Renal Effects of Captopril and Nitrendipine in Transgenic Rats With an Extra Renin Gene

Claudia Hirth-Dietrich, Johannes-Peter Stasch, Detlev Ganten, Friedrich C. Luft

Abstract We investigated the acute effects of captopril and nitrendipine on renal function and sodium excretion in hypertensive, male, heterozygous transgenic rats harboring a mouse renin gene [TGR(mRen-2)27]. Both drugs reduced blood pressure dose dependently in conscious transgenic rats. The oral ED80 for captopril was 0.5 mg/kg and 2.7 mg/kg for nitrendipine. In orally salt-loaded (20 mL/kg saline) transgenic rats captopril (0.3 to 3.0 mg/kg) reduced sodium excretion by approximately 90% in the 6 hours after administration, whereas equally antihypertensive doses of nitrendipine increased sodium excretion by approximately 100%. The antinatriuretic effect of captopril was accompanied by a reduction in creatinine clearance and a decrease in the excretion of cyclic GMP. In orally water-loaded (20 mL/kg water) transgenic rats captopril also reduced sodium excretion by more than 90%, and nitrendipine slightly increased sodium excretion. In control Sprague-Dawley rats the effects were opposite; namely, captopril tended to increase natriuresis, and nitrendipine caused a small but distinct decrease in sodium excretion. Intravenous captopril in anesthetized transgenic rats caused an antinatriuresis with a decrease in inulin clearance but not in Sprague-Dawley rats. To control for non-renin-related effects of captopril, we gave transgenic rats oral losartan. Losartan also decreased urinary sodium excretion. The results suggest a role for the renin-angiotensin system in the maintenance of glomerular filtration rate and sodium excretion in transgenic TGR(mRen-2)27 rats. (Hypertension. 1994;23:626-631.)

Key Words • captopril • nitrendipine • animals, transgenic • renin • natriuresis

Numerous genetic rat models of hypertension show abnormalities of the renin-angiotensin system. However, primary and secondary changes cannot be easily distinguished in these models, which makes it difficult to establish cause and effect. Transgenic (TG) rats carry an additional mouse renin gene [TGR(mRen-2)27]. They provide a new model of hypertension with a well-defined genetic background. TG rats are characterized by high transcription rates of the transgene in the adrenal glands, high plasma aldosterone concentrations, suppression of renal renin production, and low plasma renin activity. The finding of exaggerated natriuresis both in hypertensive patients with low plasma renin activity but high aldosterone concentrations and in TG rats suggests a clinical relevance for this new hypertension model. We therefore investigated the renal effects of antihypertensive drugs with both renin-angiotensin system–dependent and renin-angiotensin system–independent mechanisms of action in TG rats. In particular, we compared the antihypertensive and renal sodium handling effects of the angiotensin-converting enzyme inhibitor captopril and the calcium antagonist nitrendipine in TG rats. Nitrendipine was chosen because its vasodilator action is independent of the renin-angiotensin system. Both angiotensin-converting enzyme inhibitors and calcium antagonists may serve to protect the kidney from hypertension-induced renal damage, which is a prominent feature in these rats.

Methods

Animals

Experiments were performed in heterozygous male TG rats of the strain TGR(mRen-2)27 and age-matched Sprague-Dawley (SD) rats (Zentralinstitut). The animals were maintained on a standard (1% sodium by weight) diet (Altromin) and drinking water ad libitum. Food but not drinking water was withdrawn in the afternoon (4 PM) before the experiment, which started in the morning (8 AM). Captopril and Nitrendipine in Conscious Rats

Captopril or nitrendipine was suspended in aqueous tylose (0.5%) solution for oral administration. In later experiments three groups of TG rats received 1, 3, or 10 mg/kg oral losartan (DuP 753) in a protocol identical to that described above for captopril and nitrendipine. The oral volume was 5 mL/kg. An additional water loading (20 mL/kg PO tap water) or sodium loading (20 mL/kg PO 0.9% NaCl solution) was performed for the diuresis experiments in conscious rats. The loads were given immediately after drug administration, and the rats were placed into metabolic cages to which they had already become accustomed. They remained in the cages without access to food or drinking water for 6 hours. Because the duration of action is shorter for nitrendipine than for captopril, the 6-hour collection period was subdivided into 0- to 2-hour and 2- to 6-hour periods. Blood was taken from the retro-orbital plexus with rats under ether anesthesia immediately at the end of urine collections for the determination of creatinine clearance. Blood pressure was measured by a tail-cuff method in conscious rats prewarmed to 36°C in thermostatic cages to which they had become accustomed. After determination of the pretreatment value, drugs were administered in 5 mL/kg body wt tylose solution without additional fluid loading, and blood pressure was determined at 1, 2, 4, and 6 hours after gavage.
Intravenous Captopril in Anesthetized Rats

