Contribution of endothelin to renal vascular tone and autoregulation in the conscious dog

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Berthold, Heike, Klaus Münter, Armin Just, Hartmut R. Kirchheim, and Heimo Ehmke. Contribution of endothelin to renal vascular tone and autoregulation in the conscious dog. Am. J. Physiol. 276 (Renal Physiol. 45): F417–F424, 1999.—Exogenous endothelin-1 (ET-1) is a strong vasoconstrictor in the canine kidney and causes a decrease in renal blood flow (RBF) by stimulating the ETA receptor subtype. The aim of the present study was to investigate the role of endogenously generated ET-1 in renal hemodynamics under physiological conditions. In six conscious foxhounds, the time course of the effects of the selective ETA receptor antagonist LU-135252 (10 mg/kg iv) on mean arterial blood pressure (MAP), heart rate (HR), RBF, and glomerular filtration rate (GFR), as well as its effects on renal autoregulation, were examined. LU-135252 increased RBF by 20% (from 270 ± 21 to 323 ± 41 ml/min, P < 0.05) and HR from 76 ± 5 to 97 ± 8 beats/min (P < 0.05), but did not alter MAP, GFR, or autoregulation of RBF and GFR. Since a number of interactions between ET-1 and the renin-angiotensin system have been reported previously, experiments were repeated during angiotensin converting enzyme (ACE) inhibition by trandolapril (2 mg/kg iv). When ETA receptor blockade was combined with ACE inhibition, which by itself had no effects on renal hemodynamics, marked changes were observed: MAP decreased from 91 ± 4 to 80 ± 5 mmHg (P < 0.05), HR increased from 85 ± 5 to 102 ± 11 beats/min (P < 0.05), and RBF increased from 278 ± 23 to 412 ± 45 ml/min (P < 0.05). Despite a pronounced decrease in renal vascular resistance over the entire pressure range investigated (40–100 mmHg), the capacity of the kidneys to autoregulate RBF was not impaired. The GFR remained completely unaffected at all pressure levels. These results demonstrate that endogenously generated ET-1 contributes significantly to renal vascular tone but does not interfere with the mechanisms of renal autoregulation. If ETA receptors are blocked, then the vasoconstrictor effects of ET-1 in the kidney are compensated for to a large extent by an augmented influence of ANG II. Thus ET-1 and ANG II appear to constitute a major interrelated vasoconstrictor system in the control of RBF.

ET\textsubscript{A} receptor blockade; renal hemodynamics; renal autoregulation; renin-angiotensin system

THE VASCULAR ENDOTHELIUM modulates the basal vascular tone by releasing vasoactive factors acting on smooth muscle cells in a paracrine way. In the kidney, this influence is of particular importance, because changes in renal hemodynamics affect renal excretory function. Glomerular filtration rate (GFR) is altered by changes of pre- and postglomerular resistances, and the capacity of urine concentration or dilution is affected by the level of medullary blood flow. Moreover, paracrine vasoactive factors may interact with the ability of the kidney to autoregulate renal blood flow (RBF) and GFR.

Endothelin-1 (ET-1), the major isoform of the ET family of peptides, is released by various endothelial cells of the kidney (38) and has abundant binding sites at renal cortical and medullary vessels (19). Therefore, it may exert a considerable effect on renal function by influencing vasomotor tone. In general, intrarenally infused ET-1 leads to a long-lasting reduction in RBF (7, 39), which in the dog is mediated by the ETA receptor subtype (9). Depending on the dose, ET-1 has been shown to affect both pre- and postglomerular resistance vessels, thereby altering GFR (25, 36). Correspondingly, in the nonfiltering dog kidney, ET-1 infused at low doses (<1 ng·kg\textsuperscript{-1}·min\textsuperscript{-1}) into the renal artery produced decreases in RBF without changes in glomerular hydraulic pressure, whereas at higher doses it reduced both RBF and glomerular hydraulic pressure (25). Because of its prominent vasoconstrictor effect, ET-1 is thought to be involved in the development of a number of cardiovascular and renal diseases associated with endothelial dysfunction (4, 17). Long-term pathophysiological elevations in circulating ET-1 were found to be associated with a twofold increase of total peripheral and renal vascular resistances and a considerable reduction of renal plasma flow (43).

