Blockade of Angiotensin II Type 1 Receptor and Not of Endothelin Receptor Prevents Hypertension and Cardiovascular Disease in Transgenic (mREN2)27 Rats via Adrenocortical Steroid–Independent Mechanisms

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Abstract—We investigated the role of angiotensin II (Ang II) and endothelin-1 (ET-1) in transgenic (mREN2)27 rats, a model of the monogenic renin-dependent form of severe hypertension and cardiovascular disease. Four-week-old heterozygous male transgenic (mREN2)27 rats (n=24) were matched according to body weight (BW) and blood pressure (BP) and randomly allocated to receive a placebo (group P), the mixed endothelin type A and B receptor antagonist bosentan (100 mg/kg BW PO, group B), the Ang II type 1–specific receptor antagonist irbesartan (50 mg/kg BW PO, group I), or the endothelin type A–selective antagonist BMS-182874 (52 mg/kg BW PO, group BMS). After 4 weeks of treatment, during which BW and BP were measured weekly, animals were euthanized, and the heart, left ventricle, right ventricle, adrenal gland, brain, and kidney were weighed. The plasma levels of adrenocortical steroids were measured by high-performance liquid chromatography. The tension responses of ET-free segments of the thoracic aorta to 5×10^{-6} mmol/L phenylephrine, 60 mmol/L KCl, and cumulative doses of ET-1 were assessed. The density of ET-1 receptor subtypes in the aorta and vascular structural changes in the mesenteric arterioles (100 to 200 μm ID) were also measured with autoradiography and myography, respectively. Compared with all other groups, group I rats showed significantly (P<0.001) lower systolic BP (group I, 161±6 mm Hg; group P, 269±6 mm Hg; group B, 275±17 mm Hg; and group BMS, 254±21 mm Hg), left ventricular weight (2.28±0.15 versus 3.71±0.26, 3.38±0.27, and 3.96±0.51 mg/g BW, respectively), tension responses to vasoconstrictors, and normalized media thickness of the mesenteric arterioles (22.3±0.6 versus 25.3±0.5, 25.5±0.7, and 24.1±1.5 μm, respectively). Compared with levels in group P (78±25 pmol/mL), plasma aldosterone levels were significantly decreased in group B (51±11 pmol/mL) and group I (40±16 pmol/mL). Thus, endogenous ET-1 and Ang II contribute to the regulation of aldosterone, but only Ang II is crucial for the development of hypertension and related target organ damage via the Ang II type 1 receptor. Endogenous Ang II does not appear to enhance cardiovascular production of ET-1 in this model of hypertension within the time span of our experiment. (Arterioscler Thromb Vasc Biol. 2000;20:949-956.)

Key Words: hypertension ■ angiotensin ■ target organ damage ■ endothelin ■ receptor antagonists

The very potent vasoconstrictor endothelin-1 (ET-1) plays an important role in different forms of arterial hypertension (see Reference 1 for a review). Molecular data and in vitro and in vivo findings2–4 have indicated that angiotensin II (Ang II) can turn on transcription of the preproET-1 gene and biosynthesis of ET-1 in different cell types, including cultured vascular smooth muscle cells (VSMCs)5–7 and endothelial cells (ECs),8 by acting on Ang II type 1 (AT1) receptors linked to activation of transcription via activator protein-1/protein kinase C–mediated mechanisms.7–11 ET-1 might be involved in mediating renin-dependent hypertension and also cardiovascular damage (CVD), because it was found to contribute to the hypertrophic response to Ang II.10–14 However, data concerning the interactions between Ang II and ET-1 in hypertension are conflicting (see Reference 4 for a review). Studies in rats with Ang II infusion and administration of bosentan suggested that ET-1 could importantly contribute to renal and systemic vasoconstriction and thus to arterial hypertension.15,16 Bosentan was also reported to prevent the increase in heart weight, albuminuria, and carotid medial thickness (MT) induced by exogenous Ang II, thereby suggesting a participation of ET-1 in hypertension-related
CVD. However, only a modest or no blood pressure (BP)-lowering effect of a mixed endothelin type A and B (ETa/ETb) receptor antagonist in a rat model of renovascular hypertension was seen.26 Thus, although exogenous Ang II infusion stimulates ET-1 production in the vasculature and thereby induces an ET-1-dependent elevation of BP and vascular hypertrophy, paradoxically, there seems to be only a modest or negligible ET-1-dependent component in models of hypertension due to enhanced endogenous Ang II generation.1

