Effects of Chronic ET<sub>A</sub>-Receptor Blockade in Angiotensin II–Induced Hypertension

Livius V d’Uschio, Pierre Moreau, Sidney Shaw, Hnoyuki Takase, Matthias Barton, Thomas F Luscher

Abstract Angiotensin II, a constrictor and mitogen of vascular smooth muscle cells, affects the release of endothelium-derived factors such as nitric oxide or endothelin-1. This study investigated the influence of endothelin-1, using the selective endothelin A receptor antagonist LU135252, on blood pressure and endothelial function in angiotensin II–induced hypertension in the rat. Two weeks of angiotensin II administration (200 ng/kg per minute) increased systolic blood pressure (+35 ± 5 mm Hg, tail cuff method) compared with placebo (P < 0.05). LU135252 alone did not affect systolic pressure but lowered the angiotensin II–induced pressure increase (P < 0.05). In isolated aortic rings, endothelium-dependent relaxations to acetylcholine were reduced in the angiotensin II group (P < 0.05 versus placebo) and improved by concomitant chronic LU135252 treatment (P < 0.05 versus angiotensin II). Blood pressure elevation strongly correlated with impaired endothelium-dependent relaxations to acetylcholine (r = −0.967). LU135252 did not affect endothelium-independent relaxations to sodium nitroprusside, which were diminished after angiotensin II treatment (P < 0.05). In quiescent rings, chronic angiotensin II administration enhanced endothelium-dependent contractions to acetylcholine, which were reduced by LU135252 (P < 0.05). Impaired contractions to endothelin-1 and norepinephrine in the angiotensin II group were normalized after treatment with LU135252 (P < 0.05). Thus, chronic therapy with LU135252 partially prevents angiotensin II–induced hypertension and the alternations of the endothelial function observed in this experimental model (Hypertension. 1997;29(part 2):435-441.)

Key Words • angiotensin II • endothelium • ET receptors • endothelins • LU135252 • aorta

The renin-angiotensin system and endothelium-derived vasoactive substances are important regulators of the cardiovascular system. The endothelium is a source of vasodilators such as nitric oxide and prostacyclin, as well as vasoconstrictors such as prostaglandin H₂/thromboxane A₂ and ET-1. ET-1, a potent vasoconstrictor and mitogen, is generated from its precursor molecule big endothelin by endothelin-converting enzyme. Interestingly, nitric oxide as well as prostacyclin inhibit ET-1 production via a cGMP-dependent mechanism. ET-1 exerts its biological effects via activation of specific receptors. ET<sub>A</sub> receptors on VSMC cause vasoconstriction and proliferation, although ET<sub>B</sub> receptors also contribute to these effects. Endothelial cells express only ET<sub>B</sub> receptors linked to the formation of nitric oxide and prostacyclin. Previous studies with bosentan, a combined ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist, found no blood pressure–lowering effect in spontaneously hypertensive and WKY rats or in the two-kidney, one-clip model. In contrast, selective ET<sub>B</sub>-receptor blockade did lower blood pressure in renin hypertensive rats (partial renal ablation). Hence, selective ET<sub>A</sub>-receptor blockade may be particularly effective in angiotensin-dependent hypertension.

Ang II also plays an important role as an autocrine and a paracrine mediator in the regulation of vascular tone. Endothelial cells express angiotensin receptors that may modulate vascular tone via the release of vasoactive agents such as nitric oxide, ET-1, and prostaglandins in response to Ang II. Furthermore, Ang II stimulates the proliferation and migration of VSMC via AT₁ receptor.

In cultured endothelial cells, Ang II stimulates the expression of preproendothelin messenger RNA. In perfused mesenteric resistance arteries of spontaneously hypertensive rats, short-term application of Ang II (100 nmol/L) increases the local vascular production of ET-1 and enhances vasoconstrictor responses to norepinephrine. In human coronary arteries, a potentating effect of exogenous ET-1 on contractions to norepinephrine and to serotonin has been demonstrated. This potentating mechanism might be of great physiological and pathophysiological importance, as under most circumstances circulating levels of ET-1 are quite low.

