Hemodynamic, renal, and endocrine responses to acute ET<sub>A</sub> blockade at different ANG II plasma levels

Willehad Boemke, Berthold Hocher, Nora Schleyer, Martin Otto Krebs, and Gabriele Kaczmarczyk. Hemodynamic, renal, and endocrine responses to acute ET<sub>A</sub> blockade at different ANG II plasma levels. Am J Physiol Regulatory Integrative Comp Physiol 280: R1322–R1331, 2001.—Angiotensin (ANG) II effects may be partly mediated by endothelin (ET)-1. This study analyses the hemodynamic, renal, and hormonal responses of acute ET<sub>A</sub> receptor antagonism (LU-135252) at two ANG II plasma levels in eight conscious dogs. Protocol 1 involved a 60-min baseline, followed by two doses of ANG II for 60 min each (4 and 20 ng·kg<sup>-1</sup>·min<sup>-1</sup>), termed ANG II 4 (slightly increased) and ANG II 20 (pathophysiologically increased ANG II plasma concentration). Protocol 2 was the same as protocol 1 but included 15 mg/kg iv LU-135252 after the baseline period. Protocol 3 was a 3-h time control. ANG II without LU-135252 did not increase plasma big ET-1 and ET-1, whereas LU-135252 increased ET-1 transiently after injection. This transient ET-1 increase was not reflected in urinary ET-1 excretion. The ANG II induced decreases in sodium, water, and potassium excretion, glomerular filtration rate, and fractional sodium excretion were not different with and without LU-135252. Mean arterial pressure increased during ANG II and was not lower with LU-135252 (~6 mmHg, not significant). Most importantly, during ANG II 20 LU-135252 prevented the decrease in cardiac output. Simultaneously, systemic vascular resistance increased 40% less, pulmonary vascular resistance was maintained at baseline levels, and central venous and wedge pressure were lower. Because ANG II stimulated endothelin de novo synthesis should just have started after 2 h of ANG II infusion, there must be mechanisms other than blocking the coupling of de novo synthesized endothelins to the ET<sub>A</sub> receptors to explain the effects of acute ET<sub>A</sub> receptor inhibition in our setting.

THE ACTIONS OF ANGIOTENSIN (ANG) II are manifold, e.g., it dose dependently increases total peripheral resistance and mean arterial pressure (MAP); stimulates aldosterone and antidiuretic hormone release; affects vascular, glomerular, and tubular renal function; causes salt appetite; increases sympathetic discharge; and facilitates adrenergic transmission and neurotransmission (24). Chronically, it induces vascular proliferation and plays an important part in cardiovascular and renal disease (24). Recently it was demonstrated that ANG II may increase prepro-endothelin (ET)-1 mRNA, ET-1 gene expression, and ET-1 release in endothelial cells, vascular smooth muscle cells, cardiomyocytes, and mesangial cells (8, 9, 11, 22, 30). Monoclonal antibodies to ET-1, endothelin-converting enzyme inhibitors, or ET<sub>A</sub>-receptor blockers reduced or prevented the hypertrophic and mitogenic effects of ANG II in vitro and in vivo (11, 18, 22). In addition, long-term studies in rats demonstrated that the ANG II-induced increase in vascular and kidney ET-1 content and the increase in functional endothelin-converting enzyme can be totally inhibited by ET<sub>A</sub> receptor blockade (3). Accordingly, there is substantial evidence that ET-1 contributes to or mediates part of the ANG II effects.

With an integrative approach, the present study investigates for the first time on conscious healthy dogs the influence of acute ET<sub>A</sub>-receptor blockade on acute ANG II-induced changes in pulmonary and systemic hemodynamics, plasma hormones, and renal excretions. ANG II was infused at two different rates to differentiate between the effects of slightly and pathophysiologically increased plasma ANG II concentrations (10).

