Brain Ouabain-Like Activity and the Sympathoexcitatory and Pressor Effects of Central Sodium in Rats

Bing S. Huang, Eef Harmsen, Huilian Yu, and Frans H.H. Leenen

Intracerebroventricularly infused hypertonic saline elicits sympathoexcitatory and pressor effects. To clarify the mechanisms mediating these effects, we evaluated blood pressure (BP), heart rate (HR), and renal sympathetic nerve activity (RSNA) responses to intracerebroventricular administration of 0.3 M NaCl, ouabain, and rat hypothalamic and pituitary extracts containing ouabain-like activity (OLA) in conscious Wistar rats, before and after intracerebroventricular preinjection of digoxin-specific antibody Fab (DAF) fragments. To exclude modulatory effects of arginine vasopressin (AVP), treatment with DAF fragments was in all experiments preceded by intravenous injection of the AVP antagonist (β-mercapto-β,β-cyclopentamethylenepropionyl1,-o-Me-Tyr1,-Arg8)AVP. After AVP antagonist pretreatment, 0.3 M NaCl i.c.v. at 3.8 μl/min for 10 minutes caused simultaneous increases in BP, RSNA, and HR. After AVP antagonist pretreatment, intracerebroventricular injections of 0.3 and 1.0 μg/1 μl ouabain or the OLA equivalent to 1 μg ouabain/2 μl elicited similar significant increases in BP, HR, and RSNA. After pretreatment with AVP antagonist and DAF fragments (66 μg/4 μl i.c.v.), BP, HR, and RSNA responses to 0.3 M NaCl, ouabain, and OLA were all significantly diminished. In contrast, combined AVP blockade and DAF fragments did not affect the BP response to intracerebroventricular angiotensin II, the BP, HR, and RSNA responses to intravenous sodium nitroprusside. Intracerebroventricularly injected γ-globulins (66 μg/4 μl) did not affect the responses to 0.3 M NaCl, ouabain, or OLA. These data demonstrate that the effects of intracerebroventricularly infused hypertonic saline share, at least in part, a common mechanism with the effects of intracerebroventricular ouabain/OLA; i.e., brain OLA appears to be involved in the sympathoexcitatory and pressor effects of intracerebroventricular hypertonic saline. (Circulation Research 1992;71:1059–1066)

Key Words • central hypertonic saline • brain ouabain-like activity • ouabain • renal sympathetic nerve activity • digoxin-specific antibody Fab fragments • Na⁺,K⁺-ATPase activity

High sodium intake exaggerates the development of hypertension in rats genetically predisposed to hypertension; this response is partially due to central effects increasing sympathetic outflow and blood pressure (BP).1,2 The primary mechanisms through which high sodium intake causes these sympathoexcitatory responses have so far not been elucidated. One possibility is that high sodium intake intermittently or chronically increases sodium concentrations in the cerebrospinal fluid (CSF), resulting in an activation of not only central osmotic receptors but also central sodium receptors,3,4 leading to increased sympathetic outflow and arterial BP. We recently provided indirect evidence that brain ouabain-like activity (OLA) may play a primary role in the different responses to high sodium intake in spontaneously hypertensive rats (SHR) versus Wistar-Kyoto (WKY) control rats.5 In the present study, we provide more direct evidence for a possible role of brain OLA in the sympathoexcitatory and pressor responses to increases in central sodium.

