Angiotensin Type 2 Receptor–Mediated Hypotension in Angiotensin Type-1 Receptor–Blocked Rats

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Abstract—The type-2 (AT2) angiotensin (Ang) II receptor has been characterized as potentially counterregulatory to the actions of Ang II at its type-1 (AT1) receptor. We investigated the effects of Ang II and CGP-42112A (CGP), a selective peptide AT2 receptor agonist, on blood pressure (BP) in rats with or without pharmacological blockade of the AT1 receptor with losartan (LOS) or valsartan (VAL). In anesthetized rats (n=5 per group) receiving normal sodium intake, Ang II (200 pmol/kg per minute IV) alone increased BP from a control of 112±3 to 168±7 mm Hg (P<0.001) and LOS (30 mg/kg) alone decreased BP to 89±7 mm Hg (P<0.0001 from control). Ang II administered together with LOS decreased BP further to 71±4 mm Hg (P<0.00001 from control and LOS alone). AT2 receptor antagonist PD 123,319 (PD) completely blocked the hypotensive response to LOS combined with Ang II (P=NS from control). In conscious rats (n=5 per group) receiving normal sodium intake, VAL (10 mg/kg) alone decreased BP from a control of 98±5 to 86±3 mm Hg (P<0.00001). Ang II combined with VAL induced a consistent, highly significant decline in BP for 6 days to a nadir of 69±3 mm Hg (P<0.01 versus daily VAL alone). PD completely blocked the chronic hypotensive response to the combination of Ang II and VAL to control levels before VAL administration. In another study in conscious rats (n=5 per group), CGP (70 μg/kg per minute) also decreased BP in VAL-treated conscious rats. BP was 119±3 mm Hg during the control period, decreased to 86±6 mm Hg during 3 days of VAL alone (P<0.00001) and decreased further to 65±7 mm Hg (P<0.001 from daily VAL alone) with 7 days of CGP in the presence of VAL. In the absence of VAL, CGP decreased BP for 4 consecutive days, and this response was blocked by PD. Also, the CGP-induced decrease in BP over a 7-day period was blocked by Nω-nitro-L-arginine methyl ester, an inhibitor of NO synthase. The results strongly suggest that the AT2 receptor induces a systemic vasodilator response mediated by NO that counterbalances the vasoconstrictor action of Ang II at the AT1 receptor. (Hypertension. 2001;38:1272-1277.)

Key Words: angiotensin II □ receptors, angiotensin II □ blood pressure □ losartan

Angiotensin (Ang) II is a pleiotropic vasoactive peptide that acts at 2 known Ang II receptors, type 1 (AT1) and type 2 (AT2). The actions of AT2 receptors at AT1 receptors are well characterized. However, the physiological actions of Ang II at AT2 receptors have been difficult to elicit, at least in part because AT2 receptors have a low degree of expression compared with that of AT1 receptors.1–3

Recent evidence suggests that AT2 receptors may play a role in the reduction of arterial blood pressure (BP).4–15 In the mouse, targeted disruption of the AT2 receptor gene increased BP slightly and induced pressor sensitivity to Ang II, both acutely and chronically.4–7 In the rat, the depressor phase of the biphasic BP response to Ang III (des-aspartyl[1–Ang II]) was blocked by AT2 receptor blockade with PD 123,319 (PD), which also enhanced the pressor phase,8 and AT2 receptor blockade augmented the pressor effect of Ang II.9 Tsutsumi et al10 demonstrated that overexpression of the AT2 receptor in vascular smooth muscle cells of transgenic mice blunted the pressor response to Ang II. This study also demonstrated that pharmacological blockade of the AT1 receptor resulted in a depressor response to Ang II in both AT2 receptor overexpressing and wild-type mice.10 In the spontaneously hypertensive rat (SHR), Barber et al11 showed that AT1 receptor stimulation with CGP-42112 induced a depressor response during simultaneous AT1 receptor blockade. Matrougui et al12 found that activation of AT1 receptors by endogenous Ang II is involved in flow-induced dilatation of rat mesenteric resistance arteries. Touyz et al13 further showed that AT1 receptors are involved in mitigating Ang II–induced contraction of small mesenteric arteries in SHR. In Ang II–dependent hypertension in rats, the hypotensive response to AT1 receptor blockade was eliminated by AT2 receptor blockade.14 Tamura et al15 demonstrated AT2 receptor–mediated BP regulation in hypertensive rats fed a purified synthetic diet. Taken together, these studies4–15 suggest that the AT2 receptor may act as a vasodilator pathway counter-regulatory to the vasoconstrictor actions of Ang II through the AT1 receptor.

