Stimulation of the Renin-Angiotensin System by Endothelin Subtype A Receptor Blockade in Conscious Dogs

Heike Berthold, Klaus Münter, Armin Just, Hartmut R. Kirchheim, Heimo Ehmke

Abstract—Previous studies in dogs have shown additive or even synergistic effects of combined angiotensin-converting enzyme inhibition and either nonselective endothelin subtype A/B (ET_{AB}) or selective endothelin subtype A (ET_{A}) receptor blockade on renal vascular resistance and mean arterial blood pressure. A possible mechanism underlying this interaction may be a stimulation of the renin-angiotensin system during endothelin (ET) receptor blockade. We therefore investigated whether plasma renin activity and renin release are regulated by the ET_{A} receptor. Experiments were made in conscious, chronically instrumented dogs receiving either saline or the selective ET_{A} receptor antagonist LU 135252 (10 mg/kg IV). Eighty to 100 minutes after the administration of LU 135252 (n=5), heart rate (99±7 versus 81±6 bpm; P<0.05) and renal blood flow (327±40 versus 278±36 mL/min; P<0.05) were increased significantly, whereas mean arterial blood pressure tended to be lower (93±5 versus 105±7 mm Hg). These changes were associated with a 2-fold increase in plasma renin activity (0.74±0.12 versus 0.37±0.10 ng angiotensin I per milliliter per hour; P<0.05). Measurements of renin release at various renal perfusion pressures (n=5) with the use of a vascular occluder implanted around the left renal artery revealed that ET_{A} receptor blockade did not alter renin release at resting renal perfusion pressure (78±25 versus 71±39 U/min) but strongly enhanced the sensitivity of pressure-dependent renin release <80 mm Hg ∼2.2-fold. In conclusion, selective ET_{A} receptor blockade is associated with a stimulation of the circulating renin-angiotensin system, which results from both a sensitization of pressure-dependent renin release and a larger proportion of blood pressure values falling into the low pressure range, where renin release is stimulated. These findings strengthen the view that ET and the renin-angiotensin system closely interact to regulate vascular resistance and provide a physiological basis for synergistic hypotensive effects of a combined blockade of both pressor systems. (Hypertension. 1999;33:1420-1424.)

Key Words: endothelin receptors, endothelin renal blood flow renin-angiotensin system renin

Exogenous endothelin (ET), predominantly by activating endothelin subtype A (ET_{A}) receptors, is a strong constrictor of renal and systemic vessels, but the relevance of endogenous ET for the maintenance of basal vascular tone and blood pressure is still controversial. In healthy subjects, short-term blockade of ET_{A} receptors\(^1\) had no effects on blood pressure and renal blood flow (RBF). In addition, nonselective blockade of ET_{A} and endothelin subtype B (ET_{B}) receptors did not affect blood pressure in anesthetized dogs.\(^2\) In contrast, intra-arterial infusion of an ET_{A} receptor blocker in normotensive humans caused strong increases in forearm blood flow, indicating a pronounced reduction in vascular resistance.\(^3,4\)

An interaction of ET and the renin-angiotensin system could account for the lack of large blood pressure changes during systemic ET receptor blockade. In 2 recent investigations in normotensive dogs, we observed significant falls in mean arterial blood pressure (MAP)\(^5,6\) and large increases in RBF\(^5\) when the ET_{A} receptor blockade was combined with angiotensin-converting enzyme (ACE) inhibition. Similarly, nonselective ET_{AB} receptor blockade by bosentan exerted an additional hypotensive effect in hypertensive dogs during ACE inhibition.\(^7\) Combined administration of bosentan and an ACE inhibitor also resulted in pronounced hypotensive effects in rats with chronic heart failure.\(^8\) The mechanisms underlying this interaction between ET receptor blockade and the renin-angiotensin system are still unclear. Because previous investigations have demonstrated that exogenous ET may inhibit basal or stimulated renin release in isolated perfused kidneys\(^9\) and in anesthetized rats\(^10\) and dogs,\(^11\) it is conceivable that ET_{A} receptor blockade may have stimulatory effects on renin release. Consequently, elevated angiotensin II concentrations may take over the vasoconstrictor effects of ET, thus allowing for maintenance of vascular tone and blood pressure during ET_{A} receptor blockade. Accordingly, the aim of the present study was to investigate the influence of a selective ET_{A} receptor blockade on the renin-angiotensin system in conscious, normotensive dogs.