For the inulin experiments rats were anesthetized with 100 mg/kg IP thiopental sodium (Trapanal, Byk-Gulden) for simultaneous measurement of blood pressure and renal electrolyte excretion. Arterial blood pressure was continuously monitored via a femoral artery catheter. Infusions and injections were made through a catheter placed in the jugular vein. A tracheostomy was performed to facilitate spontaneous breathing. Urine was collected with a bladder catheter inserted through a suprapubic incision. After completion of surgery the rats received a priming injection of 5 mL/kg saline (0.9% NaCl) that was followed by a continuous infusion of saline containing 20 mg/mL inulin at a rate of 2.3 mL/h throughout the experiment. The rectal temperature of the rats was kept at 37±1°C with warming lamps.

After surgery rats were allowed an equilibration period of 1 hour before urine collection was begun in preweighed vials. After two control sampling periods lasting 20 minutes each, captopril (0.3 mg/kg) was administered intravenously in a saline solution containing 20% polyethylene glycol 400 (administration volume 1 mL/kg). Controls were injected with vehicle only. Urine was collected for three additional periods lasting 20 minutes (immediately after injection) and 30 minutes, respectively. Blood samples for inulin clearance were obtained 20 minutes before drug administration as well as 20, 50, and 80 minutes thereafter.

Laboratory Parameters

The concentration of cyclic GMP (cGMP) in urine was determined by radioimmunoassay (Immunobiological Laboratories). Urinary sodium and potassium concentrations were determined by flame photometry. Creatinine concentrations in urine and blood were determined according to methods outlined elsewhere.8,9

Statistics

Results are presented as mean±SEM. Significance of drug effects was assessed using the unpaired t test and one-way ANOVA. If not otherwise noted, values were compared with the corresponding control values of the same rat strain. A value of P<.05 was considered significant.

Results

Oral Captopril and Nitrendipine in Conscious Rats

Fig 1A shows the dose-response curves (maximum antihypertensive effect) of captopril and nitrendipine in TG rats. Captopril at 0.1 to 1.0 mg/kg PO dose-dependently reduced blood pressure. The calculated dose for 20% reduction (ED20) was 0.5 mg/kg PO. The dose-response curve for captopril leveled off at a maximal reduction of 60 to 70 mm Hg, which corresponds to systolic blood pressure values of 150 to 160 mm Hg. The dose-response curve for nitrendipine was steeper, and the ED20 was higher (2.7 mg/kg PO) than for captopril. At a dose of 3 mg/kg the antihypertensive effect of both drugs was approximately equal. Fig 1B shows the time course of blood pressure reduction by this dose. The effect of captopril subsided only slightly during 6 hours, whereas the effect of nitrendipine was reduced by approximately 50% 4 to 6 hours after administration.

Fig 2 shows the dose-response curves for the effects of captopril and nitrendipine on sodium excretion in salt-loaded TG rats 0 to 6 hours after administration. Captopril reduced sodium excretion to approximately 10% with doses of 0.3 mg/kg PO or higher. On the other
hand, nitrendipine dose-dependently increased sodium excretion up to 200% compared with controls. The effects on natriuresis became evident at doses (0.1 mg/kg for captopril, 1 mg/kg for nitrendipine) that reduced blood pressure by less than 20 mm Hg.

To further characterize the antinatriuretic effect of captopril, we measured creatinine clearance in sodium-loaded TG rats 0 to 6 hours after the oral administration of 3 mg/kg. Creatinine clearance was 1.47±0.07 mL/min in the control group (n=7) and 0.87±0.10 mL/min (P<.002) in the captopril group (n=8). Thus, the antinatriuretic effect of captopril was accompanied by a marked reduction in glomerular filtration rate. The antinatriuretic effect of captopril was more prominent (almost three times control values after 3 mg/kg PO) 0 to 2 hours after administration than in the second collection period (122% of control).

