Late-Onset Endothelin-A Receptor Blockade Reduces Podocyte Injury in Homozygous Ren-2 Rats Despite Severe Hypertension

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Abstract—We have recently found in male homozygous hypertensive Ren-2 transgenic rats (TGRs) fed a high-salt diet that early onset selective endothelin (ET) A (ET\textsubscript{A}) or nonselective ET\textsubscript{A}/ET B (ET\textsubscript{B}) receptor blockade improved survival rate and reduced proteinuria, glomerulosclerosis, and cardiac hypertrophy, whereas selective ET\textsubscript{A} receptor blockade also significantly attenuated the rise in blood pressure. Because antihypertensive therapy in general is known to be more efficient when started at early age, our study was performed to determine whether onset of ET receptor blockade at a later age in animals with established hypertension will have similar protective effects as does early-onset therapy. Male homozygous TGRs and age-matched normotensive Hannover Sprague–Dawley rats were fed a high-salt diet between days 51 and 90 of age. TGRs received vehicle (untreated), the selective ET\textsubscript{A} receptor blocker atrasentan (ABT-627), or the nonselective ET\textsubscript{A}/ET\textsubscript{B} receptor blocker bosentan. Survival rates in untreated and bosentan-treated TGRs were 50% and 64%, respectively, whereas with atrasentan, survival rate of TGR was 96%, thus, similar to 93% in Hannover Sprague–Dawley rats. From day 60 on, systolic blood pressure in atrasentan-treated TGRs was transiently lower (P<0.05) than in untreated or bosentan-treated TGRs. Glomerular podocyte injury was substantially reduced with atrasentan treatment independent of severe hypertension and strongly correlated with survival (P<0.001). Our data indicate that in homozygous TGR ET receptors play an important role also in established hypertension. Selective ET\textsubscript{A} receptor blockade not only reduces podocyte injury and end-organ damage but also improves growth and survival independently of hypertension. (Hypertension. 2006;48:965-971.)

Key Words: endothelin-1 $\bullet$ ET\textsubscript{A} and ET\textsubscript{B} receptors $\bullet$ bosentan $\bullet$ atrasentan (ABT-627), homozygous transgenic Ren-2 rats $\bullet$ hypertension $\bullet$ end-organ damage $\bullet$ survival rate

The hypertensive rat strain transgenic for the mouse Ren-2 renin gene ([TGR] strain name TGR[mRen2][27]) is a valuable monogenetic model of renin-dependent and thus angiotensin II (Ang II)–dependent hypertension, which exhibits typical signs of fulminant hypertension, that is, reduced glomerular filtration rate and proteinuria associated with glomerulosclerosis. Moreover, it carries a salt-sensitive component.

Endothelin (ET)-1 is known to be one of the most powerful vasoconstrictors and also a mitogen in vivo and in vitro. The beneficial effects of ET receptor blockers in modulating target organ damage are attributed to their antiproliferative actions. Numerous studies have shown that the ET system plays an important role in the pathogenesis of high blood pressure (BP) in salt-sensitive models of hypertension and in associated end-organ damage. For the detrimental effects of ET-1 in the development of hypertension, activation of ETA (ET\textsubscript{A}) receptors may be responsible, whereas the role of ET B (ET\textsubscript{B}) receptors may be the mediation of peripheral vasorelaxation and the renal tubular natriuresis. Nonselective blockade, therefore, inhibits not only the deleterious effects of ET-1 mediated by ET\textsubscript{A} receptors but also concomitantly blocks its antihypertensive effects mediated by ET\textsubscript{B} receptors. However, because at present only conflicting data regarding selective ET\textsubscript{A} and nonselective ET\textsubscript{A/B} receptor blockade are available, the relative beneficial effects of selective versus nonselective ET receptor blockade remain to be elucidated.

Several lines of evidence indicate that Ang II stimulates the release of ET-1, and it is known that the ET system plays an important role in the pathogenesis of hypertension and accompanying end-organ damage in salt-dependent and in Ang II–dependent models of hypertension induced by exogenous administration of Ang II.

Dietary sodium plays an important role in the pathogenesis of hypertension not only in humans but also in salt-sensitive

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models of hypertension. Thus, on one hand, the increased sodium intake exerts detrimental cardiac effects and leads to dysfunction of vascular endothelium, which, through the release of ET-1, contributes to vascular changes found in salt-sensitive hypertension. On the other hand, selective ET$_A$ receptor blockade lowers BP predominantly in salt-dependent models of hypertension. Moreover, a growing body of evidence shows that on high salt intake there is a substantial impact of ET$_B$ receptors in promoting higher sodium excretion from the body.