Because all previously published studies reported AT2 receptor–mediated hypotensive effects in a hypertensive an-
inal model, the present study was designed to demonstrate the role of the AT$_2$ receptor in the regulation of BP in the normal rat. We investigated the effects of Ang II and CGP-42112A (CGP), a selective peptide AT$_2$ receptor agonist, on BP in rats both acutely and chronically with or without pharmacological blockade of the AT$_1$ receptor with losartan (LOS) or valsartan (VAL), respectively. We selected these 2 agents because we wanted to demonstrate results with 2 different AT$_1$ receptor blockers. Valsartan was used for the chronic studies because it has at least 5-fold-higher affinity for the AT$_2$ receptor than losartan has, ensuring an effective long-term AT$_1$ receptor blockade. The results suggest that stimulation of the AT$_2$ receptor induces hypotension, probably through stimulation of NO production, in the AT$_1$ receptor–blocked rat.

Methods

Animal Preparation

The experiments, which were approved by the University of Virginia Animal Research Committee, were conducted in 10- to 12-week-old Sprague-Dawley rats (Harlan, Teklad). For acute experiments, rats were placed under general anesthesia with ketamine (80 mg/kg IM) and xylazine (8 mg/kg IM), which resulted in deep anesthesia for 90-minute control and experimental periods, and a heparinized polyethylene tube was inserted into the right femoral vein for administration of pharmacological agents and into the right carotid artery for direct arterial BP monitoring. For chronic experiments in conscious animals, osmotic minipumps were implanted in the interscapular region with the animals under short-term anesthesia with ketamine (40 mg/kg IM) and xylazine (4 mg/kg IM). Rats were housed under controlled conditions (temperature, 21 ± 1°C; humidity, 60 ± 10%; and light, 8 to 20 hours). Experiments were initiated at the same time each day to prevent any diurnal variation in BP.

BP Measurement

For acute experiments in anesthetized rats, systolic BP (SBP) was measured by the direct intracarotid method with the use of a BP analyzer (Micromed Inc). Blood pressures were recorded continuously and averaged for 30-minute periods. For chronic experiments in conscious rats, SBP was measured by the tail-cuff method with an automated sphygmomanometer (model 679, IITC/Life Sciences Instruments). Blood pressures were recorded at 10-minute intervals for 30 minutes at the same time each day (model 179 Apollo Recorder, Life Sciences Instruments), and values were averaged each day as previously published.14,16

Acute Effects of Ang II and AT$_2$ Receptor Blockade on BP in AT$_1$ Receptor–Blocked Anesthetized Rats

Rats (n=5 in each group) were studied on normal sodium intake for 11 days. On experimental day 0, basal SBP was monitored. At 8 AM on experimental day 1, a subcutaneous infusion of VAL (10 mg/kg per day) was initiated and continued for 9 days through a micro-osmotic pump. SBP was monitored daily. In one group of rats (n=5), at 8 AM on experimental day 4, in addition to the infusion of VAL, an infusion of Ang II at 100 pmol/kg per minute was initiated and continued through 8 AM on day 10. In another group (n=5), in addition to the infusion of VAL, an infusion of Ang II and PD at 50 µg/kg per minute was initiated at 8 AM on day 4 and continued through 8 AM on day 10. In another group (n=5), rats received vehicle instead of VAL on days 1 to 11. In this group, an infusion of Ang II alone was initiated at 8 AM on day 4 and continued until 8 AM on day 10. At 8 AM on day 10 in all groups, the infusion of all pharmacological agents was discontinued by removing the pump, and SBP was monitored for 2 additional days (experimental days 10 and 11). For purposes of data analysis, days 1 to 3 (VAL alone) were designated as period 1 and days 4 to 9 (different pharmacological agents) were designated as period 2.