Fig 3 shows a direct comparison of the effects of both drugs (3 mg/kg PO) in water-loaded SD and TG rats. In SD rats, 3 mg/kg oral captopril did not alter natriuresis in the first 2-hour collection period and even slightly increased natriuresis in the second collection period. In sharp contrast to the effects in control rats, captopril diminished the natriuresis during both collection periods in TG rats. Nitrendipine was antinatriuretic in water-loaded SD rats during the first 2 hours, with no indication of rebound during the following 4 hours. In TG rats nitrendipine was not antinatriuretic during the period of its main antihypertensive effect (0 to 2 hours after administration). Effects on urine volume, potassium excretion, sodium-potassium ratio, and cGMP are shown in Table 1. Captopril reduced urine volume by 50%. On the other hand, nitrendipine almost doubled urine volume. Changes in potassium excretion paralleled changes in urine volume and were less pronounced than changes in sodium excretion. This fact was reflected in the effects on the urinary sodium-potassium ratio. Captopril diminished this ratio in salt-loaded TG rats, whereas nitrendipine significantly increased the sodium-potassium ratio. The excretion of cGMP was higher after salt loading than after water loading. Captopril reduced the high cGMP excretion of sodium-loaded TG rats, whereas nitrendipine slightly increased the low cGMP excretion of water-loaded TG rats. Under both experimental conditions, captopril-treated rats tended to excrete less cGMP than nitrendipine-treated rats.

Table 2 gives the effects of the angiotensin II (Ang II) antagonist losartan (1 to 10 mg/kg PO), which showed a similar pattern of renal effects in TG rats as captopril. Losartan diminished natriuresis but reduced urine volume and kaliuresis only slightly.

**Intravenous Captopril in Anesthetized Rats**

In anesthetized TG rats mean blood pressure averaged 145 mm Hg (systolic blood pressure before injection, 201±6 mm Hg in the vehicle group and 208±10 mm Hg in the captopril group). Vehicle injection transiently increased blood pressure by approximately 15 mm Hg. Intravenous captopril (0.3 mg/kg) significantly

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**Table 1. Effects of Captopril (3 mg/kg PO) and Nitrendipine (3 mg/kg PO) on Urine Volume, Potassium Excretion, Sodium-Potassium Ratio, and Cyclic GMP Excretion 0 to 2 Hours After Administration**

<table>
<thead>
<tr>
<th></th>
<th>Water-loaded TG rats</th>
<th>Sodium-loaded TG rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V, mL/kg</td>
<td>U,KV, μmol/kg</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>14.4±1.4</td>
<td>138±19</td>
</tr>
<tr>
<td>Captopril (n=10)</td>
<td>7.4±1.2*</td>
<td>80±14*</td>
</tr>
<tr>
<td>Nitrendipine (n=10)</td>
<td>27.1±3.1*</td>
<td>323±64*</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>14.5±2</td>
<td>214±23</td>
</tr>
<tr>
<td>Captopril (n=9)</td>
<td>7.5±1.0*</td>
<td>100±15*</td>
</tr>
<tr>
<td>Nitrendipine (n=10)</td>
<td>25.1±2.8*</td>
<td>404±66*</td>
</tr>
</tbody>
</table>

V indicates urine volume; U,KV, urinary potassium excretion; cGMP, cyclic GMP; and TG, transgenic. Rats were aged 22 weeks.

*P<.05 vs corresponding control.
†P<.05 vs corresponding captopril group.
Sprague-Dawley (SD) and transgenic (TG) rats. *P<.05, TG vs vehicle injection caused a slight increase in sodium excretion slightly. After Administration

**TABLE 2. Effects of Losartan (DuP 753) on Urine Volume and Urinary Sodium and Potassium Excretions in Water-Loaded Transgenic Rats 0 to 6 Hours After Administration**

<table>
<thead>
<tr>
<th>Losartan, mg/kg PO</th>
<th>V, mL/kg</th>
<th>(U_{\text{Na}}V), (\mu\text{mol/kg})</th>
<th>(U_{\text{K}}V), (\mu\text{mol/kg})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.1±1.5</td>
<td>239±58</td>
<td>498±78</td>
</tr>
<tr>
<td>1</td>
<td>26.1±0.8</td>
<td>86±32*</td>
<td>558±79</td>
</tr>
<tr>
<td>3</td>
<td>21.9±1.1*</td>
<td>26±7*</td>
<td>475±87</td>
</tr>
<tr>
<td>10</td>
<td>18.9±2.1*</td>
<td>72±32*</td>
<td>380±98</td>
</tr>
</tbody>
</table>

V indicates urine volume; \(U_{\text{Na}}V\), urinary sodium excretion; and \(U_{\text{K}}V\), urinary potassium excretion. n=8 per group. Rats were aged 11 weeks. *P<.05.