The transgenic (mREN2)27 rat (TGR) is a model of severe arterial hypertension created by the insertion into the rat genome of the mouse REN-2 gene coding for renin in the submaxillary gland.23 Being characterized by overexpression of the transgene in tissues, such as the adrenal cortex and the arterial wall,3,4,12–14,22 the TGR is a paradigm of endogenous Ang II–dependent hypertension suitable for investigating this paradox. Furthermore, because it shows vascular hypertrophy in excess of that expected on the basis of BP elevation,26 the TGR is a useful model in which to test the effects on CVD of pharmacological blockade of different pressor systems.

It has also been proposed that Ang II–driven aldosterone oversecretion could contribute to raising BP in this model, although this contention remains controversial.27 Limited and conflicting information on the role of ET-1 in TGR is in fact available, and there are no data on its effect on aldosterone. Male HanRen2/Edin rats, derived from crossing homozygous TGRs with Edinburgh Sprague-Dawley rats, quite commonly develop (74% incidence) the malignant phase of hypertension, which is associated with an increased aldosterone. Male HanRen2/Edin rats, derived from crossing homozygous TGRs with Edinburgh Sprague-Dawley rats, quite commonly develop (74% incidence) the malignant phase of hypertension, which is associated with an increased aldosterone.

To this end, we have compared the effect of the AT1 receptor–selective antagonist BMS-182874 15 and of the mixed ETa/ETb receptor antagonist bosentan14 on BP and survival of the TGR is a useful model in which to test the effects on CVD of pharmacological blockade of different pressor systems.

Thus, we investigated the relative roles of Ang II, ET-1, and adrenal steroids in hypertension and related CVD in TGRs. To this end, we have compared the effect of the AT1 receptor–selective antagonist irbesartan with that of the ETa receptor–selective antagonist BMS-18287415 and of the mixed ETa/ETb receptor antagonist bosentan14 on BP, myocardi and vascular hypertrophy, aortic tension responses to vasoconstrictors, arterial wall endothelin receptor subtype density, and plasma adrenocortical steroid levels.

Methods

Animals and Experimental Design
Male heterozygous TGRs, aged 3 weeks, were purchased from M & B (Ry, Denmark). After their arrival, they were allocated to individual cages and allowed to accommodate to the housing conditions (air conditioning at a temperature of 21°C and a light/dark cycle of 12/12 hours) for 1 week. At 4 weeks of age, after body weight (BW) and systolic BP (tail-cuff method) were measured, they were BW- and BP-matched and randomly allocated to the 4 different treatment groups: group P received a placebo; group B, the mixed ETa/ETb receptor antagonist bosentan (100 mg/kg BW); group I, the AT1 receptor–selective antagonist irbesartan (50 mg/kg BW); and group BMS, the ETa receptor–selective antagonist BMS-182874 (52 mg/kg BW PO).15 Bosentan was a generous gift of Dr Martine Clozel (Actelion Ltd, Allschwil, Switzerland), and BMS-182874 and irbesartan were kindly provided by Dr James Powell of Bristol Myers Squibb (Princeton, NJ). To minimize variability in dosage intake during each treatment, which was given individually to each rat as a chocolate-flavored tablet prepared from a 2% agarose gel added to 15 g of chocolate cream (Nutella) and to a solution of each drug, was visually verified daily by one investigator (G.M.). Bosentan and BMS-182874 were dissolved in distilled water under continuous stirring and added to 2% agarose gel at 37°C to obtain a gel with the required final concentration of the drug. Irbesartan was dissolved in distilled water and ethanol (4:1) under continuous stirring and added to the same agarose gel to obtain a gel with a final concentration of the drug ranging between 0.37% and 1.9%. The agarose gel added with BMS-182874, bosentan, or irbesartan was cut into pieces of proper size to obtain the amount required to provide each individual rat with its BW-tailored daily dosage of the drug. The last dosing of each drug was administered at least 24 hours before euthanasia. Two additional groups of untreated Sprague-Dawley normotensive rats were investigated at 10 to 12 weeks of age for determination of the normal values of plasma steroids and of the indices of mesenteric arteriolar structure. The animal handling and the protocol of the study followed the guidelines for animal studies at our institution.

