Brain sodium channels and ouabainlike compounds mediate central aldosterone-induced hypertension

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Wang, Hao, Bing S. Huang, and Frans H. H. Leenen. Brain sodium channels and ouabainlike compounds mediate central aldosterone-induced hypertension. Am J Physiol Heart Circ Physiol 285: H2516–H2523, 2003. First published August 21, 2003; 10.1152/ajpheart.00299.2003.—Central nervous system (CNS) effects of mineralocorticoids participate in the development of salt-sensitive hypertension. In the brain, mineralocorticoids activate amiloride-sensitive sodium channels, and we hypothesized that this would lead to increased release of ouabainlike compounds (OLC) and thereby sympathetic hyperactivity and hypertension. In conscious Wistar rats, intracerebroventricular infusion of aldosterone at 300 or 900 ng/h in artificial cerebrospinal fluid (aCSF) with 0.145 M Na⁺ for 2 h did not change baseline mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), or heart rate (HR). Intracerebroventricular infusion of aCSF containing 0.16 M Na⁺ (versus 0.145 M Na⁺ in regular aCSF) did not change MAP or RSNA, but significant increases in MAP, RSNA, and HR were observed after intracerebroventricular infusion of aldosterone at 300 ng/h for 2 h. Intracerebroventricular infusion of aCSF containing 0.3 M Na⁺ increased MAP, RSNA, and HR significantly more after intracerebroventricular infusion of aldosterone versus vehicle. After intracerebroventricular infusion of aldosterone, the MAP, RSNA, and HR responses to intracerebroventricular infusion of aCSF containing 0.16 M Na⁺ were blocked by blockade of brain OLC with intracerebroventricular infusion of Fab fragments or of brain sodium channels with intracerebroventricular benzamil. Chronic intracerebroventricular infusion of aldosterone at 25 ng/h in aCSF with 0.15 M Na⁺ for 2 wk increased MAP by 15–20 mmHg and increased hypothalamic OLC by 30% and pituitary OLC by 60%. Benzamil blocked all these responses to aldosterone. These findings indicate that in the brain, mineralocorticoids activate brain sodium channels, with small increases in CSF Na⁺ leading to increases in brain OLC, sympathetic outflow, and blood pressure.

Benzamil; blood pressure

NEURAL MECHANISMS play a major role in the development of salt-induced hypertension in genetic models such as Dahl salt-sensitive (S) rats or spontaneously hypertensive rats (SHR) (2) and in mineralocorticoid hypertension in rats (8). Intracerebroventricular infusion of a mineralocorticoid receptor (MR) antagonist blocks the development of hypertension in DOCA salt-treated rats (27), as well as in Dahl S rats and SHR on high salt intake (9, 26). Continuous intracerebroventricular infusion of aldosterone at low rates in rats causes a significant increase in blood pressure (BP), whereas the same dose is ineffective when given systemically (6). Chronic intracerebroventricular infusion of a MR antagonist blocks this type of hypertension (6) as well as the hypertension by long-term subcutaneous infusion of a high dose of aldosterone (8). Altogether, these findings suggest that central actions of mineralocorticoids such as aldosterone play a critical role in high-salt or mineralocorticoid-induced hypertension. However, the mechanisms and pathways through which aldosterone in the brain induces hypertension have not yet been clarified.

Brain amiloride-sensitive sodium channels, which can be blocked by amiloride and its analog benzamil hydrochloride may mediate the central aldosterone-induced hypertension. Intracerebroventricular infusion of benzamil, which blocks amiloride-sensitive sodium channels more specifically than amiloride (20), blocks central aldosterone-induced hypertension (10), as well as salt-induced hypertension in Dahl S rats (11, 30), SHR (24), and DOCA-salt hypertension (19, 24).