Because ET-1 acts as a paracrine hormone rather than a circulating one (38), it is difficult to draw conclusions about its physiological role from experiments in which plasma concentrations of ET-1 are elevated to pathophysiological or pharmacological levels. Therefore, in the present study, an ET-1 receptor antagonist was used to investigate the physiological role of endogenously generated ET-1 in the regulation of renal vascular tone. Since in the dog, as in humans, the ETA receptor subtype seems to be the predominant receptor mediating hemodynamic effects, a selective ETA receptor blocker (LU-135252) was used. Furthermore, because a number of studies have reported several interactions between ET-1 and the renin-angiotensin system (3, 8, 10, 11, 14, 15, 18, 35), we additionally investigated the effects of a combined blockade of both systems.

METHODS

Animals

Experiments were made in six conscious foxhounds of either sex weighing 29–33 kg. The dogs received a commer-
Germany) and N2O (0.5 l/min). Surgery was performed under 20 mg/kg iv; Sanofi, Libourne Cedex, France) and maintained with pentobarbitone sodium (Nembutal, 58.8–1.0%; Zeneca, Plankstadt, Germany) and N2O (0.5 l/min). Surgery was performed under sterile conditions. A Silastic catheter was implanted into the left renal artery. A Silastic catheter was implanted into the left renal vein. The ovari (or spermatic) vein was ligated. An inflatable cuff was placed around the left renal artery proximal to the tip of the renal artery catheter. An ultrasound transit-time flow probe (6 mm diameter; Transonic Systems, Ithaca, NY) was fixed around the left renal artery between its origin from the aorta and the cuff. The flow probe was wrapped with Dacron velour material (Protograft; Braun-Dexon, Spanenberg, Germany) to prevent ingrowth of fatty tissue and to enhance stabilization of the probe after healing. No surgery was performed on the right kidney. The catheters and cuff leads were led subcutaneously to the dog’s neck and brought out through the skin. The first 9 days after surgery the dogs received a combination of benzathine benzylpenicillin and sulfadiazine (Tardomyocel, 0.3 ml 3 sc; Bayer, Leverkusen, Germany) every third day. The arterial catheters were flushed every third day with sterile saline and filled with a solution of heparin (1,700 IU/mI; Braun). The venous catheter was flushed and filled daily.

Circulatory Measurements

Blood pressure was measured via the implanted catheters in the abdominal aorta and the renal artery using Statham pressure transducers (P23Db) and Gould pressure processors. Heart rate (HR) was recorded instantaneously with a rate meter (Gould pressure processor). RBF was measured using the implanted flow probe connected to a flowmeter (Transonic T106 or T108). The flow signals were passed through a 10 Hz-filter (Transonic). An analog recorder (Gould 2600) was used to directly display arterial blood pressure, renal perfusion pressure (RPP), RBF, and HR. Data were sampled with 1 or 5 Hz and stored on-line (IBM PC 386) after analog-to-digital conversion.

Renal Hemodynamic Measurements

GFR was determined by combined measurements of creatinine extraction, hematocrit (Hct, microrube centrifugation) and RBF. An oral dose of 3 mg creatinine was given 90 min before the start of the experiment, and another dose of 1.5 mg was given 20 min after the experiment had begun (i.e., after the control period). Creatinine plasma concentrations reached maximal values (1,000–1,200 µmol/I) 2–3 h after the first dose and fell to values of 400–600 µmol/I at the end of the experiment. Blood samples were taken from the arterial and venous catheter simultaneously and collected in chilled tubes. Arterial and venous concentrations of creatinine (Carter and CV) were determined by an automatic creatinine analyzer (Creatinine Analyzer 2; Beckman, Munich, Germany). GFR was calculated according to the equation

\[
GFR = \frac{RBF \times (1 - Hct) \times (CA - CV)}{CA}
\]

Renal vascular resistance (RVR) was derived by dividing the driving force (RPP − RPP̉) by RBF. RPP̉ refers to RPP at zero RBF and was obtained by complete occlusion of the renal artery.

Drugs

LU-135252 was used as a selective ETA receptor antagonist (Kᵢ for ETA, 1.4 nM; Kᵢ for ETB, 184 nM; see Ref. 30) at a dose of 10 mg/kg. This dose completely inhibited the vasoconstrictor response to 0.75 nmol/kg ET-1 in anesthetized dogs (n = 3–4; data not shown). Plasma ET-1 levels are not affected by this dose of LU-135252, indicating absence of a significant ETB receptor blockade (13).