In cardiovascular diseases such as hypertension, heart failure, and renal failure, the renin-angiotensin system is activated. However, the contribution of ET-1 to Ang II–induced hypertension is not known. Therefore, we investigated the effect of chronic ET<sub>A</sub>-receptor blockade with a selective and orally active ET<sub>A</sub>-receptor antagonist LU 135252 on Ang II–induced hypertension with special emphasis on endothelium-dependent and -independent vascular reactivity of isolated rat aorta.

Methods

Animal Preparations

Male normotensive WKY rats at 9 weeks of age were obtained from IFFA Credo (L’Arbresle, France) and held at the animal facilities of University Hospital Bern for 1 week. Rats were distributed in random order in four groups: a placebo group (fed with standard chow and water), an Ang II group, which received...
the peptide via subcutaneously implanted osmotic mini-pumps (model 2002, Alzet Corp) releasing a constant dose of 200 ng kg⁻¹ min⁻¹ during 14 days, an LU135252 group (≈50 mg kg⁻¹ d⁻¹ LU135252, which was mixed with the powdered chow), and an Ang II+LU135252 group. The dose of LU135252 blocking ETA receptors was based on previous studies by the manufacturer. The chow intake was controlled daily, and the dose of LU135252 averaged 47 ± 3 mg kg⁻¹ d⁻¹ in the LU135252 group and 55 ± 4 mg kg⁻¹ d⁻¹ in the Ang II+LU135252 group (NS). Systolic arterial pressure and heart rate were measured by a tail-cuff method (Model LE 5000, Letica), and body weight of the rats was monitored before and on the 14th day of treatment (Table 1). Housing facilities and all experimental protocols were approved by the local authorities for animal research (Kommission für Tierversuche des Kantons Bern, Bern, Switzerland).

The rats were anesthetized (thiopental, 50 mg/kg body wt IP) and decapitated. The thoracic aorta was dissected and placed in a laminar flow chamber. Aortic rings were incubated with or without SQ30741 (a prostaglandin E₂ receptor antagonist, 10⁻⁷ mol/L) for 30 minutes. The solutions were kept at 37°C and aerated continuously with 95% O₂ and 5% CO₂ gas. After an equilibration period of 30 minutes, the rings were stretched progressively to their optimal tension in response to 100 mmol/L KCl (2 ± 0.2 g). Then the stretched ring segments were equilibrated for 30 minutes before the experiment.

**Protocols**

For the studies of endothelium-dependent relaxations, aortic rings were incubated with or without SQ30741 (a prostanoid H₂-receptor antagonist, 10⁻⁷ mol/L) for 30 minutes or superoxide dismutase (a superoxide anion scavenger, 150 U/mL) for 5 minutes. Rings were precontracted with norepinephrine (2 × 10⁻⁷ mol/L) and then relaxed with acetylcholine (10⁻⁹ to 10⁻⁴ mol/L) for endothelium-independent relaxation. Rings were precontracted with norepinephrine (2 × 10⁻⁷ mol/L) and relaxed with 10⁻¹⁰ to 10⁻⁵ mol/L sodium nitroprusside.

To study endothelium-dependent relaxations, aortic rings were treated with L-NAME (10⁻⁴ mol/L, 30 minutes to inhibit nitric oxide formation) alone or with SQ30741 (10⁻³ mol/L). Cumulative concentrations of acetylcholine (10⁻⁹ to 10⁻⁴ mol/L) were then added to the organ baths.

**Measurement of ET-1 Plasma Levels**

Arterial blood samples were obtained through a catheter inserted in the left femoral artery from anesthetized rats. The blood was immediately transferred to a tube containing EDTA and centrifuged at 4°C for 10 minutes. Plasma was separated at 4°C and kept at −80°C until assay.

