Role of Tissue Renin in the Pathophysiology of Hypertension in TGR(mREN2)27 Rats

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A transgenic rat line, TGR(mREN2)27, was established by introducing the murine Ren-2 gene into the genome of rats by microinjection techniques. These rats exhibit severe hypertension, making them an interesting model in which to study the role of renin in the pathophysiology of hypertension. However, although the additional renin gene is the only genetic difference compared with control rats, the exact mechanism of hypertension in TGR(mREN2)27 rats is still unclear. It cannot be attributed to a stimulation of the endocrine renin-angiotensin system or to an overexpression of renin in the kidney, since plasma and kidney renin and renin gene expression in the kidney are low in these animals. Here we describe recent progress made toward elucidating mechanisms of hypertension in TGR(mREN2)27 rats.

1) TGR(mREN2)27 rats were bred to homozygosity. The development of high blood pressure in homozygous rats is accelerated compared with that of heterozygous rats. This is paralleled by a higher mortality rate in homozygous TGR(mREN2)27 rats. Blood pressure and mortality rate of homozygous transgenic rats were effectively reduced by 10 mg captopril per kilogram body weight. 2) Treatment of 8-week-old heterozygous TGR(mREN2)27 rats with 10 mg/kg body wt per day of the angiotensin II receptor antagonist DuP 753 for 4.5 weeks normalized blood pressure. After withdrawal of the drug, blood pressure increased rapidly, reaching control levels after 3 weeks. In another group of TGR(mREN2)27 rats treated with 0.5 mg/kg per day, there was no change in blood pressure. Plasma renin and plasma angiotensin II were significantly higher in the high-dose group compared with the low-dose group. These data indicate that angiotensin II plays a major role in hypertension of TGR(mREN2)27 rats. 3) Because the activity of the plasma renin-angiotensin system is reduced in TGR(mREN2)27 rats but the pharmacological interventions with captopril and DuP 753 suggest an important role of angiotensin II for hypertension, our interest focused on tissue renin-angiotensin systems. By Northern hybridization, highest transgene expression was detected in the adrenal gland followed by thymus, tissues of the gastrointestinal and genital tracts, kidney, brain, and lung. No expression was found in the liver and submandibular gland. 4) Compared with Sprague-Dawley rats, urinary glucocorticoid and mineralocorticoid excretion was significantly enhanced in TGR(mREN2)27 rats up to an age of 18 weeks, suggesting that corticoids may be involved in the pathogenesis of hypertension in TGR(mREN2)27 rats. Treatment of 4- and 18-week-old TGR(mREN2)27 rats with the mineralocorticoid receptor antagonist spironolactone, however, did not influence blood pressure. The high expression of Ren-2 in the adrenal glands and the corticosteroid excretion analyses point to an important role of a local adrenal renin-angiotensin system in the pathophysiology of hypertension in TGR(mREN2)27 rats.

Key Words • renin-angiotensin system • genetic hypertension • rats, transgenic • spontaneously hypertensive rats • adrenal glands • mineralocorticoids • glucocorticoids • DuP 753 • spironolactone

For more than two decades, hypertensive rat strains like spontaneously hypertensive rats (SHR) have been useful tools for the study of mechanisms involved in the development of hypertension. The value of these models, however, is reduced by the fact that the genetic basis of their hypertension is undefined and therefore currently the subject of intensive investigations. Several candidate genes have been tested by recently developed genetic methods for their contribution to hypertension development in SHR and humans. These also include the renin gene because of the well-established role of the renin-angiotensin system (RAS) in cardiovascular regulation. Recently, we chose another approach to elucidate the effect of this gene on blood pressure regulation by introducing an additional renin gene, the murine Ren-2 gene, into the germ line of rats. Expression of the transgene led to fulminant hypertension in the resulting transgenic rat strain, TGR(mREN2)27. Because, with the exception of a dramatically increased prorenin, all components of the plasma RAS are unchanged or even lowered in these animals, we suggest that local tissue RASs might play a predominant role in the etiology of hypertension in this transgenic animal model. Thus, TGR(mREN2)27 rats exhibiting a defined genetic basis for their hypertension offer the possibility to study the contribution of a single gene to the regulation of blood pressure. In addition,
TGR(mREN2)27 rats appear to represent a useful model system for investigating the functions of tissue RASs. This article describes the recent progress made toward elucidating the mechanism by which the transgene elicits the increase in blood pressure in TGR (mREN2)27 rats.