Chronic Effects of CGP, AT$_2$ Receptor Blockade, or NO Synthase Inhibition on BP in AT$_1$ Receptor–Blocked and Unblocked Conscious Rats

Rats (n=5 in each group) were placed on normal sodium intake for 13 days. On experimental day 0, basal SBP was monitored. At 8 AM on experimental day 1 in one group of rats (n=5), a subcutaneous infusion of VAL (10 mg/kg per day) was initiated and continued for 10 days by micro-osmotic pump. SBP was monitored daily. In another group of rats (n=5), at 8 AM on experimental day 4, in addition to the infusion of VAL, an infusion of CGP at 70 pmol/kg per minute was initiated and continued through 8 AM on day 11. In a third group of rats (n=5), the CGP infusion was combined with PD (50 µg/kg per minute). At 8 AM on day 11, in all groups, the infusion of pharmacological agents was discontinued and SBP was monitored for 3 additional days (experimental days 11 to 13). In another group of rats (n=5), an infusion of CGP alone was initiated at 8 AM on day 1 and continued until 8 AM on day 5. In another group, the latter study was repeated except that CGP was combined with PD. In another group of rats (n=5) studied in metabolic balance at low sodium intake (0.04% dietary sodium), CGP was infused at 70 pmol/kg per minute for 7 days. In another group of rats (n=5) receiving low sodium intake, CGP was infused together with N$_2$-nitro-L-arginine methyl ester (L-NAME) at 100 ng/kg per minute for 7 days. For purposes of data analysis, days 1 to 3 (VAL alone) were designated as period 1; and days 4 to 9 (different pharmacological agents), as period 2.

Pharmacological Agents

Ang II amide [ASN-Val]$^1$–Ang II (Norvartis), an AT$_1$ and AT$_2$ receptor agonist, and CGP, a selective AT$_2$ receptor agonist (IC$_{50}$ 5×10$^{-10}$ mol/L and 2×10$^{-8}$ mol/L for AT$_1$ and AT$_2$, respectively), were used for these studies. PD (Parke-Davis), a specific AT$_1$ receptor antagonist (IC$_{50}$ 2×10$^{-8}$ mol/L and >1×10$^{-4}$ mol/L for AT$_2$ and AT$_1$, respectively, was used to block the AT$_1$ receptor. LOS, a specific, potent inhibitor of AT$_1$ receptors (IC$_{50}$ 3×10$^{-9}$ and 7×10$^{-7}$ mol/L for AT$_1$ and AT$_2$, respectively) was used for acute studies in anesthetized animals. VAL, a highly potent, selective antagonist (K$_i$ 2.38 nmol/L for the AT$_2$ receptor) with 30-fold binding affinity of LOS, was used for the chronic studies in conscious animals.18 L-NAME, a specific inhibitor of NO synthase and of NO formation, was purchased from Sigma Chemical Co.

Statistical Analysis

Comparisons among vehicle, AT$_1$ receptor blocker (LOS or VAL), AT$_2$ receptor blocker (PD), AT$_1$, and AT$_2$, receptor agonist (Ang II), and AT$_1$, receptor agonist (CGP) were estimated by ANOVA, including a repeated-measures term, by using the general linear models procedure of the statistical analysis system. Multiple comparisons of individual pairs of effect means were conducted by the use of least-squares means pooled variance. Data are expressed as mean±SEM. Statistical significance was identified at a level of P<0.05.
Results

Effects of Acute Ang II and PD Infusion on BP in AT₁ Receptor–Blocked Conscious Rats

As illustrated in Figure 1, VAL alone decreased SBP from control values of 119±5 mm Hg to 90±7 mm Hg (P<0.001 vs control) on days 1 to 3 of VAL infusion and continued to decrease SBP throughout the VAL infusion (days 4 to 9). Addition of CGP to VAL-infused rats did not decrease SBP further on day 4. Thereafter, however, CGP decreased BP each day (days 5 to 9) significantly compared with values for VAL alone. The nadir of the SBP response to CGP was 65±7 mm Hg (P<0.001 from daily VAL alone and P<0.01 from daily VAL alone days 1 to 3) on day 9. On days 10 to 13 after discontinuation of CGP, SBP rose to levels intermediate between control (day 0) and VAL alone (days 1 to 3). Combination of PD with CGP and VAL completely blocked the hypotensive response to CGP and restored SBP to control values before VAL infusion.