In our previous studies, we established that in the brain ouabainlike compounds (OLC) mediate salt-sensitive hypertension (2). High salt intake in Dahl S rats or chronic intracerebroventricular infusion of Na⁺-rich artificial cerebrospinal fluid (aCSF) in Wistar rats or Dahl S rats increases brain OLC (30). Presumably by inhibiting neuronal Na⁺-K⁺-ATPase in brain areas such as the median preoptic nucleus (2), OLC activates central pathways leading to sympathetic hyperactivity and hypertension, because both can be prevented by the blockade of OLC in the brain with antibody Fab fragments, which bind plant-derived ouabain or OLC with high affinity (13, 14). Brain amiloride-sensitive sodium channels appear to mediate the increases in OLC production/release, because intracerebroventricular infusion of benzamil inhibits the increases in brain OLC and hypertension induced by high salt intake in Dahl S rats or by chronic intracerebroventricular infusion of Na⁺-rich aCSF in Wistar rats (30).

From these findings, we hypothesized that in the brain, mineralocorticoids activate brain sodium channels, enhancing responses to small increases in CSF.
Na\(^+\), and thereby increase brain OLC, which subsequently increases sympathetic activity and BP. To test this hypothesis, we evaluated whether 1) in normotensive rats, short-term intracerebroventricular infusion of aldosterone increases the responses of sympathetic activity and BP to small increases in CSF Na\(^+\), and benzamil or blockade of OLC blocks these responses; and 2) chronic intracerebroventricular infusion of aldosterone in aCSF with 0.15 M Na\(^+\) (just above the physiological concentration) increases brain OLC and BP in normotensive rats, and benzamil blocks these responses.

**METHODS**

Male Wistar rats weighing 200–250 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 a diet of standard laboratory chow and tap water ad libitum. All procedures were carried out according to the guidelines of the University of Ottawa Animal Committee for the use and care of laboratory animals. Acute and chronic experiments were started after 1 wk of acclimatization of the animals.

**Acute Experiments**

With the rat under halothane anesthesia, a 23-gauge, stainless steel cannula was implanted and fixed to the skull for intracerebroventricular infusions into the right lateral cerebroventricle (15, 16). At least 1 wk after intracerebroventricular surgery, with the rat under halothane anesthesia, the right femoral artery and vein were cannulated. Under the same anesthesia, through a flank incision, a pair of silver electrodes (A-M System, Everett, WA) was placed around and fixed to the left renal nerve with silicone rubber (SilGel 604, Wacker; Munich, Germany) for measurement of renal sympathetic nerve activity (RSNA) as described previously (15, 16). The catheters and electrodes were tunneled subcutaneously and externalized on the back of the neck. At least 4 h after recovery from the anesthesia, the rat was placed in a small cage. The intra-arterial catheter was connected to a pressure transducer for recordings of BP and heart rate (HR). The electrodes were linked to a Grass P511 bandpass amplifier, and the amplified and filtered RSNA signals were channeled to a rectifying voltage integrator (Grass 7P10) for measurement of integrated voltage signals of RSNA. At the end of each experiment, noise for RSNA was measured 20 min after the rat had been killed by an intravenous overdose of pentobarbital sodium, and the actual activity was determined by subtracting noise from the total activity (15, 16). Changes in RSNA were expressed as the percentage of resting RSNA. Intracerebroventricular infusions were performed through a “L”-shaped stainless steel cannula, which had been inserted into the ventricle via the guide cannula and connected either to a 20-μl Hamilton microsyringe or a 500-μl volume Hamilton microsyringe mounted on a Sage 355 infusion pump.

Baseline BP, HR, and RSNA were recorded after 30 min of stabilization. In one group of rats (n = 5), aldosterone dissolved in vehicle (2% alcohol, 98% aCSF, and 0.145 M Na\(^+\)) was infused intracerebroventricularly at 300 ng·10 μl·h\(^{-1}\) for 2 h followed by 900 ng·10 μl·h\(^{-1}\) for another 2 h. In a parallel group of rats, vehicle (10 μl/h) was infused intracerebroventricularly for 4 h. These (high) rates of short-term infusions of aldosterone were in preliminary dose-range studies found to enhance responses to CSF Na\(^+\).

In the second experiment, 5 min after intravenous injection of the arginine vasopressin (AVP) antagonist [30 μg/kg, D-(CH2)5-Tyr-(Me)AVP, Sigma Chemical)] (15, 16), aCSF containing 0.3 M Na\(^+\) was infused intracerebroventricularly at 3.8 μl/min for 5 min. Twenty minutes after the responses had disappeared, aldosterone (300 ng·10 μl·h\(^{-1}\)) was infused intracerebroventricularly for 2 h, followed by intracerebroventricular injection of antibody Fab fragments (Digibind, Glaxo Wellcome, Toronto, Canada) or nonspecific γ-globulins as the control (132 μg in 4 μl aCSF for 5 h for each group) instead of Fab fragments and γ-globulins.