Because this is an acute study its results should not be influenced by changes in vascular structure that develop during long-term ANG II infusion. In addition, by investigating the acute effects of ET<sub>A</sub> blockade at two different ANG II plasma concentrations, ANG II-plasma-level-dependent differences of ET<sub>A</sub> blockade on hemodynamics and/or renal function may be revealed.

MATERIALS AND METHODS

The study was approved by the Berlin Animal Protection Committee in accordance with the German Animal Protection Law (approval No. G 014598). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, 85–23, revised 1996.

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Address for reprint requests and other correspondence: W. Boemke, AG Experimentelle Anaesthesia, Campus Virchow-Klinikum, Medizinische Fakultät der Charité, Augustenburger Platz 1 D-13353 Berlin, Germany (E-mail: willehad.boemke@charite.de).
Animal Maintenance

Eight pure-bred female beagle dogs about 2 yr old, 13.6 ± 0.3 kg body wt, were selected from the central animal laborato-
ries of the Biomedical Research Center of the Charité. The
dogs were vaccinated, dewormed, and tested for their social behavior and tolerance to minor experimental pro-
dedures. They were kept under standardized conditions: air-
conditioned environment (21°C, humidity 55–60%), spacious
animal quarters during the day, and individual compart-
ments (5 m²) during the night. Their physical health was
controlled by daily check-ups, including measurements of
body temperature and body weight.

Over a period of 3–4 wk the dogs were accustomed to the
experimental conditions and trained to lie on a padded ani-
mal table for up to 5 h.

Dietary Regimen

To equilibrate the preexperimental input-output balance
and to establish a similar preexperimental activation of the
renin-ANG-aldosterone system (RAAS), the dogs were fed a
standardized diet beginning at least 5 days before the stud-
ies. The diet consisted of minced beef (12 g) and boiled rice
(58 g). It contained 91 ml water, 2.5 mmol sodium, and 3.5
mmol potassium (all values given per kilogram body wt
daily). The calories supplied with this diet (277 kJ kg body
wt⁻¹·day⁻¹) were sufficient to keep the body weight of the
dogs constant. The food mash was offered once a day at 2 PM,
and the intake was finished by all dogs within 1 h.

Experimental Protocols

Three experimental protocols of 3-h duration were per-
formed on each of the eight dogs in a total of 24 experiments.

Because the dogs were fasting for about 18 h before the
start of the experiments, a special fluid intake regimen was
necessary: first, to establish constant excretion rates for
urine volume and sodium throughout the observation period
(see protocol 3, time control) and second, to guarantee that
excretion rates remain within a measurable range even dur-
ing ANG II infusion (see protocols 1 and 2).

To achieve this objective, 30 ml/kg of a balanced electrolyte
solution (Ionosteril, Braun Melsungen, Germany; containing,
in mmol/l, 137 sodium, 4 potassium, 110 chloride, and 36.8
acetate) were given orally at 7:30 AM (i.e., 390 ml in a 13-kg
dog). One hour later (at 8:30 AM) an intravenous infusion of the
same balanced electrolyte solution was started and con-
tinued throughout the protocols (0.125 ml·kg body
wt⁻¹·min⁻¹, i.e., 98 ml/h in a 13-kg dog).

Within the 1 h between the oral bolus and the start of the
intravenous infusion a self-retaining bladder catheter was
inserted through the urethra, a pulmonary artery catheter
(5-F, No. 1322F5, Baxter, Unterschleissheim, Germany) in-
serted via the right external jugular vein, and an arterial line
(20 G, No. 4225–8, Ohmeda, Erlangen, Germany) advanced
into the abdominal aorta via the femoral artery. For the
latter procedures local anesthesia (5 ml of 1% lignocaine)
was applied to the puncture sites. After catheter insertion the
dogs were placed on a padded animal table and positioned on
their right side. The pressure transducers were adjusted to
the level of the right atrium. The distance between trans-
ducer and table was recorded and used for all of the following
experiments in this individual dog. The dogs were then
allowed to equilibrate for more than 60 min. At 9:50 AM the
experimental protocols were started. Three protocols were
performed at random.