Effects of Chronic CGP and PD Infusion in AT₁ Receptor–Blocked Conscious Rats

As shown in Figure 3, VAL alone decreased SBP from control values of 119±5 mm Hg to 90±7 mm Hg (P<0.001 vs control) on days 1 to 3 of VAL infusion and continued to decrease SBP throughout the VAL infusion (days 4 to 9). Addition of CGP to VAL-infused rats did not decrease SBP further on day 4. Thereafter, however, CGP decreased BP each day (days 5 to 9) significantly compared with values for VAL alone. The nadir of the SBP response to CGP was 65±7 mm Hg (P<0.001 from daily VAL alone and P<0.01 from daily VAL alone days 1 to 3) on day 9. On days 10 to 13 after discontinuation of CGP, SBP rose to values intermediate between control (day 0) and VAL alone (days 1 to 3). Combination of PD with CGP and VAL completely blocked the hypotensive response to CGP and restored SBP to control values before VAL infusion.

Figure 1. SBP of anesthetized rats (n=5 per group) measured by direct intracarotid method during 30-minute control period and 2 consecutive 30-minute experimental periods (periods 1 and 2). Slanted striped bars indicate pressures when Ang II was infused together with LOS for both periods. Cross-hatched bars indicate data when 200 pmol/kg per minute Ang II was infused together with LOS was infused alone for 2 periods. Solid black bars indicate pressures when 30 mg/kg PD was added to the combination of LOS + Ang II in period 2. Stippled bars indicate data when Ang II alone was infused for 2 periods. Data represent mean±1 SEM. *P<0.001; **P<0.0001, +++P<0.00001 from control; *P<0.00001 from LOS alone; **P<0.000001 from LOS + Ang II.

Figure 2. SBP of conscious rats (n=5 per group) measured daily by tail-cuff method. Values are shown for control period (day 0) during which vehicle was infused; for day 3 of 3-day period (days 1 to 3) during which VAL was infused at 10 mg/kg per day; and for 5-day experimental period (days 5 to 9) during which VAL alone (white bars) or VAL + Ang II (100 pmol/kg per minute) (solid black bars) or VAL + Ang II + PD (50 μg/kg per minute) (striped bars) or Ang II alone (black stippled bars) was infused. All pharmacological agents were discontinued at end of day 9, and post–control period was conducted during which vehicle was infused (days 10 and 11). Data represent mean±1 SEM. *P<0.05; **P<0.01 vs VAL alone (day 3); #P<0.05, ##P<0.01 vs daily VAL alone; +P<0.01, ++P<0.001 from control (day 0).
Figure 4 summarizes chronic SBP responses to VAL alone, VAL + Ang II or CGP, VAL + Ang II + PD, and VAL + CGP + PD. The hypotensive response to VAL was significantly enhanced by Ang II or CGP, and in each case SBP was restored to control values in the presence of PD.

Effects of Chronic CGP and PD Infusion in Conscious Rats During Normal Sodium Intake in Absence of AT₁ Receptor Blockade
As demonstrated in Figure 5, CGP decreased SBP from a control of 104 ± 3 mm Hg to 94 ± 2 mm Hg (P < 0.001) on
day 1 and to a nadir of 87±3 mm Hg (P<0.0001) on day 4. Coadministration of PD with CGP completely abrogated the hypotensive response to CGP on days 1 to 4.

Effects of Chronic CGP and L-NAME in Conscious Sodium-Restricted Rats in Absence of AT1 Receptor Blockade
As shown in Figure 6, CGP decreased SBP from a control of 102±3 to a nadir of 78±3 mm Hg (P<0.0001) on day 3. Except for day 1, SBP values were lower in response to CGP alone than to CGP+L-NAME (P<0.0001). Except for day 2, SBP values in response to CGP+L-NAME were not significantly different from control values.

Discussion
This study demonstrates that stimulation of the AT2 receptor decreases systemic arterial pressure in the normal rat. In the adult rat, AT2 receptors are present only in low copy, and Ang II increases BP by an action at AT1 receptors; therefore we reasoned that an AT2 receptor action on BP would best be magnified by stimulating the AT2 receptor during functional absence of the AT1 receptor (AT1 receptor blockade). In both acute studies in anesthetized animals and chronic studies in conscious animals, Ang II decreased BP in the presence of AT1 receptor blockade. This action of Ang II was due to stimulation of AT2 receptors because it was blocked completely by the specific AT2 receptor antagonist PD. The conclusion that AT2 receptor stimulation is vasodilatory was corroborated by additional observations showing that the selective AT2 receptor agonist CGP also decreased BP in AT1 receptor-blocked animals and that this action of CGP was also blocked by PD. Further evidence supporting the vasodilator action of the AT2 receptor included the CGP-mediated decrease in BP in conscious animals in the absence of AT1 receptor blockade and its prevention by PD. Taken altogether, these data confirm and extend previous observations4–15 that AT2 receptors play a role in the regulation of BP in the genetically altered mouse, the SHR, and both the renal vascular and the synthetic diet–fed hypertensive rat. The data are consistent with the concept that the AT2 receptor may stimulate a vasodilator pathway, which is counterregulatory to the pressor action of Ang II at the AT1 receptor. In the absence of cardiac output measurements, it remains possible that at least part of the depressor response to AT2 receptor stimulation could

