Increased Central Angiotensin and Osmotic Responses in the Ren-2 Transgenic Rat

Tatsuya Nishioka, Michael F. Callahan, Ping Li, Carlos M. Ferrario, Detlev Ganten, Mariana Morris

Abstract—We previously demonstrated that the Ren-2 transgenic (TG) rat is sensitive to salt, showing a sodium-induced pressor response. The present studies determined the effect of central stimulation with hypertonic saline (HS) and angiotensin II (Ang II) on mean arterial pressure (MAP), heart rate (HR), and plasma vasopressin. HS (1 mol/L NaCl, 5 μL) or Ang II (100 ng, 5 μL) was injected into the lateral ventricle of conscious male TG and control rats. The pressor responses to HS and Ang were greater in TG than in control rats, increases of 42±4 and 41±4 mm Hg versus 25±3 and 18±2 mm Hg (HS and Ang II and TG and control rats, respectively). The TG rats also showed an increased vasopressin response to Ang II, peak levels of 14±3 versus 28±3 pg/mL (control versus TG rats). HS increased plasma vasopressin levels, although the group responses were not different. HR was not significantly altered by either stimulus. Results demonstrate an increased responsiveness to intraventricular HS and Ang II in Ren-2 transgenic rats, suggesting a relationship between the enhanced angiotensinergic drive and central cardiovascular and vasopressin responses. (Hypertension. 1999;33[part II]:385-388.)

Key Words: genetics ■ sodium ■ brain ■ vasopressin ■ blood pressure ■ rats, transgenic

The Ren-2 transgenic (TG) rat is a model of genetic hypertension that shows increases in renin and angiotensin in brain and peripheral tissues as the result of overexpression of the mouse renin gene.1–3 There is substantial evidence that links the renin-angiotensin system (RAS) to salt and water balance.4–6 This has led to studies in the Ren-2 rat to determine whether these animals, which show elevated endogenous RAS activation, demonstrate changes in response to sodium and angiotensin. Results from our laboratory indicate that male TG rats show an increased response to sodium whether administered orally or intravenously. With consumption of 2% NaCl there was an increase in blood pressure, vasopressin secretion, hypothalamic vasopressin mRNA levels, and brain angiotensin receptors.7–10 Intravenous administration of NaCl produced a greater increase in blood pressure, even though the TG animals excreted the salt load with a time course not different from that of the control animals.11 Likewise, a comparison of the pressure-induced natriuresis between TG and deoxycorticosterone acetate (DOCA)/salt hypertensive rats showed no differences between the groups, indicating a lack of relationship between the RAS and the renal excretory responses.12 Differences were not observed in the pressor response to peripherally administered angiotensin II (Ang II) in TG rats, although in vitro studies have suggested an increase in vascular reactivity.13,14

With regard to central nervous system (CNS) alterations in TG animals, brain levels of angiotensin peptides were increased, whereas angiotensin receptors were unchanged.2,8 The central infusion of Ang II in anesthetized female TG rats increased plasma vasopressin level but did not change blood pressure.15 However, a role for endogenous brain angiotensin receptors in the maintenance of salt-sensitive hypertension was suggested in studies in which male TG rats consumed 2% NaCl.8 Injection of an angiotensin AT1 receptor antisense oligonucleotide into the hypothalamic paraventricular nucleus reduced receptors and blood pressure but only in the salt-treated TG rats. These findings raise questions about the interactions between sodium and angiotensin in the control of blood pressure in the TG model. To explore the idea that endogenous changes in the brain RAS alters CNS responses, we evaluated the effect of cerebral ventricular injection of hypertonic saline and Ang II on blood pressure, heart rate (HR), and plasma vasopressin in conscious male TG rats.

Methods
Male heterozygous (mRen-2)27 transgenic (Ren-2 TG) and control rats were obtained from the breeding colony established at the Hypertension Center of Wake Forest University Medical Center. The breeding colony was developed from descendants of the transgenic strain produced by Mullins and colleagues.3 The control strain was the Hannover Sprague-Dawley, the same line as that used to create the transgenic line. TG (496±14 g) and control (478±10 g) rats were housed singly in cages with free access to food and water. All
Experimental protocols were approved by the Wake Forest University Animal Care and Use Committee.