Protocol 1. After a baseline period of 60 min without ANG
II infusion, two increasing doses of ANG II (4 and 20
ng·kg⁻¹·min⁻¹; Sigma, Deisenhofen, Germany), termed
ANG II 4 and ANG II 20, were infused intravenously for 60
min each. The ANG II was dissolved in Ionosteril and
 infused at a rate of 0.018 ml·kg body wt⁻¹·min⁻¹. The constant
Ionosteril infusion rate of 0.125 ml·kg body wt⁻¹·min⁻¹ was
corrected for this rate. After the cardiovascular, renal, and
endocrine systems were allowed to equilibrate to the new
rate of ANG II infusion for 40 min, the last 20 min of each
60-min period were evaluated.

Protocol 2. This protocol is the same as protocol 1 but with
the selective ETα receptor antagonist LU-135252 [Knoll,
Ludwigshafen, Germany; inhibitory constant for ETα, 1.4
nmol/l, and for ETβ, 184 nmol/l (19) applied as an intrave-
nous bolus injection (15 mg/kg body wt) after the baseline
period, 5 min before the start of the ANG II infusion.

Protocol 3. Time control over 3 h: only Ionosteril containing
no ANG II and LU-135252 was infused.

Additional studies. On four separate dogs it was tested
whether the bolus injection of 15 mg/kg body wt LU-135252
alone, i.e., without ANG II, had an effect on plasma renin
activity (PRA) or plasma ET-1 concentrations.

Blood withdrawn during the experiments was instantly
replaced with the same amount of the dog’s own blood, which
had been collected into a bag (Biopack CPDA-1, Biotrans,
Dreieich, Germany) about 1 wk before the experiments and
stored at 4°C.

For the individual dog the recovery period between the
different protocols was at least 2 wk.

Pilot study. The effectiveness of the intravenous bolus
injection of LU-135252 (15 mg/kg) was tested on three dogs in
which the blood pressure response toward a continuous in-
travenous infusion of 30 ng ET-1·kg body wt⁻¹·min⁻¹ (Sigma)
was analyzed over a period of 40 min with and without
ETα blockade (Fig. 1).

Measurements and Calculations

Central venous pressure (CVP), MAP, pulmonary artery
pressure (PAP), and heart rate were continuously recorded.
Cardiac output (CO) was measured using the thermodilution
technique (5-ml injection volume, average of three values;
Vigilance, Baxter, Unterschleissheim, Germany). Systemic
(SVR) and pulmonary vascular resistance (PVR) were calcu-
lated as follows: SVR = (MAP – CVP)/CO; PVR = (mean
PAP – PCWP)/CO, where PCWP is pulmonary capillary
wedge pressure.

Urine was collected during the 20-min study intervals (air
wash-out). In urine, sodium, and potassium (flame photom-
etry; Eppendorf, Hamburg, Germany), ET-1 and big ET-1
(ELISA; Biomedica, Vienna, Austria), osmolality (freezing
point depression; osmometer Roebling, Berlin, Germany),
and creatinine concentration (modified Jaffé reaction; creat-
inine analyzer 2, Beckman, Brea, CA) were determined.
Glomerular filtration rate (GFR) was assessed by exogenous
creatinine clearance: 1.4 g creatinine dissolved in 50 ml
dextrose 5% in water was infused over 30 min before the start
of the experiments, followed by 224 mg/h (8 ml/h) throughout
the experiment. Creatinine clearance (ClCr
on) was calculated as
ClCr
on = ([Crea]U·UV/[Crea]Pl)·100, where [Crea]U
is urinary creatinine concentration, UV is urinary excretion
rate, [Crea]Pl is plasma creatinine concentration, [x]Pl is ur-
inary concentration of substance x, and [x]UV is plasma con-
centration of substance x.
Plasma samples were taken at the end of each hour to measure plasma sodium, potassium, and creatinine concentrations, osmolality, blood gases (ABL 505, OSM 3 Hemoximeter, Radiometer, Copenhagen, Denmark), and plasma hormones.