Increased blood pressure by 15 to 30 mm Hg compared with the control group. In SD rats captopril had little effect on blood pressure (mean blood pressure, 110±3 mm Hg before captopril and 113±3 mm Hg 50 minutes after captopril).

Fig 4 shows the effects of captopril on sodium excretion in TG and SD rats. Sodium excretion of SD rats remained constant throughout the experiment. Sodium excretion tended to be higher in TG rats during the control periods compared with SD rats and was increased substantially by vehicle injection. Captopril prevented this increase in sodium excretion and diminished natriuresis in TG rats in all observation periods. On the other hand, in SD rats captopril increased sodium excretion slightly.

Fig 5 shows the effects of captopril on inulin clearance in SD rats (A) and TG rats (B). During the control periods the values for TG and SD rats were not different. Vehicle injection caused a slight increase in inulin clearance in SD rats and a significantly greater increase in TG rats. With respect to the vehicle control, captopril slightly reduced inulin clearance in SD rats but caused a pronounced reduction by more than 50% in TG rats.

**Discussion**

Both captopril and nitrendipine effectively lowered blood pressure in TG rats. The dose-response curves for the peak antihypertensive effect were different in slope as well as ED\(_{50}\) (captopril, 0.5 mg/kg; nitrendipine, 2.7 mg/kg) and intersected at a dose of approximately 3 mg/kg PO. However, the drugs had very different effects on renal sodium excretion. Captopril decreased sodium excretion in both water- and sodium-loaded TG rats, whereas nitrendipine increased sodium excretion in sodium-loaded TG rats. To a lesser extent this effect was also noted in water-loaded TG rats.

In normotensive SD rats the effects of both drugs were diametrically opposed. Nitrendipine had a small but significant antinatriuretic effect in water-loaded SD rats, whereas captopril was not antinatriuretic and even caused a slight natriuresis in the late collection period.

The effects of nitrendipine in TG and SD rats can be readily explained by the known natriuretic properties of calcium antagonists that may be counteracted by blood pressure reduction, especially in normotensive rats.10 The effects of captopril in SD rats are also consistent with earlier reports.11 However, the profound antinatriuresis caused by captopril in TG rats was unexpected. The response to captopril may permit some insight into renal sodium handling in this animal model of hypertension, which resembles human hypertension with low plasma renin activity and high plasma aldosterone values. Because the Ang II antagonist losartan also diminished sodium excretion in TG rats, the antinatriuresis appears to be due to inhibition of the renin-angiotensin system in TG rats and cannot be attributed to other effects of captopril.

The antinatriuretic effect of renin-angiotensin system inhibition in TG rats cannot be easily reconciled with the known effects of the renin-angiotensin system. In rats the renal response to angiotensin is biphasic12,13: lower doses stimulate sodium reabsorption and higher doses promote natriuresis. High local Ang II concentrations may have been present in the renal vasculature of TG rats; however, the uniform antinatriuretic response to captopril over a wide dose range would make this explanation appear unlikely. Moreover, both natriuretic and antinatriuretic doses of angiotensin uniformly reduce glomerular filtration rate.12 In our experiments the antinatriuretic effect of captopril was associated with a decreased rather than an increased glomerular filtration rate in TG rats.

The natriuresis caused by large doses of Ang II reverts to an antinatriuresis when increases in renal arterial pressure are prevented.14 It is possible that the reduction of systemic blood pressure caused by captopril in TG rats was responsible for the observed antinatriuresis. However, the antinatriuretic and natriuretic effects of captopril and nitrendipine, respectively, paralleled the antihypertensive effects in relation to both dose and time. Thus, the antinatriuretic effect of captopril cannot be solely attributed to blood pressure reduction.

