responses to Na⁺-rich aCSF can be prevented by CNS blockade of OLC or AT₁-receptors (21), whereas CNS blockade of ENaC (by benzamil) prevents the increase in brain OLC as well as the sympathetic excitation and hypertension (42). Whether these responses to central Na⁺ can also be prevented by CNS blockade of MR has not yet been investigated.

The mineralocorticoid hormone aldosterone is a major activator of MR and ENaC. It appears that aldosterone can be synthesized in the brain (13). In adrenalectomized Wistar rats, aldosterone remains present in the brain (13). Blockade of aldosterone biosynthesis in the CNS by intracerebroventricular infusion of the 3β-hydroxysteroid dehydrogenase blocker trilostane prevents hypertension in Dahl S rats on high salt intake (15). These studies suggest that aldosterone in the CNS mediates the hypertension in Dahl S rats on high salt. It has not yet been clarified whether increasing CSF [Na⁺] increases aldosterone and thereby activates MR-ENaC in the brain.

Accordingly, the present study was designed to elucidate in Wistar rats whether: 1) increasing CSF [Na⁺] by intracerebroventricular infusion of Na⁺-rich aCSF increases aldosterone, OLC, and ACE and AT₁-receptor binding densities in the brain; and 2) CNS blockade of MR prevents activation of these central pressor mechanisms. Together with our previous study (42), these studies would provide evidence to support the functional role of MR and ENaC in CNS Na⁺-transport mechanisms downstream to the choroid plexus.

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METHODS

Male Wistar rats weighing 150–200 g (Charles River, Montreal, Canada) were housed two per cage in a climatized room on a 12:12-h light:dark cycle at constant room temperature and humidity and given standard laboratory chow (120 µmol Na+/g) and tap water ad libitum. The study was carried out in accordance with the guidelines of the Canadian Council on Animal Care, which conform to National Institutes of Health guidelines and was approved by the University of Ottawa Animal Care Committee.

Protocol 1: Role of Brain MR in Effects of Na⁺-Rich aCSF

Intracerebroventricular cannulation and infusion of drugs. After 1 wk of acclimatization, under halothane anesthesia and with the use of a stereotaxic frame (Harvard Apparatus), a 23-gauge right-angled stainless steel cannula was implanted into the left lateral cerebral ventricle and fixed to the skull of the rat with acrylic cement. The cannula was placed 0.4-mm posterior and 1.4-mm lateral to the bregma. The lower end (shorter arm) of the cannula was at a depth of 3.5 mm from the dura, and the upper end (longer arm) was connected to an osmotic minipump (model 2ML2, Alza, Palo Alto, CA) for chronic intracerebroventricular infusion at 5 µl/h for 14 days. The pumps were filled with aCSF with 150 mmol/l Na⁺ or aCSF containing 800 mmol/l Na⁺ (Na⁺-rich aCSF) alone or combined with spironolactone (100 ng/h) (n = 7–8 rats/group) and implanted subcutaneously on the back of the rats. Considering the secretion rate of Ottawa Animal Care Committee.

Protocol 2: Intracerebroventricular Na⁺-Rich aCSF and Brain Aldosterone

In two sets of Wistar rats, the same intracerebroventricular cannulation and minipump (model 2ML2) implantation as above were performed. In the first set of rats, minipumps were filled with aCSF (n = 5) or Na⁺-rich aCSF (n = 7). After 2 wk, a catheter was inserted into the left femoral artery. About 3–4 h later, resting BP and HR were recorded as described above. Three milliters of blood were then collected from conscious rats and placed into prechilled microtubes containing either EDTA alone or EDTA plus 1,10-phenanthroline for assay of plasma renin activity (PRA) or ANG II concentration. The rats were then euthanized by decapitation, and brain tissue was collected, frozen, and stored at −80°C. The whole hypothalamus was dissected for measurement of aldosterone content.