For the measurement of tension responses, immediately after the rats were euthanized, the thoracic aortas were cleaned of connective tissue and cut into rings of 2-mm length. The same segment of thoracic aorta from all animals was used for all measurements for purposes of consistency. Rings were deprived of endothelium by gently rubbing the lumen with the tip of round-nose pliers. Two steel hooks were inserted into the lumen of each ring, which was then vertically placed in an organ bath filled with 15 mL of physiological salt solution (PSS) aerated with 95% O2 and 5% CO2 and maintained at 35°C (pH 7.35 to 7.40). One of the hooks was connected to an isometric transducer coupled to a pen recorder (Battaglia-Rangoni TRB/200/2) for monitoring of developed tension. The composition of the PSS was as follows (mmol/L): NaCl 125, KCl 5, CaCl2 2.7, MgSO4 1, KH2PO4 1.2, NaHCO3 25, and glucose 11. High K+-PSS was made by increasing the KCl concentration to 60 mmol/L without substituting for NaCl. Rings were stretched passively to achieve a resting tension of 1 g and then allowed to equilibrate for at least 60 minutes, during which they were washed every 15 minutes, before starting the experiment. Each ring was then repeatedly stimulated with 10 μmol/L phenylephrine until a reproducible response was obtained. The removal of endothelium was verified by a lack of relaxation of phenylephrine-contracted rings to 10−5 mol/L carbamoylcholine. All rings were at first exposed to the contractile stimuli in the following sequence: 5×10−6 mol/L phenylephrine, 60 mmol/L K+, and then cumulative concentrations (2×10−7 to 4×10−4 mol/L) of ET-1. A 60-minute washout was applied between 1 stimulus and the following stimulus. All these measurements were performed by an investigator (S.B.), who was kept unaware of the group treatment.

