Responses to Central Na\(^+\) and Ouabain Are Attenuated in Transgenic Rats Deficient in Brain Angiotensinogen

Bing S. Huang, Detlev Ganten, Frans H.H. Leenen

Abstract—Studies with angiotensin (Ang) II type 1 receptor blockers suggest that the brain renin-angiotensin system contributes to sodium-induced sympathoexcitation and hypertension. To provide more specific evidence for the involvement of Ang II, locally produced in the brain, transgenic rats were used, which express an antisense RNA against angiotensinogen mRNA specifically in the brain, reducing angiotensinogen levels in the brain by \(>90\%\). In freely moving transgenic rats and Sprague-Dawley rats as control animals, blood pressure and heart rate responses to intracerebroventricular infusion (3.8 \(\mu\)L/min for 10 minutes) of artificial cerebrospinal fluid and Na\(^+\)-rich artificial cerebrospinal fluid (containing 0.2, 0.3, and 0.45 mol/L Na\(^+\)) as well as intracerebroventricular injection of ouabain (0.3 and 0.6 \(\mu\)g/2 \(\mu\)L) were assessed. Central infusion of Na\(^+\)-rich artificial cerebrospinal fluid increased blood pressure and heart rate in a dose-related manner. However, the peak increases by each dose of Na\(^+\) were attenuated by 50% to 70% in the transgenic versus Sprague-Dawley rats. Increases in blood pressure and heart rate in response to ouabain at both doses were attenuated by 55% to 70% in the transgenic versus Sprague-Dawley rats. In the hypothalamus, Ang I level was markedly lower (31 ± 9 versus 76 ± 13 pg/g, \(P<0.05\)) and Ang II level tended to be lower in the transgenic versus Sprague-Dawley rats. These results indicate that the production of angiotensins in the brain is decreased in transgenic rats. The attenuated sympathoexcitatory and pressor responses to ouabain and Na\(^+\)-rich artificial cerebrospinal fluid in transgenic rats support the concept that the local brain renin-angiotensin system, that is, locally produced Ang II, plays an important role in the sympathoexcitatory effects of ouabain and sodium. (Hypertension. 2001;37[part 2]:683-686.)

Key Words: angiotensinogen ■ antisense elements ■ brain ■ DNA ■ sodium ■ ouabain

There is extensive evidence supporting a major role for neural mechanisms in salt-induced hypertension in Dahl salt-sensitive (S) rats receiving high salt intake (for review, see Reference 1). Dahl S rats receiving high salt intake demonstrate sympathetic hyperactivity and hypertension as well as impaired baroreflex function compared with Dahl salt-resistant (R) rats.2,3 These central effects of high salt can be prevented by blockade of ouabainlike activity ("ouabain")2,3 or the renin-angiotensin system (RAS)2 in the brain. Salt-induced sympathoexcitation and hypertension in salt-sensitive rats can be mimicked in normotensive rats by central sodium loading,4,5 which can also be prevented by blockade of "ouabain" or angiotensin (Ang) II type 1 (AT\(_1\)) receptors in the brain.5 These findings support the concept that in salt-sensitive rats receiving high salt, increased sodium in the cerebrospinal fluid (CSF) activates central pathways involving "ouabain" and AT\(_1\) receptors, causing sympathoexcitation and hypertension. Increased responsiveness to CSF Na\(^+\) in Dahl S versus R rats probably is one of the mechanisms through which high salt leads to hypertension only in Dahl S but not R rats.6

Thus far, central administration of AT\(_1\)-receptor antagonists such as losartan has been used to assess the role of the RAS in the brain in salt-sensitive hypertension.3,5 This approach has its limitations: (1) the antagonists will block brain AT\(_1\) receptors both inside and outside the blood-brain barrier and therefore do not differentiate between central effects of circulating versus locally produced Ang II; and (2) AT\(_1\)-receptor blockade can lead to unopposed, even enhanced stimulation of other Ang II receptors such as AT\(_2\) and AT\(_4\). These limitations may lead to overestimation of the role of a local brain RAS and brain AT\(_1\) receptors in salt-sensitive hypertension.

