Depressor Role of Angiotensin AT$_2$ Receptors in the (mRen-2)27 Transgenic Rat

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

The (mRen-2)27 transgenic rat (Tg$^+$), a hypertensive model dependent on increased expression of the renin angiotensin system, was used to explore the role of angiotensin AT$_2$ receptors in the control of cardiovascular and renal excretory function. Experiments tested the effect of blockade of AT$_2$ receptors on basal blood pressure and the pressor, renal excretory, and vasopressin (VP) responses to intravenous hypertonic saline (HS). Chronically catheterized male Tg$^+$ and normotensive Sprague-Dawley rats (Tg$^-$) were housed in metabolic cages. PD123319 (AT$_2$ antagonist) or 0.9% NaCl was given by intravenous bolus (3 mg/kg) followed by infusion (50 μg/kg/min). Blockade of AT$_2$ receptors both in Tg$^+$ and Tg$^-$ rats produced no change in basal mean arterial pressure (MAP). The pressor response to intravenous HS (10% NaCl; 325 μL/100 g body weight) was significantly greater in Tg$^+$ than in Tg$^-$ rats. PD123319 did not affect the peak rise in MAP but extended the time course of the response only in Tg$^+$ rats. MAP was increased 39 ± 4 and 36 ± 3 mm Hg in Tg$^+$ rats with and without the antagonist as compared to 20 ± 2 and 24 ± 2 mm Hg in Tg$^-$ rats. In the antagonist-treated Tg$^+$ rats, MAP remained elevated for 60 min as compared to 5 min for Tg$^+$ control or Tg$^-$ control or antagonist-treated rats. Hypertonic saline caused similar increases in plasma Na, VP, and in the natriuretic and diuretic responses in both Tg$^+$ and Tg$^-$ rats, with no effect of antagonist treatment. These results demonstrate that Tg$^+$ rats are sensitive to the effects of peripheral osmotic stimulation showing an increased pressor response, not attributed to greater secretion of VP or diminished natriuresis. These data also suggest that angiotensin AT$_2$ receptors play a depressor role in the sodium-induced pressor response in this model. Am J Hypertens 1998;11:357–362 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Osmotic stimulation, sodium, blood pressure, AT$_2$ antagonist, PD123319, vasopressin, renal function.

Angiotensin II (Ang II) is a vasoactive peptide known to be important in the development of hypertension through its vasoconstrictor action on blood vessels, renal effects on salt and water retention, and central endocrine and neurogenic actions. Ang II interacts with at least two types of receptors (AT$_1$ and AT$_2$). Most of the cardiovascular and endocrine actions of Ang II are mediated by AT$_1$ receptors, distributed in the cardiovascular system, kidney, adrenal gland, and brain.$^1$

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AT₂ receptors are localized in fetal tissues, adrenal gland, kidney, and brain; however, their function and biochemical mediators are uncertain.²,³ Studies using the selective AT₂ ligands (PD123317 or PD123319) have suggested that AT₂ receptors enhance renal tubular sodium reabsorption.⁴,⁵ A depressor role for the AT₂ receptor was suggested by the increase in mice lacking the AT₂ receptor⁶ and the blockade of the depressor response to Ang II by the AT₂ antagonist.⁷

One useful model for the study of angiotensinergic function is the (mRen-2)²⁷ transgenic rat (Tg⁺). This hypertensive model shows increased expression of the mouse renin gene with associated increases in Ang peptides in plasma, brain, and other tissues.⁸–¹⁴ Blood pressure in Tg⁺ rats is reduced by angiotensin converting enzyme inhibitors or AT₁ receptor antagonists, suggesting that the hypertension is dependent on Ang II.¹⁵ These rats are also sensitive to the effects of NaCl, showing an increase in blood pressure with consumption of 2% NaCl as well as alterations in urinary sodium excretion,¹⁶ a decrease in blood pressure on a low salt diet,¹⁷ and a decrease in salt-induced hypertension produced by Ang AT₁ antisense.¹⁸ Although it is clear that chronic activation of AT₁ receptors is involved in the hypertension and salt sensitivity, it is also plausible that the cardiovascular changes may result from an imbalance between AT₁ and AT₂ receptors. To test the hypothesis that AT₂ receptors are involved in the control of blood pressure and sodium excretion, we evaluated the effect of AT₂ receptor antagonism on basal mean arterial pressure (MAP) and heart rate (HR) and the cardiovascular, renal excretory, and plasma vasopressin (VP) responses to an intravenous sodium load in male Tg⁺ and Tg⁻ rats. The experiments were conducted using the specific AT₂ antagonist PD123319 infused intravenously in chronically catheterized rats with monitoring of blood pressure, HR, and urinary volume and electrolyte excretion.

