Brain Renin-Angiotensin System and Ouabain-Induced Sympathetic Hyperactivity and Hypertension in Wistar Rats

Bing S. Huang, Frans H.H. Leenen

Abstract—In Dahl salt-sensitive rats on a high salt diet or normotensive rats with chronic central infusion of sodium, increased brain “ouabain” results in sympathetic hyperactivity and hypertension, possibly by activating the brain renin-angiotensin system. In the present study, we tested whether the hypertension caused by exogenous ouabain also depends on activation of brain renin-angiotensin system. In Wistar rats, ouabain (50 μg/d) was infused subcutaneously for 14 days with the use of osmotic minipumps. Concomitantly, in one group, the angiotensin II type 1 receptor blocker losartan (1 mg/kg per day) was infused intracerebroventricularly. On day 15, mean arterial pressure, heart rate, central venous pressure, and renal sympathetic nerve activity were recorded in conscious rats at rest and in response to air-jet stress, intracerebroventricular injection of the α₁-agonist guanabenz (25 and 75 μg) or angiotensin II (30 ng), acute volume expansion, and ramp changes of blood pressure by ±50 mm Hg with phenylephrine and nitroprusside. Compared with control rats, in rats treated with ouabain, resting mean arterial pressure was significantly increased (111±4 versus 93±3 mm Hg; P<0.05), and increases or decreases in mean arterial pressure, heart rate, and renal sympathetic nerve activity in response to air stress or guanabenz were enhanced significantly. These effects of ouabain were prevented when losartan was given concomitantly. Maximal slopes of arterial baroreflex control of renal sympathetic nerve activity and heart rate tended to be decreased in ouabain-treated versus control rats and were significantly increased in ouabain-treated rats with versus without losartan. No differences in cardiopulmonary baroreflex function were detected. It seems that by day 14 to 15, the central effect of ouabain on baroreflex control prevails over its peripheral sensitizing effect on baroreceptors, leading to a tendency of desensitization. These results indicate that chronic administration of ouabain activates the brain renin-angiotensin system, resulting in decreased sympathoinhibition and increased sympathoexcitation, impairment of baroreflex function, and hypertension. (Hypertension. 1999;34:107-112.)

Key Words: baroreflex ■ renal nerves ■ stress, air-jet ■ guanabenz ■ renin-angiotensin system

Increased sympathetic activity mediates salt-sensitive hypertension in spontaneously hypertensive rats (SHR) and Dahl salt-sensitive rats.1,2 The sympathoexcitatory and pressor effects of high salt are prevented by either chronic intracerebroventricular administration of an angiotensin II type 1 (AT₁) receptor antagonist3,4 or antibody Fab fragments,3,4 binding ouabain and endogenous ouabain-like activity (“ouabain”).5,6 Therefore, in Dahl salt-sensitive rats and SHR on high salt, both brain angiotensin II (Ang II) and brain “ouabain” contribute to sympathetic hyperactivity and hypertension.3,4 We postulated that brain “ouabain” exerts its effects possibly by activation of the brain renin angiotensin system (RAS),8,9 since blockade of brain AT₁ receptors blocks the responses to acute intracerebroventricular ouabain, but not vice versa.8

Chronic central or peripheral administration of ouabain induces hypertension in normotensive rats.9,10 The hypertension may result from increased sympathetic tone,10 as estimated indirectly by ganglionic blockade, or from inhibition of Na⁺, K⁺-ATPase activity in blood vessels,9 leading to an increase in total peripheral resistance. Chronically administered ouabain increases the content of ouabain in several brain areas10 that are closely related to central cardiovascular regulation. This increase of ouabain in the brain appears to mediate the increase in blood pressure (BP), since the hypertensive effect of ouabain can be prevented by concomitant intracerebroventricular infusion of Fab fragments.10 Acute intracerebroventricularly administered ouabain elicits sympathoexcitatory and pressor effects likely through an activation of brain Ang II pathways.8 However, it is unclear at present whether chronic peripheral administration of ouabain also activates the brain RAS and thereby causes sympathetic hyperactivity and hypertension.

The goal of the present study is to clarify whether the pattern of central changes induced by chronic infusion of ouabain in Wistar rats resembles the pattern of changes induced by high salt intake in salt-sensitive rats. We investigated therefore (1) whether chronic exogenous ouabain treat-
ment causes sympathetic hyperactivity and impairment of baroreflex function and (2) whether activation of brain RAS mediates such central effects of exogenous ouabain. Sympathetic activity was evaluated by recording changes in renal sympathetic nerve activity (RSNA) in response to air stress and acute intracerebroventricular infusion of an α1-adrenoceptor agonist and by estimating arterial and cardiopulmonary baroreflex function. Chronic intracerebroventricular infusion of the AT1 receptor blocker losartan was used to block the brain RAS.