**Animals**

TGR(mREN2)27 rats heterozygous and homozygous for the Ren-2 transgene and stroke-prone SHR were kept in the animal facility of our institute with free access to standard laboratory diet under alternating 12-hour light/dark cycles. Homozygosity of a transgenic animal was checked by ensuring that all litters of crosses between this animal and Sprague-Dawley rats contained exclusively transgenic animals. Sprague-Dawley rats were obtained from the Zentralinstitut für Versuchstierkunde, Hannover, FRG.

Blood pressure determinations were performed by tail plethysmography during light short-term anesthesia as described previously.

**DuP 753 Treatment**

Groups of four heterozygous TGR(mREN2)27 rats were treated with either 0.5 or 10 mg DuP 753 per kilogram body weight per day by adding the potassium salt of this drug to the drinking water for 4.5 weeks. Then DuP 753 treatment was stopped. Blood pressure recordings were continued for 3 weeks. Blood pressure was determined one time before, four times during, and six times after cessation of treatment. Blood was withdrawn retro-orbitally one time before, after 2 and 4 weeks of treatment, and 3 weeks after cessation of treatment. Plasma prorenin levels were calculated by subtraction of renin activity from total plasma renin activity determined after trypsin activation. Concentrations of renin and angiotensin II (Ang II) were determined as described previously.

**Spironolactone Treatment**

Groups of 10 male heterozygous TGR(mREN2)27 rats, Sprague-Dawley rats, and stroke-prone SHR (age, 18 weeks) received spironolactone in a daily dose of 50 mg/kg body wt per day added in micronized form to normal rat powder chow (Boehringer Mannheim, FRG) for 4 weeks. In a second experiment, 10 male TGR(mREN2)27 rats were given the same dose of the drug directly after weaning at 4 weeks of age for 3 weeks; 10 untreated TGR(mREN2)27 rats served as controls. Blood pressure was measured before and once a week during treatment.

**Northern Blot Analysis**

Total RNA was isolated from tissues by the method of Auffray and Rougeon. For Northern blotting the RNA was denatured in 17 mM NaH2PO4 (pH 7.0), 1 M glyoxal, and 9 mM dimethyl/sulfoxide at 50°C for 50 minutes, cooled on ice, and electrophoresed on a 1.25% agarose gel in 10 mM sodium phosphate (pH 6.8). The RNA was transferred to Nytran nylon membranes by vacuum blotting. The membranes were incubated for 2 hours at 80°C and prehybridized for 4 hours at 62°C in 50% formamide, 5x SSC, 1% sodium dodecyl sulfate (SDS), 5 x Denhardt’s solution, and 200 μg/ml denatured salmon sperm DNA followed by hybridization overnight under the same conditions in the presence of 10% dextran sulfate and 5x10⁶ cpm of a 32P-labeled cRNA probe prepared by transcription of the Ren-2 cDNA clone pSLM with SP6-polymerase in the presence of labeled UTP. Filters were washed for 15 minutes with 2x SSC and 0.1% SDS at room temperature and 2 hours with 0.1x SSC and 0.1% SDS at 65°C followed by autoradiography.

**Statistical Analysis**

Between-group differences in blood pressure and plasma renin and Ang II data were analyzed by the nonparametric Mann-Whitney U test. Statistical analysis was performed by use of standard statistics software (CRUNCH), which was installed on an IBM personal computer. Values of p<0.05 were considered to indicate significance.