In the second set of rats, the minipumps were filled with either aCSF (group 1, n = 6) or Na⁺-rich aCSF (groups 2 and 3, n = 6–8) for intracerebroventricular infusion for 2 wk. Another osmotic pump (model 2002) was implanted in rats of groups 1 and 3 for simultaneous subcutaneous infusion of spironolactone at the same rate as used for intracerebroventricular infusion (100 ng/h). Resting BP and HR were then recorded, and 2 ml of blood were collected for measurement of aldosterone and electrolytes. The rats were then anesthetized, and 100- to 200-µl CSF samples were taken for measurements of electrolytes, as described in protocol 1. Rats were euthanized by decapitation, and brain tissue from group 1 and 2 was collected, frozen, and stored as described above, for measurement of aldosterone content in the hypothalamus. The hypothalamic aldosterone content in the corresponding rat groups from the two sets of rats were similar: 0.9 ± 0.1 vs. 0.8 ± 0.1 pg/mg in the two groups with aCSF and 1.3 ± 0.2 vs. 1.1 ± 0.1 pg/mg in the two groups with Na⁺-rich aCSF. Results were therefore combined for the two aCSF groups and the two Na⁺-rich aCSF groups.

Plasma and tissue aldosterone were measured by radioimmunoassay (RIA) according to Brochu et al. (2), with minor modifications. Briefly, plasma was applied onto preconditioned C18 cartridges (Water Sep-Pak plus no. 020515), which were then washed with 10 ml of distilled water, followed by 4 ml of 12% methanol. The aldosterone was eluted with 4 ml 80% methanol. The eluates, along with standard aliquots, were dried by using a Savant Speed Vac vacuum concentrator and then redissolved in phosphate-buffered saline containing 0.5% BSA for the RIA. Aldosterone antiserum (ICN Pharmaceuticals no. 07-108216, 1:30,000 dilution) and 125I-labeled aldosterone (ICN no. 07-108226, 5– 6,000 counts per tube) were added to the tubes, and the tubes were redissolved in 3 ml 0.1% trifluoroacetic acid and centrifuged, the supernatant was separated by centrifugation in the Sorvall RT 6000B centrifuge (3,000 rpm) at 4°C for 30 min, the supernatant was counted by using a Canberra-Packard AutoGamma counter. Unknowns were determined from the standard curve. For the assay of hypothalamic aldosterone, the tissues were weighed and homogenized in 10 volumes 100% methanol using a polytron (Brinkmann Instruments). The supernatant was separated by centrifugation in the Sorvall RT 6000B centrifuge for 30 min and dried in the Savant Speed-Vac. The residues were redissolved in 3 ml 0.1% trifluoroacetic acid and centrifuged, and the supernatants were applied to preconditioned cartridges and
assayed as described for plasma. 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 0.09% and 0.14% for corticosterone and deoxycorticosterone acetate (DOCA), respectively. Parallelism of the assay was demonstrated.

As described previously (27), plasma ANG II concentration was measured by RIA after extraction on C18 Sep-Pak cartridges and separation by HPLC, and PRA was determined by RIA to measure the ANG I generated during incubation at 37°C for 1 h.

Protocol 3: Activation of OLC and Components of the Renin-Angiotensin System by Na⁺-Rich aCSF

Wistar rats were instrumented with intracerebroventricular cannulation and minipump. The minipumps were filled with either (1) aCSF plus antibody Fab fragments (Fab, Digibind, Glaxo Wellcome, n = 8), (2) aCSF plus γ-globulins (γ-glob, 5 nM, Sigma) as control for the administration of Fab, (3) Na⁺-rich aCSF plus Fab (n = 8), or (4) Na⁺-rich aCSF plus γ-glob (n = 7) for intracerebroventricular infusion for 2 wk.

After 2 wk, rest and HR were recorded through a catheter placed in the carotid artery ~20 h earlier. The animals were decapitated, the brain was removed and quickly frozen in 2-methylbutane at −20 to −30°C and then stored at −80°C. Autoradiography was performed as recently described in detail (39). Briefly, serial cryostat 20-μm sections were mounted onto Superfrost Plus microscope slides (VWR, West Chester, PA) and stored at −80°C. To assess AT₂-receptor binding, sections were preincubated in 5 mM Na₂EDTA, 0.2% BSA, and 0.4 mM bacitracin (Sigma) at room temperature for 15 min and then incubated in the same buffer with 0.3 μCi/ml 125I-Sar⁶,Ile¹⁰-ANG II (2,176 Ci/ml; Dept. of Pharmacology and Re-search Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi) plus antibody Fab fragments (Digibind). A 10 mM phosphate incubation buffer binding was determined in the presence of 100 mM EDTA, which completely reversed nonspecific binding, which was <2–5% in control and experimental rats. ACE and AT₁-receptor binding densities were measured bilaterally in coronal cryostat sections of the brain containing the organum vasculosum laminae terminalis (OVLT), the median preoptic nucleus (MnPO), the subfornical organ (SFO), and the paraventricular nucleus (PVN) (from bregma: 0.20 to −0.26 mm; 0.26 to −0.4 mm; −0.80 to −1.40 mm; and −1.6 to −2.12 mm, respectively). For each rat, four to six sections containing the nucleus of interest were quantified and presented as average density for the entire nucleus. The localization of these nuclei was defined according to the rat brain atlas of Paxinos and Watson (31).