Chronic intracerebroventricular infusion with hypertonic saline elevates BP in normotensive rats.3 In anesthetized rats, intracerebroventricular infusion with hypertonic saline increases BP, heart rate (HR), and sympathetic nerve activity.6 These responses depend on an increase in the Na⁺ concentration in the cerebrospinal fluid and not on changes in osmolality.7,8 Part of the early pressor response results from increased arginine vasopressin (AVP) release, but the subsequent maintenance of pressor effect is associated with increased sympathetic nerve activity and can be abolished by ganglionic blockade3,4,8 and not by AVP or angiotensin II blockade. In conscious rats, intracerebroventricular injection of ouabain5,9 or rat hypothalamic extracts10 elicits similar pressor and sympathoexcitatory responses. No studies have so far been done to clarify the possible role of brain OLA in mediating the effects of intracerebroventricularly infused hypertonic saline by

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using a specific antagonist. Digoxin-specific antibody Fab (DAF) fragments (Digibind) have been used for the treatment of digitalis intoxication for several years. These Fab fragments bind digoxin, digitoxin, and other glycosides with high affinity and reverse the inhibition of Na⁺,K⁺-ATPase. OLA may actually be an analogue of the digitalis glycoside ouabain. The present study was undertaken to determine whether brain OLA is involved in the sympathoexcitatory and pressor effects of intracerebroventricularly infused hypertonic saline. For this, we evaluated in conscious rats, BP, HR, and renal sympathetic nerve activity (RSNA) responses to intracerebroventricularly administered hypertonic saline, ouabain, and rat hypothalamic and pituitary extracts containing OLA before and after intracerebroventricular preinjection with DAF fragments. To assess the specificity of blockade, responses to intracerebroventricular angiotensin II and carbachol, to intravenous sodium nitropusside, and to air stress were evaluated before and after the administration of DAF fragments. The results indicate that brain OLA plays a major role in the sympathoexcitatory and pressor responses to increases in central sodium.

**Materials and Methods**

Male Wistar rats weighing 180–200 g (Charles River, Montreal, Canada) were housed under constant room temperature (24°C) and in a 12-hour light/12-hour dark cycle and given normal rat chow and water ad libitum for 3 days before surgery.

**Surgical Procedure**

Implantation of a chronic intracerebroventricular cannula under sodium pentobarbital anesthesia and verification of the placement were described previously. Briefly, via a stereotaxic frame, a 23-gauge needle was fixed on the skull of the rat with acrylic cement (over the left lateral ventricle, 0.3 mm posterior and 1.4 mm lateral to the bregma). The lower end of the guide needle was approximately 0.5 mm above the ventricle, and the upper end was sealed with a stylet. At least 1 week after the surgery, with the rats under halothane anesthesia, catheters were placed in the right femoral artery and jugular vein. With an additional intravenous injection of sodium pentobarbital, a pair of platinum electrodes was placed around the left renal nerve through a left flank incision. The electrodes and the catheters were then tunneled under the skin to the back of the neck. After recovery from the anesthesia (5 hours), the rats were placed in a small cage in which they could move back and forth for the experiments.

**BP, HR, and RSNA Recordings**

As described previously, the intra-arterial catheter was connected to a transducer, and BP and HR were monitored through a polygraph (model 7E, Grass Instrument Co., Quincy, Mass.) and a Grass 7P44 tachograph. The electrodes were connected to a Grass P511 bandpass amplifier, and RSNA (spikes per second) was counted by means of a nerve traffic analyzer. The output of counts, BP, and HR were digitized through a microcomputer.

