AT$_2$ Receptor Stimulation Increases Aortic Cyclic GMP in SHRSP by a Kinin-Dependent Mechanism

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Abstract—In the present study we tested the hypothesis whether an angiotensin AT$_2$ receptor-mediated stimulation of the bradykinin (BK)/nitric oxide (NO) system can account for the effects of AT$_1$ receptor antagonism on aortic cGMP described previously in SHRSP. Adult SHRSP were treated for 4 hours with angiotensin II (ANG II) (30 ng/kg per min IV) or vehicle (0.9% NaCl IV). Animals were pretreated with vehicle, losartan (100 mg/kg PO), PD 123319 (30 mg/kg IV), losartan plus PD 123319, icatibant (500 µg/kg IV), N$^{G}$-nitro-L-arginine methyl ester (L-NAME, 1 mg/kg IV), or minoxidil (3 mg/kg IV). Mean arterial blood pressure (MAP) was continuously monitored over the 4-hour experimental period, and plasma ANG II and aortic cGMP were measured by RIA at the end of the study. ANG II infusion over 4 hours raised MAP by about 20 mm Hg. Losartan alone or losartan plus ANG II as well as minoxidil plus ANG II markedly reduced blood pressure when compared to vehicle-treated or ANG II-treated animals, respectively. Plasma levels of ANG II were increased 2-fold by ANG II infusion alone or by ANG II in combination with icatibant, L-NAME, or minoxidil. The increase in plasma ANG II levels was even more pronounced after losartan treatment. Aortic cGMP content was significantly increased by ANG II, losartan, losartan plus ANG II, and minoxidil plus ANG II by 60%, 45%, and 52%, respectively (P<0.05). The effects of ANG II and of losartan plus ANG II on aortic cGMP content were both blocked by cotreatment with the AT$_2$ receptor antagonist PD 123319. Icatibant and L-NAME abolished the effects of ANG II on aortic cGMP. Our results demonstrate the following: (1) ANG II increases aortic cGMP by an AT$_2$ receptor-mediated action because the effect could be prevented by an AT$_2$ receptor antagonist; (2) the effect of ANG II was not secondary to blood pressure increase because it remained under reduction of MAP with minoxidil; (3) losartan increased aortic cGMP most likely by increasing plasma ANG II levels with a subsequent stimulation of AT$_2$ receptors; and (4) the effects of AT$_2$ receptor stimulation were mediated by BK and, subsequently, NO because they were abolished by B$_2$ receptor blockade as well as by NO synthase inhibition.

Key Words: angiotensin II n AT$_1$ receptor antagonist n AT$_2$ receptor n bradykinin n nitric oxide n cGMP n losartan

The renin-angiotensin system (RAS), with its effector peptide angiotensin II (ANG II), plays an important role in the cardiovascular, electrolyte, and fluid homeostasis and has been implicated as a causative factor in the development of hypertension.$^{1}$ Inhibitors of the RAS such as angiotensin-converting enzyme (ACE) inhibitors and the nonpeptide antagonists of the AT$_1$ receptor are a major breakthrough in the treatment of hypertension and other cardiovascular diseases.$^{2-4}$ AT$_1$ antagonists such as losartan interfer with the RAS by a specific blockade of the AT$_1$ receptor which mediates most of the known actions of ANG II.$^{2-4}$ ACE inhibitors, on the other hand, not only interfere with the RAS by inhibition of ANG II generation, but, in addition, also potentiate the effects of bradykinin (BK) by inhibition of BK degradation.$^{5}$ Two major tools have been introduced in recent years to study the contribution of BK to the cardiovascular actions of ACE inhibitors: first, the combined treatment with an ACE inhibitor and with a specific BK$_2$ receptor antagonist such as icatibant; and second, the comparison of the effects of an ACE inhibitor with those of an AT$_1$ receptor antagonist which block the RAS without an interaction with kinin metabolism. With these approaches, several investigators have demonstrated that a number of actions of ACE inhibitors is related to cardiovascular control can be blocked by BK$_2$ receptor antagonism and thus be related to kinins.$^{5}$ For example, in a series of experiments our laboratory demonstrated that long-term treatment with ACE inhibitors improved cardiac function and metabolism in hearts from SHRSP and preserved endothelial function as indicated by an increased vascular nitric oxide (NO) production.$^{6}$ All these effects could be prevented by BK$_2$ receptor antagonism, suggesting that they were BK mediated. On the other hand, we observed that the AT$_1$ antagonist losartan produced strikingly similar effects as the ACE inhibitor under identical treatment conditions.$^{6}$ These results indirectly pointed that losartan, like the ACE inhibitor, can also interact with the kinin/NO system. One possible mechanism for such an interaction could be related to a stimulation of non-AT$_1$ receptors, particularly the AT$_2$ receptor, because losartan at therapeutic doses leaves the AT$_1$ receptor unopposed$^{24}$ and because AT$_2$ receptor antagonism engenders an increase in plasma ANG II levels as a result of blockade of the AT$_1$ receptor-mediated negative feedback.
inhibition of renin secretion in the kidney. In line with this hypothesis are recent studies in bovine endothelial cells, isolated rat carotid arteries, canine microvessels from coronary arteries, rat aortic strips, and rat kidney, suggesting that ANG II or metabolites of ANG II can stimulate NO production. Furthermore, in a rat model of heart failure induced by myocardial infarction, Liu et al demonstrated that the decrease in left ventricular end-diastolic pressure and left ventricular systolic pressure induced by AT1 receptor antagonism was blocked by an AT2 antagonist and partially blocked by a B2 receptor antagonist. Treatment was started 2 months after myocardial infarction and was continued for another 2 months. This study indicated that the effect of the AT1 antagonist on cardiac function was dependent on the activation of AT2 receptors and was in part mediated by nitric oxide.