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Methods

Animals
Experiments were performed in 6 conscious foxhounds of either sex weighing 28 to 31 kg. The dogs received a commercially available dog diet (SSniff Spezialdiäten GmbH) containing ~100 mEq sodium per day and had free access to water. At least 10 days were allowed for recovery after the implantation surgery. All experiments were done in accordance with the national law for the care and use of research animals.

Surgical Procedures
The dogs were premedicated with atropine (0.5 mg SC; Braun) and propionylpromazine (Combelen, 0.64 mg/kg SC; Bayer). Anesthesia was introduced with sodium pentobarbital (Nembutal, 20 mg/kg IV; Sanofi) and maintained with halothane (Fluothane, 0.8% to 1.0%; Zeneca) and N₂O (0.5 L/min). Surgery was performed under sterile conditions. Through a left flank incision, polyurethane catheters were implanted into the abdominal aorta and left renal artery. A silicone elastomer catheter was implanted into the left renal vein. The ovarian or spermatic vein was ligated. An inflatable cuff was placed around the renal artery proximal to the tip of the renal artery catheter. An ultrasound transit-time flow probe (6-mm diameter; Transonic Systems) was fixed around the left renal artery between the origin of the artery from the aorta and the cuff. The flow probe was wrapped with synthetic polyester velour material (Protgraft; Braun-Dexon) to prevent ingrowth of fatty tissue and enhance probe stabilization after healing. No surgery was performed on the right kidney. The catheters and cuff leads were led subcutaneously to the incision. The dogs were premedicated with atropine (0.5 mg SC; Braun) and the cuff lead was led subcutaneously to the dog’s neck and brought out through the skin. The left 9 days after surgery, the dogs received a combination of benzathine benzylpenicillin (100,000 U IV; Zeneca) and N₂O (0.5 L/min) until recovery. Surgery was performed under sterile conditions. The right kidney was left intact as a control.

Circulatory Measurements and Blood Sampling
Blood pressure was measured in the abdominal aorta and the renal artery with the use of Statham pressure transducers (P23Db) and Gould pressure processors. Heart rate (HR) was recorded instantaneously with a rate meter (Gould pressure processor). RBF was measured with the implanted flow probe connected to a flowmeter. The flow probe signals were passed through a 10-Hz filter (Transonic). An analog recorder (Gould 2600) was used to display the measured variables. All data were sampled at 20 Hz and stored as 1-second mean values online (IBM PC 386) after analog-to-digital conversion.

For the determination of plasma renin activity (PRA) and renin release, blood samples (1 mL) were taken from the arterial and venous catheters simultaneously and collected in chilled tubes containing 6 mEq EDTA. For the estimation of PRA, the amount of angiotensin I was determined by radioimmunoassay. Renin release was calculated according to the equation

\[
\text{Renin release} = (\text{PRA} - \text{PRA}_0) \times \text{RBF} \times (1 - \text{hematocrit})
\]

where the subscripts 0 and a denote venous and arterial, respectively.

The resulting dimension for renin release is nanograms angiotensin I per milliliter per hour of incubation multiplied by milliliter per minute and is referred to as units of renin release.

Drugs
LU 135252 is a nonpeptide, selective ET₄ receptor antagonist with a plasma half-life in dogs of ~12 hours. The selectivity for ET₄ receptors, expressed as the ratio of the affinities for ET₄ over ET₆ receptors, is 131.12 LU 135252 was used at a dose of 10 mg/kg. Preliminary experiments in anesthetized dogs showed that this dose completely inhibits the vasoconstrictor response (~26±4 mm Hg) to an intravenous injection of 0.75 nmol/kg ET-1, which increases plasma ET-1 concentrations into the nanomolar range (ie, 100- to 1000-fold higher than normal).

Experimental Protocols
All experiments were performed in conscious dogs lying quietly on their right side on a bench. The dogs were connected to the recording instruments by extension cables. The renal cuff could be inflated without distracting the dog’s attention. The experiments started between 8 and 9 AM, 16 to 20 hours after the last feeding. Two experimental protocols were followed, as described below.

Time Course Experiments (n=5)
MAP, HR, RBF, and arterial PRA were examined 20 minutes before the administration of LU 135252 and for the following 100 minutes. Arterial blood samples for the determination of PRA were taken every 20 minutes. LU 135252 was given slowly as a bolus (10 mg/kg IV), dissolved in 10 mL saline. Experiments with bolus infusions of 10 mL saline served as time controls. Time control experiments were performed in the same dogs with at least 2 days left between the 2 experiments. Experiments were done in random order.