Extraction was performed by absorption on 500-mg SepPak Vac C18 cartridges (Millipore). Columns were preactivated by successive washes with 5 mL of 86% ethanol in 4% acetic acid, 5 mL of methanol, 5 mL of sterile distilled water, and 5 mL of 4% acetic acid. A 2-mL plasma sample acidified with 6 mL of 4% acetic acid was then applied on the column with the flow rate of 3 mL/min. The columns were then washed with 18 mL of sterile distilled water, 1.8 mL of ethyl acetate, and 18 mL of 24% ethanol in 4% acetic acid before ET was eluted with 86% ethanol in 4% acetic acid. The eluate was dried under nitrogen at 37°C and redissolved in 230 µL of assay buffer composed of 0.1% phosphate buffer (pH 7.4), 0.05 mol/L NaCl, 0.1% Triton X-100, 0.02% sodium azide, and 0.1% BSA. The radioimmunoassay of plasma ET was performed using synthetic human pig ET-1 (Sigma Chemical Co), a rabbit antibody against synthetic ET (Peninsula Laboratories), and ²¹²I-ET-1 (Amersham). The antibody has 100% cross-reactivity with ET-1, 7% with ET-2 and ET-3, 17% with big endothelin-1, and no cross-reactivity with other peptides. The anti-ET antibody was reconstituted according to the manufacturer’s instructions and then further diluted 1:3.5 with the assay buffer before adding 100 µL to the standards or to the reconstituted plasma samples (100 µL) analyzed in duplicate. After 24 hours of incubation, 100 µL of ²¹²I-ET-1 (10 to 12 × 10⁶ cpm per tube) was added, and incubation was allowed to continue for an additional 24 hours. The separation of bound and free antigen was performed with a second antibody method, and pellets were counted by a gamma counter (Canberra Packard).

# Table 1. Changes in Body Weight, Systolic Blood Pressure (SBP), and Heart Rate in WKY Rats After 2 Weeks of Treatment With Different Regimens

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP, mm Hg</th>
<th>Heart Rate, Beats/min</th>
<th>Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 2</td>
<td>Week 0</td>
</tr>
<tr>
<td>Placebo</td>
<td>134±5</td>
<td>136±3</td>
<td>325±5</td>
</tr>
<tr>
<td>Ang II</td>
<td>132±4</td>
<td>167±5†</td>
<td>336±8</td>
</tr>
<tr>
<td>LU</td>
<td>139±3</td>
<td>132±3</td>
<td>340±10</td>
</tr>
<tr>
<td>Ang II+LU</td>
<td>131±3</td>
<td>146±3††</td>
<td>321±9</td>
</tr>
</tbody>
</table>

*Week 0 indicates before treatment, week 2, after 2 weeks of treatment, and LU, LU135252 (ETA-receptor antagonist) Data are mean±SEM of 7 to 9 rats
†P < 0.05 vs placebo rats
‡P < 0.05 vs Ang II-treated rats (by ANOVA and Bonferroni correction)
§P < 0.05, week 0 vs week 2
In all experiments, n equals the number of rats from which blood vessels were obtained. For simple comparison between two values, paired Student’s t test was used, and for multiple comparisons results were analyzed with ANOVA followed by Bonferroni’s correction. A value of \( P < 0.05 \) was considered significant.

**Results**

**Body Weight, Systolic Blood Pressure, and Heart Rate**

Before treatment, the mean body weight did not differ between the groups of rats. After 2 weeks, body weight increased in all groups. However, in the Ang II group the weight gain was less (\( P < 0.05 \) versus placebo, Table 1). Systolic blood pressure was increased by chronic administration of Ang II (+35 ± 5 mm Hg) compared with placebo (1 ± 5 mm Hg, \( P > 0.05 \), \( n = 9 \)). The ET<sub>A</sub>-receptor antagonist LU135252 did not affect systolic pressure on its own (−7 ± 4 mm Hg, NS versus placebo, \( n = 7 \)) but in part prevented the Ang II–induced pressure rise (+15 ± 3 mm Hg, \( P < 0.05 \) versus Ang II; \( n = 8 \)). Although heart rate tended to be higher after Ang II administration, this did not reach statistical significance (Table 1).