In the present study, we investigated the effect of an acute oral treatment with the AT1 antagonist losartan on aortic cGMP content. The hypothesis was that increased plasma ANG II concentrations during AT1 receptor blockade can account for the effects of losartan on aortic cGMP by stimulation of unopposed AT2 receptors. Therefore, we compared the effects of losartan with those of exogenously administered ANG II and losartan plus ANG II. Losartan plus ANG II was continuously monitored during the 4-hour infusion period. At the end of the experimental period, 2 mL blood were withdrawn from the arterial catheter for the measurement of plasma ANG II concentrations. Immediately after blood collection, the rats were decapitated. A 20 to 30 mm segment of the proximal part of the thoracic aorta just distal to the aortic arch was excised, dissected, snap-frozen in liquid nitrogen, and stored at −20°C until cGMP content was measured.

**Methods**

Male 18- to 22-week-old (280–320 g) SHRSP bred at the Department of Pharmacology in Kiel were used for the experiments. All rats were housed at constant humidity (60±5%) and temperature (25±1°C) and kept on a 12-hour light/dark cycle. The study was performed in accordance with the guidelines for animal experiments of the University of Kiel and was approved by the German governmental office dealing with animal protection.

**Surgical Procedure**

One day before the experiments, rats were cannulated under ether anesthesia with catheters (PE-10 in PE-50 for arterial catheters, PE-25 for venous catheters, Portex Corp, Hythe, UK) in the right femoral artery for direct recording of mean arterial pressure (MAP) and in the right femoral vein for intravenous drug infusion.

**Blood Pressure Measurement**

MAP was measured directly by use of a Statham P23 Db pressure transducer (Gold Inc., Oxnard, Calif.) connected to the right femoral arterial catheter. The blood pressure signal was amplified by a Gould Brush pressure processor (Gold Inc., Oxnard, Calif.) and monitored on a Gold Brush 2400 recorder (Gold Inc., Oxnard, Calif.). MAP was recorded continuously in conscious and unrestrained animals throughout the experimental period.

**General Procedure**

One day after the surgery, the femoral artery catheter was connected to the pressure transducer, which was attached to the monitoring system. After a stabilization period of at least 30 minutes, basal MAP was recorded. Thereafter, the animals were pretreated with either vehicle, the AT1 receptor antagonist losartan, the AT2 receptor antagonist PD 123319, losartan plus PD 123319, the NO-synthase inhibitor L-NAME, the BK B2 receptor antagonist catlbant, or the arterial vasodilator mnxoidil according to the treatment groups given below followed by a 4-hour infusion of either physiological saline or ANG II. ANG II was continuously monitored during the 4-hour infusion period. At the end of the experimental period, 2 mL blood were withdrawn from the arterial catheter for the measurement of plasma ANG II concentration. Immediately after blood collection, the rats were decapitated. A 20 to 30 mm segment of the proximal part of the thoracic aorta just distal to the aortic arch was excised, dissected, snap-frozen in liquid nitrogen, and stored at −20°C until cGMP content was measured.

**Experimental Protocol**

Animals were randomly divided into 11 groups.