Determination of Pressure-Dependent Renin Release (n=5)
Renin release experiments were done 100 minutes after the administration of LU 135252 and saline, respectively. The renal artery catheter and the cuff lead were connected to an extracorporal electropneumatic control system. By controlled inflation of the cuff, renal perfusion pressure (RPP) was reduced to defined levels below the systemic blood pressure. The precision of the servo-control system was ±1 mm Hg. A 5-minute control period was allowed before RPP was servo-controlled. Then RPP was reduced in steps of 5 or 10 mm Hg to 60 mm Hg. The duration of each pressure step was 5 minutes. In the last 30 seconds, arterial and venous blood samples were taken for the determination of renin release.

Data Analysis and Statistics
For the analysis of the time course, mean values of MAP, HR, and RBF were calculated over 20-minute periods. The effects of LU 135252 versus saline were analyzed by 2-way ANOVA. If significant changes were detected, mean values obtained during the last experimental period (80 to 100 minutes) were analyzed by the paired Student t test.

For the analysis of pressure-dependent renin release, renin release was calculated for each pressure step in single dogs. Then mean values over all dogs were calculated. Two-way ANOVA was used to determine whether significant changes occurred between renin release during control conditions and during ET₄ receptor blockade.

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

Results
Baseline values before the administration of either LU 135252 or saline were not different in the 2 experimental groups (Table).

The effects of acute ET₄ receptor blockade on MAP, HR, and RBF are summarized in Figure 1. LU 135252 induced renal vasodilation (RBF, 327±40 versus 278±36 mL/min; P<0.05) together with an increase in HR (99±7 versus 81±6 bpm; P<0.05). MAP tended to be lower during ET₄ receptor...
blockade compared with saline infusion, but the difference failed to reach statistical significance.

The time course of arterial PRA in response to acute ET\textsubscript{A} receptor blockade is depicted in Figure 2. Whereas in the control experiments arterial PRA remained unchanged, it started to increase immediately after the administration of LU 135252 and remained elevated over the entire observation period. One hundred minutes after the administration of LU 135252, arterial PRA was doubled (0.74 ± 0.12 versus 0.37 ± 0.10 ng angiotensin I per milliliter per hour; \( P < 0.05 \)).

Renin release was transiently elevated after ET\textsubscript{A} receptor blockade in each dog, reaching maximum values between 20 and 80 minutes after the administration of LU 135252, and then returned to control levels. Because of the high interindividual variability of renin release and the different time courses, however, this effect did not reach statistical significance (\( P = 0.09 \)).

The effects of acute ET\textsubscript{A} receptor blockade on renin release at rest and at reduced RPP are shown in Figure 3. At resting RPP, renin release was unaffected by ET\textsubscript{A} receptor blockade (78 ± 25 versus 71 ± 39 U) and remained unaltered down to a RPP of \( \approx 80 \) mm Hg. Below 80 mm Hg, however, a strong enhancement of renin release was observed during ET\textsubscript{A} receptor blockade, indicating a sensitization of pressure-dependent renin release by a factor of \( \approx 2.2 \).

Discussion

Previous studies have shown pronounced hypotensive effects of a blockade of ET receptors combined with an ACE inhibition in normotensive dogs\textsuperscript{5,6} and in animal models of chronic heart failure\textsuperscript{8} and hypertension.\textsuperscript{7} In contrast, both nonselective ET\textsubscript{A/B} or selective ET\textsubscript{A} receptor blockade\textsuperscript{2,5} and ACE inhibition alone\textsuperscript{5,13} had only minor hypotensive effects in normal dogs. From the results of a recent study, we proposed a close interaction of ET and the renin-angiotensin system, such that during blockade of ET\textsubscript{A} receptors an increased effect of angiotensin II may compensate for the lack of vasoconstrictor activity of ET.\textsuperscript{5} Therefore, the aim of the present study was to test the hypothesis that the renin-angiotensin system is stimulated during ET\textsubscript{A} receptor blockade. In accordance with this hypothesis, we observed an increase of arterial PRA during ET\textsubscript{A} receptor blockade; 100 minutes after the administration of LU 135252, PRA was doubled. Until now, only a few studies have been performed to determine the effects of ET receptor blockade on the renin-angiotensin system. Schricker et al\textsuperscript{14} investigated the effects of administration of bosentan for 2 days on PRA in 2-kidney, 1 clip hypertensive rats. Neither renal mRNA nor PRA was found to be altered. Similar results after bosentan were obtained in patients with mild to moderate essential hypertension.\textsuperscript{15} A possible explanation for the discrepancy to the present study may be the different selectivities of the ET
receptor blockade. It is conceivable that ET exerts an inhibitory effect on renin release via the ET$_A$ receptor subtype and a stimulatory effect via the ET$_B$ receptor subtype, secondary to the release of nitric oxide. During nonselective ET$_{A/B}$ receptor blockade, the removal of both inhibitory and stimulatory effects may be balanced, leading to an unchanged PRA. In a recent in vitro investigation, however, bosentan has been shown to increase renin secretion from isolated afferent arterioles even after nitric oxide synthase inhibition by N$^\omega$-nitro-L-arginine methyl ester (L-NAME).$^{16}$ Therefore, a more likely explanation for the discrepant results of the present investigation and those of Schricker et al$^{14}$ and Krum et al$^{15}$ appears to be that in the latter studies the ET system was blocked chronically, which may result in secondary adaptive changes in the regulation of renin release. Differences between acute and chronic effects of ET receptor blockade on the renin-angiotensin system may also explain the failure of ACE inhibition to sensitize blood pressure to the hypotensive effects of ET$_A$ receptor antagonists in hypertensive rats.$^{17,18}$ Furthermore, species differences may exist in the regulation of renin release by ET.