Proteinuria may be caused by defects of podocytes that maintain intact filtration barrier and control glomerular basement membrane (GBM) turnover under normal conditions, of the endothelial cells, and of the GBM itself. Podocytes are reported to be injured in many types of proteinuric renal diseases, including nephrotic syndrome, diabetic nephropathy, and lupus nephritis. Only a few studies explored the involvement of podocyte damage in experimental hypertensive glomerulopathy.

In our previous study, we have shown that early treatment both with bosentan, a nonselective ET$_A$/ET$_B$ receptor antagonist, and with atrasentan, a selective ET$_A$ receptor blocker, improved survival rate and ameliorated end-organ damage in homozygous male TGRs fed a high-salt diet (HS), but only atrasentan attenuated the rise in BP. It is well known that young animals are more susceptible to various hypertensive stimuli than adult animals; therefore, interventions made in early life are usually more effective.

Thus, in the present study, our objective was, first, to evaluate whether selective ET$_A$ or nonselective ET$_A$/B receptor blockade in homozygous TGRs on a high-salt regimen will have similar protective effects on survival, end-organ damage, and BP when started in adult rats with established hypertension as when applied at an early stage before BP had risen and, second, whether selective ET$_A$ receptor blockade is superior to nonselective ET$_A$/ET$_B$ receptor blockade under these experimental conditions.

Methods

The protocols in the present study are in adherence to the Guide for the Care and Use of Laboratory Animals and were approved by Czech Animal Care and Use Committee (protocols 79/2001 and 923/2003).

Animals

Homozygous male TGRs and their normotensive Hanover Sprague–Dawley control rats (HanSD) were housed at 25°C under a 12 hour light/dark cycle and had free access to chow and water. All of the animals used in this study were bred at the Center for Experimental Medicine of the Institute for Clinical and Experimental Medicine from stock animals supplied from the Max Delbrück Center for Molecular Medicine (Berlin, Germany).

Experimental Design

At the age of 51 days, HanSD rats and 3 groups of TGRs were placed on a HS diet (2% NaCl). At the same time, 1 group of TGRs received no drug (untreated TGRs), the 2 other groups of TGRs received treatment with either the nonselective ET$_A$/ET$_B$ receptor blocker bosentan (Actelion) or the selective ET$_A$ receptor blocker atrasentan (ABT-627; Abbott). Bosentan was mixed into the diet in an amount depending on the actual food intake to achieve a final consumption of 100 mg kg$^{-1}$ day$^{-1}$. This dose was validated previously in our laboratory to effectively block ET$_A$ receptors. Atrasentan was added to the drinking fluid; the dose was adjusted weekly to provide a dose of 5 mg kg$^{-1}$ day$^{-1}$, which is accepted and confirmed by various groups of investigators to effectively block ET$_A$ receptors. The following experimental groups were investigated:

1. male HanSD rats + HS (n=14); 2. male TGR + HS, untreated (n=18); 3. male TGR + HS + bosentan (n=14); and 4. male TGR + HS + atrasentan (ABT-627; n=24).

Determination of BP, Protein Excretion, and Tissue Weight

From the age of 32 days on, rats were weighed and systolic BP (SBP) was measured once weekly by the tail-cuff method validated previously in our laboratory. At the age of 50 and 80 days, animals were individually housed in metabolic cages, and measurements of fluid consumption and urine excretion, as well as protein excretion, were monitored over 24 hours. Urinary protein determination was performed using the biuret method (Lachema).

On termination of the experiment (day 90), animals were weighed and anesthetized with thiopental sodium (50 mg kg$^{-1}$), and mean arterial pressure (MAP) was monitored directly in the carotid artery using the data acquisition system PowerLab (ADInstruments). Kidneys and hearts were rapidly removed and weighed. Ratios of kidney weight (KW)/body weight (BW) and heart weight (HW)/BW were used as indices of organ hypertrophy.

Determination of Tissue ET-1 Concentrations

After dissection on ice-cold plates, tissues from the left cardiac ventricle and right kidney cortex were immediately frozen in liquid nitrogen for ET-1 determination by ELISA (Amersham).