The renin-angiotensin system was blocked by the angiotensin converting enzyme (ACE) inhibitor trandolaprilat (2 mg/kg). In preliminary experiments, this dose was found to significantly suppress the pressor response to exogenous ANG I by 74 ± 6% 2 h after administration of trandolaprilat (n = 6; data not shown).

Hoe-140 was continuously infused intravenously at a rate of 3 µg·kg⁻¹·min⁻¹ for 10 min followed by a constant rate of 0.5 µg·kg⁻¹·min⁻¹ until the end of the experiment. Hoe-140 is a highly potent bradykinin B₂ receptor antagonist that completely blocks the effects of endogenous bradykinin at doses of <10 µg/kg without affecting mean arterial blood pressure (MAP) or HR in doses up to 0.1 mg/kg (44).

Experimental Protocols

All experiments were carried out in conscious dogs lying quietly on their right side on a bench. The dogs were connected to the recording instruments via extension cables. The renal cuff could be inflated without attracting the dog’s attention. The experiments started between 8:00 and 9:00 AM, 16–20 h after the last feeding. Two experimental protocols were followed.

Time course. The effects of ETA receptor blockade and ACE inhibition on renal and systemic hemodynamics were studied over a period of 100 min. MAP, RBF, and HR were measured, and mean values over 20-min periods were calculated. At the end of each 20-min period, arterial and venous blood samples (1 ml) were taken to determine creatinine extraction for the calculation of GFR. During the first 20 min, baseline values were obtained, before the experimental infusions were given. The time course experiments were carried out in five dogs under control conditions, during ETA receptor blockade with LU-135252, during ACE inhibition with trandolaprilat, and during combined ETA receptor blockade and ACE inhibition. All drugs were given slowly (in 2 min each) as injections, dissolved in 10 ml saline. In a further series of experiments in three dogs, bradykinin receptors were blocked by Hoe-140 in addition to the combined administration of LU-135252 and trandolaprilat.

Renal autoregulation. The renal arterial catheter and the cuff lead were connected to an extravascular electromechanical control system. By controlled inflation of the cuff, this system allowed us to reduce RPP to any desired level below systemic blood pressure. The precision of the servo-control system was below ±1 mmHg. After one 5-min control period, RPP was servo-controlled. RPP was reduced in steps of 5 or 10 mmHg down to 40 mmHg. The duration of each pressure step was 5 min. The first 2 min were allowed to obtain stable conditions, then mean values for MAP, RPP, HR, and RBF were measured over the last 3 min. Arterial and venous blood samples were taken in the last 30 s of each pressure step to
determine creatinine concentration. After the last pressure step, the cuff was inflated maximally to reduce RBF to zero for determination of RPP0. Experiments were performed after the time course experiments during control conditions, during ETα receptor blockade, during ACE inhibition, and during combined ETα receptor blockade and ACE inhibition.

Data Analysis and Statistics

Time course. Averages of hemodynamic and renal function measurements were calculated for each 20-min period during the various experimental conditions over all dogs. The data were statistically evaluated by two-way ANOVA to determine whether any significant change occurred during the course of the experiment. If significance was detected, then the Student-Newman-Keuls test for multiple comparisons was used to determine which values were significantly different from baseline. For the analysis of the effects of Hoe-140, MAP, HR, and RBF were averaged over 40 min (60–100 min after the start of infusion). These averages were statistically compared with the baseline values by the paired Student’s t-test.

Renal autoregulation. For each single dog, measurements of RBF and GFR were plotted against the corresponding RPP. Linear regressions were calculated for the low pressure (subautoregulatory) range. Since poor correlations were usually found in the high pressure (autoregulatory) range, these data were averaged and termed “autoregulatory plateau.” To decide whether a data point was to be attributed to the plateau or the subautoregulatory range, the magnitude of the correlation coefficient of the regression line was taken as a criterion. The lower limits of autoregulation were obtained by the intersection of the subautoregulatory regression line with the autoregulatory plateau. The characteristics of the autoregulation curves (plateau, lower limit, subautoregulatory function) were determined for each individual dog under the various conditions. Averages over all dogs were statistically evaluated (vs. control conditions) by the Bonferroni test for repeated comparisons. In Fig. 3 and 4, data of RBF, GFR, and RVR were averaged over all dogs and plotted against RPP. Differences between control and experimental groups were statistically evaluated by two-way ANOVA followed by the Bonferroni test.

Differences at the 5% level were considered statistically significant. All data are presented as mean ± SE.