Blood samples for plasma hormone determination were placed into precooled Na-EDTA vials and centrifuged at 4°C. The separated plasma was stored at −20°C until analysis. Commercially available radioimmunoassays were used to measure plasma concentrations of aldosterone (AldoCtk-2, Sorin, Sallugia, Italy), ANG II (Eurodiagnostika, Arnhem, The Netherlands), PRA, expressed as nanograms of ANG I generated per milliliter of plasma per hour of incubation (ng ANG I·ml⁻¹·h⁻¹; New England Nuclear, North Billerica, MA), and atrial natriuretic peptide (its Production B.V., Wijchen, The Netherlands). Endothelin (1−21) and big endothelin (1−38) were determined in both plasma and urine using ELISA kits (Biomedica BI-20052, BI-20072, Vienna, Austria) (13). In dog plasma interfering effects because of different matrices were eliminated by precipitation with a

![Fig. 1. Effectiveness of the intravenous bolus injection of LU-135252 (15 mg/kg) during continuous intravenous infusion of endothelin (ET)-1 (30 ng·kg body wt⁻¹·min⁻¹). Mean arterial pressure without (●) and with (○) LU-135252. Means ± SE of 3 dogs.](http://ajpregu.physiology.org/)

![Fig. 2. Plasma hormones during baseline and during angiotensin (ANG) II 4 and 20 ng·kg body wt⁻¹·min⁻¹ without (●) and with (○) LU-135252 (LU), and during a 3-h time control (◆). Means ± SE, n = 8, P < 0.05. With vs. *without ET-A antagonist, vs. †control and baseline, and vs. ‡ANG II 4.](http://ajpregu.physiology.org/)
mixture of acetone and precipitating agent additive. Urine samples were collected in cooled containers and stored at −20°C. In time controls no urinary ET-1 and no urinary and plasma big endothelin were determined.

**Statistical Analysis**

Differences over time and between the three protocols were evaluated by analysis of variance for repeated measures (NCSS 97, Kaysville, UT). Post hoc testing of the means was performed with Fisher’s least-significant difference test. Statistical significance was assumed at $P < 0.05$. Values are means ± SE ($n = 8$).

**RESULTS**

**Effectiveness of $ET_A$ Blockade**

The effectiveness of the LU-135252 dosage used was tested in pilot studies (Fig. 1). After 40 min of ET-1 infusion (30 ng·kg$^{-1}$·min$^{-1}$) MAP had increased from 87 ± 2 at baseline to 125 ± 6 mmHg in the non-$ET_A$-antagonized dogs, whereas in the LU-135252 dogs MAP remained at baseline levels of 91 ± 2 mmHg throughout the entire ET-1 infusion period (Fig. 1).

**Plasma Hormones**

Only in the ANG II 4 period without LU-135252 was the PRA lower than in the baseline period ($P > 0.05$), whereas, compared with the respective time control period, PRA was lower during both the ANG II 4 and ANG II 20 period, with and without $ET_A$ receptor blockade ($P < 0.05$; Fig. 2). The plasma concentrations of ANG II, aldosterone, and atrial natriuretic peptide (ANP) increased with increasing ANG II infusion rates, independent of whether the dogs received the $ET_A$ antagonist ($P < 0.05$). In the $ET_A$-blocked dogs, however, the increase in aldosterone was ~90 pmol/l less during ANG II 20 ($P < 0.05$) and the increase in ANP concentrations ~27 ng/l less during ANG II 4 ($P < 0.05$; Fig. 2).