Histological Examination

The left kidney was quickly removed, fixed in 4% buffered formaldehyde, dehydrated, and embedded. Paraffin sections were stained with hematoxylin/eosin and periodic acid–Schiff reaction and examined using a Nicon Eclipse E 600 light microscope. Slides were evaluated in a blind fashion. As described previously, 100 glomeruli per section were selected randomly, and the degree of glomerular damage was evaluated using a semiquantitative scoring method (grade 0: normal glomeruli; grade 1: sclerotic area ≤25% of total glomerular area or distinct adhesion present between capillary tuft and Bowman’s capsule; grade 2: sclerotic area between 25% and 50%; grade 3: sclerotic area 50% to 75%; and grade 4: sclerotic area 75% to 100% of total glomerular area). The glomerulosclerosis index was calculated using the following formula: glomerulosclerosis index = (1×n$_1$) + (2×n$_2$) + (3×n$_3$) + (4×n$_4$) + n$_5$, where n$_i$ is the number of glomeruli in each grade of glomerulosclerosis.

Electron Microscopy and Measurement of GBM Thickness

Small portions of the renal cortex of formalin-fixed and paraffin-embedded kidneys were selectively removed and used for electron microscopic examinations. Two animals from each group were examined. Samples were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. Sections were examined using a Philips EM 280 /Morgagni transmission electron microscope (FEI Company). Evaluations were performed by 2 independent observers in a blinded fashion. Thickness of the GBM was measured in 5 glomeruli per rat. The average of 50 measurements per glomerulus was taken. The perpendicular distance from the endothelial cell boundary to the epithelial cell boundary of the peripheral basement membrane was measured. Areas of wrinkled GBM and bevelled sections were excluded. The GBM measurements were taken using Analysis version 3.2 (Build 765 GmbH).

Statistical Analysis

Statistical analysis of data were performed using Graph-Pad Prism software (Graph Pad Software). Group comparisons were determined by 2-way ANOVA. Statistical comparisons of the results obtained for HWs and KWs and for ET-1 concentrations were made by 1-way ANOVA. The relationship between podocyte damage and...
survival rate was evaluated using least-squares linear regression analysis. Unless otherwise stated, values are expressed as mean±SEM, and "n" represents the number of animals. A P value <0.05 was considered significant.

Results

Systolic and Mean Arterial BP
In HanSD rats, SBP remained within the normotensive range throughout the whole experimental period (Figure 1A). In the 3 groups of TGRs, SBP rose gradually after weaning until the age of 46 days. Thus, just before changing to a high salt intake on day 51, SBP was 198.1±6.1 mm Hg in subsequently untreated rats, 201.0±5.4 mm Hg in rats subsequently treated with bosentan, and 192.0±3.8 mm Hg in rats subsequently treated with atrasentan. At the age of 60 days, SBP was 198.6±5.5 and 209.2±5.6 mm Hg in untreated and bosentan-treated TGRs, respectively, whereas treatment with atrasentan resulted in a significantly lower SBP (173.9±4.3 mm Hg; P<0.05) but did not reach SBP of HanSD rats (151.3±5.5 mm Hg). At day 81 of age, SBP was 151.3±5.2 mm Hg in HanSD rats and 210.8±3.4, 210.7±8.5, and 200.0±5.2 mm Hg in the surviving untreated, bosentan-treated, and atrasentan-treated TGRs, respectively.

At the end of the experiment on day 90, MAP in the surviving rats was not different among HanSD, bosentan-treated, and atrasentan-treated TGRs (153.6±6.5, 159.3±10.5, and 165.3±5.2 mm Hg, respectively). In untreated TGRs, MAP of 200.7±18.9 mm Hg was higher but not significantly different from the other groups of TGRs.

Survival Rate
Survival rate in HanSD rats was 93%, and in atrasentan-treated TGRs it was 96%, being significantly different from untreated and bosentan-treated TGRs (P<0.01; Figure 1B), which started to die progressively from days 60 and 53 of age on, respectively. Survival rates were 50% and 64%, respectively, on termination of the experiment.

Protein Excretion
There were no significant differences in protein excretion between the TGR groups at the age of 50 days before treatment (Figure 2A). At the age of 80 days, untreated TGRs had significantly greater proteinuria than HanSD rats (32.95±2.0 versus 13.75±2.22 mg of protein per 24 hours; P<0.05; Figure 2B). Both bosentan and atrasentan decreased protein excretion to Figure 1. SBP (A) and survival rates (B) during the course of the experiment in homozygous male Ren-2 TGRs (+/+) on high salt intake (HS). #P<0.01 vs unmarked values, P<0.05; *P<0.05 atrasentan (ABT-627) vs untreated and bosentan-treated TGRs.