METHODS

Animals Male heterozygous (mRen2)²⁷ Tg⁺ and Tg⁻ rats were obtained from the breeding colony established at the Hypertension Center of the Wake Forest University Medical Center. Tg⁻ littermates were produced by breeding male heterozygous Tg⁺ rats with female rats from the Hannover Sprague-Dawley strain used to create the transgenic line. The genetic lineage of each rat was determined by the polymerase chain reaction amplification of tissue samples. Phenotypic expression of the transgene was initially determined by measurement of blood pressure by tail-cuff plethysmography. Tg⁺ rats (440 ± 15 g body weight) and Tg⁻ rats (395 ± 32 g body weight) were housed singly in cages on a 12-h light–dark cycle with free access to food and water. All procedures used in this study were approved by the University Animal Care and Use Committee.

Surgery Rats were anesthetized with intramuscular ketamine/xylazine (71.6 mg/kg). A polyethylene catheter formed from PE-10 tubing connected to PE-60 was inserted into the abdominal aorta for measurement of MAP and HR, and blood sampling. Another PE-10 catheter was inserted into the jugular vein for drug and saline administration. The catheters, filled with heparinized saline (50 U/mL), were exteriorized, protected by a steel spring, and secured at the back of the neck. Immediately after the surgery, the rats were placed in metabolic cages for the chronic monitoring of metabolic and cardiovascular parameters. The urine was collected in a tapered centrifuge tube, the cage design preventing contamination with food or fecal material. The arterial catheter was connected to a swivel and a pressure transducer (Microswitch, Freeport, IL) attached to the top of the cage. Catheter patency was maintained by a constant infusion of heparinized saline (50 U/mL, 1.8 mL/24 h). The catheter–swivel system allowed the rat to move freely in the metabolic cage and to have easy access to food and water.

Experimental Protocol The experiment was performed in conscious, unrestrained rats after fluid intake and urinary excretion had stabilized after surgery (minimum of 5 days). The protocol combined pretreatment with saline or AT₂ antagonist followed by an intravenous hypertonic saline (HS) challenge. Each rat was used for two consecutive experiments with a 2-day interval between tests. In the first experiment, rats were given either isotonic saline (0.9% NaCl) or the AT₂ antagonist PD123319 (Parke-Davis, Ann Arbor, MI) dissolved in 0.9% NaCl. In the second experiment, the alternative pretreatment was performed. MAP and HR were monitored throughout the experiment.

Isotonic saline or PD123319 was initially administered by an intravenous bolus injection (volume of 1 mL/kg, dose of PD123319; 3 mg/kg) followed by infusion at 4.6 μL/min (PD123319; 50 μg/kg/min) for 2 h through the jugular vein. The infusion rate of PD123319 (50 μg/kg/min) produces plasma concentrations with a high selectivity for the AT₂ receptor.¹⁹ Twenty minutes after the start of the saline or drug administration, HS (10% NaCl, 325 μL/100g body weight) was given by intravenous infusion over a 5-min period as an osmotic stimulation. Blood samples (1.2 to 1.5 mL) were taken 1 h before drug administration and 5 min after the end of HS infusion. The blood was replaced with an equal volume of 0.9% NaCl. Urine was collected beginning with the HS in-
TABLE 1. MEAN ARTERIAL PRESSURE (MAP) (mm Hg) BEFORE AND AFTER PRETREATMENT

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After Pretreatment</th>
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<tbody>
<tr>
<td>Tg− control</td>
<td>81 ± 3</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>Tg+ PD123319</td>
<td>78 ± 2</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>Tg+ control</td>
<td>126 ± 13</td>
<td>121 ± 14</td>
</tr>
<tr>
<td>Tg+ PD123319</td>
<td>123 ± 8</td>
<td>120 ± 7</td>
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</table>

Values are mean ± SEM.