ET-1 concentrations in $ET_A$-antagonized dogs increased from 0.47 ± 0.03 during baseline to 3.0 ± 0.44 pmol/l during ANG II 4 ($P < 0.05$). During ANG II 20 the elevated ET-1 concentration had markedly decreased again (0.94 ± 0.17 pmol/l; $P < 0.05$; Fig. 3). In arterial and mixed venous blood the ET-1 plasma concentrations were not different (Fig. 3). The increase in plasma ET-1 was not paralleled in plasma big endothelin concentrations (Fig. 3).

In experiments conducted on four separate dogs it was tested whether the bolus injection of 15 mg/kg body wt LU-135252 alone, i.e., without ANG II, had an effect on PRA or plasma ET-1 concentrations. It was found that PRA remained constant after LU-135252 administration (1.5 ± 0.15 at baseline, 1.6 ± 0.23 1 h after LU-135252, and 1.5 ± 0.20 ng ANG I·ml$^{-1}$·h$^{-1}$ 2 h after LU-135252), whereas ET-1 concentrations transiently increased (0.9 ± 0.19 at baseline, 3.3 ± 0.54 pmol/l 1 h after LU-135252, and 1.6 ± 0.32 pmol/l 2 h after LU-135252), similar to the increase observed...
when LU-135252 was combined with ANG II infusion. MAP remained stable during the 2-h observation period following the application of LU-135252 alone (range 90–100 mmHg).

Other Plasma Values and Blood Gases

Plasma sodium (range 142–147 mmol/l), potassium (3.22–3.50 mmol/l), and osmolality (299–305 mosmol/l) remained unchanged throughout the three protocols. Also unchanged were arterial pH, 7.38–7.42; arterial carbon dioxide pressure (35–38 mmHg), 4.7–5.1 kPa; arterial oxygen pressure (86–99 mmHg), 11.5–13.2 kPa; oxygen saturation, 95–97%; and plasma bicarbonate concentration, 22–23 mmol/l. In the ET$_A$ antagonized dogs, mixed venous oxygen saturation did not decrease during ANG II 20 (74 ± 1% baseline vs. 71 ± 1% ANG II 20), whereas it was lower when ANG II 20 was infused without the ET$_A$ antagonist (baseline 74 ± 1% vs. 68 ± 2% ANG II 20; $P < 0.05$).

Urinary Excretion

ANG II infusion decreased urine volume, sodium, and potassium excretion. There was no difference between the dogs that received the ET$_A$ receptor antagonist and those that did not (Fig. 4). In the time control protocol, potassium excretion during hour 2 and 3 was also decreased compared with the first hour (baseline period; $P < 0.05$) and was not significantly higher than in the ANG II protocols with and without LU-135252. GFR was only slightly decreased during the ANG II infusion periods compared with the baseline hour, with and without LU-135252 ($P < 0.05$; Fig. 4). Urine osmolality was 330 mosmol/l during baseline, ANG II 4, and time control, and increased to 485 mosmol/l during ANG II 20 ($P < 0.05$). There was no difference between LU-135252 treated and untreated dogs.

URINARY ET-1 and big ET-1 excretion paralleled the time courses of urine volume, sodium, and potassium excretion (Figs. 4 and 5). There was no difference in the endothelin excretion rates between ET$_A$ blocked and unblocked dogs. However, fractional ET-1 excretion during ANG II 4 with LU-135252 was lower. This resulted from the transiently increased plasma ET-1 concentration measured during ANG II 4 (see Fig. 3).

Hemodynamics

MAP increased with increasing ANG II infusion rates and was ~35 mmHg higher during ANG II 20 than during baseline and time controls (100 ± 3 mmHg; $P < 0.05$). Acute ET$_A$ blockade did not significantly reduce the ANG II-induced MAP increase [−5–6 mmHg; not significant (NS); Fig. 6].