**ET-1 Plasma Levels**

Plasma concentrations of ET-1 tended to increase after chronic Ang II treatment (4 ± 0.5 versus placebo group. \( 2.6 ± 0.1 \) pg/mL, NS) In rats treated chronically with either LU135252 alone or in combination with Ang II, the levels of ET-1 were markedly increased (6.7 ± 0.4 and 8.0 ± 0.7 pg/mL, respectively) compared with placebo (\( P < 0.05 \), \( n = 6 \) to 8).

**Endothelium-Dependent Relaxations**

The concentration-relaxation curves of acetylcholine (10<sup>−9</sup> to 10<sup>−4</sup> mol/L) after precontraction with norepinephrine (2 × 10<sup>−7</sup> mol/L) were biphasic in all groups’ relaxations at lower concentration were followed by contractions at higher concentrations of acetylcholine (Fig 1A). In contrast, in aortas pretreated with SQ30741, the maximal relaxations to acetylcholine were significantly improved, and contractions at higher concentration were abolished in all groups (\( P < 0.05 \), Fig 1B). Maximal responses to acetylcholine were diminished after chronic treatment with Ang II compared with placebo (\( P < 0.05 \), \( n = 9 \)), irrespective of the presence or absence of SQ30741. These attenuated relaxations were improved after concomitant administration of LU135252 in the presence or absence of SQ30741 (\( P < 0.05 \) versus Ang II group, \( n = 8 \)). Acute incubation of vessels with superoxide dismutase did not affect maximal response to acetylcholine (data not shown, \( n = 7 \)). Precontraction with norepinephrine tended to be slightly decreased in rings treated with either SQ30741 or superoxide dismutase compared with untreated rings, but this did not reach statistical significance. Furthermore, precontraction to norepinephrine was not different between placebo-, Ang II-, and Ang II + LU135252-treated rats (NS). In contrast, the contraction was slightly higher in the group treated with LU135252 alone (\( P < 0.05 \)). The sensitivity of concentration-response curves to acetylcholine did not differ between control vessels and rings preincubated with either SQ30741 or superoxide dismutase or in any of the treatment groups.

**Data Analysis**

For statistical analysis, the sensitivity of the vessels to the drugs were expressed as negative logarithm of the concentration that caused half-maximal relaxation or contraction (pD<sub>2</sub> value). Maximal contraction or relaxation (expressed as percentage of precontraction) and AUC (in arbitrary units from 0 to 1000) were determined for each individual concentration-response curve by nonlinear regression analysis using MatLab software. The contractions were expressed as percentage of the maximal response to KCl (100 mmol/L), which was obtained at the beginning of each experiment. Results are given as mean ± SEM
Relation of Systolic Blood Pressure and Endothelial-Dependent Relaxations

In untreated aortic rings from animals of the Ang II group, in which an increase in systolic pressure was observed, endothelium-dependent relaxation to acetylcholine was impaired. Endothelial dysfunction and the rise in systolic pressure in this group were prevented almost completely by chronic LU135252 administration. Systolic pressure changes correlated highly with endothelium-dependent relaxations ($r = -0.967$, Fig 2).

Endothelium-Independent Relaxations

Sodium nitroprusside caused concentration-dependent relaxations that were shifted threefold to the right after chronic Ang II treatment ($pD_2 7.13$, $P < 0.05$ versus placebo 7.61; $n=7$, Fig 3). Concomitant treatment with LU135252 did not affect the sensitivity to sodium nitroprusside ($pD_2 7.16$; NS) but normalized maximal relaxation (104 ± 1%), which was impaired in the Ang II group (100 ± 1%, $P < 0.05$).