For the measurements of microvascular structure, mesenteric vessels corresponding to the second branch (~140 to 200 μm of average diameter in relaxed conditions, 2 mm long) were dissected from the surrounding fat from each rat. Vessels were mounted as a ring preparation on an isometric myograph (410 A, JP Trading) by threading them onto 2 stainless-steel wires (40-μm diameter). The wires were attached to a force transducer and a micrometer, respectively, as previously described by Mulvany and colleagues.32,33 Vessels were treated with 5% CO2 in O2. The vessel internal circumference was set to give a wall tension of 0.1 nN/mm. Vessel wall thickness (WT) and MT were measured at 12 different sites that were then averaged; measurement was accomplished by using a light microscope with an
immersion lens (Laboratory 20, Carl Zeiss S.P.A.) at ×600 magnification, which provides a resolution of 0.2 μm. Lower magnification was used for measurement of the distance between the wires and length of the blood vessel. The resting tension–internal circumference relation was determined, and vessels were set to the normalized length of the blood vessel. The resting tension–internal circumference was used for measurement of the distance between the wires and under a transmural pressure of 100 mm Hg, as described previously. From L₁, the normalized internal diameter was calculated. It was assumed that the cross-sectional area remains constant when the vessel is extended to L₁, and WT and MT were automatically calculated in normalized conditions. Measurements of WT and MT of blood vessels in normalized conditions (vessels extended to L₁) were obtained by assuming a constant wall and media volume from the wall and media cross-sectional areas calculated from WT and MT measured in unstretched vessels, as previously described. All these measurements were carried out by investigators (D.R., E.P.) who were unaware of the group treatment. Morphological results from 2 different blood vessels in each rat were averaged to provide 1 mean observation per animal. Endothelin receptor density was measured by autoradiography on 10- to 12-μm-thick sections of the iliac artery wall. They were cut in a cryostat (Leitz 1720 Digital) at −20°C and processed according to Kuhar and Palacios et al. Autoradiography was performed as reported. In brief, ET-1 binding sites were labeled in vitro by incubation for 120 minutes with 100 pmol/L 125I-ET-1 (Amersham Laboratories, specific activity 2000 Ci/mmol) at room temperature; nonspecific binding was determined by adding 1 μmol/L unlabeled ET-1. Selective displacement of 125I-ET-1 was studied by adding 1 μmol/L either BQ-123 or BQ-788 (both from Neosystem Laboratories). Reaction was terminated by washing the samples 3 times in cold 50 nmol/L Tris-HCl buffer. After they were rinsed in distilled water, the sections were rapidly dried, fixed in paraformaldehyde vapors at 80°C for 120 minutes, and then coated with NTB2 Kodak Nuclear emulsion (Eastman Kodak). The autoradiograms were exposed for 2 weeks at 4°C and then developed with undiluted D19 Kodak developer. Quantification of ETₐ and ETᵦ receptor density was carried out on sections treated with BQ-788 and BQ-123, respectively, by use of computer-assisted image analysis software (Casti Imaging) coupled to a Leitz Laborlux microscope, as reported. Area of at least 50,000 μm² in 8 to 16 sections for each receptor subtype from each rat were examined. Serum creatinine was measured with a standard biochemical technique. Plasma adrenocortical steroids were measured by quantitative high-performance liquid chromatography, as previously reported. The sensitivity of our assay system was 1 pmol/L, and the average intra-assay and interassay coefficients were 5% and 8%, respectively.

Statistical Analysis

Results are expressed as mean±SD or mean±SEM. Comparisons were carried out with a 1-way ANOVA followed by the Bonferroni post hoc test or with the Kruskall-Wallis test for variables not normally distributed. Plasma adrenocortical steroid levels were analyzed after log transformation. To investigate the relation between normalized MT of mesenteric arteries (as a dependent variable) and the other variables, a stepwise regression analysis (backward method with an inclusion cutoff value of 0.05) was used. The Pearson correlation coefficient was also estimated to assess the relation of individual variables. A value of P<0.05 was considered statistically significant. All analyses were carried out with the SPSS for Windows statistical package (version 8.0, SPSS Inc).

Results

BW and BP

The changes in BW and systolic BP of the 4 groups are shown in Figure 1. No significant difference of BW between groups was observed at any time during the study period (panel A). At variance, compared with all other groups, the animals receiving irbesartan showed a markedly lower systolic BP starting from the end of the first week of treatment. By the end of the study period, systolic BP was, on average, 100 mm Hg lower in group I compared with the other 3 groups (panel B).

Organ Weight and Biochemical Variables

The effect of the 4 treatments on the weight of different organs normalized per BW is summarized in Table 1. A significantly lower weight of the heart in toto and of the LV but not of the right ventricle and all the other organs was seen in group I compared with the other groups. Of interest, kidney weight and serum creatinine also tended to be lower, albeit not significantly, in group I compared with the other groups. The highest serum creatinine levels, total heart weight, and left ventricular (LV) weight were seen in the BMS group.

Plasma Adrenocortical Steroids

The normal plasma levels of steroids measured in a group of untreated Sprague-Dawley normotensive rats are shown for comparison in Figure 2 (legend). In the treatment groups, plasma aldosterone levels were significantly decreased with the administration of bosentan and irbesartan by 35% and 49%, respectively; deoxycorticosterone was significantly lower in group I compared with group BMS (Figure 2). No significant relation between the systolic BP and plasma adrenocortical steroid levels was found with stepwise regression analysis.