**Experimental Protocol**

The rats were allowed to rest for 30 minutes after the catheters and electrodes were connected to the transducer and amplifier. All protocols involved pretreatment with a long-acting AVP antagonist to exclude the modulatory effects of AVP, in particular after the administration of intracerebroventricular hypertonic saline. The following injection or infusion protocols were performed for individual groups of rats: For protocol A, five minutes after intravenous injection of 30 μg/kg AVP antagonist [β-mercapto-β,β-cyclopentamethylenepropionyl]-ο-Me-Tyr²,Arg⁴ AVP, termed d-(CH₂)₂-Tyr-(Me)AVP, 0.3 μg/3 μl and 1 μg/1 μl ouabain were injected intracerebroventricularly after a 20-minute interval. After the responses had subsided, the rats rested for 1 hour. DAF fragments (66 μg/4 μl) were then administered via two intracerebroventricular injections (33 μg/2 μl each) separated by a 5-minute interval. Ten minutes after the completion of DAF fragment injection, intravenous injection of AVP antagonist (30 μg/kg) was repeated, followed by intracerebroventricular injection of the two doses of ouabain. For protocol B, the intracerebroventricular ouabain injections at two doses in protocol A were substituted with a single intracerebroventricular dose of hypothalamic and pituitary extract containing OLA (equivalent to 1 μg ouabain/2 μl), both before and after DAF fragment injection. The injections of AVP antagonist and DAF fragments as well as corresponding intervals were the same as those in protocol A. For protocol C, the intracerebroventricular ouabain injections in protocol A were substituted with an intracerebroventricular infusion of 0.3 M NaCl at 3.8 μl/min for 10 minutes, both before and after DAF fragment injection. The rest of the injections and the intervals were the same as those in protocol A. For protocols D–F, the injections or infusions used in protocols A–C, respectively, were repeated while using γ-globulins (66 μg/4 μl) as a substitute for the DAF fragments. For protocol G, without applying DAF fragments and AVP antagonist, 0.3 M NaCl was infused intracerebroventricularly at 3.8 μl/min for 15 minutes. After the responses had subsided, the rats were allowed to rest for 1 hour. Then, after intravenous AVP antagonist injection (30 μg/kg), intracerebroventricular injection of 3 μl of 0.15 M (0.9%) NaCl, intravenous injection of 3 μg ouabain, intravenous infusion of 0.3 M NaCl at 3.8 μl/min for 10 minutes, and intracerebroventricular infusion of 0.15 M NaCl at 3.8 μl/min for 10 minutes were performed at 20-minute intervals. For protocol H, the same procedure used in protocol A was repeated, but angiotensin II (10 ng and 100 ng) was used instead of ouabain. For protocol K, a standardized stress was performed by a jet of air blowing on the face of the rats for 30 seconds as described previously. Ten minutes after the stress, the vasodilator sodium nitropusside was infused intravenously at progressively increasing doses (5–100 μg/kg per minute) to induce a ramp decline in mean arterial pressure (MAP) with a maximum decrease of 50 mm Hg over 3 minutes. Twenty minutes after BP, HR, and RSNA responses had normalized, the AVP antagonist (30 μg/kg i.v.) was injected, followed after 5 minutes by intracerebroventricular injection of the cholinomimetic carbachol (25 ng/2 μl) over a period of 20 seconds.
After MAP, RSNA, and HR had returned to basal levels, the rats were allowed to rest for at least 1 hour. Subsequently, DAF fragments (66 μg/4 μl) were injected intracerebroventricularly as in protocol A, and the air stress and nitroprusside infusion were repeated. Ten minutes after the administration of additional intracerebroventricular DAF fragments (66 μg/2 μl) and intravenous AVP antagonist, the responses to intracerebroventricular carbachol were again assessed.

Intracerebroventricular injection or infusion was performed by use of a Hamilton microsyringe with a 10-μl volume or a Hamilton syringe with a 500-μl volume mounted on a Sage 355 infusion pump via a 26-gauge L-shaped needle placed into the lateral ventricle (3.8 mm below the dura) along the guide needle. The following drugs were purchased from Sigma Chemical Co., St. Louis, Mo.: ouabain, γ-globulins, carbachol, sodium nitroprusside, angiotensin II, and the AVP antagonist D-(CH3)2Tyr-(Me)AVP.