Surprisingly, renin release at resting RPP was only transiently affected by ET$_A$ receptor blockade. Only when RPP was reduced was a strong enhancement of the sensitivity of pressure-dependent renin release disclosed, suggesting a more prominent regulatory role of renal ET at low perfusion pressures. This interpretation would be in accordance with the results from a study in anesthetized dogs, in which ET infused intravenously into denervated kidneys inhibited renin release precipitated by renal artery constriction.$^{11}$ Similarly, in the isolated rat kidney$^9$ and the nonfiltering canine kidney,$^{19}$ ET elicited an inhibitory effect on renin release at a constant renal artery pressure of 80 mm Hg, which was abolished if the kidney was perfused with a Ca$^{2+}$-free solution.$^9$ In contrast, in some early studies ET had been found to stimulate renin release,$^{20,21}$ although at pharmacological concentrations. Thus, this stimulatory action may not reflect the physiological effect of ET.

The mechanism by which renin release is altered by ET$_A$ receptor blockade is not clear. Because ET$_A$ receptor blockade was associated with an increase in HR and a reduction in MAP, the enhancement of renin release may be mediated by a reflex increase in sympathetic activity due to an unloading of arterial baroreceptors. However, several studies have shown that both direct$^{22,23}$ and reflex$^{24,25}$ stimulation of renal sympathetic nerve activity as well as humoral activation of intrarenal adrenoceptors$^{25–27}$ cause a parallel shift of the relation between RPP and renin release without affecting the sensitivity of renin release in the lower pressure range. By contrast, ET$_A$ receptor blockade increased the sensitivity of the pressure-dependent mechanism of renin release without major effects on the threshold pressure of renin release. Therefore, it seems more likely that the enhancement of renin release resulted from a direct effect of renal ET at the level of juxtaglomerular cells. One possibility may be the release of a direct inhibitory effect of ET on juxtaglomerular cells at lower perfusion pressures. For example, ET may tonically antagonize the effects of nitric oxide on renin release. Previous studies have demonstrated that endogenously generated nitric oxide increases the sensitivity of pressure-dependent renin release.$^{28,29}$ Alternatively, it is conceivable that the renal ET production may be enhanced at low perfusion pressures, but it remains to be shown whether this actually occurs under physiological conditions. Finally, the macula densa mechanism of renin release may be altered during ET$_A$ receptor blockade. Low, nonpressor doses of ET induced a natriuresis in dogs$^{30}$ and in rats,$^{31}$ an action that may be mediated by an inhibition of proximal tubular sodium reabsorption.$^{31}$ Accordingly, if ET$_A$ receptor blockade should cause an increase in sodium reabsorption, the reduced sodium chloride concentration at the macula densa would be expected to stimulate renin release. However, if ET exerted a tonic inhibitory action on renin release by this mechanism, one would rather expect an elevation of renin release over the entire pressure range during the ET$_A$ receptor blockade. Nevertheless, in any case the slight decrease in MAP will contribute to the increase in PRA, inasmuch as a greater proportion of pressure values are falling into the lower pressure range and consequently stimulate renin release.

Taken together, the present study shows that selective ET$_A$ receptor blockade is associated with a stimulation of the renin-angiotensin system, which results from both a sensitization of pressure-dependent renin release and a larger proportion of pressure values falling into the lower pressure range. These findings are in agreement with the suggestion of a strong interaction of ET and the renin-angiotensin system in the maintenance of basal vascular tone and provide a physiological basis for the synergistic hypotensive effects of a combined blockade of both vasoconstrictor systems.

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References


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