Data show MAP before and after the intravenous administration of 0.9% NaCl (control, n = 5) or PD123319 (3 mg/kg, n = 8).

fusion for 2, 6, and 24 h. Immediately after collection, urine volume was recorded and a 1-mL sample was taken for later measurement of sodium concentration.

Sodium concentration was measured by flame photometry (IL943 Flame Photometer, Lexington, MA) in plasma and urine. Plasma samples were extracted using acetone precipitation and petroleum ether extraction. VP was measured in the lyophilized plasma extracts by a sensitive and specific radioimmunoassay.

Statistical Analysis Data were analyzed by three-way ANOVA for repeated measures on two factors followed by Student-Newman-Keuls or Bonferroni post-hoc test. A significance level of $P < .05$ was used for all analyses. All values are presented as mean ± SEM.

RESULTS

Basal blood pressure was significantly increased in Tg− rats as compared to Tg+ rats (Table 1). However, treatment with AT2 antagonist (PD123319) or isotonic saline produced no significant changes in MAP in either group (Table 1). Tg+ rats showed a significantly greater change in MAP after HS stimulation than Tg− rats (peak response of $36 ± 3 \text{ mm Hg}$, $P < .05$) (Figure 1). Although the AT2 antagonist produced no difference in the pressor response to the intravenous sodium load, there was an alteration in the time course in Tg+ rats treated with PD123319 (Figure 1). Statistical analysis revealed an interaction between genetic background and drug pretreatment. PD123319 extended the pressor response in the Tg+ (change in MAP; $14 ± 4 \text{ mm Hg}$ at 15 min, $12 ± 5 \text{ mm Hg}$ at 20 min, $9 ± 2 \text{ mm Hg}$ at 30 min, $8 ± 7 \text{ mm Hg}$ at 45 min, and $11 ± 3 \text{ mm Hg}$ at 60 min, control $v$ antagonist, $P < .05$). Hypertonic saline stimulation with or without drug treatment caused no significant change in HR either in Tg− or Tg+ rats (Table 2).

The intravenous infusion of HS caused a diuresis and natriuresis that were not different between the groups or drug treatments (Figure 2). Most of the load was excreted within 2 h at which time $1.3 ± 0.4 \text{ mEq}$ of sodium were excreted in the urine of saline-treated Tg− compared to $1.4 ± 0.4 \text{ mEq}$ in saline-treated Tg+ rats. Pretreatment with PD123319 did not change the excretion pattern in either Tg− or Tg+ rats.

Basal plasma VP levels were not significantly different between Tg− and Tg+ rats (Figure 3). Plasma VP levels were markedly increased at 5 min after the osmotic stimulation both in Tg− and Tg+ rats; however, the levels reached were not significantly different ($9.1 ± 1.6 \text{ pg/mL, Tg− v Tg+}$). Pretreatment with PD123319 did not significantly affect the plasma VP responses in either group. Plasma sodium concentration was significantly increased 5 min after hypertonic stimulation. The levels and time course were not different between Tg+ or Tg− or with treatment.

DISCUSSION

Our results in the mRen-2 Tg+ rat provide evidence for a depressor role for AT2 receptors in the blood pressure response to hypertonic sodium chloride stimulation. Blockade of AT2 receptors with PD123319 altered the time course of the osmotic-induced change in blood pressure, extending the pressor response in Tg+ but not in Tg− rats. Vascular AT2 receptors do not appear to be involved in tonic blood pressure control or renal excretory function as the antagonist had no effect on basal MAP or the ability to excrete a salt load.