**Extraction and Quantitation of Brain OLA**

Hypothalamic and pituitary extracts were prepared by using a revised method of Takahashi et al. Whole brain and pituitary were removed from 50 male Wistar rats (weighing 250–300 g) immediately after decapitation and frozen at −20°C. The hypothalamic tissue was obtained by dissecting the brain at 4°C according to the method of Glowinski and Iversen. After the pituitary and hypothalamus had been weighed, 10 vol distilled water was added, and the tissue was homogenized with a Polytron homogenizer (Brinkmann Instruments, Canada). The homogenates were centrifuged at 15,000 rpm for 30 minutes, and the supernatant was run through a Sep-Pak C18 column (Waters-Millipore, Milford, Mass.) preconditioned with 10 ml methanol and washed with 10 ml distilled water. The column was then washed again with 10 ml distilled water and 2 ml of 10% acetonitrile. The OLA was eluted from the column with 60% acetonitrile (4 ml). The eluate extracted from the tissue of each rat was pooled, lyophilized, and later dissolved with distilled water for quantitation of the activity and intracerebroventricular injection.

The assay for Na⁺,K⁺-ATPase activity for the quantitation of OLA in crude tissue extracts was similar to that of Takahashi et al. Briefly, the activity was determined by measuring 32P liberated from [γ-32P]ATP (NEN Research Products, Boston), which was hydrolyzed by ouabain-sensitive Na⁺,K⁺-ATPase (Sigma) with or without the presence of specific amounts of ouabain or brain extracts. The reaction mixture was incubated at 37°C for 30 minutes, and charcoal was added in the cooled solution. After being mixed thoroughly for 20 minutes in an ice bath, the mixture was centrifuged (Eppendorf centrifuge, Eppendorf Inc., Fremont, Calif.) for 40 minutes at 3,000 rpm at 4°C. The supernatant was mixed with scintillator (Aquasol-II, New England Nuclear, Inc.), and the radioactivity of the sample was counted.

**Data Analysis**

As described previously, arterial baroreflex curves induced by intravenous sodium nitroprusside (i.e., HR-MAP and RSNA-MAP curves) were constructed by means of linear regression. The gain of the baroreflex was expressed as the percent increase in RSNA or the increase in HR per unit decrease in MAP over the linear portion of the baroreflex curve. Statistically significant differences between the responses before and after the administration of DAF fragments were determined by paired t test. Significances for other comparisons were determined by analysis of variance followed by Duncan’s multiple-range test. The level of statistical significance was set at p < 0.05.

**Results**

**Intracerebroventricular Infusion of 0.3 M NaCl**

Without pretreatment with the AVP antagonist, intracerebroventricular infusion of 0.3 M NaCl caused increases in MAP and RSNA within 2 minutes of the start of the infusion, reaching plateau levels within 5 minutes. HR, however, decreased for the first 5 minutes of saline infusion, then returned to and passed baseline, and reached a plateau level that was significantly higher than the resting level. All responses returned to resting levels a few minutes after the termination of the infusion.

The AVP antagonist injected intravenously at 30 μg/kg caused transient decreases in BP, HR, and RSNA that were not statistically significant compared with the resting levels. When intracerebroventricular infusion of 0.3 M NaCl was performed 5 minutes after the intravenous injection of the AVP antagonist, the initial decrease in HR was no longer observed. BP, HR, and RSNA increased simultaneously within 2 minutes after infusion, reached plateau levels, and returned to the baselines 2–3 minutes after the termination of infusion (Figure 1). The peak values of responses were significantly higher than the resting levels (Figure 2) and similar in magnitudes to those without AVP antagonist injection. After AVP antagonist pretreatment, intracerebroventricular infusion of 0.15 M (or 0.9%) NaCl at 3.8 μl/min for 15 minutes did not cause any changes in BP, HR, and RSNA.

Intracerebroventricular injection of 66 μg/4 μl DAF fragments did not affect the resting MAP, HR, and RSNA. After pretreatment with intracerebroventricular DAF fragments and intravenous AVP antagonist, intracerebroventricular infusion of 0.3 M NaCl caused only slight increases in BP, HR, and RSNA, which were significantly smaller in magnitude compared with the responses without DAF fragment pretreatment (Figure 2). The increases in BP, HR, and RSNA in response to 0.3 M NaCl i.e.v. before and after pretreatment with 66 μg γ-globulins were similar (Figure 2).