Fig. 4. Renal function parameters during baseline and during ANG II 4 and 20 ng·kg$^{-1}$·min$^{-1}$ without (solid bars) and with (open bars) LU, and during a 3-h time control (hatched bars). Means ± SE, n = 8, $P < 0.05$. For glomerular filtration rate and potassium excretion rate the † indicates vs. baseline only; otherwise † indicates vs. both baseline and time control.
Heart rate with ANG II alone was 87 ± 6 beats/min during baseline and 80 ± 6 beats/min during ANG II 20 (NS). With LU-135252 heart rate was 85 ± 3 during baseline and 88 ± 6 beats/min during ANG II 20 (NS). In time controls average heart rate was 82 ± 3 beats/min. Overall, heart rate was not different among the three protocols during the respective time periods.

CVP and PCWP both increased with increasing ANG II infusion rates (P < 0.05) but by about 2 mmHg less in the LU-135252-treated dogs (Fig. 6; P < 0.05).

Without ET_\text{A} blockage cardiac output decreased markedly during ANG II 20 (P < 0.05; Fig. 7), whereas with the ET_\text{A} antagonist, cardiac output remained stable in the range of baseline values (P < 0.05). During ANG II 20, LU-135252 blunted the ANG II-induced increase in systemic vascular resistance by about 40% (∼15 mmHg·min^{-1}·m^{-1} = 1,200 dynes·s·cm^{-5}) (P < 0.05; Fig. 6). Pulmonary vascular resistance in the LU-135252 dogs remained at baseline levels even during ANG II 20, whereas it increased without the ET_\text{A} blocker (P < 0.05; Fig. 6).

DISCUSSION

In conscious dogs two levels of plasma ANG II concentration were established by intravenous ANG II infusion, slightly elevated (ANG II 4) and pathophysiologically elevated (ANG II 20). The integrated cardiovascular, renal, and endocrine responses to acute ET_\text{A} receptor blockade were determined. It was found that during ANG II administration renal function was unaltered by acute ET_\text{A} receptor blockade. In contrast, acute ET_\text{A} blockade had distinct effects on hemodynamic parameters, dependent on the rate of ANG II infusion. ET_\text{A} antagonism at pathophysiologically high ANG II plasma concentrations (ANG II 20) maintained cardiac output and blunted the ANG II-induced increase in systemic and pulmonary vascular resistance. The blood pressure lowering effect of acute ET_\text{A} blockade was insignificant. There were no changes in plasma ET-1 and big ET-1 concentrations when ANG II was infused alone, whereas additional ET_\text{A} receptor blockade transiently increased plasma ET-1 but not big ET-1 concentrations.

Methodological Aspects

To avoid endogenous stimulation of the RAAS the activity of the RAAS was strictly controlled in this study: 1) prior to the study by a standardized water and sodium intake and 2) during the study by the standardized fluid regimen and avoidance of stress to the animals.

In the current study the higher ANG II infusion rate (ANG II 20) yielded pathophysiologically, but not “pharmacologically,” high ANG II plasma concentrations of about 140 pmol/l. Plasma ANG II levels this high are found, e.g., in severe congestive heart failure, Bartter’s syndrome, Addison's disease, or after large hemorrhage or severe salt depletion (24).

Because of the short-term nature of our experiments the results should not be influenced by structural vascular changes that occur when ANG is administered over several weeks (18). In addition, endothelin synthesis from ANG II-stimulated cells may require several hours. Thus compared with long-term experiments, endothelin de novo synthesis should only have a minor part for the effects observed after acute ET_\text{A} blockade in our setting. Considering this it may be speculated that, apart from endothelin de novo synthesis, ANG may increase the sensitivity of the vascular tissue to ET_\text{A} receptor stimulation by endothelins and...
that this effect can be influenced and/or antagonized by ET_{A} receptor blockade.