Methods
Male Wistar rats (150 to 200 g; Charles River, Montreal, Canada) were housed in animal rooms with a 12-hour light/dark cycle and an ambient room temperature of 23±2°C. All rats received a diet of standard laboratory chow and tap water ad libitum. The entire study was conducted in accordance with the guidelines of the University of Ottawa Animal Care Committee.

After 5 to 7 days of acclimatization, under anesthesia with halothane inhalation, a guide cannula (23 gauge, stainless steel tubing) was implanted just above the left lateral cerebral ventricle and fixed on the skull of the rat. The cannula was 0.5 mm posterior and 1.4 mm lateral to the bregma, and its lower end was ~0.3 mm above the ventricle. In 2 groups of rats (n=8 each), an osmotic minipump (Alzet, model 2002) filled with ouabain dissolved in saline was implanted subcutaneously on the back. The infusion rate of ouabain was 50 µg in 12 µL per day. In 1 group of rats treated with ouabain subcutaneously, an L-shaped cannula (23 gauge, stainless steel tubing) was implanted into the right lateral cerebral ventricle (3.5 mm deep from dura) and fixed on the skull. By means of polyethylene tubing (PE-50 fused to PE-60), the intracerebroventricular cannula was connected to another osmotic pump (Alzet 2002) subcutaneously filled with losartan dissolved in artificial cerebrospinal fluid (aCSF), with an infusion rate of 1 mg/kg in 12 µL per day. The surgery was scheduled so that the final experiment for each rat was performed after 14 days of losartan and/or ouabain infusion. Previous studies9,10 demonstrated that in rats, chronic administration of ouabain induces hypertension with a delay of several days. In rats, resting BP was significantly increased by ouabain administered subcutaneously for 14 to 21 days9 but not for 1 to 7 days.10,11 One group of rats (n=8) was assigned as control without either ouabain or losartan treatment. The rationale for this dose of losartan was previously outlined, and this intracerebroventricular dose has no measurable peripheral effects.3 On 3 to 4 different occasions 1 week before the final experiment, the rats were trained to stay in a smaller experimental cage, in which the rat can move back and forth, for 1 to 2 hours.

In the early morning of the final part of the experiment, under halothane inhalation, the right femoral vein and artery were cannulated with PE-10 fused to PE-50 tubing filled with heparinized saline. PE-50 tubing was inserted into right jugular vein with its tip advanced down to the level of right atrium. With methohexital sodium (Brevital, 30 mg/kg IV, supplemented with 10 mg/kg as needed; Eli Lilly Canada Inc), through a flank incision1 a pair of silver electrodes (A-M System, Inc) was placed around and fixed to the left renal nerve with silicone rubber (SilGil 604, Wacker). At least 4 hours after recovery from the anesthesia, the rat was placed in the experimental cage. The intra-arterial catheter and the catheter in the jugular vein were connected to pressure transducers, and BP, HR, and central venous pressure (CVP) were recorded through a polygraph (model 7E, Grass Instrument Co) and a Grass 7P44 tachograph. The electrodes were linked to a Grass P511 bandpass amplifier. The amplified (gain, 10 000 to 50 000) and filtered (bandwidth, 30 to 1000 Hz) RSNA signals were channeled to a rectifying voltage integrator (model 7P10, Grass Instrument Co). The integrated voltage signals (expressed in millivolts), together with BP, HR, and CVP signals, were then fed into an online computer equipped with a Grass data acquisition and analysis program (Polyview 2.0) for display, storage, and later analysis of both analog and digital data. The changes in RSNA were expressed as percentage of resting RSNA. The actual RSNA was determined by subtracting noise from the total activity. The noise was recorded ~20 minutes after the rats had been killed at the end of the experiment.3

After a 30-minute stabilization period, resting MAP, HR, CVP, and RSNA were recorded for 10 minutes. An environmental stress was provided twice at a 10-minute interval by blowing the face of the rat with a jet of air (1 to 1.5 psi) for 30 seconds from a tube located 2 to 3 cm in front of the rat. The average of peak responses in MAP, HR, and RSNA to the 2 applications of stress was used for analysis.

After a 20-minute rest, phenylephrine (5 to 50 µg/min) dissolved in normal saline was infused intravenously through an infusion pump (model 355, Sage Instruments) to achieve a ramp increase in MAP with a maximum of 50 mm Hg over 1 to 2 minutes. Twenty minutes after the responses had subsided, nitroprusside sodium (5 to 100 µg/min IV) was infused, inducing a ramp decrease in MAP with a maximum of 50 mm Hg over 1 to 2 minutes. Infusion rates were <0.08 ml/min.