**Intracerebroventricular Ouabain**

Intracerebroventricular ouabain 5 minutes after intravenous AVP antagonist injection increased BP, HR, and RSNA in a dose-related fashion. The responses began 1 minute after ouabain injection (Figure 1), reached plateau levels within 5 minutes, and returned to resting levels at 20 minutes after the injection. Peak increases in MAP, HR, and RSNA after intracerebroventricular injections of 0.3 and 1.0 μg ouabain are shown in Figure 3. All responses were significantly different from the resting values. No behavioral changes were observed when 0.3 μg ouabain was injected. The rats moved back and forth after 1.0 μg ouabain, resulting in some fluctuation in MAP, HR, and RSNA.
Intracerebroventricular injection of 3 μl of 0.15 M (0.9%) NaCl or intravenous injection of 3 μg ouabain did not cause any responses.

After DAF fragments and AVP antagonist injection, the MAP, HR, and RSNA responses to ouabain injection were all abolished or diminished significantly compared with the responses to intracerebroventricular ouabain at the same doses without DAF fragments (Figure 3). Behavioral changes to intracerebroventricular ouabain were also no longer observed. After γ-globulins (66 μg/4 μl) were injected intracerebroventricularly, ouabain injection elicited significant increases in MAP, HR, and RSNA, similar to intracerebroventricular ouabain alone (Figure 3).

**Intracerebroventricular Brain OLA**

After intravenous injection of AVP antagonist, intracerebroventricular injection of brain OLA at the dose equivalent to 1 μg ouabain/2 μl caused simultaneous increases in BP, HR, and RSNA. The time course of responses was similar to that of the responses to ouabain. All the responses were significantly different from

**FIGURE 1.** Tracings of blood pressure (BP), heart rate (HR), and renal sympathetic nerve activity (RSNA) in response to intracerebroventricular infusion of 0.3 M NaCl (3.8 μl/min) and intracerebroventricular injection of ouabain (1 μg/1 μl) and the brain ouabain-like activity (OLA) equivalent to 1 μg ouabain. Intracerebroventricular infusion and injection were performed 5 minutes after the intravenous injection of vasopressin antagonist (30 μg/kg).

**FIGURE 2.** Bar graphs showing peak mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) responses to intracerebroventricular infusion of 0.3 M NaCl (3.8 μl/min) for 10 minutes, before and after intracerebroventricular pretreatment with digoxin-specific antibody Fab fragments (66 μg/4 μl) or γ-globulins (66 μg/4 μl). Intracerebroventricular infusions were performed after intravenous injection of vasopressin antagonist. Values are mean ± SEM (n=7–9). *p<0.05 vs. resting values. **p<0.05 vs. NaCl only.
basal levels (Figure 4). The magnitudes of BP and RSNA increases were significantly smaller than the increase elicited by 1.0 µg ouabain (+18±2 versus +27±5 mm Hg and +49±2% versus +68±5% [resting], p<0.05 for both). Behavioral changes were also observed. The rats looked slightly agitated, but there was no fluctuation in BP, HR, and RSNA.

An intracerebroventricular preinjection with DAF fragments abolished or significantly diminished the BP, HR, and RSNA responses to intracerebroventricular brain OLA (Figure 4). Behavioral changes were no longer observed either. Intracerebroventricular OLA injection after γ-globulin administration elicited significant increases in MAP, HR, and RSNA, similar to intracerebroventricular OLA alone (Figure 4).

Responses to Intracerebroventricular Angiotensin II, Intracerebroventricular Carbachol, Air Stress, and Intravenous Nitroprusside

Intracerebroventricular injection of 10 and 100 ng angiotensin II caused a dose-related increase in MAP (+15±4 and +26±6 mm Hg, respectively, p<0.05 for...
both). Changes in HR and RSNA after intracerebroventricular angiotensin II were not significant compared with the resting values. Pretreatment with intravenous AVP antagonist and intracerebroventricular DAF fragments did not affect the pressor response to intracerebroventricular angiotensin II.