To more specifically assess the role of locally produced Ang II, in the present study we used transgenic rats deficient in brain angiotensinogen (AOGEN).7,8 By incorporating AOGEN antisense driven by a glial fibrillary acidic protein promoter in the transgene that is injected into rat germ cells, antisense RNA against AOGEN mRNA is expressed specifically in the brain, and the brain AOGEN in these transgenic rats [TGR(AsrAOGEN)] is reduced markedly.7 The main goal of this study was, by using these TGRs as an animal model, to assess the specific role of locally produced Ang II in the central sympathoexcitatory effects of high salt.
model with low activity of the brain RAS, to test the hypothesis that angiotensins generated in the brain mediate the sympathetic hyperactivity and hypertension elicited by increased CSF Na⁺ as well as central injection of ouabain. To verify the decreased activity of the brain RAS, brain Ang I and II levels were also examined.

Methods

Six TGR and 6 Sprague-Dawley (SD) rats (both male; weight, 300 to 350 g) were transferred from the Max-Delbruck Center for Molecular Medicine to the University of Ottawa Heart Institute. They were fed a standard diet and had free access to tap water. All experimental procedures were carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee for the use and care of laboratory animals.

Five to 7 days after arrival, under halothane inhalation, a 23-gauge, stainless steel guide cannula was implanted above the left lateral cerebroventricle and fixed to the skull of the rat. At least 1 week after surgery, under halothane, the left carotid artery and right jugular vein were catheterized with polyethylene tubing. Approximately 20 hours after catheterization, in freely moving rats, the arterial catheter was connected to pressure transducers, and blood pressure (BP) and heart rate (HR) were monitored by a Grass 7E polygraph and an on-line computer equipped with a Grass data acquisition program (Polyview 2.0). For intracerebroventricular (ICV) administration, a 26-gauge needle was inserted into the guide cannula so that its tip protruded 1 mm into the lateral ventricle during the administration. The needle was connected to a Hamilton microsyringe with volume of 20 or 500 μL for injection and infusions, respectively. Thirty minutes after the lines had been connected, baseline BP and HR were measured. The following solutions were then given by ICV infusion with a Sage 355 infusion pump at 3.8 μL/min for 10 minutes at 30-minute intervals: artificial cerebrospinal fluid (aCSF); and aCSF containing 0.2, 0.3, or 0.45 mol/L Na⁺.

Angiotensin II antibody had 55% cross-reactivity with Ang III and 0.1% cross-reactivity with Ang I. Ang III was not included in the analyses. High-performance liquid chromatography, as previously described, was used as the post hoc test. Linear regression was used to calculate the slopes of Na⁺-dose-dependent mean arterial pressure (MAP) and HR responses to ICV aCSF and Na⁺-rich aCSF containing 0.2, 0.3, and 0.45 mol/L Na⁺ in TGR and SD rats. Values are mean ± SEM.

Responses to ICV Infusions of Na⁺-Rich aCSF

 Pretreatment with the AVP antagonist by intravenous administration transiently decreased MAP and HR. Infusion of aCSF at 3.8 μL/min ICV for 10 minutes did not affect baseline BP and HR significantly. In contrast, an minute after ICV infusion of aCSF containing 0.2, 0.3, or 0.45 mol/L Na⁺, MAP and HR started rising, reached plateau levels in another 1 to 2 minutes, and returned to baseline levels within 2 minutes after termination of ICV infusions. The peak increases in MAP and HR in response to the 3 doses of Na⁺-rich aCSF were all significant versus baseline and were in a Na⁺-concentration–dependent manner (Figure 1). Peak MAP and HR responses to the 3 doses of Na⁺-rich aCSF were all markedly attenuated in TGR versus SD rats. For both MAP and HR responses, slopes of linear regression were significantly attenuated in TGR versus SD rats (−41 ± 3 versus −97 ± 11 mm Hg/M Na⁺ and −105 ± 15 versus 194 ± 15 bpm/M, respectively, P < 0.05 for both).