- **Group 1 control** (n=14), rats were pretreated orally by gavage with distilled water (1 mL) or by intravenous bolus injection of physiological saline (1 mL) followed by an intravenous infusion of physiological saline (1 mL/h).
- **Group 2 ANG II** (30 ng/kg per min) (n=25), rats were pretreated orally by gavage with distilled water (1 mL) or by IV bolus injection of physiological saline (1 mL) followed by an IV infusion of ANG II (30 ng/kg per min) dissolved in physiological saline.
- **Group 3 Losartan** (n=7), rats were pretreated orally by gavage (1 mL) with losartan (100 mg/kg) followed by an IV infusion of physiological saline (1 mL/h).
- **Group 4 Losartan plus ANG II** (n=7), rats were pretreated orally by gavage (1 mL) with losartan (100 mg/kg) followed by an IV infusion of ANG II (30 ng/kg per min, 1 mL/h).
- **Group 5 PD 123319 (n=8)**, rats were pretreated with PD 123319 (30 mg/kg per mL physiological saline, IV bolus injection) followed by an IV infusion of physiological saline (1 mL/h).
- **Group 6 PD 123319 plus ANG II** (n=8), rats were pretreated intravenously with PD 123319 (30 mg/kg per mL physiological saline, IV bolus injection) followed by an IV infusion of ANG II (30 ng/kg per min, 1 mL/h).
- **Group 7 Losartan plus PD 123319 plus ANG II** (n=8), rats were pretreated intravenously by gavage with losartan (100 mg/kg) and intravenously with PD 123319 (30 mg/kg per mL physiological saline, IV bolus injection) followed by an IV infusion of ANG II (30 ng/kg per min, 1 mL/h).
- **Group 8 L-NAME plus ANG II** (n=10), rats were pretreated intravenously with L-NAME (1 mg/kg per 0.1 mL physiological saline, IV bolus injection) followed by an IV infusion of ANG II (30 ng/kg per min, 1 mL/h).
- **Group 9 Catlbant plus ANG II** (n=10), rats were pretreated intravenously with catlbant (0.5 mg/kg per 0.1 mL physiological saline, IV bolus injection) followed by an IV infusion of ANG II (30 ng/kg per min, 1 mL/h).
- **Group 10 Minoxidil plus ANG II** (n=11), rats were pretreated intravenously with minoxidil (3 mg/kg per 0.1 mL physiological...
Plasma ANG II Measurements

Blood (2 mL) was withdrawn from the aortal catheter directly into a syringe containing 0.1 mL minibar solution (25 mmol/L. L 10 phenanthroline, 125 mmol/L EDTA, 0.2 g/L neomycin sulfate, and 1% ethanol) at 4°C. The samples were immediately centrifuged at 6000g and 4°C for 10 minutes. Plasma was separated, snap frozen in liquid nitrogen, and stored at −80°C until analyzed. Plasma ANG II immunoreactivity was determined by radioimmunoassay. Directly, plasma (50 μL) was incubated with 450 μL of 0.1 mol/L Tris-acetate buffer (pH 7.4), containing 0.1% BSA and 0.1% Triton X-100, 50 μL tritiated [3H]-ANG II (4000 cpm), and 50 μL antiserum 925 (a gift of Dr. Peter Admiraal, Rotterdam, The Netherlands), final dilution in the assay 7 × 10^{-6} for 20 hours at 4°C. The antibody-antigen complex was separated from “free” ANG II by addition of dextran-coated charcoal and subsequent centrifugation. Free radioactive ANG II (pellet) was counted in a gamma counter (1470 Wizard, Wallac, Turku, Finland). The evaluation of the samples was carried out by computer analysis, and standard curves were smoothed with spline functions. Standards ranged from 1 to 250 fmol/tube, and the detection limit was 0.5 to 0.8 pg ANG II/tube. The ANG II RIA showed cross-reactivities of 100% for ANG(4–8), 73% for ANG(3–8), 55% for ANG III, and <0.2% for ANG I, ANG(2–10), and ANG(1–7). The antiserum is sensitive toward 1:10 phenthanthrene, an inhibitor used to prevent ANG II degradation. The inhibitor gave nonspecific interference in the RIA of 4.5 pg/tube. The ANG II data have been corrected accordingly. Results are expressed as picograms per milliliter (pg/mL).