FIGURE 1. Change in MAP (5, 15, 30, 45, and 60 min) after intravenous hypertonic saline stimulation. Tg− (n = 5) and Tg+ (n = 8) male rats were pretreated with 0.9% NaCl (control) or PD123319. * $P < .05$ compared with Tg− rats, † $P < .05$ compared with Tg+ control.
One of the first actions attributed to an AT$_2$ receptor-mediated function was the diuresis and natriuresis produced by volume expansion or blood pressure increase. In the rat, approximately 5% to 10% of renal Ang II receptors are of the AT$_2$ subtype, and AT$_2$ antagonists decreased renal electrolyte absorption in a microperfusion study and inhibited the natriuresis and diuresis produced by an increase in blood pressure. Likewise, in the dog, administration of an AT$_2$ antagonist increased urine volume and free water clearance without affecting renal hemodynamics. Different conclusions were reached in the studies of Macari and colleagues, which showed no effect of AT$_2$ antagonists on renal parameters in rats when lower doses of antagonist were administered. Indeed, our study supports the latter results as AT$_2$ receptor blockade had no effect on renal excretory function, with control and PD 123319-treated Tg$^+$ and Tg$^-$ rats showing similar patterns of salt and water excretion after a sodium load. The data further suggest that accentuation of the pressor phase in the antagonist-treated Tg$^+$ rats cannot be attributed to a deficit in renal excretory function.

Although most Ang II-induced vascular effects are thought to be mediated by AT$_1$ receptors, there is growing evidence for a role of AT$_2$ receptors in the control of blood pressure. Ang II injection produces a biphasic blood pressure response (increase followed by decrease), thought to be mediated by a sequential contribution of AT$_1$ and AT$_2$ receptors. Blockade of AT$_1$ receptors with losartan eliminated the increase in MAP and increased the depressor response; whereas, blockade of AT$_2$ receptors attenuated the depressor phase and increased the blood pressure rise. These results are intriguing because of the similar blood pressure response pattern seen after AT$_2$ blockade in Tg$^+$ rats (ie, no alteration in the pressor phase but an attenuation of the recovery from the blood pressure increase). We suggest that the long-lasting pressor response in AT$_2$ antagonist-treated Tg$^+$ rats is related to the depressor function of AT$_2$ receptors. Studies in AT$_2$ receptor knockout mice strains provide further support for a role of AT$_2$ receptors in blood pressure control. Although MAP was elevated in one AT$_2$ knockout strain but not in another, both strains showed an increased pressor response to peripheral Ang II injection. Thus, AT$_2$ receptor input may act to counterbalance the pressor (AT$_1$) effects of Ang II. Results presented in a recent abstract showed growing evidence for a role of AT$_2$ receptors in the control of blood pressure. Ang II injection produces a biphasic blood pressure response (increase followed by decrease), thought to be mediated by a sequential contribution of AT$_1$ and AT$_2$ receptors. Blockade of AT$_1$ receptors with losartan eliminated the increase in MAP and increased the depressor response; whereas, blockade of AT$_2$ receptors attenuated the depressor phase and increased the blood pressure rise. These results are intriguing because of the similar blood pressure response pattern seen after AT$_2$ blockade in Tg$^+$ rats (ie, no alteration in the pressor phase but an attenuation of the recovery from the blood pressure increase). We suggest that the long-lasting pressor response in AT$_2$ antagonist-treated Tg$^+$ rats is related to the depressor function of AT$_2$ receptors. Studies in AT$_2$ receptor knockout mice strains provide further support for a role of AT$_2$ receptors in blood pressure control. Although MAP was elevated in one AT$_2$ knockout strain but not in another, both strains showed an increased pressor response to peripheral Ang II injection. Thus, AT$_2$ receptor input may act to counterbalance the pressor (AT$_1$) effects of Ang II. Results presented in a recent abstract showed

**FIGURE 2.** Effect of AT$_2$ antagonist on the diuretic and natriuretic responses to intravenous hypertonic saline. Tg$^-$ (n = 5) and Tg$^+$ (n = 8) male rats were pretreated with 0.9% NaCl (control) or PD123319. There were no significant differences among the groups.