Endothelium-Dependent Contractions

During acute L-NAME ($10^{-4}$ mol/L) incubation, quiescent aortic rings with endothelium exhibited enhanced contractions to acetylcholine ($10^{-10}$ to $10^{-8}$ mol/L) in the Ang II group ($P < 0.05$ versus placebo; $n=7$, Fig 4). This increase in contraction was prevented by concomitant chronic administration of LU135252 ($P < 0.05$, $n=7$).

TABLE 2. Maximal Response, Sensitivity, and AUC From Concentration-Dependent Contractions to Different Vasoactive Agents in Aortas of WKY Rats After 2 Weeks of Treatment With Different Regimens

<table>
<thead>
<tr>
<th>Condition</th>
<th>Group</th>
<th>Max, %</th>
<th>$pD_2$</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>U46619</td>
<td>Placebo</td>
<td>137 ± 4</td>
<td>7.3 ± 0.1</td>
<td>178 ± 6</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>139 ± 4</td>
<td>7.4 ± 0.1</td>
<td>169 ± 0</td>
</tr>
<tr>
<td></td>
<td>LU</td>
<td>127 ± 2</td>
<td>7.4 ± 0.1</td>
<td>176 ± 11</td>
</tr>
<tr>
<td></td>
<td>Ang II + LU</td>
<td>127 ± 5</td>
<td>7.7 ± 0.1*</td>
<td>204 ± 11</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Placebo</td>
<td>111 ± 4</td>
<td>7.6 ± 0.1</td>
<td>280 ± 13</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>85 ± 6*</td>
<td>7.5 ± 0.1</td>
<td>201 ± 20*</td>
</tr>
<tr>
<td></td>
<td>LU</td>
<td>113 ± 2</td>
<td>7.7 ± 0.1</td>
<td>302 ± 11</td>
</tr>
<tr>
<td></td>
<td>Ang II + LU</td>
<td>94 ± 5</td>
<td>7.9 ± 0.1†</td>
<td>270 ± 17†</td>
</tr>
<tr>
<td>ET-1</td>
<td>Placebo</td>
<td>135 ± 4</td>
<td>8.2 ± 0.1</td>
<td>168 ± 5</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>114 ± 9*</td>
<td>8.1 ± 0.1</td>
<td>133 ± 12*</td>
</tr>
<tr>
<td></td>
<td>LU</td>
<td>140 ± 2</td>
<td>8.3 ± 0.1</td>
<td>185 ± 5</td>
</tr>
<tr>
<td></td>
<td>Ang II + LU</td>
<td>132 ± 3t</td>
<td>8.3 ± 0.1</td>
<td>169 ± 6t</td>
</tr>
</tbody>
</table>

Max % indicates maximal response to agonist (percent of 100 mmol/L KCl), $pD_2$, log of the agonist concentration (mol/L) causing half-maximal contraction, AUC, area under the concentration-response curve (arbitrary units, 0 to 1000), and LU, LU135252 (ET$_4$ receptor antagonist). Data are mean ± SEM of 7 to 8 rats.

After acute incubation with the thromboxane/endoperoxide receptor antagonist SQ30741 ($10^{-7}$ mol/L), contractions to acetylcholine were abolished in all groups ($P < 0.05$, data not shown, $n=7$ per group). Concentration-response curves to the thromboxane analogue U46619 ($10^{-10}$ to $10^{-7}$ mol/L) were slightly shifted to the left in the Ang II+LU135252 group ($P < 0.05$ versus placebo, $n=7$ to 8, Table 2). In contrast, maximal contractions to U46619 were comparable in all groups.

Contractions of Vascular Smooth Muscle

Contractions to 100 mmol/L KCl did not differ among placebo (3.8 ± 0.1 mN/mm), Ang II (3.8 ± 0.1 mN/mm), and Ang II+LU135252-treated (3.8 ± 0.1 mN/mm) groups but were slightly reduced in the LU135252 group (3.4 ± 0.1 mN/mm, $P < 0.05$).