Aortic cGMP Content

cGMP in the aorta was determined by a specific RIA (Amersham Buchler, Braunschweig, Germany). The frozen aortas were each weighted, pulverized in liquid nitrogen by mortar and pestle, and transferred into 2 mL ethanol. The samples were sonicated for 10 seconds at 4°C and centrifuged for 15 minutes at 6000g. The supernatant was lyophilized and reconstituted in 0.5 mL sodium acetate buffer (0.05 mol/L, pH 5.8) containing 0.1% sodium azide. After addition of 25 μL of an acetylation reagent (one part acetic anhydride and two parts methanol), the samples were vortexed, and aliquots of 100 μL were transferred into tubes containing 100 μL antiserum (rabbit anti-cGMP serum) after incubation for 1 hour at room temperature, 100 μL tracer ([3H]2′-o-succinyl-cGMP tyrosine methyl ester) was added, and the samples were incubated for another 15 to 18 hours at 4°C. Then, the samples were incubated for 10 minutes with 500 μL of a second antibody, Amerlex M (anti-rabbit antibody from donkey) after centrifugation for 10 minutes at 1500g, the supernatant was discarded, and the radioactivity in the pellet was counted in a gamma counter. cGMP content was expressed as femtomoles per gram fresh weight (fmol/g).

Drugs

Losartan potassium (2-n-buty-4-chloro-5-hydroxy-methyl-1-(2′-[1H-tetrazol-5-yl]biphenyl-4-yl)methyl)imidazole, potassium salt) was obtained from DuPont Merck (Wilmington, Del.) PD 123319 [[(4-[dimethylamino]-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid, trifluoroacetate, monohydrate] was a gift from Dr. Joan Kelser) (Park-Davis, Ann Arbor, Mich.) Icabitant (Hoe 140) (D-Arg[1Hyp3, Thr5, D-Tyr7, Orn8]-bradykinin) was obtained from Hoechst Marion Rousell (Frankfurt, Germany) L-NNAME and minoxidil were purchased from Sigma Chemical Co (Deisenhofen, Germany).

Figure 1. Upper panel, Line graph showing effects of the AT1 receptor antagonist losartan (L) (100 mg/kg PO), losartan plus the AT2 receptor antagonist PD 123319 (PD) (30 mg/kg IV) or vehicle (distilled water PO) followed by a 4-hour IV infusion with either physiological saline or ANG II (30 ng/kg per min) on MAP in SHRSP. Values represent mean±SEM. Overall effects of losartan plus saline, vehicle plus ANG II, losartan plus ANG II, and losartan plus PD 123319 plus ANG II were significantly different from vehicle plus saline (P<0.05). Lower panel, Line graph showing effect of minoxidil (3 mg/kg PO), followed by a 4-hour IV infusion with either physiological saline or ANG II (30 ng/kg per min) on MAP in SHRSP. Values represent mean±SEM. Overall effects of minoxidil plus saline were significantly different from vehicle plus saline (P<0.05).

Statistics

All results are presented as mean±SEM. Data were subjected to analysis of variance (ANOVA). Significant differences between groups were further analyzed with Turkey’s method for multiple comparisons. Analysis of blood pressure data was performed by ANOVA with repeated measures using the SYSTAT statistical software. When a significant difference was found between groups, the univariate F-test was used for analysis of differences between the control group and various treatment groups. A significance level of P<0.05 was accepted.

Results

Blood Pressure Measurements

MAP was continuously monitored during the 4-hour infusion period under control conditions, that is during a 4-hour infusion of vehicle (physiological saline), MAP remained constant at 184±3±7 mm Hg ANG II infusion (30 ng/kg per min) over 4 hours raised MAP by 19±1±4 mm Hg Losartan alone as well as losartan in combination with an ANG II infusion (30 ng/kg per min) markedly reduced MAP by 36±2±3 mm Hg and 44±6±2 mm Hg when compared with vehicle-treated or ANG II–treated animals, respectively (Fig 1). Combined blockade of AT1 and AT2 receptors with losartan

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AT₂ Receptors and the Kinin/NO System