Statistical Analysis

For comparisons of daily water intake, a repeated-measures analysis of variance was performed. When the F-value was significant, a Duncan’s test was performed for multiple comparisons. For comparisons of multiple groups, two-way ANOVA was performed followed by a Student-Newman-Keuls test to determine which treatments were significantly different. Student’s t-test was performed to compare two groups. Statistical significance was defined as P < 0.05.

RESULTS

Intracerebroventricular Na⁺-Rich aCSF and Brain MR

All groups of rats developed normally over the period of the study. No differences in body weight and the weights of the brain, heart, kidney, and adrenal gland were observed among rats receiving different treatments (data not shown). In rats with intracerebroventricular infusion of aCSF, intracerebroventricular spironolactone did not affect any of the parameters evaluated (Figs. 1–3, Table 1).

Water intake. The water intake of rats with intracerebroventricular infusion of Na⁺-rich aCSF tended to be increased compared with other groups (P = 0.07–0.1) (Fig. 1A). When corrected for body weight at the end of the experiment, water intake was significantly decreased by intracerebroventricular infusion of spironolactone in rats treated with intracerebroventricular Na⁺-rich aCSF (Fig. 1B).

Table 1. Plasma and CSF electrolytes and hematocrit in Wistar rats after a 2-wk intracerebroventricular infusion of aCSF or Na⁺-rich aCSF combined with intracerebroventricular (protocol 1) or subcutaneous (protocol 2) infusion of spironolactone

<table>
<thead>
<tr>
<th></th>
<th>aCSF + Veh</th>
<th>aCSF + Spiro</th>
<th>Na⁺-Rich aCSF + Veh</th>
<th>Na⁺-Rich aCSF + Spiro</th>
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<tbody>
<tr>
<td><strong>Protocol 1</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hematocrit, %</td>
<td>42.8 ± 2.2</td>
<td>41.6 ± 1.4</td>
<td>40.2 ± 1.2</td>
<td>41.5 ± 1.4</td>
</tr>
<tr>
<td>Plasma Na⁺, mmol/l</td>
<td>140 ± 1</td>
<td>141 ± 1</td>
<td>140 ± 1</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Plasma K⁺, mmol/l</td>
<td>4.7 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>4.7 ± 0.1</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Plasma Cl⁻, mmol/l</td>
<td>101 ± 1</td>
<td>102 ± 1</td>
<td>101 ± 1</td>
<td>101 ± 0.8</td>
</tr>
<tr>
<td>CSF Na⁺, mmol/l</td>
<td>152 ± 3</td>
<td>150 ± 3</td>
<td>155 ± 3*</td>
<td>155 ± 2*</td>
</tr>
<tr>
<td>CSF K⁺, mmol/l</td>
<td>2.6 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>CSF Cl⁻, mmol/l</td>
<td>119 ± 3</td>
<td>121 ± 2</td>
<td>123 ± 3</td>
<td>123 ± 2</td>
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<tr>
<td><strong>Protocol 2</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CSF Na⁺, mmol/l</td>
<td>150 ± 2</td>
<td>154 ± 1*</td>
<td>155 ± 2*</td>
<td>155 ± 2*</td>
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<tr>
<td>CSF K⁺, mmol/l</td>
<td>2.6 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>CSF Cl⁻, mmol/l</td>
<td>121 ± 2</td>
<td>124 ± 2</td>
<td>122 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6–8 rats/group. aCSF, cerebral spinal fluid; aCSF, artificial CSF; Veh, vehicle; Spiro, spironolactone. Infusion rate of spiro was 100 ng/h. *P < 0.05, Na⁺-rich aCSF vs. aCSF.