**Results**

**Blood Pressure Is Dependent on the Transgene Dose**

The originally described hypertensive TGR(mREN2) 27 rats were heterozygous, i.e., they contained copies of the transgene on only a single chromosome. The line has now been bred to homozygosity. This doubling of the transgene dose led to a further significant increase in blood pressure compared with that of heterozygous animals (Figure 1). The higher blood pressure levels in homozygous rats are paralleled by a higher mortality rate of homozygous TGR(mREN2)27 rats if they are not chronically treated with converting enzyme inhibitors. In a group of 10 homozygous animals, captopril was withdrawn after weaning at 4 weeks of age. Five of these animals had already died before reaching the age of 14 weeks. Captopril (10 mg/kg body wt) lowered...
Influence of the Angiotensin II Receptor Antagonist DuP 753 on Blood Pressure and the Plasma Renin-Angiotensin System

If the activity of the transgene product renin is responsible for the elevation of blood pressure in TGR(mREN2)27 rats, inhibitors of the RAS should markedly reduce hypertension. In fact, we could show that the converting enzyme inhibitor captopril reduces blood pressure to approximately normotensive levels in heterozygous animals.2 This effect could, however, also be caused by the kinin-potentiating activity of this drug. Therefore, we tested the action of the Ang II receptor type I antagonist DuP 7539 on the development of hypertension in 8-week-old heterozygous male TGR(mREN2)27 rats. At this age, blood pressure has not yet reached maximal values and any effect of this drug on the development and maintenance of hypertension should be easily detectable. Two different doses were applied (0.5 and 10 mg/kg body wt). The 10 mg/kg dose was shown to normalize blood pressure in renal hypertensive rats, while doses lower than 1 mg/kg resulted in only slight reductions of mean arterial pressure.10 After 4.5 weeks of treatment blood pressure was normalized in the high-dose group but remained constantly high in the low-dose group, while it increased slightly in the controls (p<0.05 between both treated groups, Figure 2). Then the drug was withdrawn, resulting in rapid increases of blood pressure that reached values of untreated TGR(mREN2)27 rats after 3 weeks (Figure 2).

Plasma renin activity (Figure 3, top panel) was 13-fold and plasma Ang II concentration (Figure 3, bottom panel) was 15-fold elevated in the high-dose group compared with the low-dose group after 4 weeks of treatment (p<0.05) and returned to control values after withdrawal of the drug. Plasma prorenin concentration did not change significantly with DuP 753 (869±302 versus 1,555±722 ng angiotensin I per milliliter per hour, p>0.05).

Tissue-Specific Transgene Expression

The reduction of blood pressure by DuP 753 shows that Ang II plays a major role in the development of hypertension in TGR(mREN2)27 rats. Because the plasma RAS is decreased in activity we suggest that one or several tissue RASs are responsible for the increase in blood pressure. To evaluate which organs might be involved, we investigated the Ren-2 mRNA levels in several tissues of the transgenic rats by Northern blotting. Highest transgene expression was detected in the adrenal gland followed by thymus, parts of the gastrointestinal and genital tracts, kidney, brain, and lung (Figure 4). No expression could be detected in the liver and submandibular gland. Because the rat renin mRNA slightly cross-hybridizes to the Ren-2 probe, we confirmed the results of the Northern blot by a more sensitive and species-specific RNase-protection assay.11 Table 1 shows that, with the exception of the submandibular gland, the expression pattern in TGR(mREN2)27 rats parallels the pattern of the Ren-2 gene expression in DBA/2 mice, from which this gene is originally derived, and is markedly different from rat renin gene expression, e.g., in the liver and adrenal gland.12-15 The lowered expression in the kidney is probably caused by the high blood pressure. These results indicate that the gene is under the correct tissue-specific regulation.

Role of Adrenal Steroids in the Development of Hypertension in TGR(mREN2)27 Rats

The adrenal gland exhibits the highest transgene expression in TGR(mREN2)27 rats and is one of the organs in which the transgene is much more highly expressed than the rat renin gene (Table 1). This points to a stimulated adrenal RAS. Because Ang II is a well-known stimulator of adrenal steroid synthesis, we measured adrenal steroids in the urine of these animals. Compared with control Sprague-Dawley rats, mineral-
ocorticoids as well as glucocorticoids were significantly enhanced in young animals up to an age of 18 weeks but were unchanged in older rats. To evaluate the role of the increased mineralocorticoids in TGR(mREN2)27 rats for the development and maintenance of hypertension, we treated ten 4-week-old and ten 18-week-old animals with the mineralocorticoid receptor antagonist spironolactone. Ten 18-week-old Sprague-Dawley rats and 10 stroke-prone SHR of the same age were treated with the same dose of the drug. Blood pressure was not reduced in adult animals irrespective of the strain from which they were derived (Figure 5, top panel), and there was no difference in blood pressure development in young treated TGR(mREN2)27 rats compared with untreated rats (Figure 5, bottom panel). Thus, spironolactone has no effect on blood pressure development and maintenance in TGR(mREN2)27 rats.