When interpreting the results species differences have to be considered too. It is known that, e.g., the distribution and density of the different endothelin receptors are dependent on the species and the organ studied. In addition, experiments performed during anesthesia may yield different results because they may change or debilitate the physiological response toward the experimental stimuli, e.g., because of the effects of the anesthetic per se or because of perioperative stress. Studies in healthy conscious animals, as in our study, seem to be preferable because they leave all the regulatory mechanisms intact. Furthermore, the kind of endothelin receptor blocker has to be taken into account. There are ET_{A}, ET_{B}, and nonselective ET_{A/B} receptor antagonists. The ET_{A} antagonists predominantly block ET_{A} receptors but may also antagonize ET_{B} receptors, especially when high dosages are being used. LU-135252 is a selective, nonpeptide ET_{A} antagonist with a plasma half-life of ~12 h. Its selectivity for the ET_{A} receptors is 131 (expressed as the ratio of the affinities for the ET_{A} over the ET_{B} receptors) (20). The dosage used in our experiments was shown to totally prevent an ET-1-induced increase in blood pressure (Fig. 1).

**Hormonal Response**

Because of the continuous extracellular volume expansion by the electrolyte infusion, overall PRA values were low. Compared with time controls, PRA values were only slightly further suppressed by ANG II infusion, with the higher ANG II dose having no additional effect. During ANG II infusion, plasma ANG II concentrations increased markedly and to the same extent in both LU-135252-treated and -untreated dogs (Fig. 2). Aldosterone concentrations also increased but were lower during ANG II 20 in the LU-135252-treated dogs (Fig. 2). Inasmuch as plasma potassium concentrations were unaltered, this finding is probably due to ET_{A} receptor blockade, inhibiting the aldosterone secreta-
There still is some controversy in the literature, however, of whether it is the ETA or the ETB receptor or both that mediate the ET-1 effects on aldosterone secretion in vivo (12, 15, 25, 26).

ANP concentrations increased with increasing ANG II plasma concentrations but were somewhat lower during ETA blockade in the ANG II infusion period. Because central venous and wedge pressure were lower in the LU-135252-treated dogs, this finding may be explained by lower atrial pressures during ETA blockade (Fig. 6). In addition, ETA antagonism also has been shown to dose dependently reduce ET-1-stimulated ANP increase in rat atrial myocytes (28). Our results in healthy dogs resemble results obtained in canine experimental heart failure models in which the RAAS is endogenously stimulated. In these studies ANP was either found lower after ETA blockade (4, 29) or remained unchanged (17).

ET-1 and big ET-1 plasma concentrations did not increase when ANG II was infused alone, i.e., without ETA blockade. This may be due to the fact, that the endothelins act primarily as paracrine and/or autocrine hormones. Therefore, an activation of the endothelin system by ANG II must not necessarily be reflected in increased plasma ET-1 levels. In contrast, a marked transient increase in ET-1 was found 1 h after LU-135252 injection during the ANG II 4 period (Fig. 3). The same transient increase was observed when the same dose of LU-135252 was injected in other dogs of ours but no ANG was infused (see Plasma Hormones in Results). A possible explanation for this finding could be that LU-135252 initially not only blocked ETA but also ETB receptors. ETB receptors in the lung have been shown to serve as clearance receptors for circulating endothelins (7). On the other hand, it is also conceivable that the antagonist initially displaced ET-1 from its vascular receptors, leading to a spillover into the plasma. This possibility is more likely, because ET-1 plasma concentrations were already markedly decreased 2 h after injection, although the half-life of LU-135252 in dogs is ~12 h.

Kidney Function

There was no major difference in urine volume, sodium, and potassium excretion, as well as GFR between the dogs that received the ETA antagonist and those that did not. Non-ETA receptor-mediated effects of ANG II seem to govern the marked reduction in urine volume, sodium, and potassium excretion during ANG II infusion, overriding the slightly higher sodium excretion rates reported from ETA-antagonized anesthetized dogs that received the ETA antagonist BQ-123 (6) but no ANG II infusion. When applying LU-135252 at different rates in rats and dogs, Cernacek et al. (5) found excretory function changed in rats but not in dogs, stating that there may be interspecies differences in the role of endogenous endothelins in the regulation of renal function, probably because of a different renal receptor profile and distribution (5).