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Chronic intracerebroventricular infusion of Na\textsuperscript{+}/H\textsubscript{11}00\textsubscript{1}-rich aCSF increased CSF \([\text{Na}\textsuperscript{+}/H\textsubscript{11}]\) by 5 mmol/l (\(P<0.05\) compared with aCSF). This increase was not affected by spironolactone. There were no differences in CSF K\textsuperscript{+}/H\textsubscript{11} and Cl\textsuperscript{−}/H\textsubscript{12}, plasma Na\textsuperscript{+}/H\textsubscript{11}, K\textsuperscript{+}/H\textsubscript{11}, and Cl\textsuperscript{−}/H\textsubscript{12}, or hematocrit among the groups (Table 1).

Blood pressure and heart rate. Intracerebroventricular infusion of Na\textsuperscript{+}/H\textsubscript{11}-rich aCSF for 2 wk significantly increased systolic and diastolic BP (not shown) as well as mean arterial BP (Fig. 2). Spironolactone blocked this increase. HR was also increased by chronic intracerebroventricular infusion of Na\textsuperscript{+}-rich aCSF, and intracerebroventricular spironolactone prevented this increase as well (Fig. 2).

Brain OLC. In rats with intracerebroventricular infusion of Na\textsuperscript{+}-rich aCSF versus aCSF, OLC content in the hypothalamus and pituitary were significantly higher (15 ± 2 vs. 10 ± 1 ng/g and 85 ± 7 vs. 62 ± 6 ng/g, respectively, \(P<0.05\) for both). Intracerebroventricular infusion of spironolactone prevented the increase in OLC content induced by Na\textsuperscript{+}-rich aCSF (Fig. 3, A and B).

Peripheral OLC. In rats with intracerebroventricular infusion of Na\textsuperscript{+}-rich aCSF versus aCSF, OLC content in the adrenal gland and plasma were significantly lower (11 ± 2 vs. 22 ± 3 ng/g and 0.70 ± 0.04 vs. 1.00 ± 0.06 ng/ml, respectively, \(P<0.05\) for both). Spironolactone prevented this decrease in adrenal and plasma OLC induced by Na\textsuperscript{+}-rich aCSF (Fig. 3, C and D).

Intracerebroventricular Na\textsuperscript{+}-Rich aCSF and Brain Aldosterone

For rats in protocol 2, gain in body weight was similar among the groups (data not shown). A similar increase in water intake as in protocol 1 was observed in rats with intracerebroventricular Na\textsuperscript{+}-rich aCSF, and subcutaneous spironolactone at the low rate of 100 ng/h did not affect this increase in water intake (data not shown).

CSF [Na\textsuperscript{+}] increased by 4–5 mmol/l (\(P<0.05\)) in rats treated with intracerebroventricular infusion of Na\textsuperscript{+}-rich aCSF with or without subcutaneous spironolactone. There were no differences in CSF K\textsuperscript{+} and Cl\textsuperscript{−}/H\textsubscript{12}, as well as plasma Na\textsuperscript{+}, K\textsuperscript{+}, and Cl\textsuperscript{−}, and hematocrit (not shown), among the groups.

Resting mean arterial pressure (MAP) and HR of rats with intracerebroventricular Na\textsuperscript{+}-rich aCSF were significantly higher than those of rats with intracerebroventricular aCSF plus subcutaneous spironolactone (129 ± 2 vs. 106 ± 3 mmHg and 443 ± 13 vs. 393 ± 12 beats/min, respectively, \(P<0.05\) for both) similar to the increases in protocol 1 (shown in Fig. 2). Subcutaneous infusion of spironolactone at the low rate did...
not affect the Na\(^+\)-rich aCSF-induced increases in BP and HR (133 ± 3 vs. 129 ± 2 mmHg and 448 ± 17 vs. 443 ± 13 beats/min, not significant for both).