Figure 2. Protein excretion at the age of 50 days before treatment (A) and at the age of 80 days (B) in homozygous male Ren-2 TGRs (+/+) on high salt intake (HS) during treatment. *P<0.05 vs normotensive HanSD (−/−); #P<0.05 vs untreated TGRs.
that found in control HanSD rats (14.70±2.75 and 21.06±1.38 mg of protein per 24 hours, respectively; *P* value not significant).

**BW**

During the study, HanSD rats weighed statistically more than each group of TGRs. Among the TGR groups, BWs of untreated TGRs were the lowest with reduced weight gain starting on day 39 of age. By day 90 of age, BW of HanSD rats (354.3±8.2 g) was significantly (*P* 0.01) higher than that of untreated and bosentan-treated TGRs (227.6±18.1 and 240.0±10.1 g, respectively). In atrasentan-treated TGRs, it was higher than in the other groups of TGRs (310.7±5.7 g; *P* 0.01) but did not reach that of HanSD rats.

**HWs and KWs**

In untreated TGRs, the ratio HW/BW was 4.15±0.08 as compared with 3.13±0.09 in HanSD rats (*P*<0.01), but its increase was significantly attenuated by bosentan or atrasentan treatment (3.67±0.04 and 3.61±0.06, respectively; *P*<0.01). Similarly, the ratio of left ventricular weight to BW was 3.08±0.09 in untreated TGRs versus 2.21±0.08 in HanSD rats, and its rise was significantly attenuated by bosentan or atrasentan treatment (2.65±0.04 and 2.58±0.06, respectively; *P*<0.01). There was no difference in the ratios of KW/BW among all of the TGR groups of rats at the end of the experiment (4.74±0.18, 4.62±0.22, and 4.11±0.15 in untreated, bosentan-treated, and atrasentan-treated TGRs, respectively).

**Glomerulosclerosis Index**

At the end of the experiment, we did not find statistically significant differences in the glomerulosclerosis indices between HanSD rats (0.149±0.016) and the surviving untreated (0.152±0.024), bosentan-treated (0.140±0.018), and atrasentan-treated TGRs (0.160±0.022). Only mild changes of renal parenchyma and mild hyalinosis of afferent arterioles were observed in all 4 groups of rats. Glomerular involvement ranged from slight basement membrane wrinkling to mild focal mesangial expansion. Total glomerular collapse was present only sporadically.

**Ultrastructural Examination and GBM Measurement**

The only major morphological differences between the groups were seen in the thickness of GBM and in podocyte alterations. As compared with HanSD rats (Figure 3A), TGRs fed a HS diet showed the most striking glomerular changes on the ultrastructural level; that is, irregular thickening, partial disintegration, and focal wrinkling of the GBM were found. The foot processes of podocytes seemed wider than normal and revealed patchy fusion. Sporadically, podocytes contained electron dense granules and lipid droplets in their basal parts (Figure 3B). TGRs treated with bosentan showed only moderate podocyte abnormalities; fusion of foot processes was nearly absent. Foot processes seemed to be thinner and longer. The appearance of lipid droplets and vacuoles within the podocyte cytoplasm was not prevented by bosentan (Figure 3C). Podocytes of atrasentan-treated animals showed a normal structure and an orderly arrangement (Figure 3D) and resembled those of control HanSD rats. A strong correlation (*P*<0.001) between podocyte injury (x=GBM width) and survival rate (y) in rats from all 4 groups has been found (*y*=168−0.69 *x*; *r*²=0.7295; *P*<0.0001).

The width of the GBM on electron microscopic sections in untreated (157.9±2.0 nm) and bosentan-treated (148.6±2.0 nm) TGRs was significantly greater than in HanSD rats (111.0±0.9 nm). We did not find significant differences between atrasentan-treated TGRs (115.2±1.1 nm) and HanSD rats.
The effects of pharmacological interruption of the ET system have not been studied in homozygous Ren-2 transgenic rats at a time when arterial hypertension is firmly established. In the present study we, therefore, addressed 2 questions: (1) whether ET receptor blockade has a similar protective effect on hypertension, end-organ damage, and survival in homozygous TGRs on a high salt intake when started at a later stage in life as when started immediately after weaning; and (2) whether nonselective ET_{A/B} or selective ET_{A} receptor blockade was more effective.

