Both Brain Angiotensin II and “Ouabain” Contribute to Sympathoexcitation and Hypertension in Dahl S Rats on High Salt Intake

Bing S. Huang, Frans H.H. Leenen

Abstract—Dahl salt-sensitive (Dahl S) and salt-resistant (Dahl R) rats from 5 to 9 weeks of age received a regular or high salt diet and concomitantly an intracerebroventricular infusion of the angiotensin type 1 blocker losartan (1 mg · kg \(^{-1}\) · d \(^{-1}\)) or antibody Fab fragments, which bind ouabain and related steroids with high affinity, or \(\gamma\)-globulins as control (200 µg/d for both). At 9 weeks of age, blood pressure (BP), heart rate (HR), central venous pressure, and renal sympathetic nerve activity were recorded in conscious rats at rest and in response to air stress and to intracerebroventricular \(\alpha_2\)-agonist guanabenz (50 µg) and ouabain (0.5 µg). Baroreflex function was assessed by acute volume expansion with intravenous 5% dextrose and ramp changes of BP by ±50 mm Hg induced by intravenous phenylephrine and sodium nitroprusside. In Dahl S but not R rats, high salt significantly increased BP and HR; enhanced BP, HR, and renal sympathetic nerve activity responses to air stress and guanabenz; and attenuated cardiopulmonary and arterial baroreflex control of renal sympathetic nerve activity and HR. Both losartan and Fab fragments prevented these responses to high salt to a similar extent in Dahl S rats but had no effect in Dahl R rats on high salt. Sympathoexcitatory responses to ouabain were attenuated in Dahl S on high versus regular salt and were abolished in Dahl R or S treated with losartan or Fab fragments. Consistent with previous studies in SHR, the present data indicate that in Dahl S on high salt, both brain “ouabain” and angiotensin II contribute to decreased sympathoinhibition and increased sympathoexcitation, impairment of baroreflex, and therefore hypertension. (Hypertension. 1998;32:1028-1033.)

Key Words: sympathetic nervous system ■ baroreflex ■ losartan ■ stress ■ guanabenz ■ antibody Fab fragments

Neural mechanisms leading to sympathetic hyperactivity play a major role in the pathogenesis of salt-dependent hypertension in Dahl salt-sensitive (Dahl S) rats. In Dahl S rats, high salt intake enhances sympathoexcitatory responses and attenuates sympathoinhibitory responses.2 Arterial3,4 and cardiopulmonary5 baroreflex control of efferent sympathetic nerve activity and/or heart rate (HR) are impaired in Dahl S rats, even before the commencement of high salt intake.3,5 High salt intake also causes sympathetic hyperactivity6 and impairment of baroreflex function7 in spontaneously hypertensive rats (SHR), another model of salt-sensitive hypertension. In a series of studies we have shown that high salt intake increases ouabain-like activity (“ouabain”)8,9 in several brain areas and that in both SHR and Dahl S rats, blockade of brain “ouabain” prevents sympathoexcitation, baroreflex impairment, and exaggeration of hypertension.2,4,6,9 In SHR, it appears that high dietary salt increases brain “ouabain” and the latter exerts its sympathoexcitatory and pressor effects via the brain renin-angiotensin system (RAS).5 Whether brain angiotensin II (Ang II) is also involved in the impairment of baroreflex function in SHR by high salt diet has not yet been examined. Little is known about the involvement of the brain RAS in the development of salt-sensitive hypertension in Dahl S rats. In Dahl S on high salt diet, acute intracerebroventricular (ICV) injection of the angiotensin type 1 (AT\(_1\)) receptor blocker losartan did not decrease BP,10 but chronic ICV administration of the AT\(_1\) blocker CV-11974 prevented the development of hypertension.11 Whether in Dahl S rats brain Ang II is involved in the sympathoexcitation and impairment of baroreflex function by high salt intake has not yet been evaluated. In the present study, we examined in Dahl S rats whether both brain Ang II and “ouabain” contribute to the impairment of arterial and cardiopulmonary baroreflex function, as well as the sympathetic hyperactivity and hypertension caused by high dietary salt intake.

Methods

Male 4- to 5-week-old Dahl R and S rats of the newly reestablished colony at Harlan Sprague-Dawley Inc (Indianapolis, Ind) were housed in constant room temperature and humidity with a 12-hour light/dark cycle. The study was carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee. After 3 to 5 days of adaptation, with rats under sodium pentobarbital anesthesia, a 23-gauge guide needle was fixed on the skull over

Received June 17, 1998; first decision July 1, 1998; revision accepted July 15, 1998.