RESULTS

Time Course

The effects of ETα receptor blockade on MAP, HR, RBF, and GFR are illustrated in Fig. 1. The baseline values (time 0) did not differ from control conditions.

Although MAP was not significantly altered by ETα receptor blockade, HR significantly increased from 76 ± 5 to 93 ± 8 beats/min after 80 min, and to 97 ± 8 beats/min after 100 min (P < 0.05). RBF slowly increased over the entire observation period from 270 ± 21 to 323 ± 41 ml/min after 100 min (P < 0.05). GFR was not affected. Under control conditions, all variables remained stable over the experimental period.

The effects of ACE inhibition and combined ETα receptor blockade and ACE inhibition on MAP, HR, RBF, and GFR are shown in Fig. 2. MAP was not altered by ACE inhibition alone, but decreased significantly by ~10 mmHg after the 20th min until the end of the experiment (from 91 ± 5 to 80 ± 5 mmHg after 100 min; P < 0.05) during combined ETα receptor blockade and ACE inhibition. Along this blood pressure reduction, HR rose immediately and remained elevated until the end of the experiment (from 85 ± 5 to 102 ± 11 beats/min after 100 min; P < 0.05). During ACE inhibition, HR was transiently increased over the first 20 min (from 75 ± 4 to 91 ± 5 beats/min; P < 0.05) but then returned to its baseline level. Similarly, RBF only transiently increased during the first 60 min (from 261 ± 35 to 287 ± 38 ml/min after 60 min; P < 0.05) after ACE inhibition. In contrast, combined ETα receptor blockade and ACE inhibition immediately increased RBF by ~50% to a new steady-state level (from 278 ± 23 to 412 ± 45 ml/min after 100 min; P < 0.05). Despite the marked changes in RBF, the GFR remained unaffected in both experimental groups.
The effects of combined ETA receptor blockade and ACE inhibition on MAP, HR, and RBF were not altered by coinfusion of the bradykinin receptor antagonist Hoe-140 (Fig. 3). The increase in RBF was very similar with and without blockade of bradykinin receptors (351 ± 45 to 477 ± 69 vs. 309 ± 27 to 452 ± 55 ml/min after 100 min; P < 0.05 for both conditions).

Renal Autoregulation

The effects of the experimental interventions on renal autoregulation are depicted in Figs. 4 and 5, and summarized in Tables 1 and 2. RBF and GFR were autoregulated down to 66 ± 3 and 72 ± 2 mmHg, respectively, in the control group. RVR decreased steadily within the autoregulatory range from 0.33 ± 0.05 to 0.20 ± 0.05 mmHg·ml⁻¹·min⁻¹ (P < 0.05). In the subautoregulatory range, RVR remained constant. Neither ETA receptor blockade (Fig. 4) nor ACE inhibition alone (Fig. 5) had significant effects on autoregulation of RBF or GFR. Over all pressure steps, RVR was slightly decreased during ETA receptor blockade. ACE inhibition had no influence on RVR. The combined ETA receptor blockade and ACE inhibition significantly elevated RBF in the autoregulatory plateau (from 280 ± 39 to 428 ± 43 ml/min; P < 0.05; Table 1 and Fig. 5), whereas the lower limit of RBF autoregulation remained unchanged. Although basal RVR was largely reduced, it further decreased with lower RPP values, thus allowing for a normal RBF autoregulation. In contrast to RBF, the GFR remained completely unaffected at all RPPs.

DISCUSSION

ET-1 is a strong vasoconstrictor in the systemic and renal circulation. In the dog, this effect is exerted via ETA receptors, whereas the ETB receptor appears to mediate predominantly the diuretic and natriuretic responses (5, 9). By using the selective ETA receptor antagonist Hoe-140, the effects on MAP, HR, RBF, and GFR were investigated (Fig. 3). The increase in RBF was very similar with and without blockade of bradykinin receptors (351 ± 45 to 477 ± 69 vs. 309 ± 27 to 452 ± 55 ml/min after 100 min; P < 0.05 for both conditions).

Renal Autoregulation

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antagonist LU-135252, the present study demonstrates that endogenously generated ET-1 is an important modulator of renal hemodynamics under physiological conditions. An intravenous bolus injection of LU-135252 caused an increase in RBF by 20%. This weak effect seems to argue against an important contribution of ET-1 to normal renal vascular tone. When the ETA receptor blockade was combined with ACE inhibition, however, a long-lasting elevation of RBF by 50% was observed.