Subsequently, the rat rested for 20 minutes after a cannula for intracerebroventricular injection filled with guanabenz had been inserted into the intracerebroventricular guide cannula. With a 10-minute interval, 2 doses (25 and 75 µg/2.5 to 7.5 µL aCSF) of guanabenz were injected intracerebroventricularly over 10 to 30 seconds. Thirty minutes after the responses to guanabenz had disappeared, Ang II (30 ng/2 µL aCSF) was injected intracerebroventricularly.

After the responses to Ang II had subsided, the rats rested for 20 minutes, and acute volume expansion was performed with intravenous infusion of 5% dextrose at 2 rates (3 and 10 ml/kg in 30 seconds) with a 10-minute interval. The rat was then killed by an overdose of intravenously administered pentobarbital. The accuracy of intracerebroventricular cannulation was checked through autopsy with an intracerebroventricular injection of methylene blue.

In all 3 groups of rats, there were no significant differences for the resting BP, HR, or RSNA immediately before the application of each stimulus versus those at the very beginning of the experiment. In control, ouabain, and ouabain plus losartan groups, the differences between resting MAP, HR, and RSNA before the last stimulus (volume expansion) and those at the beginning of the experiment were ~5±3, 2±4, and 4±3 mm Hg; 15±10, 21±2, and 12±9 bpm; and 7±4%, 4±5%, and ~3–4% of original resting level (P=NS for all, respectively).

To assess arterial baroreflex function, responses of RSNA were expressed as percentage of resting RSNA, and changes in both RSNA (∆RSNA) and HR (∆HR) in response to increases and decreases in MAP were analyzed together as a logistic model with the use of the logistic equation ∆RSNA=−P1+P2/[1+e^(P3−MAP−P4)]12 Cardiopulmonary baroreflex function was evaluated by the gain of the reflex, i.e., the slope of the relationships between ∆RSNA or ∆HR and corresponding CVP analyzed by linear regression, combining the 2 rates of volume expansion. Differences among groups were evaluated by ANOVA. When F ratios were significant, a Duncan multirange test was followed. Statistical significance was defined as P<0.05.

Results
After treatment for 2 weeks, compared with control rats, the resting MAP was significantly increased in rats treated with ouabain but was not changed in rats treated with ouabain plus intracerebroventricular losartan (Table 1). There were no significant differences in resting HR and CVP among the 3 groups of rats. The body weight gain over the 2 weeks of treatment was similar in all groups.

Responses to Air Stress
Air stress caused rapid increases in RSNA, MAP, and HR (Figure 1). In rats treated with ouabain, peak increases in RSNA, MAP, and HR were approximately twice those in
control rats. These enhanced responses did not develop when losartan was administered intracerebroventricularly.

Responses to Intracerebroventricular Guanabenz
After intracerebroventricular administration of guanabenz at either dose, MAP, RSNA, and HR decreased and reached a plateau within 4 to 6 minutes (Figure 2). The peak responses were dose related. In rats treated with ouabain, maximum decreases in RSNA, MAP, and HR were twice those in control rats. These differences in the maximum responses were not seen when losartan was administered intracerebroventricularly in rats treated with ouabain.

When the responses of MAP as well as HR to air stress and intracerebroventricular guanabenz were expressed as percentage of their resting values, respectively, the responses changed in patterns similar to those observed for absolute values (data not shown).

Responses to Intracerebroventricular Ang II
Intracerebroventricular injection of 30 ng Ang II increased MAP and decreased RSNA and HR in the 2 groups without losartan, and there was no significant difference in the extent of responses between these 2 groups (Table 1). Intracerebroventricular Ang II did not elicit significant responses in rats treated with losartan.

Arterial and Cardiopulmonary Baroreflex
Figure 3 shows the RSNA and HR responses to arterial baroreceptor (de)activation elicited by decreasing/increasing BP by intravenous nitroprusside and phenylephrine. Compared with control rats, ouabain-treated rats showed a tendency for desensitization in baroreflex control of RSNA and HR, as reflected by the maximal slopes of the baroreflex curves (Table 2). The maximum slope of baroreflex control of either RSNA or HR was significantly increased in ouabain-treated rats with versus without intracerebroventricular losartan (Table 2).

Volume expansion caused increases in CVP and decreases in RSNA and HR. The maximum increase in MAP was 3 mm Hg in all groups of rats. As shown in Table 2, no significant differences in cardiopulmonary baroreflex control of RSNA or HR were detected among the 3 groups of rats.