An explanation for our findings may reside in the renal microcirculation. Nitrendipine causes vasodilatation predominantly in the preglomerular vessels,15 which in turn increases the hydrostatic pressure for glomerular filtration. An increase in hydrostatic filtration pressure...
can also be achieved by postglomerular vasoconstriction. The vasoconstriction exerted by Ang II is more pronounced in postglomerular than preglomerular vessels,16,17 which in turn creates an increased glomerular filtration pressure. Blockade of Ang II by saralasin causes dilatation in the efferent but not the afferent glomerular arteriole.18 Angiotensin-converting enzyme inhibition would therefore result in reduced glomerular filtration pressure, even in the face of high vascular Ang II levels.19

Measurements of whole-kidney glomerular filtration rate gave no indication of hyperfiltration in TG compared with SD rats; however, the reduction in glomerular filtration rate caused by captopril was specific for TG rats. In normotensive sodium-replete animals captopril usually has no effect on glomerular filtration rate, although under conditions of sodium depletion decreases in glomerular filtration rate have been observed.20 In spontaneously hypertensive rats (SHR) renal plasma flow and glomerular filtration rate increased after captopril.21 However, in renovascular hypertension significant decreases in renal plasma flow and glomerular filtration rate were observed after captopril treatment.22 The integrity of the renin-angiotensin system appears critical for the maintenance of renal function under these conditions.

Atrial natriuretic peptide (ANP) represents another natriuretic mechanism that is influenced differently by angiotensin-converting enzyme inhibitors and calcium antagonists. Captopril acutely reduces ANP plasma levels in SD rats23 and humans,24 whereas calcium antagonists may cause a slight increase in ANP plasma levels.25,26 We observed that the excretion of the second messenger cGMP, a specific marker for activation of the ANP system,27 was influenced differently by captopril and nifedipine. Captopril decreased cGMP excretion compared with control in sodium-loaded TG rats, whereas nifedipine slightly increased cGMP excretion in water-loaded TG rats. Under both experimental conditions cGMP excretion tended to be lower in the captopril than the nifedipine group. cGMP excretion was enhanced in sodium-loaded TG rats compared with SD rats,28 consistent with an activation of the ANP system in this strain. Gaillard et al29 reported opposite effects of nifedipine and enalapril on the natriuretic response to ANP in healthy volunteers. ANP-stimulated natriuresis was potentiated by nifedipine and reduced by enalapril. Although the effect of nifedipine seems to depend on the dosage of ANP,29 the two classes of drugs appear to modulate the effects of ANP differently. For instance, nifedipine potentiated ANP effects in rabbits,30 and captopril reduced the natriuretic effects of an ANP infusion in humans.31 However, captopril did not influence the natriuretic effects of ANP in the rat, by either bolus32 or infusion.33 The effects of angiotensin-converting enzyme inhibitors depend in part on renin-angiotensin system activation. After infusion of Ang II the natriuretic response to ANP was enhanced in both normotensive rats34 and healthy volunteers.35 Because the putative tissue renin-angiotensin system appears activated in TG rats, an inhibition of the natriuresis enhancement by Ang II might serve to explain the differential effects of captopril in TG and SD rats.

We did not specifically examine the exaggerated natriuresis of hypertension; however, our data speak indirectly to that issue. Willis36 gave oral 0.9% saline loads (2% body weight) to treated and untreated SHR. He found that the exaggerated natriuresis was independent of blood pressure. Our anesthetized rats received 0.9% saline intravenously at 5 mL/kg, with a maintenance infusion of 2.3 mL/h. This dose was considerably less than that given by DiBona and Rios37 when they showed that the exaggerated natriuresis was related to decreased salt reabsorption in Henle’s loop. Nevertheless, even at this modest dose a prompt exaggerated natriuresis was observed in TG control rats compared with all SD control rats and both treated groups. These data suggest that TG rats handle salt differently than SHR and other previously described models. In humans the exaggerated natriuresis is inversely related to plasma renin activity and directly related to plasma aldosterone values.3 Interestingly, TG rats also have low renin values, increased expression of renin in the adrenal gland, and elevated aldosterone values.2 Salt balance in TG rats appears to be modulated differentially than in previously described models. These differences may well be in part responsible for the findings we
observed. In-depth micropuncture studies will be necessary to elucidate these observations further.

In summary, our experiments indicated that the renin-angiotensin system may closely regulate natriuresis and maintain glomerular filtration rate in TG rats. Blockade of this system with either captopril or losartan was antinatriuretic and decreased glomerular filtration rate. Instead, nitrrendipine enhanced natriuresis in these rats. The chronic effects of these drugs on salt balance, renal function, and structure in TG rats are unknown. They may have relevance on the course of glomerulosclerosis, a prominent feature of TG rats. Further studies are warranted to explore these issues.

References


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Hypertension. 1994;23:626-631
doi: 10.1161/01.HYP.23.5.626

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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