In rats treated with intracerebroventricular infusion of Na\(^+\)-rich aCSF, hypothalamic aldosterone content was increased by \(\sim 33\%\) (\(P = 0.05\)) (Fig. 4). There were no significant differences in plasma aldosterone concentration among rats with intracerebroventricular aCSF plus subcutaneous spironolactone at the low rate versus intracerebroventricular Na\(^+\)-rich aCSF (1.1 ± 0.2 vs. 1.3 ± 0.1 ng/ml). PRA and plasma Ang II tended to be higher in rats with intracerebroventricular Na\(^+\)-rich aCSF versus intracerebroventricular aCSF (6.0 ± 1.8 vs. 3.2 ± 0.5 ng·ml\(^{-1}\)·h\(^{-1}\) and 4.5 ± 0.4 vs. 3.4 ± 0.7 pg/ml, respectively, \(P = 0.08\) and 0.1).

**Na\(^+\)-Rich aCSF and Components of Brain RAS**

In this protocol, intracerebroventricular infusion of Na\(^+\)-rich aCSF similarly increased resting MAP and HR compared with intracerebroventricular infusion of aCSF as in protocols 1 and 2: 119 ± 3 vs. 94 ± 3 mmHg, and 442 ± 10 vs. 402 ± 11 beats/min, respectively (\(P < 0.05\) for both). Concomitant intracerebroventricular infusion of Fab fragments prevented these increases by Na\(^+\)-rich aCSF (MAP: 98 ± 3 mmHg and HR: 411 ± 8 beats/min).

Figure 5 shows representative autoradiograph images of ACE and AT\(_1\)-receptor binding densities in the PVN of the four groups of rats. In rats infused with intracerebroventricular aCSF, intracerebroventricular infusion of Fab fragments did not cause significant changes in ACE and AT\(_1\)-receptor densities in the four assessed brain nuclei. Intracerebroventricular infusion of Na\(^+\)-rich aCSF increased ACE densities by \(\sim 40–50\%\) in the SFO, MnPO, and PVN and caused a nearly twofold increase in the OVLT. AT\(_1\)-receptor densities increased to a lesser extent by 40–50% in the OVLT and SFO and by 10–20% in the MnPO and PVN (Table 2). All the increases in both ACE and AT\(_1\)-receptor densities were prevented by concomitant intracerebroventricular infusion of the Fab fragments.

**DISCUSSION**

The present study in Wistar rats provides as new findings that: 1) increases in CSF [Na\(^+\)] by chronic intracerebroventricular infusion of Na\(^+\)-rich aCSF are associated with increases in aldosterone and OLC content in the hypothalamus as well as in ACE and AT\(_1\)-receptor densities in the four assessed brain nuclei. Intracerebroventricular infusion of Na\(^+\)-rich aCSF increased ACE densities by \(\sim 40–50\%\) in the SFO, MnPO, and PVN and caused a nearly twofold increase in the OVLT. AT\(_1\)-receptor densities increased to a lesser extent by 40–50% in the OVLT and SFO and by 10–20% in the MnPO and PVN (Table 2). All the increases in both ACE and AT\(_1\)-receptor densities were prevented by concomitant intracerebroventricular infusion of the Fab fragments.
or Na\textsuperscript{+}-rich aCSF combined with intracerebroventricular γ-globulins or Fab fragments.

Table 2. ACE and AT\textsubscript{1}-receptor binding densities in various brain nuclei in Wistar rats after a 2-wk intracerebroventricular infusion of aCSF or Na\textsuperscript{+}-rich aCSF combined with intracerebroventricular γ-globulins or Fab fragments

<table>
<thead>
<tr>
<th></th>
<th>OVLT</th>
<th>SFO</th>
<th>MnPO</th>
<th>PVN</th>
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<tr>
<td>ACE density, fmol/g</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>aCSF + γ-globulins</td>
<td>2.008±54</td>
<td>6.748±112</td>
<td>837±22</td>
<td>1,192±56</td>
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<tr>
<td>aCSF + Fab fragments</td>
<td>1.918±80</td>
<td>6.571±145</td>
<td>779±31</td>
<td>1,138±62</td>
</tr>
<tr>
<td>Na\textsuperscript{+}-rich aCSF + γ-globulins</td>
<td>3.825±110*</td>
<td>9.487±150*</td>
<td>1,261±72*</td>
<td>1,473±63*</td>
</tr>
<tr>
<td>Na\textsuperscript{+}-rich aCSF + Fab fragments</td>
<td>2.146±51</td>
<td>6.829±136</td>
<td>778±26</td>
<td>1,209±44</td>
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<tr>
<td>AT\textsubscript{1}-receptor density, fmol/mg</td>
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<tr>
<td>aCSF + γ-globulins</td>
<td>404±18</td>
<td>644±27</td>
<td>326±15</td>
<td>350±10</td>
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<tr>
<td>aCSF + Fab fragments</td>
<td>437±20</td>
<td>627±31</td>
<td>335±12</td>
<td>369±13</td>
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<td>Na\textsuperscript{+}-rich aCSF + γ-globulins</td>
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<td>903±34*</td>
<td>423±17*</td>
<td>391±11*</td>
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<tr>
<td>Na\textsuperscript{+}-rich aCSF + Fab fragments</td>
<td>443±21</td>
<td>671±36</td>
<td>335±17</td>
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</table>