**Chronic Experiments**

**Intracerebroventricular cannulation and infusion of drugs.** With the rat under halothane anesthesia, a 23-gauge right-angled stainless steel cannula was implanted into the left lateral cerebral ventricle and fixed to the skull with acrylic cement. 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 vehicle with 0.15 M Na\(^+\) just above the physiological range of CSF Na\(^+\) (0.144–0.145 M Na\(^+\)) (23) or aldosterone (100 ng·kg·h\(^{-1}\))(1.6, 25) alone or combined with benzamil (4.0 μg·kg·h\(^{-1}\))(1.1) and implanted subcutaneously on the back of the rats.

**Arterial BP and HR measurements.** Thirteen days after the intracerebroventricular cannulation, carotid arterial cannulation was performed with the rat under halothane anesthesia. A PE-50 catheter filled with heparinized saline (100 IU/ml) was inserted into the left carotid artery. The next morning, the carotid arterial catheters were connected to pressure transducers. Rats were allowed to rest for about 30 min, and then BP and HR were recorded for 30 min. After the BP and HR measurements, blood was withdrawn from the arterial line in the conscious rats into an ice-chilled tube containing EDTA and centrifuged at 3,000 g for 10 min. Plasma electrolytes were determined using ion-selective electrodes (model 917, Hitachi electrode).

**ELISA for OLC**

Endogenous OLCs were extracted from brain regions and plasma and the levels quantified by ELISA (17, 31). The anti-olivocerebellar antibody was raised from rabbits immunized with the commercially available cardenolide ouabain conjugated with bovine serum albumin. This antibody has a high antibody titer (1:16×10\(^5\)), full cross-reactivity with ouabain, 8% cross-reactivity with digoxin, and minimal cross-reactivity with numerous common endogenous steroids and pep-
tides (31). In addition, the OLC is eluted with 25% acetonitrile in water from Sep-Pak C18 cartridges, and common adrenocortical steroids such as corticosterone or aldosterone are too hydrophobic to be eluted at this acetonitrile concentration.

Statistical Analysis

Values are expressed as means ± SE. Within one group, for responses of MAP, RSNA, and HR following intracerebroventricular infusions, repeated-measures analysis of variance was performed followed by a Duncan’s test for multiple comparisons. Differences between groups were evaluated by one-way ANOVA followed by Newman-Keuls post hoc multiple comparison. The level of significance was set at a value of \( P < 0.05 \).

RESULTS

Acute Experiments

In Wistar rats, 2-h intracerebroventricular infusions of aldosterone at 300 or 900 ng/h or vehicle for aldosterone in 0.145 M \( \text{Na}^+ \) aCSF did not change baseline mean arterial pressure (MAP), RSNA, and HR (Table 1).

Within 1–2 min after the start of intracerebroventricular infusion of aCSF containing 0.3 M \( \text{Na}^+ \) at 3.8 \( \mu \text{l/min}, \) MAP, RSNA, and HR started to increase, reached a plateau within 3–4 min, and returned to the resting levels within 2–3 min after the termination of infusion (Fig. 1). After intracerebroventricular infusion of aldosterone vehicle for 2 h, intracerebroventricular infusion of aCSF containing 0.3 M \( \text{Na}^+ \) elicited similar MAP, RSNA, and HR responses as those before intracerebroventricular vehicle. In contrast, after intracerebroventricular infusion of aldosterone at 300 ng/h for 2 h, intracerebroventricular infusion of aCSF containing 0.3 M \( \text{Na}^+ \) elicited significantly larger increases in MAP, RSNA, and HR than those before intracerebroventricular aldosterone (Fig. 1). MAP, RSNA, and HR remained significantly higher up to 4–5 min after the termination of the infusion.