Table 1. Characteristics of RBF autoregulation during control conditions, ETA receptor blockade, ACE inhibition, and combined ETA receptor blockade and ACE inhibition

<table>
<thead>
<tr>
<th></th>
<th>Plateau, mL/min</th>
<th>Lower Limit of Autoregulation, mmHg</th>
<th>Slope of Subautoregulatory Function Curve</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>280 ± 49</td>
<td>66 ± 3</td>
<td>5.6 ± 0.8</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>ETA receptor blockade</td>
<td>332 ± 47</td>
<td>67 ± 3</td>
<td>6.8 ± 1.0</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>287 ± 38</td>
<td>59 ± 3</td>
<td>6.5 ± 1.2</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>ETA receptor blockade + ACE inhibition</td>
<td>428 ± 43*</td>
<td>65 ± 3</td>
<td>8.9 ± 1.0*</td>
<td>1.00 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. ACE, angiotensin converting enzyme; RBF, renal blood flow. *P < 0.05 compared with control.

Table 2. Characteristics of GFR autoregulation during control conditions, ETA receptor blockade, ACE inhibition, and combined ETA receptor blockade and ACE inhibition

<table>
<thead>
<tr>
<th></th>
<th>Plateau, mL/min</th>
<th>Lower Limit of Autoregulation, mmHg</th>
<th>Slope of Subautoregulatory Function Curve</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39 ± 4</td>
<td>72 ± 2</td>
<td>1.5 ± 0.3</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>ETA receptor blockade</td>
<td>44 ± 5</td>
<td>71 ± 2</td>
<td>1.6 ± 0.2</td>
<td>0.95 ± 0.03</td>
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<tr>
<td>ACE inhibition</td>
<td>38 ± 6</td>
<td>67 ± 1</td>
<td>1.5 ± 0.4</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>ETA receptor blockade + ACE inhibition</td>
<td>49 ± 8</td>
<td>74 ± 3</td>
<td>1.8 ± 0.4</td>
<td>0.92 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. GFR, glomerular filtration rate.

Fig. 4. Effects of ETA receptor blockade on renal autoregulation (control, ●; ETA receptor blockade, △). RBF, GFR, and renal vascular resistance (RVR) were averaged over 5 dogs during each pressure step. Values are means ± SE.

Fig. 5. Effects of ACE inhibition and of combined ETA receptor blockade and ACE inhibition on renal autoregulation (control, ●; ACE inhibition, □; combined ETA receptor blockade and ACE inhibition, ○). RBF, GFR, and RVR were averaged over 5 dogs during each pressure step. Values are means ± SE. *P < 0.05 vs. control.
Since ACE inhibition by itself did not alter RBF, this result cannot be attributed to an additive effect, suggesting important interactions between ET-1 and ANG II in the control of RBF. The importance of the renin-angiotensin system for the maintenance of MAP during ETA receptor blockade is less clear. Both ETA receptor blockade and ACE inhibition alone had only minor effects on MAP, and the decrease of MAP after combined ETA receptor blockade and ACE inhibition appears to be rather additive. Similar effects have been recently reported for the nonselective ETAB receptor antagonist bosentan in hypertensive dogs (12). It should be noted that HR considerably increased during ETA receptor blockade, indicating that in addition to the renin-angiotensin system other neurohumoral mechanisms were activated to preserve MAP. Further experiments are necessary to clarify this issue.

Previous studies have demonstrated that the vascular production of ET is stimulated by ANG II at several cellular levels. ANG II has been shown to increase proET-1 mRNA (10), ET converting enzyme activity (3), and ET-1 release (15) in endothelial cells. Conversely, chronic inhibition of ANG II formation by ACE inhibition prevented the stimulation of ET-1 release that is characteristically observed after chronic thoracic inferior vena caval constriction in dogs (8). Accordingly, ET-1 may act as an important mediator of the physiological actions of ANG II. This suggestion is supported by a number of recent studies which demonstrated that in rats ETA receptor blockade largely prevented the effects of chronically infused ANG II on MAP, RBF, and vascular responsiveness (14, 18, 35). Similar results were obtained in dogs, where the nonselective ETAB receptor blocker bosentan caused a significantly stronger MAP reduction in animals with ANG II-dependent hypertension than in normotensive controls (11). Thus the present finding that the effects of ETA receptor blockade were most pronounced during ACE inhibition was rather unexpected. Possible explanations for this synergy may be that the importance of ET-1 as a mediator of the effects of ANG II depends on the level of activation of the renin-angiotensin system or on the mechanism that causes an increase in circulating ANG II.