From the University of Ottawa Heart Institute, Ottawa, Ontario, Canada.

Correspondence to Frans H.H. Leenen, MD, PhD, FRCP(C), Hypertension Unit, H360, Division of Cardiology, University of Ottawa Heart Institute, 40 Ruskin St, Ottawa, Ontario K1Y 4W7, Canada. E-mail fleenen@ohi-net.heartinst.on.ca

© 1998 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org

1028
the right lateral cerebroventricle, and a 23-gauge stainless steel right-angled cannula was implanted into the left lateral ventricle, as previously described.2 The latter was connected to an osmotic minipump (model 2002; rate, 12 μL/d; Alzet Corp) for chronic ICV infusion of the AT1 blocker losartan (1 mg · kg-1 · d-1), Merck Research Laboratories), or antibody Fab fragments (Digibind, Glaxo Wellcome Inc) or γ-globulins (Sigma Chemical Co) as control (200 μg/d for both). After surgery, rats were provided with tap water and either regular rat chow (101 μmol Na/g, RNA) or high salt rat chow (1370 μmol Na/g, HNa), both from Harlan Sprague-Dawley Inc, for 4 weeks. Dahl R and S rats were each divided into 4 groups: Dahl R/RNa/γ-globulin (n = 7); Dahl R/RHa/γ-globulin (n = 14); Dahl R/RHa/Fab (n = 8); Dahl S/RNa/γ-globulin (n = 8); and Dahl S/RNa/γ-globulin (n = 8); Dahl S/RNa/Fab (n = 8); and Dahl S/RHa/losartan (n = 8). At 7 weeks of age, with rats under halothane anesthesia, the pumps were replaced with new ones filled with original compounds for ICV infusion for another 2 weeks.

On the day of the experiment, with rats under halothane anesthesia, catheters were placed into a femoral artery and vein and into the right jugular vein advanced down to the level of the right atrium. With methohexital sodium (Brevital, 30 mg/kg IV, supplemented with 10 mg/kg as needed; Eli Lilly Canada Inc), through a flank incision, 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 ~4 hours after recovery from anesthesia, rats were placed in a testing cage that permitted movement back and forth. The intraarterial catheter and the catheter in the jugular vein were connected to a transducer, 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, and renal sympathetic nerve activity (RSNA, spikes per second) was counted by a nerve traffic analyzer (model 706C, University of Iowa Bioengineering) and digitalized. The RSNA was determined by subtracting noise from the total activity.

The noise was determined after the rats had been killed at the end of the experiment.2 After a 30-minute stabilization period, basal mean arterial pressure (MAP), HR, CVP, and RSNA were recorded. A standardized air stress was then provided for 30 seconds twice at 10-minute intervals, using an air stream (1 to 1.5 psi) directed into the face of the rat.2 Ten minutes after the responses to air stress had subsided, phenylephrine was infused at increasing rates (5 to 50 μg · kg-1 · min-1 · IV) to achieve a ramp increase in MAP with a maximum of 50 mm Hg over 1 to 2 minutes. Ten minutes after return to baseline, nitroprusside was infused (5 to 100 μg · kg-1 · min-1 · IV) to induce a ramp MAP decrease with a maximum of ~50 mm Hg over 1 to 2 minutes. Infusion rate was <0.08 ml/minute for both.

After rats had rested for 20 minutes, guanabenz (Sigma) in artificial cerebrospinal fluid (aCSF; 50 μg/5 μL per minute) was injected ICV using a 26-gauge needle and a Hamilton microsyringe (20 μL volume).2 Twenty minutes after the responses to guanabenz had disappeared, 2 doses of 5% dextrose solution (3.3 and 10.0 ml/kg body wt, IV over 30 seconds) were infused at an interval of 5 minutes. Thirty minutes after the disappearance of the responses to the volume expansion, ouabain (Sigma, 0.5 μg/2 μL aCSF) was injected ICV.

Responses of RSNA were expressed as percentage of baseline. To evaluate the arterial baroreflex function, changes in RSNA (∆RSNA) or HR (∆HR) at 5-mm Hg incremental increases and decreases in MAP were analyzed together as a logistic model, using the logistic equation \[ \Delta RSNA = P1 + P2/(1 + e^{-(\Delta MAP - P3)}) \]. Cardiopulmonary baroreflex function was evaluated by the gain of the reflex, ie, the slope of the relation between ∆RSNA or ∆HR and corresponding CVP analyzed by linear regression, combining the 2 rates of volume expansion.2 Two-way ANOVA was performed for all data. When F ratios were significant, a Duncan multi-range test was performed. Statistical significance was defined as P<0.05.