Tension Response of Endothelium-Free Aortic Rings

Compared with aortic rings of the other groups, the aortic rings of group I and BMS showed a significantly lower
tension response to the highest concentration (4×10⁻⁶ mol/L) of ET-1 (Figure 3) as well as to 5×10⁻⁶ mol/L phenylephrine and 60 mmol/L KCl (not shown). The tension response curve to ET-1 of the BMS group tended to be shifted to the left, but no significant difference of EC₅₀ between groups was found. Highly significant correlations between the responses to the maximal concentration of ET-1 and to phenylephrine (Figure 4A) and KCl (Figure 4B) were seen.

Vascular Structural Changes

The normal values of the indices of mesenteric arteriolar structure measured in a group of untreated Sprague-Dawley normotensive rats are shown for comparison in Table 2. In the 4 treatment groups, normalized MT was significantly decreased by ~12% by irbesartan, and a lower normalized intimal thickness was also found in group I compared with group BMS (Table 2). No significant differences in the remaining indices of vascular structure examined were found. MT was found to be directly tightly related to systolic BP (r=0.618, P<0.01) and LV weight (r=0.383, P<0.05). A stepwise regression analysis showed that only systolic BP remained in the model (β=0.618, t=3.605, P=0.002) and accounted for ~30% of the variance of the MT variance (adjusted R²=0.282, F=12.99, P=0.002). A significant direct correlation between MT of the mesenteric arterioles and LV weight was seen (r=0.507, P=0.012); however, in the BMS group this relation tended to be inverse, albeit not statistically significant (data not shown).

Endothelin Receptor Subtype Density

The density of ETA and ETB receptor subtypes in the iliac artery walls of the rats of the 4 experimental groups is shown in Figure 5. A significantly higher density of ETA receptor was observed in the irbesartan-treated rat arteries compared with the animals receiving placebo, when the total binding (not shown) and when the specific ETA binding were both considered. No significant difference of ETB receptor density among groups was found.

Figure 2. Plasma levels of adrenocortical steroids measured by high-performance liquid chromatography. Significantly lower plasma aldosterone levels were observed in the irbesartan-treated (group I) and bosentan-treated (group B) rats compared with the placebo group (group P, *P<0.05). †P<0.05 vs group BMS. Values are mean±SEM. Normal values obtained in 12 normotensive Sprague-Dawley controls were as follows (pmol/L): aldosterone 23.2±1.7, 18OH-corticosterone 21.2±2.4, corticosterone 210±9, 18OH-deoxycorticosterone (18OH-DOC) 35.8±2.3, and deoxycorticosterone (DOC) 27.1±4.7.

Figure 3. Tension-response curve to cumulative concentrations of ET-1 of endothelium-free thoracic aorta rings of TGRs of the 4 experimental groups. A significantly lower tension response to the highest concentration of ET-1 was observed in the irbesartan-treated (▼) and BMS-182874-treated (●) groups compared with the placebo-treated (■) and bosentan-treated (◆) groups. However, no significant change of EC₅₀ was found.