It is generally accepted that increased dietary sodium intake causes hypertension in salt-sensitive humans and animals and induces endothelial dysfunction through enhanced ET-1 production, which may be stimulated by Ang II. Although increased sodium intake should decrease Ang II production, our previous experiments have shown that it does not decrease plasma or kidney Ang II levels in conscious male TGRs between 32 and 90 days of age. Thus, we conclude that the activated circulating and tissue renin–angiotensin system in conscious TGRs is not modulated by or is relatively unresponsive to changes in sodium balance. We also suggest that in TGRs, the enhanced plasma and kidney Ang II would increase ET-1 production, which could be one of the potential mechanisms contributing to the development of hypertension and/or organ damage in TGRs.

We have demonstrated recently that treatment both with the unselective ET_{A/B} receptor blocker bosentan or with the selective ET_{A} receptor blocker atrasentan, when started immediately after weaning, markedly improved survival rates of homozygous TGRs and that atrasentan but not bosentan, in addition, induced a significant decrease in BP. Also, selective ET_{A} blockade had substantial nephroprotective and cardioprotective effects that were superior to unselective receptor blockade. Our present findings are partly compatible with the results of our previous study. ETA receptor blockade with atrasentan substantially prolonged survival, improved BW, and, in contrast to nonselective blockade with bosentan, also transiently decreased SBP. This only temporary decrease of BP is surprising, because the dose used is generally accepted as sufficient to block ETA receptors. Both atrasentan and bosentan partially reduced cardiac hypertrophy and normalized protein excretion. The most important difference from our previous findings is that, in the present study, bosentan was much less effective in reducing the mortality rate than atrasentan. However, this finding is in accordance with previous results of Rothermund et al who found no improvement of survival in heterozygous Ren-2 rats receiving a nonselective ET_{A/B} receptor blocker. This lack of effect may be related either to the later onset of drug treatment and/or, more likely, the later introduction of the high-salt regimen, which resulted in a lesser rise in tissue ET-1 concentration. The greater efficacy of selective ETA receptor than nonselective ET_{A/B} receptor blockade in improving survival rate may have resulted rather from its antiproliferative than from its antivasoconstrictor actions. There exist 2 types of ET_{B} receptors, namely, ET_{B1} and ET_{B2} receptors, mediating either vasodilation or vasoconstriction, respectively. Our results favor the possibility that vasoconstrictory ET_{B2} receptors may play a less important role in BP regulation, because concomitant blockade of ETA and ET_{B2} receptors did not lower BP in contrast to the effect of the ETA antagonist, which suggests a substantial effect of vasoconstrictory ET_{B1} receptors. However, conflicting results concerning this issue have been obtained. Although Matsumura et al found in deoxycorticosterone acetate salt rats that selective ET_{B} blockade has no vasomotor effect, referring to the negligible impact of both vasodilatory ET_{B} receptors on endothelium.

**Figure 4.** ET-1 concentrations in kidney cortex (A) and in left heart ventricle (B) in HanSD rats (−/−) and in homozygous male Ren-2 TGRs (+/+) on high salt intake (HS) without and with treatment with bosentan or atrasentan (ABT-627).  *P<0.05 vs normotensive HanSD (−/−); #P<0.05 vs untreated TGRs (+/+).
and vasoconstrictory ET<sub>B</sub> receptors on vascular smooth muscle cells, a major role for vasodilatory ET<sub>B</sub> receptors was found in hamsters<sup>30</sup> rat<sup>31</sup> and humans,<sup>32</sup> where their blockade resulted in hypertension.