Discussion
The present study demonstrates the significant new finding that in Wistar rats the sympathoexcitatory as well as pressor effects of chronic treatment with ouabain are mediated by the brain RAS.

Ouabain-Induced Hypertension
The role of endogenous ouabainlike activity (“ouabain”) in the development of hypertension has been supported by the finding that chronic treatment with exogenous ouabain induces hypertension in normotensive rats. With a delay of several days, intravenous, subcutaneous, or intracerebroventricu-
tricular administration of ouabain all induce hypertension in normotensive rats. It has been postulated that chronic ouabain treatment increases plasma ouabain concentration, inhibits Na$^+$, K$^+$-ATPase activity in blood vessels, increases total peripheral resistance, and therefore induces hypertension. However, regardless of the route of administration, chronic ouabain increases the brain content of ouabain in rats. Blockade of ouabain in the brain by intracerebroventricular Fab fragments prevents ouabain-induced hypertension, indicating a paramount role of ouabain in the central nervous system. Ganglionic blockade normalizes ouabain-induced hypertension, indicating that sympathetic activity is essential. The present study demonstrates the sympathoexcitatory effect of ouabain, showing that both sympathoexcitatory responses to air-jet stress and sympathoinhibitory responses to intracerebroventricular guanabenz are enhanced in ouabain-treated rats. The enhanced responses to guanabenz in ouabain-treated rats are consistent with a decreased activity in sympathoinhibitory pathways, which results in enhanced responses to an exogenous $\alpha_2$-adrenoceptor agonist. These findings support a central sympathoexcitatory and pressor role of chronically administered ouabain.

In intact Wistar rats, sympathetic hyperactivity and hypertension develop ≈ 1 week after the commencement of ouabain treatment. In Wistar rats with chronic sinoaortic denervation, the delay in the onset of ouabain-induced hypertension is markedly shortened, suggesting that several days are needed before the central sympathoexcitatory effects of ouabain prevail over its putative sensitizing effects on peripheral baroreceptors. In Wistar rats, chronic treatment with ouabain for 2 weeks tends to desensitize arterial baroreflex control of RSNA and HR, and the baroreflex sensitivity is significantly improved in ouabain-treated rats with versus without intracerebroventricular losartan. Changes in baroreflex function appear to be determined by the balance of central and peripheral effects of ouabain. Peripherally, ouabain sensitizes baroreceptors by inhibiting membrane Na$^+$, K$^+$-ATPase activity and therefore tends to decrease sympathetic outflow. These results suggest that, in rats, during chronic treatment the central desensitizing effect of ouabain becomes stronger than its peripheral sensitizing effect.

**Ouabain Activates Brain RAS**

In rats the pressor/sympathoexcitatory response to acute intracerebroventricular ouabain can be blocked by intracerebroventricular pretreatment with the Ang II receptor blocker saralasin or the angiotensin-converting enzyme inhibitor captopril. Intracerebroventricular losartan markedly attenuates sympathoexcitatory and pressor responses to acute intracerebroventricular injection of Ang II, ouabain, or brain extracts containing “ouabain.” Pretreatment with intracerebroventricular antibody Fab fragments blocks only the effects of ouabain and “ouabain” but not those of Ang II. These studies suggest that activation of brain AT$_1$ receptors occurs in the pathways mediating the effects of acute intracerebroventricular ouabain. Intracerebroventricular Fab fragments also prevent the development of hypertension in rats with chronic ouabain treatment. The present results demonstrate that intracerebroventricular losartan also prevents the central effects of chronic ouabain, ie, increased activity in sympathoexcitatory pathways, decreased activity in sympathoinhibitory pathways, and impairment of arterial baroreflex function as well as hypertension. Losartan is a specific AT$_1$ blocker without agonist effects. Most of the central physiological effects of Ang II are mediated through AT$_1$ receptor stimulation. Whereas chronic losartan abolishes the acute responses to intracerebroventricular Ang II, the vehicle (aCSF) per se administered chronically or acutely has no effects on the responses to Ang II. Therefore, similar to acute intracerebroventricular administration of ouabain, a chronic increase in brain ouabain as a result of chronic subcutaneous administration of ouabain appears to activate brain pathways involving AT$_1$ receptors, leading to sympathoexcitation and hypertension. Brain areas where brain ouabain and brain RAS may interact have not yet been defined. In rat brain, nerve fibers of ouabain-immunopositive neurons and Ang II receptors or other components of the brain RAS coexist in several hypothalamic areas, such as the anteroventral third ventricle, including the organum vasculosum of the lamina terminalis and the subfornical organ. In Wistar rats, the pressor response to acute intracerebroventricular ouabain is attenuated by losartan in the median preoptic nucleus. Losartan or Fab fragments in the median preoptic nucleus significantly decrease BP in SHR with high but not with regular salt intake, suggesting that the median preoptic nucleus is one of the areas in which brain “ouabain” interacts with the brain RAS. On the other hand, it is worthwhile to note that acute or chronic intracerebroventricularly administered Ang II in rats or dogs increases plasma