Figure 2. Upper panel, Bar graph showing effects of the AT₂ receptor antagonist losartan (L) (100 mg/kg PO) or vehicle (distilled water PO) followed by a 4-hour IV infusion with physiological saline (open bar and closed bar on the left) and effects of vehicle (distilled water PO), the AT₂ receptor antagonist PD 123319 (PD) (30 mg/kg IV bolus injection), losartan (100 mg/kg PO), and losartan (100 mg/kg PO) plus PD 123319 (30 mg/kg IV bolus injection) followed by a 4-hour IV infusion with ANG II (30 ng/kg per min) on plasma ANG II concentrations in SHRSP. Lower panel, Bar graph showing effects of a 4-hour IV infusion with ANG II (30 ng/kg per min) in rats pretreated with either vehicle (distilled water PO), the BK B₂ antagonist icatibant (0.5 mg/kg IV bolus injection), the NO-synthase inhibitor L-NAME (1 mg/kg IV bolus injection), or the arterial vasodilator minoxidil (3 mg/kg PO) compared with the effect of a 4-hour IV infusion with physiological saline in rats pretreated with vehicle (distilled water PO) on plasma ANG II concentrations in SHRSP. Values represent mean±SEM. *P<05 versus vehicle plus saline-treated animals. #P<05 versus vehicle plus ANG II and losartan + ANG II-treated rats.

Figure 3. Upper panel, Bar graph showing effects of the AT₂ receptor antagonist losartan (L) (100 mg/kg PO) or vehicle (distilled water PO) followed by a 4-hour IV infusion with physiological saline (open bar and closed bar on the left) and effects of vehicle (distilled water PO), the AT₂ receptor antagonist PD 123319 (PD) (30 mg/kg IV bolus injection), losartan (100 mg/kg PO), and losartan (100 mg/kg PO) plus PD 123319 (30 mg/kg IV bolus injection) followed by a 4-hour IV infusion with ANG II (30 ng/kg per min) on aortic cGMP concentrations in SHRSP. Lower panel, Bar graph showing effects of a 4-hour IV infusion with ANG II (30 ng/kg per min) in rats pretreated with either vehicle (distilled water PO), the BK B₂ antagonist icatibant (0.5 mg/kg IV bolus injection), the NO-synthase inhibitor L-NAME (1 mg/kg IV bolus injection), or the arterial vasodilator minoxidil (3 mg/kg PO) compared to the effect of a 4-hour IV infusion with physiological saline in rats pretreated with vehicle (distilled water PO) on aortic cGMP concentrations in SHRSP. Values represent mean±SEM. *P<05 versus vehicle + saline-treated animals. #P<05 versus vehicle + ANG II and losartan + ANG II-treated rats.

Plasma ANG II Concentrations

Plasma concentrations of ANG II were increased about 2-fold by ANG II infusion (30 ng/kg per min) alone or by infusion of 30 ng/kg per min ANG II in combination with PD 123319, icatibant, L-NAME, or minoxidil (Fig 2). The increase in plasma ANG II levels was even greater after treatment with losartan alone (3.5-fold increase) or losartan in combination with ANG II infusion (2.6-fold increase). A marked 8-fold increase in plasma ANG II concentration was observed during infusion of 30 ng/kg per min ANG II and concomitant blockade of AT₁ as well as AT₂ receptors with losartan and PD 123319, respectively. The low dose of 3 ng/kg per min ANG II did not affect plasma levels of ANG II when compared with vehicle-treated controls (70 ± 13 vs. 92 ± 7 pg/mL in vehicle treated rats).

Aortic cGMP

Aortic cGMP content was significantly increased after a 4-hour infusion of ANG II (30 ng/kg per min) as well as 4 hours after oral pretreatment of rats with losartan by 60% and 45%, respectively, when compared with vehicle-treated controls (Fig 3). The combination of AT₁ blockade with losartan plus infusion of ANG II (30 ng/kg per min) further increased aortic cGMP content (68% increase when compared with vehicle-treated controls) (Fig 3). The effects of ANG II infusion alone and of losartan plus ANG II infusion on aortic cGMP content was abolished by blockade of AT₂ receptors with PD 123319 (Fig 3). The AT₂ receptor antagonist alone had no effect on aortic cGMP content (data not shown). Blockade of BK B₂ receptors with icatibant as well as inhibition of NO synthase with L-NAME markedly attenuated the effects of ANG II.
infusion on aortic cGMP (Fig 3) The effect of ANG II infusion (30 ng/kg per min) on aortic cGMP was not secondary to the blood pressure increase induced by ANG II because a concomitant reduction of MAP by pretreatment with minoxidil did not reduce the effect of ANG II (57% increase when compared to vehicle-treated rats) (Fig 3) A 4-hour infusion of ANG II at the lower dose of 3 ng/kg per min did not significantly alter aortic cGMP content when compared with vehicle-treated rats (20% increase versus controls) (data not shown)