Although our TGR developed strong proteinuria, on light microscopy only moderate alterations in the renal parenchyma were observed in TGRs. By electron microscopy, however, we found significant morphological differences between the experimental groups. They included thickening of the GBM and degenerative changes of podocytes. We found that treatment with atrasantan normalized the thickness of the GBM and reduced the extent of podocyte alterations. Moreover, there was a strong correlation between podocyte injury and survival, showing a great dependency of proper podocyte function on further survival of the animals. Our results are in agreement with studies of Barton et al.,<sup>33</sup> which have shown positive effects of selective ETA receptor blockade on vascular structure. Similar results were shown by Ortmann et al.<sup>34</sup> in aged Wistar rats in vivo. Our findings are also compatible with those of Matsumura et al.,<sup>29</sup> who found with ETA receptor blockade a reduction of the histopathologic changes induced by the deoxycorticosterone acetate salt regimen and support their suggestion that ETA receptor blockade has beneficial structural effects on GBM thickness and the extent of glomerulosclerosis and proteinuria. Podocytes seem, therefore, to be target cells for ET-1 as shown previously by Rebibou et al.<sup>35</sup> However, the exact mechanism(s) leading to the reduction of proteinuria by ETA receptor blockade are not yet known. On one hand, a plausible explanation for the discrepancy between undetectable glomerulosclerotic changes despite increased proteinuria may be that protein excretion rather correlates with the ultrastructural changes that precede the light microscopic changes. This is supported by the findings of Boffa et al.,<sup>36</sup> who found glomerulosclerotic changes not before 4 weeks’ duration of hypertension induced by NO deficiency. Moreover, one cannot exclude the possibility that markedly affected animals died before the end of the experiment, and only slightly affected animals survived. Although we did not find a significant proteinuria at the age of 50 days, Springgate et al.<sup>37</sup> found increased albuminuria already at the age of 2 months in heterozygous TGRs; unfortunately, they did not evaluate the presence of glomerulosclerosis at this time point. On the other hand, the effects of angiotensin-converting enzyme inhibitors cannot be attributed to measurable changes in ultrastructural components of the capillary wall but may rather be related to changes in intrinsic functional properties as shown by Macconi et al.<sup>38</sup> We suppose that pathologic changes of podocytes and of the GBM occur early in the natural course not only of diabetic nephropathy<sup>17</sup> but also of hypertension and may play an important role in microalbuminuria and macroalbuminuria. One mutual trigger mechanism for these events seems to be redox stress.<sup>17</sup> Our findings are in agreement with Nagase et al.,<sup>18</sup> who reported immunohistochemically first podocyte impairment after 2 weeks during salt loading in Dahl-sensitive rats when proteinuria was only moderately increased. Ultrastructural changes appeared 3 weeks later. These early ultrastructural changes may bear an important role in the long-term remodeling process of glomerulosclerosis. The close association between podocyte injury and proteinuria suggests that podocyte impairment underlies proteinuria and glomerulopathy in hypertensive homozygous Ren-2 rats. It is postulated that mechanical and oxidative stress are key mediators of podocyte damage.<sup>15</sup>

It is of further interest that TGRs in the present study revealed lower concentrations of ET-1 both in the renal cortex and in the left ventricle than in our previous study.<sup>24</sup> The most plausible explanation for this phenomenon may be that in the present study, TGRs developed a milder form of hypertension and hypertension-related damage because of later onset of high salt intake. This is in line with the results of Whitworth et al.,<sup>39</sup> who found significantly increased prepro–ET-1 mRNA expression only in animals with severe hypertension. Interestingly, ETA blockade strongly lowered ventricular tissue ET-1 content probably because of displacement of ET-1 from the predominating ETA receptors in this tissue in contrast to the renal tissue with predominant ET<sub>B</sub> receptors. Also, in another hypertensive rat model, namely, in the Dahl salt-sensitive rat, Barton et al.<sup>33</sup> demonstrated a decrease of ET-1 protein after treatment with the ETA blocker. Renal ET-1 concentration was not affected by selective ETA or nonselective ET receptor blockade probably because, in this organ, ET<sub>B</sub> receptors dominate, and decreased clearance of ET-1 through ET<sub>B</sub> receptors may compensate for the displacement of ET-1 from ETA (and ET<sub>B</sub>) receptors.

Collectively, in the present study BP, protein excretion, glomerulosclerosis index, HW, and tissue ET-1 content in adult TGRs were all lower than in the young TGRs of our preceding study.<sup>19</sup> These findings are compatible with the idea that young animals are more susceptible to hypertensive stimuli<sup>20</sup> (eg, HS intake) and may develop more severe hypertension and hypertension-related end-organ damage and confirm previous suggestions that the ET system is involved in hypertension and hypertension-related organ damage in TGRs on a high salt intake. Most importantly, our results also show that ultrastructural changes of podocytes precede light microscopic histological disturbances and closely correlate with survival rate. They can, therefore, serve as marker of future injury long before manifestation of proteinuria. Chronic selective ETA blockade starting at the time of established hypertension in homozygous TGRs on a high salt diet proved to have substantial protective effects on survival rate and growth with a transient attenuation of the rise in BP.

**Perspectives**

This study provides further evidence that selective ETA receptor blockade is preferential to nonselective ET receptor blockade and implies the possibility of using ultrastructural changes of podocytes as a possible prognostic marker of renal injury.

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Disclosures
None.

References
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