Responses to ICV Injection of Ouabain

Injection of 2 μL ICV aCSF did not change BP and HR. ICV injection of ouabain at both doses increased BP and HR significantly within 1 minute, reaching peak values in 2 minutes (Figure 2). The responses lasted 10 minutes for the low dose of ouabain and ∼20 minutes for the high dose. At both doses, peak MAP and HR responses were markedly attenuated in TGR versus SD rats.

Brain Ang I and II

In TGR versus SD rats, hypothalamic Ang I levels were significantly lower (31 ± 9 versus 76 ± 13 pg/g, P < 0.05), and Ang II levels tended to be lower (22 ± 7 versus 32 ± 6 pg/g, P = 0.17). Levels of Ang I+II combined were significantly lower in the hypothalamus in TGR versus SD rats (50 ± 16 versus 109 ± 14 pg/g, P < 0.05).
The major finding of the present study is that in TGR rats deficient in brain angiotensinogen and with significant decreases in hypothalamic angiotensins, BP and HR increases in response to CSF Na⁺ and ouabain are significantly attenuated.

In Dahl S rats high salt intake impairs baroreflex function and causes sympathetic hyperactivity and hypertension. Similar changes in these parameters are observed in normotensive rats with chronic central Na⁺ loading. With high salt intake, salt-sensitive rats develop sympathetic hyperactivity and hypertension, possibly because of higher CSF Na⁺ concentrations as well as higher responsiveness for a given increase in CSF Na⁺, as suggested by responses to short-term or long-term ICV infusions of hypertonic saline. This sodium-induced sympathetic hyperactivity and hypertension can be prevented by blockade of brain AT₁ receptors with losartan, or blockade of brain “ouabain.” Whereas ICV injection of losartan blocks sympathoexcitatory responses to ICV hypertonic saline, ouabain or Ang II, responses to ICV hypertonic saline and ouabain but not to Ang II are blocked by ICV ouabain antibody. On the basis of these findings, we proposed the hypothesis that in salt-sensitive rats given high salt or normotensive rats with central sodium loading, increased CSF sodium increases brain “ouabain” and the latter activates the brain RAS, leading to increases in sympathetic outflow and BP.

Thus far, the involvement of the brain RAS in salt- or ouabain-induced hypertension has been studied by central administration of AT₁-receptor antagonists such as losartan. There are some limitations regarding the use of AT₁-receptor blockers to assess the role of angiotensins produced by a local brain RAS per se. First, ICV administration of AT₁-receptor antagonists blocks AT₁ receptors not only inside the blood-brain barrier but also in the circumventricular organs with a deficient blood-brain barrier. Major AT₁ receptor-containing nuclei such as the subfornical organ (SFO), the area postrema as well as parts of the organum vasculosum of the lamina terminalis (OVLT) are activated by circulating Ang II. Other areas such as the median preoptic nucleus and the periventricular part of the OVLT respond to Ang II in the CSF, which may be derived from the circulating RAS. AT₁ blockers given by ICV administration also block these effects of circulating Ang II in the brain, leading to overestimation of the role of angiotensins produced by a local brain RAS.

Second, rat brain areas involved in cardiovascular regulation exhibit a predominance of AT₁ receptors, but AT₂ and AT₄ subtypes are also present. Central administration of an AT₁-receptor blocker will not assess the functional contribution of (enhanced) AT₂- and AT₄-receptor stimulation. Indeed, the non–AT₁-specific Ang II receptor blocker sartharan decreases the basal BP in SHR on regular salt intake, indicating the possible involvement of Ang II receptors other than AT₁ in the development of hypertension in SHR. Third, long-term ICV administration is not only technically complicated but may also lead to nonspecific activation of the brain RAS.