Discussion

The major finding of the present study is that ANG II stimulates the production of cGMP in the vascular wall by an AT2 receptor-dependent mechanism The effect of ANG II on aortic cGMP was abolished by inhibition of NO-synthase with L-NAME as well as by blockade of BK B2 receptors with icatibant These findings suggest that activation of NO synthase as well as stimulation of BK B2 receptors are both involved in the AT2 receptor-dependent actions of ANG II on aortic cGMP

The increase in aortic cGMP could be observed during conditions of increased plasma ANG II concentrations resulting from intravenous infusion of ANG II as well as during conditions of increased production of endogenous ANG II as a result of AT1 receptor blockade Both infusion of ANG II at a rate of 30 ng/kg per min as well as oral pretreatment with losartan at a dose of 100 mg/kg nearly doubled plasma ANG II concentrations Campbell et al10 recently reported a 4- to 6-fold increase in ANG II plasma levels 6 to 24 hours after intraarterial administration of 10 mg/kg losartan to Sprague-Dawley rats The effect of losartan on plasma ANG II levels may be even more pronounced after long-term treatment, as indicated by a 25-fold increase in plasma ANG II after an 8-day IP administration of 10 mg losartan twice daily 10 Despite that the dose of 100 mg/kg losartan used in the present study is in the upper range for antihypertensive treatment in rats,18 the elevated blood pressure in SHRSP was markedly lowered but not normalized

Both the effect of ANG II as well as the effect of losartan on aortic cGMP were blocked by AT2 receptor blockade with the selective AT2 receptor antagonist PD 123319 This finding suggests that ANG II mediates its effects on vascular cGMP via stimulation of the AT2 receptor in the vascular wall PD 123319 is highly selective with a KI ~ 12 nmol/L for the AT2 receptor and KI > 100 mol/L for the AT1 receptor 10-20 In the present study, the AT2 receptor antagonist PD 123319 was used at a dose of 30 mg/kg applied as an IV bolus injection In a recent study, bolus injections of PD 123319 at doses of 1 to 1000 g/kg followed by IV infusion of PD 123319 at concentrations of 1 to 1000 g/kg per min for 1 hour produced plasma concentrations of the compound that ranged from 0.1 mol/L (lowest dose) up to 10 mol/L (highest dose) Treatment of rats with PD 123319 for 10 to 14 days with PD 123319 at a dose of 30 mg/kg per day applied by osmotic minipumps resulted in plasma levels in the range of 90 to 250 nmol/L 21

Expression of AT2 receptors in rat aorta (Sprague-Dawley rats) has been demonstrated by Viswanathan et al15 by binding studies and quantitative autoradiography Their results revealed a predominance of AT2 receptors in the aorta of fetus and young (2 weeks old) rats In adult (8 weeks old) rats, approximately 30% of the receptors expressed in the aorta were AT2. This study also demonstrated that the number of ANG II receptors decreased with age, being 20 times lower in the adult rats compared with the fetus The data suggested a localization of AT2 receptors throughout the aortic wall including the medial layer, although a localization of AT2 receptors to distinct cell types was not possible Similar results were obtained by Song et al23 in WKY and SHR. Endothelial cells derived from coronary arteries of SHR express AT1 and AT2 receptors in a ratio of 80% to 20%.24 In rat aortic endothelial cells, Pueyo et al25 detected AT2 receptor expression in proliferating cells cultured without growth factors but not under normal culture conditions, that is in the presence of growth factors These results confirm recent findings that growth factors downregulate AT2 receptor expression 26 Other studies failed to detect AT1 as well as AT2 receptors in endothelial cells derived from bovine aorta27 or porcine arteries.28 Thus, the demonstration of AT2 receptor expression may largely depend on the experimental conditions. In the present study, we have not directly proven the expression of AT2 receptor in the aorta However, the fact that treatment with the specific AT2 receptor antagonist PD 123319 blocked the effects of ANG II alone and also the effects of the combination of ANG II and losartan on aortic cGMP content suggests the presence of AT2 receptors in the rat aorta under the conditions of our experiments

The finding that losartan can stimulate NO production by an AT2 receptor-mediated and BK B2-dependent mechanism provides a direct explanation for results obtained in recent studies in SHRSP 6-9 In these studies, we treated SHRSP long-term with the ACE inhibitor ramipril as well as with the AT1 antagonist losartan (30 mg/kg per day) and determined the effect of treatment on cardiac function and metabolism in ex vivo isolated hearts and on vascular cGMP production The results revealed that the ACE inhibitor improved cardiac function and metabolism and increased aortic cGMP content, the effect being completely abolished by concomitant blockade of BK B2 receptors,67 The BK B2 receptor antagonist icatibant did not affect aortic cGMP content after chronic application using the same dose as in the present acute study On the other hand, the AT1 antagonist produced strikingly similar and, with regard to increases in aortic cGMP content, even more pronounced effects as the ACE inhibitor, suggesting a relationship between AT1 antagonism and the kimm/NO system