GFR depends on the ratio of pre- and postglomerular resistance and on the glomerular ultrafiltration coefficient (Kf). Exogenous ET-1 has been shown to influence all of these variables, although the results are conflicting. In the hydronephrotic rat kidney, ET-1 had a greater effect on the afferent than efferent arteriole (26). On the other hand, glomerular hydrostatic pressure determined by the stop-flow technique was only affected at high doses of ET-1 (25). This latter observation would be consistent with quantitatively comparable effects on pre- and postglomerular resistances at lower doses of ET-1. No changes in glomerular hydrostatic pressure in response to ET-1 were reported from micropuncture studies in rats (1); the authors attributed the decrease in single-nephron GFR to a fall in Kf. They also showed that ET-1 can induce constriction of cultured mesangial cells. Other micropuncture studies found significant decreases (24) or even increases in glomerular hydrostatic pressure (22) in response to exogenous ET-1 administration. The results of the present study, in which the blockade of ETA receptors failed to alter GFR, even after additional blockade of the renin-angiotensin system by ACE inhibition, would be consistent with a balanced effect of ET-1 on pre- and postglomerular ETA receptors at low, physiological concentrations. The failure of ACE inhibition alone to alter GFR or RBF confirms a previous study from our laboratory (34) and indicates that ANG II does not contribute to renal vascular tone in dogs on a normal salt diet.

Renal autoregulation is attributed to at least two mechanisms, the tubuloglomerular feedback (TGF) and the myogenic response, albeit different studies indicated varying influences of both mechanisms (20, 28, 29). A modification of the TGF response by ET-1 seems unlikely, since micropuncture studies in rats have shown that neither exogenous ET-1 (40) nor blockade of ETA receptors (21) affected the TGF. A possible interaction of ET-1 with the myogenic response is suggested by findings in the hydronephrotic rat kidney, where local administration of ET-1 caused a preferential constriction of the arcuate and interlobular arteries via the ETA receptor subtype (16). By this effect, ET-1 could reduce the myogenic reserve and lead to a shift of the lower limit of autoregulation toward a higher pressure level (23). In the present study, however, neither RBF nor GFR autoregulation were altered by blockade of ETA receptors. Thus, under physiological conditions the endogenous ET-1 concentration seems to be too low to interfere with renal autoregulation.

Combined ETA receptor blockade and ACE inhibition caused a very strong reduction of RVR at all pressure steps which indicates the removal of a pronounced vasoconstrictor tone. Despite the marked fall of basal RVR, the autoregulatory response remained entirely intact. While renal autoregulation is abolished completely by Ca2+ antagonists (32) and impaired by substances which interact with the TGF (20), a similar increase in basal RBF in the face of a normal renal autoregulation was observed after the administration of several vasodilators, such as bradykinin (6), nitric oxide donors (33), or dopamine (2). The mechanism(s) underlying the synergistic effects of the combined blockade of the ETA and renin-angiotensin systems on renal vascular tone remain unclear. The effects of ACE inhibition have been partly attributed to elevated bradykinin levels (45), which in turn may stimulate the release of ET-1 (27) and nitric oxide (42) from renal endothelial cells. Since the synergy between ETA receptor blockade and ACE inhibition was entirely preserved in the presence of the bradykinin receptor antagonist HOE-140, an important contribution of bradykinin to the vasodilator response seems very unlikely. An alternative possibility is that ETA receptor blockade may result in an activation of the renin-angiotensin system, either due to the release of a negative regulatory effect of ET-1 on renin secretion rate (31, 37) or secondary due to the neurohumoral activation. Since the renal circula-
tion is particularly sensitive to ANG II-induced vasoconstriction, elevated levels of ANG II may help to maintain renal vascular tone in the face of an increased synthesis of nitric oxide secondary to an enhanced binding of ET-1 to endothelial ETB receptors during ETB receptor blockade (41).

In conclusion, the results of the present study suggest that endogenously generated ET-1 exerts a tonic ETB receptor-mediated vasoconstrictor action in the kidney. During blockade of ETB receptors, an increased influence of ANG II appears to compensate to a large extent for the lack of vasoconstrictor activity of ET-1. Only when both systems are blocked, the significance of tonic ETB receptor stimulation for the renal circulation becomes apparent. Thus ET-1 appears to contribute considerably to normal renal vascular tone without impairing the autoregulatory response.

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