Intracerebroventricular carbachol caused significant increases in MAP, HR, and RSNA. In the presence of AVP antagonist, responses to intracerebroventricular carbachol were not affected by pretreatment with intracerebroventricular DAF fragments. Air stress (Table 1) elicited significant increases in MAP, HR, and RSNA. These responses were not significantly different before versus after the administration of DAF fragments. Intravenous nitroprusside (Table 2) caused a ramp decrease in MAP and parallel ramp increases in HR and RSNA. The gains of the HR–MAP and RSNA–MAP baroreflex curves as well as the peak increases in HR and RSNA were the same without and with DAF fragments (Table 2).

### Table 1. Effects of Intracerebroventricular Digoxin-Specific Antibody Fab Fragments on the Responses to Intracerebroventricular Angiotensin II and Carbachol and to Air Stress

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After DAF fragments</th>
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<tbody>
<tr>
<td>Angiotensin II (100 ng)</td>
<td></td>
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<tr>
<td>ΔMAP (mm Hg)</td>
<td>+26±6</td>
<td>+25±4</td>
</tr>
<tr>
<td>ΔRSNA (% basal)</td>
<td>+4±5</td>
<td>+3±6</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>+6±6</td>
<td>+6±7</td>
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<tr>
<td>Carbachol</td>
<td></td>
<td></td>
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<tr>
<td>ΔMAP (mm Hg)</td>
<td>+12±2</td>
<td>+14±1</td>
</tr>
<tr>
<td>ΔRSNA (% basal)</td>
<td>+38±3</td>
<td>+36±4</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>+37±4</td>
<td>+35±5</td>
</tr>
<tr>
<td>Air stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔMAP (mm Hg)</td>
<td>+8±2</td>
<td>+7±1</td>
</tr>
<tr>
<td>ΔRSNA (% basal)</td>
<td>+35±3</td>
<td>+37±3</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>+25±4</td>
<td>+23±4</td>
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DAF, digoxin-specific antibody Fab; ΔMAP, change in mean arterial pressure; ΔRSNA, change in renal sympathetic nerve activity; ΔHR, change in heart rate; bpm, beats per minute. Values are mean±SEM.

### Table 2. Effects of Intracerebroventricular Digoxin-Specific Antibody Fab Fragments on Intravenous Sodium Nitroprusside–Induced Baroreflex Responses

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After DAF fragments</th>
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<tbody>
<tr>
<td>RSNA</td>
<td></td>
<td></td>
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<tr>
<td>Gain, ΔRSNA–MAP (% resting/mm Hg)</td>
<td>-2.07±0.09</td>
<td>-2.10±0.13</td>
</tr>
<tr>
<td>Peak increase (% resting)</td>
<td>+78±4</td>
<td>+81±5</td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain, ΔHR–MAP (bpm/mm Hg)</td>
<td>-2.11±0.10</td>
<td>-2.07±0.14</td>
</tr>
<tr>
<td>Peak increase (bpm)</td>
<td>+90±4</td>
<td>+91±6</td>
</tr>
</tbody>
</table>

DAF, digoxin-specific antibody Fab; RSNA, renal sympathetic nerve activity; ΔRSNA–MAP, changes in RSNA–mean arterial pressure (MAP); HR, heart rate; ΔHR–MAP, changes in HR–MAP; bpm, beats per minute. Values are mean±SEM (n=8).

**Discussion**

The present study provides a major new finding: intracerebroventricular administration of ouabain, brain OLA, and hypertonic saline causes similar sympathoexcitatory and pressor responses, which all can be blocked by DAF fragments.