A number of recent studies have suggested the existence of a link between ANG II and the kimm/nitric oxide system in various organs including the blood vessel wall, possibly mediated by the AT2 receptor An involvement of a non-AT1 receptor mechanism in the stimulation of guanylate cyclase in aortic tissue has been described previously 14 ANG II at a concentration of 25 mmol/L stimulated cGMP production in incubated aortic segments This effect was not affected by the AT1 antagonist losartan but could be blocked by inhibition of soluble guanylate cyclase as well as by NO synthase inhibition with L-NAME 14 In bovine aortic endothelial cells (BAEC), ANG II (10-7 to 10-4 mol/L) dose-dependently stimulated cGMP production and enhanced the release of endogenous

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knnms. The effect of ANG II on cGMP was blocked by the BK B2 receptor antagonist icatibant (0.1 mol/L), as well as by the NO synthase inhibitor Nω-nitro-L-arginine (10 mol/L). Furthermore, blockade of AT1 receptors with CGP 42112A at antagonistic concentrations and with PD 123177 inhibited the accumulation of cGMP induced by ANG II. Blockade of AT1 receptors with four different selective AT1 receptor antagonists had no or only minor inhibitory effects on ANG II-induced cGMP production. Although these results favor an involvement of the AT2 receptor in ANG II-induced endothelial BK/NO production, the nature of the AT2 receptor is challenged by the fact that the AT2 antagonist MSD L-158.809 behaved like an AT2 antagonist in this experimental setting.

Several studies have demonstrated that the ANG II fragment ANG (1–7) can activate the kunn/NO system and potentiate the hypotensive effects of BK in rats. Furthermore, ANG (1–7) has been shown to dilate canine and porcine coronary arteries by a NO and BK B2 receptor dependent mechanism. The relaxation induced by ANG (1–7) was not affected by AT1 or AT2 receptor blockade. However, in one of these studies, the effects of ANG (1–7) seemed to be produced by yet unknown contaminations of the ANG (1–7) batch provided by the manufacturer and were not confirmed with other ANG (1–7) batches.

Seyed et al. recently demonstrated a NO production by measuring nitrate release of canine coronary microvessels and canine large coronary arteries in response to a number of ANG peptides including ANG II (10–5 mol/L) and ANG (1–7) (10–6 mol/L). The effects of the ANG peptides were blocked by an NO synthase inhibitor and a BK B2 receptor antagonist suggesting that the ANG-induced NO release was caused by an activation of local kunn production in the vessel wall. Curiously, this effect could not be ascribed to distinct ANG receptor subtypes, because it was blocked by AT1 as well as by AT2 receptor antagonists. Interestingly, a non-AT1, non-AT2 angiotensin receptor with a high affinity for ANG II has been described in BAEC. However, unlike in the present study, there is no significant competition of PD 123319 for the ANG (1–7) binding site in BAEC.

In a microdialysis study, Slragy et al. demonstrated an increased cGMP concentration in the renal interstitial fluid in rats on normal sodium diet after ANG II application. This effect was blocked by concomitant administration of the AT2 antagonist PD 123319. In this study, PD 123319 as well as losartan alone had no effect on renal interstitial fluid cGMP content. Furthermore, activation of the RAS by sodium depletion produced an increase in renal interstitial fluid cGMP. His effect was not changed by losartan treatment, whereas PD 123319 caused a decrease in cGMP content, suggesting that the effect was AT2 mediated.

In the present study, acute treatment with losartan at a dose of 100 mg/kg PO increased aortic cGMP content 4 hours after drug application. In contrast, in a renin-dependent model of hypertension, ie, in rats made hypertensive by coarctation of the aorta, losartan at a dose of 30 mg/kg IV did not change the cGMP content in the aorta 30 minutes after drug application. Differences in the hypertension model (aortic coarctation versus genetic hypertension) or the time point of tissue collection (0.5 hour versus 4 hours) may explain the differences between both studies.