Table 1. MAP, RSNA, and HR responses to intracerebroventricular infusion of aldosterone vehicle or aldosterone in Wistar rats

<table>
<thead>
<tr>
<th>Times of Intracerebroventricular Infusion, min</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle (10 ( \mu \text{l/h} ))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>103 ± 4</td>
<td>102 ± 3</td>
<td>104 ± 3</td>
<td>102 ± 3</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>100</td>
<td>97 ± 5</td>
<td>96 ± 4</td>
<td>100 ± 5</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>412 ± 17</td>
<td>409 ± 18</td>
<td>420 ± 16</td>
<td>409 ± 20</td>
<td>410 ± 20</td>
</tr>
<tr>
<td><strong>Aldosterone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 ng·10 ( \mu \text{l·h}^{-1} )</td>
<td>390 ng·10 ( \mu \text{l·h}^{-1} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>102 ± 3</td>
<td>99 ± 4</td>
<td>102 ± 4</td>
<td>101 ± 4</td>
<td>104 ± 4</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>100</td>
<td>97 ± 3</td>
<td>101 ± 4</td>
<td>102 ± 4</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>402 ± 12</td>
<td>405 ± 17</td>
<td>410 ± 17</td>
<td>412 ± 17</td>
<td>414 ± 17</td>
</tr>
</tbody>
</table>

Data are means ± SE; \( n = 5 \) for each. MAP, mean arterial pressure; RSNA, renal sympathetic nerve activity; HR, heart rate.

Before or after the intracerebroventricular infusion of aldosterone vehicle, intracerebroventricular infusion of aCSF containing 0.16 M \( \text{Na}^+ \) did not change resting MAP, RSNA, and HR (Fig. 2). In contrast, after intracerebroventricular infusion of aldosterone at 300 ng/h for 2 h, intracerebroventricular infusion of aCSF containing 0.16 M \( \text{Na}^+ \) caused significant increases in MAP, RSNA, and HR. After aldosterone, significant responses were observed at 2–3 min, and a plateau was reached at 4–5 min (Fig. 2).

Intracerebroventricular injection of Fab fragments, \( \gamma \)-globulins, benzamil, or benzamil vehicle did not change baseline MAP, RSNA, and HR. After intracerebroventricular infusion of aldosterone at 300 ng/h for 2 h and control pretreatments (intracerebroventricular...
aCSF, γ-globulins, or benzamil vehicle), intracerebroventricular infusion of aCSF containing 0.16 M Na⁺ caused similar significant increases in MAP, RSNA, and HR (Fig. 3). In contrast, intracerebroventricular pretreatment with Fab fragments (fully) or benzamil (nearly completely) blocked the increase in MAP, RSNA, and HR significantly after pretreatment with intracerebroventricular Aldo.

**Chronic Experiments**

All rats developed normally during the study. No differences in body weight gain, food, and water intake were observed among rats receiving different treatments (Table 2 and Fig. 4).

Chronic intracerebroventricular infusion of aldosterone in 0.15 M Na⁺ aCSF increased systolic BP (SBP), diastolic BP (DBP), and MAP by 15–20 mmHg. Benzamil blocked these increases (Fig. 5A). HR tended to be increased by chronic intracerebroventricular infusion of aldosterone (428 ± 18 compared with control 400 ± 16 beats/min, *P* > 0.05), which was prevented by benzamil (Fig. 5B).

Chronic intracerebroventricular infusion of aldosterone increased hypothalamic OLC by 30% and pituitary OLC by 60%. Benzamil prevented the responses of hypothalamic and pituitary OLC to aldosterone (Fig. 6, A and B). Neither aldosterone alone nor aldosterone combined with benzamil affected adrenal or plasma OLC levels (Fig. 6, C and D).

There were no significant differences among groups for plasma Na⁺ or plasma K⁺ concentrations (Table 2).