In Ang II–treated rats, aortic segments contracted less to norepinephrine ($10^{-10}$ to $10^{-5}$ mol/L) compared with placebo ($P < 0.05$ for maximal response and AUC). After concomitant treatment with LU135252, contractile activity was improved ($P < 0.05$ versus Ang II group for $pD_2$ and AUC, $n=7$, Table 2).
Although the in vitro responses to a single dose of Ang II (10^{-7} mol/L) tended to be enhanced in Ang II+LU135252 (31±4%) and LU135252 (32±2%) groups, there were no significant differences between them and those obtained in Ang II-treated rats (26±3%) and the placebo group (23±3%, n=7 to 8 per group). The specificity of LU135252 against ETA receptors was tested by a competitive receptor binding assay by Knoll AG.29 LU135252 selectively binds to human ETA receptors with high affinity. Up to 10 μmol/L, the drug did not show any interaction with other peptide receptors, including AT1 receptors.

The maximal contraction to ET-1 (10^{-11} to 10^{-7} mol/L) was blunted in the Ang II group (P< 0.05 versus placebo for maximal response and AUC), however, additional chronic treatment with LU135252 normalized the response (P< 0.05 versus Ang II). LU135252 alone did not affect ET-1-induced contraction (Table 2). The sensitivity to ET-1 was comparable in all groups (n=7 per group).

Discussion

The present study shows that the increase in systolic blood pressure and endothelial dysfunction after long-term administration of Ang II can be prevented in part by concomitant administration of LU135252, a selective, orally active, nonpeptide ETA-receptor antagonist.

Chronic administration of Ang II represents a well-defined and clinically important experimental model of hypertension.37,38 In previous studies with renin hypertensive rats (two-kidney, one clip hypertension), the combined ETA/ETB-receptor antagonists showed no hypertensive effects, while selective blockade of the ETA receptors did lower blood pressure.15,16 Our present experiments show that long-term treatment of WKY rats with Ang II, in addition to an increase in systolic blood pressure, also impairs endothelium-dependent relaxations to acetylcholine in the aorta. The most remarkable observation of our study is that chronic treatment with LU135252 prevented part of the increase in blood pressure and improved endothelium-dependent relaxations to acetylcholine. Ang II induces expression of preproendothelin messenger RNA and in turn ET-1 production not only in vascular endothelial cells but also in nonendothelial cells, such as VSMC or cardiomyocytes.40 In addition, ET-1 has been shown to participate in Ang II–induced vascular contractions of small arteries.27,41 Hence, this study suggests that, indeed, ET-1 mediates Ang II–induced changes in vivo, explaining the efficacy of an ETA-receptor antagonist to prevent at least part of Ang II–mediated vascular responses.

Under normal conditions, there is little activation of vascular endothelin production.7 Most likely, endothelin acts primarily as a local vascular regulator (released towards the smooth muscle rather than into the lumen) and less so as a circulating hormone, except with very high circulating levels of the peptide that are found in disease states such as atherosclerosis, heart failure, acute myocardial infarction, cardiogenic shock, and renal failure.1 In this study, plasma ET-1 levels increased only slightly after chronic administration of Ang II, but they were markedly augmented after concomitant treatment with LU135252. This could be explained by chronic blockade of ETA receptors leading to increased ET-1 plasma levels, since it has been described for other selective receptor antagonists (i.e., the AT1-receptor antagonist losartan).43 Despite increased circulating ET-1 levels, LU135252 lowered systolic blood pressure in Ang II–treated rats. This could be explained by blockade of ETA receptors by LU135252 and possibly selective activation of ETB receptors, which may release nitric oxide and prostacyclin.12,13 The latter effect may explain the improvement of endothelium-dependent relaxations by LU135252 in Ang II–induced hypertension. However, this study cannot resolve the question of whether LU135252 lowers blood pressure by improving endothelial function or vice versa. Indeed, in L-NAME–induced hypertension, concomitant treatment with LU135252 improves impaired endothelium-dependent relaxations to acetylcholine and contractions to ET-1 without lowering blood pressure,44 while the combined ETB/ET receptor antagonist bosentan had no effect.45 Taken together, these results suggest that LU135252, which blocks only ETA receptors, may have pressure-independent effects to improve endothelium-dependent relaxations.