**TABLE 2. Parameters of Arterial and Cardiopulmonary Baroreflex Function**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Ouabain</th>
<th>Ouabain + Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial baroreflex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSNA-MAP, %/mm Hg</td>
<td>-3.76±0.21</td>
<td>-3.44±0.12</td>
<td>-3.85±0.14*</td>
</tr>
<tr>
<td>HR-MAP, bpm/mm Hg</td>
<td>-3.17±0.19</td>
<td>-3.01±0.13</td>
<td>-3.33±0.12*</td>
</tr>
<tr>
<td><strong>Cardiopulmonary baroreflex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSNA-CVP, %/mm Hg</td>
<td>-14.5±1.3</td>
<td>-13.9±1.4</td>
<td>-14.7±0.9</td>
</tr>
<tr>
<td>HR-CVP, bpm/mm Hg</td>
<td>-11.2±0.9</td>
<td>-10.8±1.2</td>
<td>-11.4±1.1</td>
</tr>
</tbody>
</table>

Data are mean±SE (n=8 per group). *P<0.05 vs ouabain.
“ouabain,” suggesting that AT₁-receptor stimulation also may release “ouabain” from the brain (likely pituitary) into the circulation.26

The pathways mediating the effect of chronic ouabain and the brain RAS on baroreflex function also have not been clarified. The preoptic area,27 which is adjacent to the median preoptic nucleus and is a principal location in the hypothalamic area related to arterial baroreflex control, as well as brain stem areas such as the nucleus tractus solitarii28 may participate in the relay. In rats, endogenous Ang II and angiotensin III may exert a tonic inhibitory effect on the baroreflex function by acting on Ang II type 2 receptors in the rostral nucleus reticularis ventrolateralis.29 This, however, seems unlikely to play a role in ouabain-induced hypertension, since the effects of ouabain on baroreflex function are prevented by blockade of AT₁ receptors.

Although the present study highlights the role of the brain RAS in ouabain-induced hypertension, these findings do not exclude that other neurotransmitters, such as dopamine and γ-aminobutyric acid (GABA), may play a role in ouabain-induced hypertension as well. For example, in rats, local perfusion of ouabain into the nucleus accumbens increases extracellular dopamine and GABA levels.30

Activation of Brain RAS Induced by Endogenous “Ouabain” Versus Exogenous Ouabain

A chronic increase in central (CSF) sodium in normotensive rats3 or high dietary salt in salt-sensitive rats31,32 increases brain “ouabain,” causing sympathoexcitation and hypertension. Intracerebroventricular losartan also prevents sympathoexcitation and hypertension.5,4,7 In salt-sensitive rats with high salt intake4, and normotensive rats with chronic central sodium loading,7 arterial as well as cardiopulmonary baroreflex function become impaired, and blockade of the brain RAS by losartan also prevents this impairment. In the present study, chronic blockade of the brain RAS also blocks sympathetic hyperactivity, improves arterial baroreflex function, and prevents the development of hypertension in Wistar rats chronically treated with ouabain. These findings indicate that exogenous ouabain activates central nervous system pathways involving the brain RAS. Moreover, the patterns of central effects of endogenous “ouabain” versus exogenous ouabain are similar, except for the cardiopulmonary baroreflex function. The latter becomes impaired in endogenous “ouabain”-induced hypertension4,7 but not in exogenous ouabain-induced hypertension (the present study). An explanation for this difference is not readily apparent. Nevertheless, from the otherwise similar patterns of changes, we conclude that a chronic increase in both endogenous ouabain-like compounds and exogenous ouabain in the brain activates the brain RAS, and the latter causes sympathetic hyperactivity and hypertension.

In summary, the hypertension induced by chronic treatment with ouabain administered subcutaneously is associated with increased sympathoexcitation, decreased sympathoinhibition, and a tendency toward impairment of arterial baroreflex function. These central effects of ouabain are prevented by blockade of the brain RAS with losartan, indicating that the central effects of chronic ouabain depend on the brain RAS. The present findings support the concept proposed by us4,7 that, in rats, chronic increases in endogenous ouabain-like compounds induce sympathetic hyperactivity and hypertension through activation of the brain RAS.

Acknowledgments

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References


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