Discussion

Several lines of evidence corroborate the hypothesis that the expression of the Ren-2 transgene in TGR
Table 1. Semiquantitative Comparison of Renin Gene Expression in Several Organs of DBA/2 Mice, Rats, and Adult TGR(mREN2)27 Rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Ren-2 in adult TGR(mREN2)27</th>
<th>Ren-2 in DBA/2</th>
<th>Rat renin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>-</td>
<td>+++++</td>
<td>-</td>
</tr>
<tr>
<td>Adrenal</td>
<td>+++++</td>
<td>+++</td>
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</tr>
<tr>
<td>Testis</td>
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<tr>
<td>Liver</td>
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<td>-</td>
<td>++</td>
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<tr>
<td>Brain</td>
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<td>+</td>
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(mREN2)27 rats are responsible for the dramatic elevation of blood pressure in these animals. First, all founder animals irrespective of their genomic transgene integration site were hypertensive. The only exception was animal 26, which later turned out to be a mosaic; i.e., only part of the somatic cells were transgenic. Therefore it is unlikely that an insertional mutation accounts for hypertension in these rats. Second, all transgene-positive litters of TGR(mREN2)27 rats develop hypertension. Thus there is 100% cosegregation of the transgene and the hypertensive phenotype. Third, inhibitors of the RAS such as converting enzyme inhibitors or Ang II receptor antagonists are able to reduce mean arterial pressure with very high sensitivity (Figure 2). As expected for a renin-dependent hypertension model, even very low concentrations of these drugs have significant antihypertensive effects. The observed upregulation of plasma renin and Ang II concentration during DuP 753 treatment is probably due to the blocked inhibitory feedback by Ang II on the kidney renin secretion or to the decrement in blood pressure, which also regulates renin production by the kidney. The fact that plasma prorenin concentration did not change significantly may indicate that it is not subject to these regulations, probably because in these rats it is not mainly produced by the juxtaglomerular cells of the kidney but by adrenocortical cells and uses secretory pathways different from those of active renin.

Because the activity of the plasma RAS is not elevated in TGR(mREN2)27 rats, we hypothesized that tissue RASs are involved in the hypertensive process. Our interest first focused on the adrenal gland, because this tissue contains very high concentrations of Ren-2 mRNA (Figure 4). This probably leads to an increased local Ang II level and thereby causes the observed stimulation of adrenal steroid synthesis. We are currently investigating the regulatory mechanisms involved in this process by in vitro experiments with isolated adrenal cells.

The increased levels of mineralocorticoids measured in the urine of TGR(mREN2)27 rats during the phase of developing hypertension may be causative for the increase in blood pressure. The mineralocorticoid receptor antagonist spironolactone, however, had no effect on development and maintenance of hypertension in these transgenic animals. The side effects of this substance might obscure the receptor antagonistic effect and prevent the lowering of blood pressure. It was shown that spironolactone changes the activity of several steroid-metabolizing enzymes in the adrenal cortex and stimulates the plasma RAS. We also observed an activated plasma RAS and enhanced concentrations of urinary corticosterone and aldosterone in the treated animals. Thus, the role of the mineralocorticoids in the etiology of hypertension in TGR(mREN2)27 rats is still unresolved and is the subject of investigation in our laboratory.

Glucocorticoids or other hypertensinogenic steroids may also be involved in the development of hypertension in TGR(mREN2)27 rats. In addition, the activation of other tissue RASs such as in the brain, in the walls of resistance vessels, or in the gastrointestinal tract might be involved in this process. All these hypotheses have to be evaluated by experiments that are currently in progress.

In summary, we consider TGR(mREN2)27 rats to be a useful model in which to study the role of local RASs in different organs in physiological and pathophysiological...
ical mechanisms like adrenal steroid synthesis and hypertension. Up to now, however, we have not clearly defined the way by which the transgene elicits the increment in blood pressure in these animals.

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doi: 10.1161/01.HYP.19.6.681

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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