TABLE 1. Serum Creatinine, BW, and BW-Normalized Organ Weight in the 4 Experimental Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>NV</th>
<th>Group P (n=7)</th>
<th>Group B (n=6)</th>
<th>Group I (n=6)</th>
<th>Group BMS (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>NA</td>
<td>0.79±0.19</td>
<td>0.81±0.11</td>
<td>0.72±0.14</td>
<td>1.26±0.48</td>
</tr>
<tr>
<td>BW, g</td>
<td>408±13</td>
<td>294±33</td>
<td>293±19</td>
<td>297±25</td>
<td>310±15</td>
</tr>
<tr>
<td>Total heart, mg/g BW</td>
<td>2.62±0.3</td>
<td>5.13±0.24</td>
<td>4.47±0.07</td>
<td>3.42±0.13</td>
<td>5.67±0.31</td>
</tr>
<tr>
<td>LV, mg/g BW</td>
<td>1.87±0.01</td>
<td>3.71±0.10</td>
<td>3.38±0.11</td>
<td>2.28±0.06</td>
<td>3.97±0.23</td>
</tr>
<tr>
<td>RV, mg/g BW</td>
<td>0.74±0.01</td>
<td>0.69±0.03</td>
<td>0.66±0.03</td>
<td>0.62±0.02</td>
<td>0.77±0.07</td>
</tr>
<tr>
<td>Brain, mg/g BW</td>
<td>6.06±0.34</td>
<td>6.06±0.24</td>
<td>6.00±0.28</td>
<td>5.45±0.24</td>
<td></td>
</tr>
<tr>
<td>Right adrenal gland, mg/100g BW</td>
<td>8.54±0.50</td>
<td>8.94±0.65</td>
<td>8.05±0.40</td>
<td>7.84±0.69</td>
<td></td>
</tr>
<tr>
<td>Right kidney, mg/g BW</td>
<td>4.36±0.09</td>
<td>4.48±0.12</td>
<td>4.06±0.17</td>
<td>4.61±0.13</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean±SD. NV indicates available normal values obtained in a series of 12-week-old normotensive Sprague-Dawley rats shown for comparison; RV, right ventricle. NA indicates not available.

*p<0.01 vs group BMS; †P<0.01 vs group B; ‡P<0.001 vs groups P and BMS; §P<0.0001 vs all other groups; and ||P<0.05 vs group P.
Discussion

The role of ET-1 in experimental hypertension and the interactions between the renin-angiotensin-aldosterone system and ET-1 have been recently reviewed. Available data support the role of ET-1 in hypertension caused by exogenous Ang II, whereas data on its participation in the hypertension induced by enhanced endogenous Ang II generation are conflicting. We used the TGR, a suitable model in which to further investigate this paradox. We found that at variance with the ETA receptor–selective antagonist BMS-182874 and the mixed ETA/ETB receptor antagonist bosentan, only specific blockade of the AT1 receptor with irbesartan effectively prevented the development of hypertension (Figure 1B) and related CVD (Tables 1 and 2). Compared with the other groups, the irbesartan-treated rats had markedly lower weight of the heart, which was due to a smaller LV, and reduced thickness of the tunica media of the mesenteric arterioles. Of interest, the normalized MT showed a direct significant relation with LV weight (not shown), indicating the effectiveness of AT1 antagonism in the prevention of LV and arteriolar hypertrophy. These findings are in agreement with those obtained with losartan but not with those obtained with the nonselective ET-1 antagonist SB-209670. The latter not only lowered BP but also synergistically augmented the hypotensive effect of losartan under acute conditions in older (3 to 4 months) chronically instrumented TGRs. Several explanations might account for the lack of BP lowering and CVD prevention of ET-1 antagonists in the present study. We can exclude the possibility that our dosages were too low to block a markedly Ang II–driven ET-1 synthesis in the vessel wall, because we used the highest dosages reported. Furthermore, bosentan given orally at the same dosage used in the present study did abolish the pressor effect of exogenous big ET-1 in TGR when BP was recorded telemetrically. In addition, in the present study, bosentan did lower plasma aldosterone because of the blockade of the ETB-mediated secretagogue effect of ET-1 on this steroid, even though the expression of the REN-2 transgene and the ensuing secretion of aldosterone are markedly enhanced in the adrenal cortex of TGRs. It might be argued that the BP-lowering effect of ETA blockade could have been offset by the concomitant abolishment of ETB-mediated vasodilatation, as suggested by the increase in BP elicited by an infusion of the ETB-selective antagonist BQ-788 in humans. This possibility is unlikely, in our view, because the ETB-selective antagonist BMS-182874 did not lower BP either (Figure 1). Similarly, the possibility that ET-1 receptor antagonists lower BP only when given acutely, and not chronically, is untenable because of the lack of any indication of a BP-lowering effect after acute administration of bosentan, when BP was telemetrically recorded.