The finding that stimulation of AT2 receptors produce NO generation in the vasculature suggests an involvement of AT2 receptors in basal blood pressure maintenance as well as a contribution of AT2 receptor stimulation to the antihypertensive actions of losartan. However, at least during acute conditions as in the present study, the AT2 antagonist PD 123319 did not alter basal blood pressure when compared to vehicle-treated controls. Furthermore, the blood pressure increase induced by ANG II was not further enhanced by concomitant AT2 receptor blockade, suggesting that the AT2 receptor mediated effect of ANG II on aortic NO production does not contribute to the overall blood pressure effect. Similar findings have been obtained by Levy et al. in normal Wistar rats after 3 weeks of treatment with PD 123319 (30 mg/kg per day) alone or in combination with ANG II (120 ng/kg per min). In addition, in the present study, AT2 receptor blockade during coadministration of losartan and ANG II did not attenuate the antihypertensive action of losartan but, in contrast, tended to further decrease blood pressure in this group. However, several studies have suggested a depressor role of the AT2 receptor. In anesthetized rats, ANG II and ANG III elicit a biphasic arterial pressure response. Losartan pretreatment blocked the pressor part of the response to ANG II and ANG III, whereas the depressor response became larger. Combined blockade of AT1 and AT2 receptors abolished all responses to ANG II and ANG III. Although anesthesia might have influenced the outcome of this study, it suggested that the AT2 receptor can mediate a depressor response to ANG II.

It can be argued that the effect of ANG II on aortic cGMP is secondary to the increase in blood pressure induced by ANG II, eg, by activation of counterregulatory active vasodilatory systems such as the kunn/NO system. However, marked reduction of blood pressure by either losartan or the arterial vasodilator minoxidil did not affect the ANG II induced increase in aortic cGMP. A second approach to exclude secondary effect of blood pressure was to infuse ANG II at a dose that did not alter basal blood pressure. However, this low dose also failed to produce a significant increase in plasma ANG II and, consequently, failed to stimulate aortic cGMP content.

In conclusion, we demonstrated that losartan interacts with the kunn/NO system by stimulation of AT2 receptors secondary to a losartan–induced increase in plasma ANG II concentrations. The effects of losartan on aortic cGMP were mimicked by intravenous ANG II infusion. The activation of AT2 receptors by ANG II resulted in a BK-dependent stimulation of aortic NO release with a subsequent generation of cGMP. These AT2 receptor-dependent actions of losartan or ANG II do not seem to interfere with the acute effects of both substances on blood pressure but may help to explain some of the beneficial actions of losartan on cardiovascular function and metabolism reported recently.

References


16 LIU YH, Yand XP, Sharov VG, Nass 0, Sabbah HN, Peterson E, Car-

Munzenmaler DH, Greene AS Stnnulat~on of soluble guanylare cyclase

14 Boulanger CM, Caputo L, L&y BI Endothehal AT,-medlated release of

5 Lint W, Wierner G, Gohlke P, Unger T, Scholken~ BA Contribution of


9 Gohlke P, Lmz W, Scholkens BA, Wzemer G, Unger T Cardiac and

2 Tmunermans PBMWM, Wang PC, Chm AT, Herblm WF, Benfield P,

19 Macan D, Whitebread S, Cumun F, De Gasparo M, Levens N Renal

actions of the angiotensin AT2 receptor ligands CGP 42112 and PD

20 Gasparo M, Hixxan A, Alexander W, Cast KJ, Chiu AT, Frew M, Good-

friend T, Harding JW, Inagami T. Timmermans PBMWM Proposed

update of angiotensin receptor nomenclature Hypertension 1995,25

924–927

21 Kress JA, Major TC, Lu GH, Dave LS, Panek RL: Is there a functional


18 Wong PC, Price WA, Chiu AT, Duncan JV, Cann DJ, Wecker RR,

Johnson AL, Timmermans PBMWM Hypotensive action of DoP 783, an

angiotensin II antagonist, in spontaneously hypertensive rats Hypertension 1990,15 459–468

19 Macan D, Whitebread S, Cumun F, De Gasparo M, Levens N Renal

actions of the angiotensin AT2 receptor ligands CGP 42112 and PD

123319 after blockade of the renin-angiotensin system End J Phar~mol 1994,259 27–36

20 Gasparo M, Hixxan A, Alexander W, Cast KJ, Chiu AT, Frew M, Good-

friend T, Harding JW, Inagami T. Timmermans PBMWM Proposed

update of angiotensin receptor nomenclature Hypertension 1995,25

924–927
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