Brain AOGEN is mainly produced by astrocytes and colocalized with the intermediate filament glial fibrillary acidic protein. Because a glial fibrillary acidic protein promoter is used to drive the expression of the antisense-DNA, the present TGR rats express AOGEN antisense RNA specifically in the brain, resulting in a decrease in brain AOGEN by >90% and significant decreases in drinking responses to ICV renin in TGR versus control rats. In the present study, Ang I levels were 2 to 3 times lower and Ang II levels tended to be lower in the hypothalamus of TGR rats versus SD control animals, consistent with a low generation of angiotensins in the brain. The remaining levels of brain angiotensins in TGR rats may reflect some persistence of local production from any remaining angiotensinogen in the brain or may result from contamination from circulating or CSF angiotensins. Whether production of other angiotensins such as Ang III was also decreased in the brain of TGR rats was not assessed. It has been proposed that brain Ang II must be converted to Ang III to bind at the AT₁- and AT₂-receptor subtypes.

Responses to acute administration of both Na⁺-rich aCSF and ouabain were markedly attenuated in TGR rats, consistent with the concept that locally generated angiotensins mediate the sympathoexcitatory and pressor effects of central sodium and ouabain. However, in TGR versus SD rats, responses to Na⁺-rich aCSF or ICV ouabain were not abolished. In contrast, in our previous studies, sympathoexcitatory and/or pressor responses to acute ICV hypertonic saline or ouabain were more completely blocked by ICV losartan. In rats, an angiotensinergic mechanism within the SFO mediates responses to short-term ICV administration of hypertonic saline. The SFO also may contribute to pressor responses to circulating Ang II. It appears that in normal rats, as their main mechanism, ICV hypertonic saline or ouabain increases release of locally produced angiotensins, resulting in activation of AT₁ receptors in areas such as the SFO and thereby sympathoexcitation and hypertension. In TGR rats, local production of angiotensins in the brain is markedly decreased, but areas outside the blood-brain barrier such as the circumventricular organs SFO or OVLT may pick up angiotensins or perhaps angiotensinogen from the circulation and thus still express some angiotensin-mediated responses to sodium or ICV ouabain. Therefore, in rats, activation of areas both inside and outside the blood-brain barrier appears to be involved in the responses to sodium or ICV ouabain, and areas outside the blood-brain barrier may
obtain sufficient angiotensins from the circulation in order to respond, resulting in the partial responses noted.

These conclusions assume that peripheral cardiovascular responsiveness per se is not diminished in the TGR rats. Plasma Ang II protein concentration7 as well as plasma renin activity8 do not differ between TGR and SD rats. However, the transgenic rats do show a 35% decrease in plasma vasopressin, and some degree of diabetes insipidus as indicated by decreased urine osmolality and increased urine volume.7 However, plasma osmolality and Na+ and K+ concentrations were normal.7 These results suggest that these rats exhibit relatively normal effective blood volumes.

Lower plasma AVP levels may affect pressor responses to vasoactive agents such as Ang II. However, because vasopressin V₁ receptors were blocked in both groups of rats and responses to centrally administered sodium and ouabain are mediated by the brain and not the peripheral RAS, it is unlikely that decreased plasma AVP or mild diabetes insipidus contributed to a significant degree to the decrease in responses to central sodium and ouabain.

Summary

Compared with SD control rats, the TGR rats demonstrate clear decreases in the levels of angiotensins in the hypothalamus. This model offers a unique approach to study the functions of angiotensins locally produced in different areas of the brain. A clear decrease in sympathoexcitatory and pressor responses to CSF sodium and ouabain in the TGR rats supports the concept that angiotensins produced in the brain contribute to sympathetic hyperactivity and hypertension induced by increased brain sodium and ouabain.

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