The alternation of endothelium-dependent relaxations produced by Ang II seems to involve modifications of the responsiveness of VSMC to nitric oxide. Indeed, endothelium-independent relaxations to the nitric oxide donor sodium nitroprusside, which exerts its effects via the activation of soluble guanylyl cyclase and the subsequent formation of cGMP, were also impaired after chronic Ang II treatment. By increasing the amount of nitric oxide reaching VSMC (see above), LU135252 may improve the blunted relaxations as suggested by acetylcholine–induced relaxations in the presence of prostaglandin H2/thromboxane A2 receptor antagonist SQ30142, where the nitric oxide component of the relaxations (and not endothelium–derived contracting factors such as prostaglandin H2/thromboxane A2) is unmasked (Fig 1B). The sensitivity of sodium nitroprusside–induced relaxations, which were less in Ang II–induced hypertension, was not improved by chronic LU135252 treatments, confirming that LU135252 exerted its effects through the endothelium and not directly on the VSMC.

The contribution of other endothelium-derived factors, such as superoxide anions, appeared not to be important in the blunted endothelium-dependent relaxations, since superoxide dismutase failed to improve these relaxations. Interestingly, endothelium-dependent contractions to acetylcholine were enhanced after chronic Ang II treatment and prevented by concomitant treatment with LU135252. These results demonstrate that the ETA-receptor antagonist LU135252 indeed inhibits Ang II–induced endothelium-dependent contractions, thus confirming our previous report showing that endogenous ET-1 may regulate the release and action of endothelium–derived contracting factors (i.e., thromboxane A2/prostaglandin H2) in rat aorta.47 Reactivity of the vascular smooth muscle to an agonist acting at thromboxane A2/prostaglandin H2 receptors such as the thromboxane analogue U46619 was comparable in rats treated with placebo, Ang II, or LU135252 alone. However, combined treatment with Ang II and the ETA antagonist produced a parallel leftward shift of the contractions evoked by U46619. These findings may suggest that the release of endothelium–derived contracting factors was reduced, leading to an increased sensitivity of their receptors and/or signaling pathways.

Contractions to Ang II were not different among the groups in vitro, confirming that LU135252 does not interfere with Ang II receptors. In contrast, long-term Ang II treatment reduced vascular response to ET-1, since it has
been previously observed in other models of hypertension. Interestingly, since concomitant treatment with LU135252 normalized contractions to ET-1 and, as noted above, circulating ET-1 levels may not be an appropriate reflection of the local concentration of the peptide, a down-regulation of ET receptors and/or ineffective signal transduction pathways may be involved. Indeed, the acute effects clearly differ from those with chronic treatment of LU135252, when given to the organ chamber LU135252 concentration-dependently inhibits contractions to ET-1 in a competitive fashion (data not shown). We therefore feel that the acute effects of LU135252 do not contribute to the observed normalization of the blunted response to ET-1 with chronic therapy.

Norepinephrine-induced contractions were also reduced in Ang II–induced hypertension, while chronic ET\(_A\)-receptor blockade improved the sensitivity to norepinephrine. In the mesenteric arteries of spontaneously hypertensive rats, production of ET-1 by perfusion with Ang II might be exacerbated under hypertensive conditions associated with an exaggerated pressor response to infused angiotensin II. Thus, long-term therapy with angiotensin receptor antagonists might reduce signal transduction of the adrenergic receptors and/or ineffective signal transduction of angiotensin II receptors mediate smooth muscle proliferation and endothelin biosynthesis in rat vascular smooth muscle. 

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