Data are means ± SE; n = 7–8/group. OVLT, organum vasculosum laminae terminalis; SFO, subfornical organ; MnPO, median preoptic nucleus; PVN, paraventricular nucleus; ACE, angiotensin-converting enzyme. *P < 0.05 vs. others.
corticosterone acetaate salt-treated rats (33), as well as in Dahl S rats and SHR on high salt intake (14, 32).

Similar to our previous study (40), in the present study intracerebroventricular infusion of Na\(^+\)-rich aCSF increased OLC in the hypothalamus. Blockade of MR in the brain by intracerebroventricular infusion of spironolactone prevented this increase. Both studies together indicate that an increase in CSF [Na\(^+\)] through MR and ENaC activates OLC-producing neurons and increases OLC synthesis and release in the brain. An increase in OLC in the CNS may have two functions. First, OLC released into the CSF may inhibit the Na\(^+\)-K\(^+\)-ATPase on the CSF side in the choroid plexus and prevent further increase in CSF [Na\(^+\)], as we previously reported in Dahl S rats on high salt intake (20). Such a negative feedback mechanism may explain why in the present study a 9% increase in CSF Na\(^+\) loading by intracerebroventricular Na\(^+\)-rich aCSF caused only a 3% increase in CSF [Na\(^+\)]. On the other hand, the increases in OLC by Na\(^+\)-rich aCSF plays an essential role in the activation of the brain renin-angiotensin system leading to sympathetic hyperactivity and hypertension. Intracerebroventricular administration of antibody Fab fragments to block OLC or an AT\(_1\)-receptor blocker prevents the sympathetic hyperactivity and hypertension in Wistar rats by Na\(^+\)-rich aCSF (21). In the present study we showed that ACE and AT\(_1\)-receptor densities in brain nuclei involved in cardiovascular regulation such as OVLT, SFO, PVN, and MnPO are increased by intracerebroventricular Na\(^+\)-rich aCSF. The extent of the increases in AT\(_1\)-receptor densities is quite similar to that in Dahl S rats on high salt intake (44). Blockade of brain OLC with Fab fragments prevented the intracerebroventricular Na\(^+\)-rich aCSF-induced increases in ACE and AT\(_1\)-receptor densities, indicating that OLC is essential for the CSF Na\(^+\)-induced upregulation of ACE and AT\(_1\)-receptors in these nuclei. Whether OLC directly or indirectly upregulates ACE and AT\(_1\)-receptors is presently unclear.

Similar to our previous study with chronic intracerebroventricular aldosterone (40), in the present study increased CSF [Na\(^+\)] increased OLC in the hypothalamus and pituitary but decreased adrenal and plasma OLC. These findings suggest that the brain and peripheral OLC are not regulated in parallel and that central mechanisms may be involved in the regulation of adrenal and plasma OLC. Consistent with this concept, lesions of the anteroventral region of the third ventricle prevent the appearance of circulating Na\(^+\)-K\(^+\)-ATPase inhibitory activity in rats with DOCA-salt hypertension (35). The mechanisms by which intracerebroventricular Na\(^+\)-rich aCSF may regulate peripheral OLC are not yet clear. OLC may be released from pituitary into the circulation. In the present study intracerebroventricular infusion of Na\(^+\)-rich aCSF actually increased pituitary OLC content but pituitary release was not studied. OLC can also be synthesized and released from adrenal glands. Adrenocorticotrophic hormone (ACTH) can stimulate adrenal OLC release (18), but the effects of intracerebroventricular infusion of Na\(^+\)-rich aCSF on plasma ACTH have not yet been studied. OLC release from adrenal glands can also be regulated by peripheral Na\(^+\) (45). However, intracerebroventricular infusion of Na\(^+\)-rich aCSF did not change plasma [Na\(^+\)].