Urinary ET-1 excretion paralleled very much the time courses of urine flow and sodium excretion in our conscious dogs. In rat studies, ANG II infusion increased urinary ET-1 excretion but also urine flow (1). In these studies, the increase in urinary ET-1 was prevented by infusion of the ANG II-AT1 receptor antagonist losartan, suggesting that endothelin excretion is intimately related to the ANG system (1). In human female volunteers, 1 h of ANG II infusion decreased urinary sodium excretion but did not alter ET-1 excretion (14). Thus there may also be species differences with respect to endothelin excretion after ANG II infusion.

The results of this study in dogs suggest that the ET-1 found in the urine is not mainly plasma ET-1 that passes the glomerular filter but that this ET-1 is of renal origin and reaches the tubulus lumen via renal tubular and/or renal endothelial cells (1, 16). This is concluded because the high-ET-1 plasma concentration during ANG II 4 was not reflected in a higher urinary ET-1 excretion during this time period (fractional ET-1 excretion decreased in ETA blocked dogs during ANG II 4; Fig. 5).

Hemodynamics

Endothelin receptors are present in the high- as well as the low-pressure segment of the circulation. During acute ANG II infusion in healthy dogs, the blood pressure lowering effects of acute ETA blockade were visi-
ble more in the low-pressure segment (reduced CVP and PCWP) than in the high-pressure segment (only insignificantly reduced arterial pressure; Fig. 6). Even though blood pressure rose during ANG II infusion, heart rate was stable at baseline levels with and without the ET_A blocker. This is a common finding during exogenous ANG II receptor antagonism and is assumed to be due to an impaired baroreceptor response during ANG II infusion (for review see Ref. 23).

The most important hemodynamic finding was that at pathophysiologically high ANG II plasma concentrations cardiac output was maintained at baseline levels only in the ET_A receptor antagonized dogs, whereas it did severely decrease in the non-ET_A antagonized dogs. As previously stated, the ANG II plasma levels at ANG II 20 are in the range of those found during congestive heart failure; insofar our findings in healthy dogs support observations of improved cardiac output during acute and long-term ET_A antagonism in canine models of congestive heart failure (4, 17, 21, 29) and are also comparable to a recently published study in humans with congestive heart failure (27). In the latter study, a single oral dose of LU-135252 was applied and the patients were studied at hourly intervals for 4 h thereafter (27). The most significant hemodynamic changes were found with respect to an increase in cardiac index and a decrease in systemic and pulmonary vascular resistance (27). Similarly, a blunted decrease in cardiac output was observed during ANG infusion in a conscious rat model in which the nonselective ET_AB antagonist bosentan was applied (2).

The maintenance of baseline cardiac output at ANG II 20 in the ET_A-blocked dogs was combined with a 40% reduced increase in systemic vascular resistance (Fig. 6), a 10% improved stroke volume, and a higher mixed venous oxygen saturation compared with the dogs that received ANG II alone. Pulmonary vascular resistance also benefitted from ET_A blockade as it was maintained in the range of baseline values even during ANG II 20.

In conclusion, in conscious healthy dogs, extensively monitored with respect to hemodynamics, renal function, and endocrine response, it was demonstrated that the effects observed after acute ET_A blockade are partly dependent on the level of ANG II plasma concentrations. At clinically relevant, pathophysiologically high-ANG II plasma concentrations, acute ET_A antagonism especially improved cardiovascular function. It prevented the decrease in cardiac output and the increase in pulmonary vascular resistance and blunted the ANG II-induced increase in systemic vascular resistance.

**Perspectives**

Future studies should investigate whether the observed hemodynamic effects prove beneficial in clinical situations in which ANG II plasma concentrations are increased. It would also be interesting to see whether and how the results would differ if the ET_A blocker was applied after several hours of ANG II infusion, i.e., a time period long enough to stimulate ANG II-induced endothelin de novo synthesis but yet short enough to prevent ANG II-induced changes in the vascular structure.

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