Rats were anesthetized with ketamine/xylazine (71.6 mg/kg, IM). A 23-gauge stainless steel guide cannula was implanted into the left cerebral ventricle. The coordinates were as follows: 0.8 mm posterior to the bregma, 1.3 mm lateral to the midline, and 3.6 mm below the skull surface. The cannula was anchored to the skull with 2 stainless steel screws and dental cement. After a recovery period of 5 to 7 days, the right carotid artery was catheterized with PE-60 tubing for recording arterial blood pressure and collecting blood samples. The catheter was filled with heparinized saline (50 U/mL) and tunneled subcutaneously to exit at the back of the neck. The experiment was performed in conscious freely moving rats 2 days after the arterial catheterization.

Experimental Protocols

The arterial catheter was connected to a pressure transducer (Microswitch), and arterial blood pressure was measured with a Physiograph (model 5/6H, Gilson Medical Electronics). After a 1-hour stabilization period, hypertonic saline (HS, 1 mol/L NaCl, 5 μL) was injected intracerebroventricularly over 60 seconds with a 33-gauge stainless steel injector, which extended 1 mm below the tip of the guide cannula. One and one-half hours after the HS injection, Ang II (100 ng, 5 μL) was injected intracerebroventricularly in the same manner. Arterial blood pressure and HR were recorded for 20 minutes (5 minutes before injection and 15 minutes after injection). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third the pulse pressure. Initial experiments were conducted to determine that the order of the injection did not influence the pressor response (Ang II after HS or vice versa). Blood samples (0.8 mL) were withdrawn 15 minutes before and 15 minutes after each intracerebroventricular injection. Blood was replaced with an equal volume of isotonic saline.

Plasma samples were extracted with acetone precipitation and petroleum ether extraction. Vasopressin was measured in the plasma extracts by a sensitive and specific radioimmunoassay.

Data Analysis

Data are expressed as mean±SEM. Statistical evaluation was performed by ANOVA for repeated measures followed by Newman-Keuls post-hoc test or unpaired t test. A significance level of P<0.05 was used for all analyses.

Results

Effect of Intracerebral Injection of HS and Ang II on MAP and HR

Baseline MAP in control and TG rats was 99±4 and 154±8 mm Hg, respectively (Figure 1A). Injection of HS resulted in a significant MAP increase in both groups. The absolute increase in MAP was greater in the TG rats than in the control rats (changes of 25±3 versus 42±4 mm Hg, control versus TG rats; Figure 1B). The percentage changes were not different between the groups. Basal MAP before Ang II stimulation was comparable to MAP before HS injection (103±4 versus 156±29 mm Hg; control versus TG rats, Figure 2A). Central Ang II injection elicited significant increases in MAP in both groups, with a greater absolute pressor response (18±2 versus 41±4 mm Hg, control versus TG rats; Figure 2B) and a greater percentage change (18±2% versus 28±3% increase from baseline, control versus TG rats, P<0.02). HR was not significantly altered by either stimulus (Table).

Effects of Intracerebral Injection of HS or Ang II on Plasma Vasopressin

Baseline plasma vasopressin levels were not significantly different between the groups (Figure 3). TG rats showed an increased vasopressin response to Ang II, 14 versus 28 pg/mL (control versus TG rats, respectively; Figure 3A). Central HS produced an increase in plasma vasopressin in both groups with no significant difference in the responses (Figure 3B).

Discussion

It is well documented that central osmotic or angiotensin stimulation by intracerebral injection elicits increases in water intake, blood pressure, and plasma vasopressin.16-20 Furthermore, interactions are noted such that sodium potentiates the effects of angiotensin, and angiotensin antagonists alter the responses to osmotic stimulation.18-21 Given this scenario, it could be predicted that a chronic change in the brain RAS would alter central angiotensin and osmotic responses. Indeed, results confirmed that Ang II and HS both caused an increase in blood pressure that was greater in the TG than in control rats. This was accompanied by a greater plasma vasopressin response to angiotensin but not to hypertonic saline.