**DISCUSSION**

The present study in normotensive Wistar rats provides as major new findings that 1) intracerebroven-
tricular infusion of aldosterone with physiological CSF Na\(^+\) concentration for 4 h does not change baseline MAP, RSNA, and HR but enhances Na\(^+\)-rich aCSF-induced sympathetic hyperactivity and increases in resting BP and HR; 2) these enhanced responses to intracerebroventricular infusion of Na\(^+\)-rich aCSF after pretreatment with aldosterone are blocked by blockade of brain OLC with intracerebroventricular Fab fragments or blockade of brain sodium channels with intracerebroventricular benzamil; 3) chronic intracerebroventricular infusion of aldosterone with a small increase in CSF Na\(^+\) causes hypertension associated with increases in brain OLC; and 4) benzamil blocks the increases in brain OLC and BP induced by chronic central infusion of aldosterone. These findings suggest that in the brain, aldosterone activates benzamil-blockable sodium channels, allowing enhanced Na\(^+\) entry and thereby enhanced increases in brain OLC, sympathetic activity, and BP.

In the present study, water intake, body weight, and plasma electrolytes were not affected by chronic intracerebroventricular infusion of aldosterone. The threshold for triggering sympathetic hyperactivity and hypertension may be lower than that for salt appetite or, more likely, distinct aldosterone receptor-containing cell groups in centers for BP control (in, for example, the hypothalamus) are more accessible to intracerebroventricular-infused aldosterone than those that mediate salt appetite in the amygdala (28). Garwitz and Jones (5) reported that, in rats, 4 wk of subcutaneous administration of aldosterone at a rate of 50 ng/h had no effect on BP or saline intake, doses of 100 and 250 ng/h increased BP without affecting drinking, and doses >500 ng/h produced an increase in both BP and drinking.

The present study shows that both the acute (2 h) and chronic (2 wk) responses to intracerebroventricula-
lar infusions of aldosterone and Na\textsuperscript{+}-rich CSF can be blocked by benzamil. Benzamil is an amiloride analog that blocks amiloride-sensitive sodium channels more specifically than amiloride (20). The actual location of these benzamil-blockable sodium channels in the rat brain has not yet been ascertained. Specific mineralocorticoid binding sites have been found in rats in several areas involved in the control of sympathetic tone and BP such as anterior hypothalamus and various brain stem nuclei (3). Benzamil-blockable sodium channels in the brain include at least two types: Phe-Met-Arg-Phe-(FMRF) amide-gated sodium channels or FaNaC (17) and epithelial sodium channels or ENaC (29). Aldosterone plays a major role in the regulation of ENaC (4). As the main mechanism for this regulation, aldosterone activates the cytosolic MRs, which results in increased transcription of the genes that produce ENaC subunits (4). The genomic mechanisms for aldosterone to enhance Na\textsuperscript{+} transport include the synthesis and insertion of ENaC subunits into the cell membrane and the activation of existing Na\textsuperscript{+} channels by regulatory proteins, so-called “aldosterone-induced proteins” (4). Unlike the more extensively studied cytoplasmic MRs, MRs on the plasma membrane initiate signaling cascades that alter function in as little as a few seconds (33). This rapid aldosterone activation of amiloride-sensitive ENaC occurs through nongenomic signaling pathways (33).

Intracerebroventricular infusion of either aldosterone at 300 or 900 ng/h (with physiological, 0.145 M Na\textsuperscript{+} in vehicle) for 2 h or of aCSF containing 0.16 M Na\textsuperscript{+} (just above physiological CSF Na\textsuperscript{+}) did not change baseline MAP, RSNA, and HR in conscious Wistar rats. However, after intracerebroventricular infusion of aldosterone at 300 ng/h, intracerebroventricular infusion of aCSF containing 0.16 M Na\textsuperscript{+} caused significant increases in MAP, RSNA, and HR. Moreover, intracerebroventricular infusion of aCSF with a higher (0.3 M) Na\textsuperscript{+} alone caused significant increases in MAP, RSNA, and HR, and these increases were significantly larger after intracerebroventricular pretreatment with aldosterone. Intracerebroventricular pretreatment with benzamil largely prevented the MAP, RSNA, and HR responses to intracerebroventricular infusion of aCSF containing 0.16 M Na\textsuperscript{+} after intracerebroventricular infusion of aldosterone (Fig. 3). Because only one dose of benzamil was used, we cannot ascertain whether the remaining responses would be blocked by a higher dose or are mediated through different mechanisms. These results suggest that short-term intracerebroventricular infusion of aldosterone activates brain benzamil-blockable sodium channels, presumably ENaC, thereby causing enhanced Na\textsuperscript{+} entry and subsequent sympathoexcitation. Intracerebroventricular infusion of aldosterone in aCSF with physiological Na\textsuperscript{+} did not change baseline MAP, RSNA, or HR. These findings suggest that activation of brain sodium channels alone may not be enough to induce hypertension, and that some increase in CSF Na\textsuperscript{+} is needed for sympathoexcitation and the development of hypertension. Indeed, in the chronic study, intracerebroventricular infusion of aldosterone at a low rate in aCSF with 0.15 M Na\textsuperscript{+} (slightly above the physiological CSF Na\textsuperscript{+} concentration) increased MAP by about 20 mmHg, which also could be blocked by benzamil. These findings are consistent with previous studies in rats by Gomez-Sanchez and Gomez-Sanchez (10). In contrast, chronic intracerebroventricular infusion of aldosterone in sheep did not change BP (22). The latter studies used regular aCSF, and it is tempting to speculate that sheep may respond to intracerebroventricular aldosterone if combined with a small increase in CSF Na\textsuperscript{+}.