Figure 5. SBP of conscious rats (n=5) during normal sodium intake during vehicle control period and during 4 consecutive days of infusion of 100 μg/kg per day CGP (black bars) or CGP+PD (50 μg/kg per minute) (striped bars). Values represent mean±1 SEM. *P<0.01; **P<0.0001; ***P<0.001 from daily CGP alone.

Figure 6. SBP of sodium-restricted conscious rats (n=5) during vehicle control period and during days 1, 2, 3, 5, and 7 of infusion of CGP (100 μg/kg per day) (black bars) or CGP+L-NAME (100 ng/kg per minute) (striped bars). Values represent mean±1 SEM. *P<0.001; +P<0.0001 from control; **P<0.00001 from CGP+L-NAME; #P<0.01 from control.
be due to a depressive effect of cardiac AT$_2$ receptors. However, this is unlikely because the depressor response to CGP was blocked completely by inhibition of NO synthase, implying that the AT$_2$ receptor mediates a vasodilator pathway.

It was possible that AT$_2$ receptor–stimulated NO and cyclic GMP induce vasodilatation and hypotension in the AT$_1$ receptor–blocked rat on normal sodium intake, as is the case in sodium-restricted rats. We performed additional experiments to determine whether the AT$_2$ receptor–mediated chronic vasodilator effect could be blocked with inhibition of NO synthase. L-NAME completely blocked the depressor action of CGP, an AT$_2$ receptor agonist, in sodium-restricted rats, indicating that NO mediates the vasodilator pathway through the AT$_2$ receptor.

Several studies have failed to demonstrate AT$_2$ receptor–mediated reduction in BP in the intact rat. Studying anesthetized normal Wistar rats acutely, showed no effect of PD on BP responses to Ang II and no hypotensive response to Ang II even at 1000 pmol/kg per minute in the presence of AT$_2$ receptor blockade with ibersartan. Because these studies used a 100-fold–lower infusion rate of a different AT$_2$ receptor antagonist than used in the present study, it is possible that the AT$_2$ receptor was not fully blocked. Also in these studies, the infusion rate of PD was less than half of that used in the present study, AT$_1$ and AT$_2$ receptor antagonists were not combined, and studies in conscious animals were not performed. Whether these differences account for some or all of the differences in results is not clear.

 INTERRUPTION OF THE NEGATIVE FEEDBACK LOOP, WHEREIN ANG II INHIBITS RENIN SECRETION, WITH AN AT$_1$ RECEPTOR BLOCKER INCREASES RENIN RELEASE AND STIMULATES ANG II GENERATION. THEREFORE ANIMALS WITH LOW OR VAL SHOULD HAVE HIGH CIRCULATING ANG II, WHICH CAN STIMULATE UNBLOCKED AT$_2$ RECEPTORS. IN OUR CHRONIC STUDIES IN CONSCIOUS ANIMALS, HIGH CIRCULATING ANG II SHOULD HAVE BEEN PRESENT FOR 3 DAYS OF AT$_1$ RECEPTOR BLOCKADE BEFORE INITIATING THE ANG II OR CGP. INCREASED CIRCULATING ANG II HAS BEEN OBSERVED AFTER AT$_1$ RECEPTOR BLOCKADE IN SEVERAL STUDIES. The further reduction in BP when Ang II or CGP was added indicates that despite high circulating Ang II, AT$_2$ receptors were not fully occupied, such that additional agonist elicited a biological response.

The results of the present study have pharmacological implications for the treatment of hypertension. The results confirm previous observations that at least part of the BP-lowering effect of AT$_2$ receptor blockade is attributable to AT$_2$ receptor stimulation. In addition, the sustained reduction in BP when the AT$_2$ receptor is stimulated in the presence of AT$_1$ receptor blockade implies that combination therapy, if and when available, may be superior to AT$_1$ receptor blockade alone.

References

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