Similar to our previous studies in SHR and WKY rats, the present study in conscious Wistar rats, intracerebroventricular injection of ouabain increased BP, HR, and RSNA in a dose-dependent manner. We also show that similar sympathoexcitatory and pressor responses are elicited by intracerebroventricular rat hypothalamic/pituitary extracts containing ouabain-like activity. Intracerebroventricular OLA also induced behavioral changes similar to those induced by ouabain. The magnitudes of increases in BP and RSNA by intracerebroventricular OLA equivalent to 1 μg ouabain were approximately 30% smaller than the increase induced by 1 μg ouabain per se. Therefore, the Na+, K+-ATPase-inhibiting effect of ouabain versus OLA may not be identical when applied in vitro versus in vivo.

Since Na+, K+-ATPase from rat kidney was used for quantitation of OLA and brain ATPase is different from kidney ATPase in terms of their catalytic subunit, it is also possible that OLA binds to brain and kidney ATPase with a different affinity. Takahashi et al. reported in anesthetized rats that 1–10 μg ouabain was required intracerebroventricularly to elicit significant pressor and tachycardia responses but only 10 ng/10 μl rat brain OLA was needed intracerebroventricularly to induce similar responses. In addition to the fact that conscious versus anesthetized animals were used, the discrepancy may be due to the different methods of OLA quantitation, i.e., assay for Na+, K+-ATPase activity (present study) versus quantitation of OLA by an ouabain radioimmunoassay. The amounts of ouabain or OLA administered may appear large (0.3–1.0 μg), but it is rather likely that at the actual site of action ouabain and OLA exert their effects in concentrations several orders of magnitude lower than the ones injected intracerebroventricularly. To place these amounts in a more physiological perspective, the concentration of OLA (as assessed by assay for Na+, K+-ATPase activity) amounts to 3–4 μg/g in the hypothalamus and 40–60 μg/g in the pituitary (F.H.H. Leenen, unpublished data).

After pretreatment with the AVP antagonist, intracerebroventricular infusion of hypertonic saline increased BP, HR, and RSNA similar to the responses induced by ouabain as well as brain OLA. Several studies have demonstrated that intracerebroventricular administration of hypertonic saline induces sympathoexcitatory and pressor effects. In anesthetized rats with an intact arterial baroreflex function, intracerebroventricular infusion of 0.3 M NaCl caused elevation of BP and delayed increases in HR and sympathetic nerve activity. However, after intravenous administration of 30 μg/kg AVP antagonist, which blocks the pressor effects of vasopressin, the delay in sympathetic nerve activity and HR responses was absent, and intracerebroventricular infusion of the hypertonic saline caused concomitant increases in sympathetic nerve activity, BP, and HR. Therefore, the initial part of the pressor response appears to be a result of stimulation of...
AVP release via activation of brain osmotic or sodium receptors, and the maintenance of the response appears to be independent of AVP and associated with sympathoexcitatory effects of hypertonic saline itself. Results from the current study in conscious rats are consistent with these findings.

In rats pretreated with AVP antagonist, intracerebroventricular infusion of 0.3 M NaCl at 3.8 μl/min for 10 minutes, intracerebroventricular injection of 1 µg ouabain, or the OLA equivalent to 1 µg ouabain caused a similar pattern of increases in BP, HR, and RSNA, which is suggestive of a common mechanism. Indeed, the most striking finding of the present study is that, in rats pretreated intracerebroventricularly with DAF fragments, the sympathoexcitatory and pressor responses to 0.3 M i.c.v. NaCl, intracerebroventricular ouabain, and OLA were all abolished or significantly diminished. In the absence of the AVP antagonist, similar inhibitions of the pressor responses by DAF fragments were found (data not shown). Intracerebroventricular injection of DAF fragments per se had no effects on basal BP and RSNA and, in the presence of AVP blockade, did not affect sympathoexcitatory and/or pressor responses to intracerebroventricular angiotensin II, carbachol, intravenous sodium nitroprusside, or air stress, indicating that the blockade by the DAF fragments does not represent a generalized reduced responsiveness. Pretreatment with γ-globulins did not affect responses to any of the stimuli used. Therefore, it is likely that centrally administered hypertonic saline exerts its sympathoexcitatory and pressor effects, at least in part, via stimulating the release of endogenous brain OLA; i.e., brain OLA appears to be a mediator of the effects of intracerebroventricular hypertonic saline.