One decade ago, Hamlyn et al. (12) isolated an endogenous sodium pump inhibitor, OLC, from human plasma, which appeared to be indistinguishable from the cardiac glycoside ouabain by mass spectrometric or
biochemical means. However, its exact structure is not yet clear (18). The hypothalamus may be one of the sources of OLC (2, 18, 21, 32), and OLC in the brain was found to play a critical role in the genetic models of salt-sensitive hypertension (i.e., Dahl S rats and SHR) (2). Intracerebroventricular infusion of aldosterone enhances Na\(^+\)-rich aCSF-induced sympathetic hyperactivity and enhances the increases in BP and HR. These responses were blocked not only by intracerebroventricular benzamil but also by blockade of brain OLC with intracerebroventricular antibody Fab fragments, which bind OLC with high affinity (21), indicating that brain OLC mediates the responses to intracerebroventricular aldosterone and CSF Na\(^+\). Consistent with this concept, in the chronic study, aldosterone increased OLC in the brain as well as increased BP, and benzamil blocked both increases. In previous studies, we showed that intracerebroventricular benzamil blocks increases in brain OLC in Wistar rats by chronic intracerebroventricular infusion of Na\(^+\)-rich aCSF or in Dahl S rats on high salt intake (30). These findings support the concept that open brain benzamil-blockable sodium channels are essential for brain OLC production and release, thereby leading to sympathetic hyperactivity and hypertension in Wistar rats with chronic intracerebroventricular infusion of Na\(^+\)-rich aCSF or in Dahl S rats and SHR with high salt intake (14, 16). In the brain, aldosterone appears to activate benzamil-blockable sodium channels, resulting in increased release of brain OLC in response to small increases in CSF Na\(^+\).

Limitations of Study

The present study addresses the effects of centrally administered exogenous aldosterone and cannot be directly extrapolated to endogenous aldosterone. However, studies using MR antagonist (e.g., 9, 26) indicate that endogenous mineralocorticoids may have similar central effects. The actual source of the steroids mediating these responses (i.e., adrenals or brain) is still unknown. Similarly, intracerebroventricular infusions of agonists or antagonists provide useful general information on the central effects of, in this case, aldosterone, but further studies are clearly needed to address important aspects of cell biology and actual cascade of events on a more cellular level in the CNS. Our findings are consistent with the stimulatory effects of aldosterone on ENaC, the latter blocked by benzamil. As we previously discussed (15), benzamil is selective for these channels, but can affect other channels, and alternative approaches are required to exclude other possible explanations (19).

In summary, central infusion of aldosterone in normotensive rats, together with Na\(^+\)-rich aCSF, causes sympathetic hyperactivity and an increase in blood pressure. These cardiovascular responses can be prevented by blockade of brain sodium channels with benzamil or by blockade of brain OLC. We postulate that, in the presence of a more effective aldosterone (MR) sodium channels cascade, chronic small increases in CSF sodium by, e.g., high salt intake in Dahl S rats or SHR (15), lead to enhanced Na\(^+\) entry in relevant brain areas, thereby increase brain OLC and, subsequently, sympathetic outflow and BP.

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DISCLOSURES

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