### Results

At 9 weeks of age, Dahl S on RNA showed a modest but significant increase in MAP compared with Dahl R on RNA. In Dahl R, HNa did not increase resting MAP and HR, and treatment with losartan or Fab fragments had no effect on these parameters. In Dahl S on HNa, resting MAP and HR were significantly increased compared with in Dahl R on either diet or Dahl S on RNA (Table 1). In contrast, in Dahl S on HNa treated with either losartan or Fab fragments, resting MAP or HR did not increase and remained similar to that in Dahl S on RNA. There were no significant differences in CVP and body weight gain among the 8 groups of rats (Table 1).

### Responses to Air Stress and ICV Guanabenz

Air stress caused rapid increases in RSNA, MAP, and HR (Figure 1). In Dahl R rats, HNa did not affect these responses; neither did concomitant treatment with losartan or Fab fragments. In contrast, in Dahl S on HNa the magnitudes of increases in RSNA, MAP, and HR were 2- to 2.5-fold of those in Dahl R on either diet or Dahl S on RNA (Table 1). In contrast, in Dahl S on HNa treated with either losartan or Fab fragments, resting MAP or HR did not increase and remained similar to that in Dahl S on RNA. These enhanced responses did not develop when either losartan or Fab fragments were administered ICV.

### Peak increases in MAP, HR, and RSNA in response to air stress in rats on regular (RNA) or high (HNa) salt diet. Values are mean±SEM (n=7 to 14). *P<0.05 vs other groups. Fab indicates antibody Fab fragments; Los, losartan; glob, γ-globulins; DR, Dahl salt-resistant rats; and DS, Dahl salt-sensitive rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RNa, γ-Globulin</th>
<th>HNa, γ-Globulin</th>
<th>HNa, Fab</th>
<th>HNa, Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>119±3†</td>
<td>173±3*</td>
<td>127±4†</td>
<td>120±2†</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>411±14</td>
<td>469±11†</td>
<td>432±13</td>
<td>438±7</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>1.4±0.4</td>
<td>1.0±0.5</td>
<td>1.5±0.8</td>
<td>1.2±0.5</td>
</tr>
<tr>
<td>Gain of BW, g</td>
<td>124±11</td>
<td>114±5</td>
<td>112±5</td>
<td>120±7</td>
</tr>
</tbody>
</table>

BW indicates body weight. Data are mean±SEM. See Methods for numbers of animals.

*P<0.05 vs other groups; †P<0.05 vs Dahl R; ‡P<0.05 vs Dahl R and Dahl S on RNA.
Brain Ang II and Ouabain in Dahl S Rats

After ICV guanabenz was administered, MAP, RSNA, and HR decreased and reached a plateau within 4 to 6 minutes (Figure 2). In Dahl R rats, HNa did not affect these responses; neither did concomitant treatment with losartan or Fab fragments. In contrast, in Dahl S on HNa, maximum decreases in RSNA, MAP, and HR were 1.5- to 2-fold of those in Dahl R on either diet or Dahl S on RNa. These differences were not seen when either losartan or Fab fragments were given ICV.

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

Responses to ICV Ouabain
ICV ouabain increased RSNA, MAP, and HR (Figure 3). The responses reached plateau levels within 5 minutes of injection. In Dahl R rats, HNa did not affect these responses to ouabain. In contrast, in Dahl S on HNa, the maximum responses to ouabain were significantly attenuated compared with those in Dahl R on either diet or Dahl S on RNa. When Dahl R or S on HNa were treated with losartan or Fab fragments, responses to ouabain were markedly decreased, and RSNA and HR responses were no longer significantly different from the resting values.

Arterial Baroreflex
Arterial baroreceptor (de)activation elicited by decreasing/increasing BP by intravenous nitroprusside and phenylephrine caused the expected reflex changes in RSNA and HR (Table 2). No significant differences in the reflex parameters were found for Dahl S on RNa versus Dahl R on RNa, except that in Dahl S on RNa the ED_{50} was significantly increased, indicating that the reflex was shifted to a higher pressure level. In Dahl S, HNa significantly decreased the range of RSNA responses, as well as the maximum slope of the reflex. These changes by HNa in Dahl S rats did not develop when losartan or Fab fragments were given ICV. In Dahl S, HNa also shifted the reflex curve to a much higher pressure level, which was also prevented by ICV losartan or Fab fragments.