The Ren-2 TG rat is a model of fulminating hypertension produced by the insertion of the mouse Ren-2 gene into the rat genome.1 The transgene and its protein products are expressed in a variety of tissues, including the adrenal, testes, kidney, arteries,
HR Response to Central HS and Ang II Stimulation

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>HS Baseline</th>
<th>Ang II Baseline</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>384±17</td>
<td>368±14</td>
<td>387±11</td>
</tr>
<tr>
<td>TG</td>
<td>378±18</td>
<td>404±19</td>
<td>373±16</td>
</tr>
</tbody>
</table>

Mean±SEM. Data show heart rate (beats/min) before and after central administration of HS or Ang II.

and brain and in other tissues. Our research has focused on the study of salt sensitivity in the TG rat, exploring the idea that activation of the RAS sensitizes the system to the effects of salt consumption. Indeed, drinking 2% NaCl caused a significant increase in blood pressure in TG rats, but not in control animals. This effect occurred rapidly within hours of ingestion and continued for the duration of the exposure. The blood pressure rise was associated with increased sympathetic and vasopressin drive. CNS angiotensin receptors appeared to be involved because blood pressure was reduced by central treatment with an AT1 receptor antisense. The present study involved because blood pressure was reduced by central treatment with an AT1 receptor antisense. The present study supports the idea of CNS alterations in the TG animal with increased pressor responses noted for both angiotensin and osmotic stimuli. The results contrast with an earlier report by our group that showed a complete lack of angiotensin-induced pressor responses in the TG rats. However, there are differences between the protocols, notably the use of (1) halothane-anesthetized animals, (2) female rats, and (3) a different method and dose for angiotensin administration. For studies of the cardiovascular system, the conscious preparation is always preferable because anesthetics alter basal blood pressure and drug responses. With regard to the sex of the rats, there is evidence that the pressor response to central Ang II was diminished by estrogen. Finally, it should be noted that, although basal plasma vasopressin levels were high in the anesthetized animals, angiotensin increased vasopressin secretion, indicating that a segment of the receptor-mediated event was still present.

The primary mechanisms by which these stimuli alter blood pressure are sympathetic activation, baroreflex inhibition, and vasopressin release. The contribution of each component has been studied with the use of combinations of pharmacological antagonists. Here, we show a correlation between vasopressin secretion and blood pressure, especially for central angiotensin stimulation. However, this does not provide causal evidence. The antagonist to the vasopressin V1 pressor receptor did not alter the salt-induced increase in blood pressure in TG rats. Likewise, intravenous administration of HS caused a greater pressor response in TG rats, whereas the increase in plasma vasopressin was similar to that observed in control animals. Similarly, central osmotic stimulation caused an enhanced pressor, but not vasopressin, response. It is unlikely that the increase in peripheral vasopressin provides the total explanation for the pressor responses; rather, it is likely to be a combination of effector systems.

The pathways by which vasopressin release is stimulated by angiotensin and hypertonic saline involve interactions with osmotic and angiotensin receptors in the circumventricular organs in the forebrain with relays to the magnocellular neurons and the posterior pituitary. As mentioned previously, there is significant interaction between these systems as seen with the use of pharmacological blockade, lesions, and antisense treatment. Intracerebral Ang II and HS produced significant increases in plasma vasopressin in control and TG rats. The angiotensin response was significantly higher in the TG rats, whereas there was a tendency for an exaggerated response after HS (11.5 versus 19 pg/mL vasopressin, control versus TG rats). The experimental design may have contributed to this difference because Ang II was administered after hypertonic saline, perhaps acting to sensitize the animals to the second stimulus.

In conclusion, a genetic model in which there is continuing overexpression of the RAS provides evidence for a role of this system in the peptidergic and osmotic modulation of blood pressure and hormone secretion. The data provide further evidence for the important interrelationship between sodium and angiotensin in volume and pressure control.

Acknowledgments

This work was supported by Grants HL43178 and HL51952 from the National Institutes of Health. The authors thank Cindy Barrett.

References


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Hypertension. 1999;33:385-388
doi: 10.1161/01.HYP.33.1.385

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 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:
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