The present studies were not designed to assess the peripheral mechanisms activated by increases in CSF [Na\(^+\)] leading to hypertension. Sympathetic hyperactivity may play a major role (21), and the persistent increase in HR provides indirect support. Intracerebroventricular infusion of Na\(^+\)-rich aCSF did not change plasma aldosterone but tended to cause minor increases in PRA and plasma ANG II levels, possibly resulting from an increase in renal sympathetic drive. Other mechanisms such as vasopressin and marinobufagin (8) may play a role as well. However, in Sprague-Dawley rats intracerebroventricular infusion of hypertonic saline for 7 days increased CSF [Na\(^+\)] by 5 mmol/l and resting BP by ~15 mmHg, but plasma vasopressin remained normal and did not contribute to the chronic elevation of BP (25). Body fluid volumes and urinary excretion of water and electrolytes were not measured, but body weights, plasma electrolytes and hematocrit did not differ significantly among rats on the different treatments, making sodium and water retention less likely.

**Possible Limitations of Present Study**

Plasma aldosterone levels were ~1.1 ng/ml, which is higher than those reported by others using ELISA (~200 pg/ml) (13) or RIA (~400–600 pg/ml) (28, 47). Several factors may contribute to these different levels. First, in rats plasma aldosterone levels follow a circadian rhythm with a threefold range and highest levels at ~3:00 PM (28). In the present study, blood samples were obtained at ~2:00 to 3:00 PM, whereas the time of blood collection in other studies was not stated (13, 47). Second, surgery under general anesthesia may increase plasma aldosterone by 5–10 times for up to 12 h (9). In the present study, blood was collected through an intra-arterial line in free-moving rats 3–4 h after surgery, whereas others obtained blood either in rats under general anesthesia (13, 47) or by decapitation without anesthesia (28).

Aldosterone content was 0.9 pg/mg in the hypothalamus of rats with intracerebroventricular aCSF, whereas Gomez-Sanchez et al. (13) reported aldosterone content in the whole brain of ~0.2 pg/mg in Wistar rats on a regular salt diet. In addition to the above-mentioned factors, heterogeneity of aldosterone levels in different brain regions may explain the higher levels of aldosterone in the hypothalamus compared with whole brain.

Intracerebroventricular infusion of Na\(^+\)-rich aCSF at 4 µmol Na\(^+\)/h versus 0.75 Na\(^+\) µmol/h by intracerebroventricular infusion of aCSF increased CSF [Na\(^+\)] by 4–5 mmol/l. This increase appears as large, but is within the range, seen in Dahl S rats on a high-sodium diet (20, 30), in sheep after water deprivation (34), or in patients with hypertension on high salt intake (26).

As a continuation of our previous studies on the CNS mechanisms activated by CNS [Na\(^+\)], we used Wistar rats to assess the possible involvement of MR and aldosterone in the CNS. Further studies are needed to assess to what extent these CNS mechanisms differ in salt-sensitive and salt-resistant Dahl rats to understand the CNS mechanisms contributing to the pathophysiology of salt-sensitive hypertension.

In summary, in Wistar rats chronic intracerebroventricular infusion of Na\(^+\)-rich aCSF activates several components of the renin-angiotensin-aldosterone system in the brain, i.e., increased CSF [Na\(^+\)] increases aldosterone and OLC content in the hypothalamus and ACE and AT\(_1\)-receptor binding densities in various brain nuclei. Blockade of brain MR with intracerebroventricular spironolactone prevents the increases in brain
OLC, BP, and HR by Na⁺-rich aCSF, whereas blockade of brain OLC prevents the increases in ACE and AT₁-receptor densities. We postulate that chronic, small increases in CSF sodium first increase aldosterone and its binding to MR in neurons particularly in the hypothalamus, and thereby increase brain OLC. The latter activates the brain RAS and through stimulation of brain AT₁-receptors activates peripheral mechanisms causing hypertension.

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DISCLOSURE

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