In Dahl S, the changes in baroreflex control of HR were similar to those of RSNA. In Dahl S on HNa, the slope of reflex was significantly decreased, and the ED_{50} was further increased. ICV losartan or Fab fragments prevented these changes, and these parameters remained similar to those of Dahl S on RNa.

In Dahl R, HNa increased the maximum slope of reflex control of RSNA compared with that of Dahl R on RNa but did not increase the HR-MAP reflex slope. Losartan or Fab fragments ICV did not affect baroreflex control of either RSNA or HR in Dahl R on HNa.

Cardiopulmonary Baroreflex
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. On RNa, the gain of the reflex control of RSNA and HR tended to be decreased in Dahl S versus R rats (P=0.07 and P=0.1, respectively). In Dahl R on HNa versus RNa, the gain of baroreflex control of RSNA tended to be increased (P=0.06), but ICV losartan or Fab did not affect the reflex function. In contrast, in Dahl S, HNa significantly decreased the gain of cardiopulmonary baroreflex control of both RSNA and HR, as reflected by the lower slope of the linear relation of RSNA or HR versus CVP (Figure 4, Table 3). ICV losartan or Fab fragments prevented decreases in the gain of reflex control of RSNA or HR in Dahl S rats on HNa.

Discussion
The present study provides the major new finding that sympathoexcitation, impairment of arterial and cardiopulmonary baroreflex function, and hypertension in Dahl S rats on high salt intake are prevented by concomitant blockade of brain Ang II receptors with losartan in a similar pattern as observed after blockade of brain “ouabain.”

Brain Ang II, Sympathetic Hyperactivity, and Hypertension
In SHR, the brain RAS has been shown to contribute to the sympathetic hyperactivity and BP increase by high salt intake. The involvement of the brain RAS in the development of sympathetic hyperactivity and hypertension in
Dahl S on high salt has not been evident. Chronic ICV infusion of the AT1 receptor blocker CV-11974 prevented the development of hypertension in Dahl-Iwai salt-sensitive rats on high salt.11 The present study shows that similar to SHR on high salt, 6 in Dahl S chronic ICV infusion of losartan not only prevents the hypertension but also prevents an increase in sympathoexcitation and decrease in sympathoinhibition by high salt intake. Thus, the brain RAS appears also to be involved in the salt-induced sympathetic hyperactivity and hypertension in Dahl S rats.

**Brain Ang II and Impairment of Baroreflex Function**

In Dahl rats, high salt intake sensitizes arterial16 and cardio-pulmonary17 baroreflex function in Dahl R rats but desensitizes these baroreflex functions in Dahl S rats.4,18 We demonstrated previously4 that in Dahl S rats the impairment of arterial baroreflex control of RSNA and HR by high salt intake can be prevented by blockade of brain “ouabain.” The present study confirms this previous finding and demonstrates that in Dahl S rats blockade of brain “ouabain” also prevents the impairment of cardiopulmonary baroreflex control of RSNA and HR by high salt intake. Moreover, the present study shows that blockade of the brain RAS by losartan also prevents impairment of both arterial and cardiopulmonary baroreflex control in Dahl S rats on high salt. These observations indicate that both brain “ouabain” and the brain RAS play a major role in the salt-induced changes in baroreflex control of RSA and HR in Dahl S rats.

**Brain Ang II and Impairment of Baroreflex Function**

In Dahl rats, high salt intake sensitizes arterial16 and cardio-pulmonary17 baroreflex function in Dahl R rats but desensitizes these baroreflex functions in Dahl S rats.4,18 We demonstrated previously4 that in Dahl S rats the impairment of arterial baroreflex control of RSNA and HR by high salt intake can be prevented by blockade of brain “ouabain.” The present study confirms this previous finding and demonstrates that in Dahl S rats blockade of brain “ouabain” also prevents the impairment of cardiopulmonary baroreflex control of RSNA and HR by high salt intake. Moreover, the present study shows that blockade of the brain RAS by losartan also prevents impairment of both arterial and cardiopulmonary baroreflex control in Dahl S rats on high salt. These observations indicate that both brain “ouabain” and the brain RAS play a major role in the salt-induced changes in baroreflex control of RSNA and HR in Dahl S rats.