**TABLE 2. HEART RATE (BEATS/MIN) RESPONSE TO INTRAVENOUS HYPERTONIC SALINE STIMULATION**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>30</th>
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<tbody>
<tr>
<td>Tg$^-$ control</td>
<td>365 ± 17</td>
<td>351 ± 23</td>
<td>347 ± 19</td>
<td>351 ± 17</td>
<td>350 ± 21</td>
</tr>
<tr>
<td>Tg$^+$ PD123319</td>
<td>381 ± 26</td>
<td>392 ± 24</td>
<td>380 ± 19</td>
<td>390 ± 22</td>
<td>391 ± 22</td>
</tr>
<tr>
<td>Tg$^-$ control</td>
<td>320 ± 14</td>
<td>326 ± 12</td>
<td>323 ± 10</td>
<td>337 ± 15</td>
<td>329 ± 9</td>
</tr>
<tr>
<td>Tg$^+$ PD123319</td>
<td>339 ± 17</td>
<td>332 ± 15</td>
<td>328 ± 10</td>
<td>322 ± 14</td>
<td>331 ± 12</td>
</tr>
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</table>
an association between salt loading and a depressor function of AT2 receptors. Treatment with an AT2 antagonist produced an increase in blood pressure in rats with salt-induced hypertension, but not in controls. The preponderance of evidence indicates that AT2 receptors are not involved in the modulation of basal MAP, but instead are important when the cardiovascular system is activated as with osmotic stimulation.

One must also consider the role of the central nervous system in mediating the AT2 effects. Indeed, the various brain circumventricular structures that are sensitive to changes in plasma sodium are rich in immunoreactive Ang neurons and fibers and are responsive to the peptide mediator. The locus coeruleus participates in the regulation of cardiovascular function and also receives afferent information on osmotic status through its close association with the area postrema and neural connections with the rostral forebrain. Recent studies provide evidence that AT2 receptors in the locus coeruleus modulate neuronal firing. Treatment with the AT2 antagonist PD123319 specifically blocked Ang II-induced depression of glutamate excitability. Thus, the change in the blood pressure response to HS in the Tg+ rats may be mediated by antagonism of locally activated Ang systems. This could explain the lack of effect of the antagonist in the controls in which central nervous system angiotensinergic input may be less powerful.

Another aspect of this study that deserves discussion is the increased NaCl-induced pressor response observed in the Tg+ rats. There is substantial evidence to show that the central nervous system mediates the effects of peripherally administered HS on MAP, plasma VP, and the sympathetic nervous system. There is also support for synergistic interactions between Ang II and osmotic stimuli. For example, intravenous HS potentiated the hypertensive response to chronic intracerebroventricular infusion of Ang II and AT1 receptor antagonists blocked the response to central osmotic stimulation. The Tg+ rat, characterized by overexpression of the brain renin-angiotensin system, shows increased blood pressure and VP responses to consumption of 2% NaCl. Similarly, peripheral osmotic stimulation caused a greater pressor response in Tg+ rats although plasma sodium levels were similar between the groups. It is unlikely that circulating VP played an important role in the elevated MAP response to osmotic stimulation as there were no differences in the VP response to iv stimulation. Furthermore, a VP pressor antagonist had no effect on MAP in the salt-loaded Tg+ rats (unpublished data). With regard to the interactions between VP and AT2 receptors, the data shows no effect of antagonist treatment on osmotic-induced release, in agreement with a previous report.

In summary, two main conclusions result from this study: 1) mRen-2 Tg+ rats exhibit an increased pressor response to intravenous osmotic stimulation, an effect not attributed to VP secretion or a failure in renal excretory capacity. This supports previous findings indicating that the hypertension in the Tg+ model is sensitive to osmotic challenges and 2) AT2 antagonist-treated Tg+ rats show an accentuated pressor response to sodium stimulation, suggestive of a depressive role for AT2 receptors under these conditions.
ACKNOWLEDGMENTS

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