Purified DAF fragments were first used for the treatment of digoxin toxicity two decades ago and have since been widely used clinically. The molecular mass of DAF fragments is approximately 60 times larger than that of digoxin, and DAF fragments at a dose of approximately 60 µg may bind and neutralize 1 µg digoxin. In the current study, 60 µg DAF fragments significantly blocked the effects of 1 µg ouabain and OLA, indicating that ouabain, as an analogue of digitalis glycosides, and OLA may both be recognized by and bound to DAF fragments. This is consistent with a previous report that DAF fragments were clinically effective in the treatment of digitalis intoxication caused by overdoses of digoxin, digitoxin, and other cardiac glycosides. Because of the relatively small size of the DAF fragments, they can diffuse rapidly into the interstitial space and bind free glycosides, and because the affinity of the glycosides for DAF fragments is higher than for Na+,K+-ATPase, the glycoside molecules will be moved from the cell membrane into the extracellular space with minimal reassociation with the inhibiting sites on the β-subunit of Na+,K+-ATPase.

No study has so far evaluated the possible role of central OLA in the sympathoexcitatory and pressor effects of centrally administered sodium, because, at least in part, no specific antagonist was available. Canrenone is a primary metabolite of the antimineralocorticoid spironolactone. Studies in human red blood cells showed that canrenone is able to restimulate the blocked Na+-K+ pump when the cells are treated with high concentrations of ouabain. However, canrenone is also a partial agonist of cardiac glycosides and enhances the inhibition of the Na+-K+ pump by low doses of ouabain in these cells at physiological cell Na+ levels. Nonetheless, it lowered the BP in rats made hypertensive by reduction of renal mass, presumably by antagonizing the effects of increased endogenous OLA.

There has been no information, so far, regarding the release of brain OLA by acute or chronic elevation of CSF sodium. Beasley et al reported that intracerebroventricular infusion of artificial CSF containing 0.3 M NaCl caused natriuresis that was not related to changes in AVP, RSNA, MAP, aldosterone, or angiotensin II but presumably was due to a hypothetical hormone-inhibiting Na+,K+-ATPase. The anterovernal third ventricle area in the brain has been proposed as the area controlling plasma OLA. These studies suggested central control and/or a central origin of peripheral OLA, without addressing a possible central role for OLA. Similarly, a recent review solely focused on a peripheral role for OLA. Our studies suggest that brain OLA may play a major role in the central nervous system responses to sodium. In salt-sensitive humans with essential hypertension and Dahl salt-sensitive rats as well as WKY rats and to a lesser extent in SHR, increasing sodium intake results in elevation of CSF sodium concentration. High dietary sodium increases not only plasma and pituitary OLA in rats but also hypothalamic OLA, and the increase is greater in SHR than in WKY rats (F.H.H. Leenen, unpublished data). In SHR, high sodium intake decreases the availability of brain ouabain receptors for exogenous ouabain, presumably because of increased brain OLA concentration and increased occupancy of brain OLA (ouabain) receptors. Considering the similar sympathoexcitatory and BP elevation after the administration of 0.3 M NaCl i.c.v., ouabain, and OLA and their blockade by intracerebroventricular DAF fragments, we propose that transient or chronic increases in CSF sodium concentration may stimulate OLA release from specific regions in the CNS and initiate increases in sympathetic outflow and thereby BP. Because of the different responses of the brain OLA–Na+,K+-ATPase system to high sodium intake in SHR versus WKY rats, it is possible that genetic differences in this system determine to a large extent salt sensitivity of BP. DAF fragments appear to be a valuable tool for the elucidation of the actual (patho)physiological role of brain OLA.

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