In Wistar13 or Sprague-Dawley19 rats, chronic central sodium infusion attenuates arterial baroreflex control of RSNA and/or HR. This attenuation also can be prevented by ICV treatment with Fab fragments or losartan,13 indicating that both brain “ouabain” and brain RAS are also involved in the modulation of baroreflex function by CSF sodium. Thus, increases in CSF sodium by high salt intake in Dahl S rats20 may contribute to the impairment of baroreflex function.

In contrast to Dahl S rats, in Dahl R rats high dietary salt sensitized arterial baroreflex control of RSNA and HR, and neither blockade of brain “ouabain” nor blockade of the brain...
RAS affected this sensitization. Therefore, changes in central pathways involving brain “ouabain” or the brain RAS appear not to be involved in the enhanced baroreflex control in Dahl R rats on high salt intake.

**Brain Ang II and Brain Ouabain**

Blockade of either brain Ang II or “ouabain” prevents sodium-dependent sympathetic hyperactivity and hypertension in SHR or Dahl rats (present study). The following findings support the concept that activation of Ang II receptors is secondary to brain “ouabain” in the pathways leading to sympathetic hyperactivity and hypertension in salt-sensitive hypertension. First, in conscious Wistar rats, ICV Fab fragments block sympathoexcitatory and pressor responses to ICV hypertonic saline, ouabain, and brain “ouabain” but not to ICV Ang II, whereas ICV losartan blocks responses to hypertonic saline, ouabain, and Ang II. Second, chronic ICV treatment with losartan blunts sympathoexcitatory and pressor responses to both Ang II and ouabain ICV in SHR on high salt diet and to ouabain ICV in Dahl R rats on high salt intake chronic blockade of brain “ouabain” by ICV Fab fragments does not attenuate but enhances the responses to ICV Ang II, suggesting an upregulation of brain Ang II receptors after blockade of brain “ouabain.”

The actual pathways involving the activation of brain “ouabain” and Ang II by high salt intake have not yet been clarified. In rat brain, nerve fibers of ouabain-immunopositive neurons are found abundantly in areas such as the anteroventral third ventricle, including the organum vasculosum of the lamina terminalis and the subfornical organ. Lesions of the ventral anteroventral third ventricle attenuate pressor responses to ICV hypertonic saline, ouabain, and Ang II and prevent central sodium- and ouabain-induced hypertension in Wistar rats. Moreover, in conscious Wistar rats, pressor responses to ouabain and hypertonic saline ICV are attenuated by Fab fragments or losartan in the median preoptic nucleus (MnPO). Both Fab fragments and losartan in the MnPO significantly decreased BP in SHR on high but not on regular salt intake. These results suggest that in the MnPO both “ouabain” and Ang II mediate at least some of the responses to ICV hypertonic saline and high dietary salt in SHR. In rats the preoptic area, which is adjacent to the MnPO, is a principal location in the hypothalamus, facilitating the arterial baroreflex via the nucleus raphe magnus. Further studies are needed to clarify whether Ang II and ouabain in the preoptic area or perhaps brain stem areas such as the nucleus tractus solitarii are involved in the salt-induced desensitization of baroreflex function in SHR and Dahl S rats.

In summary, the present study demonstrates that compared with Dahl S on regular salt or Dahl R on high or regular salt diet, in Dahl S on high salt sympatheticoinhibitory activity is decreased, sympathoexcitatory responses are increased, and arterial and cardiopulmonary baroreflex control of RSNA and HR is impaired. Concomitant ICV treatment with losartan or Fab fragments prevents these changes to a similar extent. We conclude that both brain Ang II and brain “ouabain” contribute to the sympathetic hyperactivity, impairment of baroreflex control of RSNA and HR, and development of hypertension in Dahl S on high sodium. We speculate that brain Ang II receptor stimulation occurs downstream from brain “ouabain” receptor activation.

**Acknowledgments**

This study was supported by operating grant MT-11897 from the Medical Research Council of Canada and an unrestricted grant from Apotex Inc, Toronto, Canada. Digibind was a generous gift from Glaxo Wellcome Inc, Toronto, Canada, and losartan was from Merck Research Laboratories, Rahway, NJ.

**References**


Both Brain Angiotensin II and "Ouabain" Contribute to Sympathoexcitation and Hypertension in Dahl S Rats on High Salt Intake
Bing S. Huang and Frans H. H. Leenen

Hypertension. 1998;32:1028-1033
doi: 10.1161/01.HYP.32.6.1028

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/32/6/1028

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/