Table 2. Indices of Arteriolar Vascular Structural Changes in Mesenteric Arterioles of the 4 Experimental Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group P (n=7)</th>
<th>Group B (n=6)</th>
<th>Group I (n=6)</th>
<th>Group BMS (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima thickness, µm</td>
<td>1.28±0.02</td>
<td>1.32±0.02</td>
<td>1.29±0.03</td>
<td>1.24±0.03*</td>
</tr>
<tr>
<td>Media/lumen</td>
<td>0.061±0.003</td>
<td>0.129±0.002</td>
<td>0.136±0.009</td>
<td>0.115±0.008</td>
</tr>
<tr>
<td>WT, µm</td>
<td>38.7±0.99</td>
<td>42.8±0.83</td>
<td>43.6±1.0</td>
<td>39.9±2.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM (range). NV indicates normal values obtained in a series of 12-week-old normotensive Sprague-Dawley rats.

*P<0.05 vs group BMS; †P<0.05 vs groups P and B.
etritically recorded. It must be pointed out, however, that young TGRs were used in the present study and in a previous study showing no effect of bosentan, whereas older animals were used in a study reporting a hypotensive effect of SB-209670.

At variance with the available data suggesting a contribution of ET-1 when Ang II is exogenously infused, data on hypertension that is due to endogenously overproduced Ang II are controversial. In renovascular hypertensive rats, bosentan attenuated the early BP increase after clipping of a renal artery. However, it did not lower BP, nor did it prevent structural changes in arterioles of different beds in rats with chronic 2-kidney, 1-clip hypertension, even though it afforded some prevention of fibrosis in the heart. The lack of effect of ET-1 antagonism on BP and CVD is in accord with the observation that ET-1 mRNA was evidently increased in cardiovascular tissues only in the late phase of 2-kidney, 1-clip hypertension, indicating that ET-1 might attain increasing importance in the non-renin-dependent late phase of the initially renin-dependent forms of hypertension.

This contention is also in agreement with data involving a chronic canine Page model of hypertension, which had high Ang II levels and in which bosentan lowered BP when it was administered after losartan. Thus, apart from the obvious differences between exogenously and endogenously borne Ang II, it might be reasonable to assume that ET-1 becomes involved in the more advanced stages of hypertension, when endothelial dysfunction is a hallmark.

Collectively, our results are consistent with those obtained in a transgenic rat model of malignant hypertension, in which bosentan did not lower BP or prevent transition to the malignant phase of hypertension, thereby incurring a fatal outcome. Nonetheless, they differ, in part, from the results of another study in which bosentan lowered the BP in TGREN2 rats made diabetic with streptozotocin, although it failed to protect from adverse histological changes in the kidney. However, in a previous study of our group, female heterozygous TGRs were used; thus, it might be that the marked sexual dimorphism in BP of TGRs could be related to ET-1–mediated mechanisms. Additionally, it could be that diabetes, by enhancing endothelial dysfunction, anticipates the enrollment of ET-1–mediated mechanisms in raising BP.

There is a widespread belief that aldosterone plays a major role in TGR hypertension, even though spironolactone did not prevent hypertension. Our data showed that blunting of aldosterone secretion, through blockade of either the Ang II–mediated or the ET_A–mediated secretagogue effect, does not prevent hypertension and CVD and that blockade of the vascular AT_1 receptor is instead required. Thus, by demonstrating a clear-cut dissociation between lack of a hypotensive effect and the aldosterone-lowering effect of bosentan, we clarified the relative roles of Ang II, ET-1, and adrenocortical steroids in TGRs.

The comparison of the 4 groups in terms of in vitro aortic tension-response curves to ET-1 showed that irbesartan lowered the maximal response without significantly affecting the EC_50. Compared with placebo, bosentan left unchanged the tension-response curves, indicating that when given in vivo, the drug was no longer effectively blocking ET-1 receptors in vitro under our experimental conditions. This is at variance with findings with BMS-182874, which, possibly because of an affinity for the ET_A receptors that was higher than that of bosentan, was as effective as irbesartan in blunting the maximal tension-response curve to ET-1 (Figure 3) but ineffective in preventing hypertension and related CVD (Figure 1). Thus, our findings showed not only a dissociation between in vivo and in vitro hemodynamic responses to BMS-182874 but also a striking difference between the 2 ET-1 antagonists, which might have therapeutic implications.

Regarding hypertension-related CVD, the tight correlation between the maximal tension responses of aortic rings in vitro to both receptors (phenylephrine and ET-1) and to non–receptor-mediated (KCl depolarization) vasoconstricting stimuli (Figure 4) suggests that these responses were mainly dependent on changes in the efficiency of the contractile machinery, ie, on the amount and/or the phenotypic status of VSMCs, rather than changes at the receptor level. This is also supported by fact that ET_A receptor density in the iliac artery walls of the irbesartan-treated rats was increased rather than lowered (Figure 5), a finding that might appear paradoxical at first sight. However, it is likely that severe hypertension with CVD and endothelial dysfunction in the groups not receiving irbesartan are associated with enhanced ET-1 synthesis with ensuing ET_A receptor downregulation, as seen in old spontaneously hypertensive rats. A direct relation between an index of structural changes in the arterioles of the mesenteric bed (ie, the normalized MT) and the LV weight, which was mainly due to the fact that both these indices were decreased by irbesartan, was also found (not shown). This underscores the efficacy of AT_1 antagonism in preventing vascular and cardiac hypertrophy. However, it is interesting to consider that in the BMS group, the relation tended to be an inverse one, suggesting a differential effectiveness of selective ET_A antagonism on vascular and myocardial hypertrophy. This contention is in keeping with recent findings in 2-kidney, 1-clip hypertensive rats, in which ET_A and ET_B blockade prevented intracardiac artery hypertrophy and cardiac fibrosis, respectively.

Thus, collectively, our data support the concept that Ang II plays a pivotal role in determining the mass of the cardiomyocytes and the VSMCs in the media of the large arteries, such as the aorta, and of the arterioles, which are a major site of the total vascular resistance.
The serum creatinine levels were the highest in the BMS group (Table 1), a fact also deserving consideration, given the consistent reports indicating that selective ET<sub>A</sub> blockade with LU1352525 prevented renal damage in a different model of nephropathy in which, however, Ang II levels were not as high as those in the TGR model. It must be underlined that in the BMS group a wider dispersion of values was seen (Table 1). Thus, although it could be hypothesized that selective ET<sub>A</sub> receptor blockade is detrimental to renal function in this specific model of hypertension (possibly because of abolition of an ET<sub>A</sub>-mediated constriction of the efferent arterioles acting synergistically with Ang II to maintain the glomerular filtration rate), this remains to be tested in a larger series of rats. Nonetheless, the fact that serum creatinine was increased in only the BMS group compared with the P and B groups, despite similarly elevated BP values in these 3 groups, corroborates the contention of a dissociation of BP and CVD in TGRs.<sup>51</sup>

In conclusion, an AT<sub>1</sub>-selective antagonist, but neither an ET<sub>A</sub>-selective nor a mixed ET<sub>A</sub>/ET<sub>B</sub> antagonist, provided protection from hypertension and CVD in TGRs. Irbesartan also prevented the downregulation of ET<sub>A</sub> receptors associated with severe hypertension and CVD; nonetheless, it lowered the maximum tension responses of the thoracic aorta to receptor-mediated and non–receptor-mediated contractile stimuli, possibly because of the blunting of arterial medial (muscular) hypertrophy. Collectively, our data support the contentions that whereas Ang II and endogenous ET-1 contribute to the regulation of aldosterone, only the former plays a key role in the early development of hypertension and related CVD in this renin-dependent model of hypertension. Thus, our data are in accordance with those of previous studies showing that endogenous Ang II does not appear to chronically stimulate vascular and cardiac ET-1 production.<sup>18,21</sup>

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