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).1 The actions of Ang II 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 regulation of arterial blood pressure (BP).1,4–15 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-aspar-tyl[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 AT2 receptors by endogenous Ang II is involved in flow-induced dilation of rat mesenteric resistance arteries. Touyz et al13 further showed that AT2 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 AT2 receptor in the regulation of BP in the normal rat. We investigated the effects of Ang II and CGP-42112A (CGP), a selective peptide AT2 receptor agonist, on BP in rats both acutely and chronically with or without pharmacological blockade of the AT1 receptor with losartan (LOS) or valsartan (VAL), respectively. We selected these 2 agents because we wanted to demonstrate results with 2 different AT1 receptor blockers. Valsartan was used for the chronic studies because it has at least 5-fold-higher affinity for the AT1 receptor than losartan has, ensuring an effective long-term AT1 receptor blockade. The results suggest that stimulation of the AT2 receptor induces hypotension, probably through stimulation of NO production, in the AT1 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°C ± 1; 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 AT2 Receptor Blockade on BP in AT1 Receptor–Blocked Anesthetized Rats

Rats (n=5 in each group) were studied on normal sodium intake (0.28% dietary sodium). SBP was monitored during a 30-minute control period (vehicle infusion) followed by 2 consecutive 30-minute experimental periods (periods 1 and 2), during which pharmacological agents were infused. In one group, LOS (30 mg/kg) alone was infused during periods 1 and 2. In another group, Ang II (200 pmol/kg per minute) was combined with LOS during period 1 and Ang II, LOS, and PD (50 µg/kg per minute) were combined in period 2. In a third group, Ang II was combined with LOS during both periods 1 and 2. In a fourth group, Ang II was infused alone for both experimental periods.

Chronic Effects of Ang II and AT2 Receptor Blockade on BP in AT1 Receptor–Blocked Conscious Rats

Rats (n=5 in each group) were placed 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, AT2 Receptor Blockade, or NO Synthase Inhibition on BP in AT1 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 Nω-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]–Ang II (Norvartis), an AT1 and AT2 receptor agonist, and CGP, a selective AT2 receptor agonist (IC50 3 ×10−10 mol/L and 2×10−8 mol/L for AT1 and AT2 receptors, respectively), were used in this study. For chronic studies, a specific AT1 receptor antagonist (IC50 2×10−8 mol/L and >1×10−4 mol/L for AT2 receptors, respectively) was used to block the AT1 receptor. LOS, a specific, potent inhibitor of AT1 receptors (IC50 3×10−8 and 7×10−5 mol/L for AT2 and AT1 receptors, respectively) was used for acute studies in anesthetized animals. VAL, a highly potent, selective antagonist (Kd, 2.38 nmol/L for the AT1 receptor and 57.7 µmol/L for the AT2 receptor) with ≈30-fold binding affinity of LOS, was used for the chronic studies in conscious animals. 13–15 L-NAME, a selective inhibitor of NO synthase and of NO formation, was purchased from Sigma Chemical Co.

Statistical Analysis

Comparisons among vehicle, AT1 receptor blocker (LOS or VAL), AT2 receptor blocker (PD), AT1 and AT2 receptor agonist (Ang II), and AT2 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±1 SEM. Statistical significance was identified at a level of P<0.05.
Effects of Acute Ang II and PD Infusion on BP in AT$_1$ Receptor–Blocked Conscious Rats

As demonstrated in Figure 1, LOS decreased SBP from control values of 111.7±2.7 to 89.3±6.5 mm Hg ($P<0.0001$) during period 1, and SBP was unchanged at 88.7±8.7 mm Hg ($P<0.001$ from control) during period 2. During LOS infusion, Ang II decreased SBP further to 71.1±3.9 mm Hg ($P<0.00001$ from LOS alone) during period 2. Addition of PD during period 2 blocked the hypotensive response to Ang II in the presence of LOS ($P<0.00001$ from LOS+Ang II) and restored SBP to control values (112.0±5.4 mm Hg) before LOS administration. Ang II alone increased SBP to 168.2±7.4 mm Hg ($P<0.001$ from control) in period 1 and to 162.5±5.4 mm Hg ($P<0.0001$ from control) during period 2.

Effects of Chronic Ang II and PD Infusion in AT$_1$ Receptor–Blocked Conscious Rats

As shown in Figure 2, VAL alone decreased SBP from control values of 119±5 mm Hg to 90±7 mm Hg ($P<0.001$ from 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 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 pressures to control values before VAL administration.
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.

Figure 3. 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 3-day period (days 1 to 3) during which VAL alone was infused at 10 mg/kg per day; and for 6-day experimental period (days 4 to 9) during which VAL alone (white bars) or VAL+CGP (black bars) or VAL+CGP+PD (striped bars) was infused. Data represent mean±1 SEM. *P<0.01 from respective vehicle control (day 0); †P<0.001 from daily VAL alone; ‡P<0.0001 from daily VAL alone; #P<0.01 from VAL alone (days 1 to 3).

Effects of Chronic CGP and PD Infusion in Conscious Rats During Normal Sodium Intake in Absence of AT<sub>1</sub> 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...
Effects of Chronic CGP and L-NAME in Conscious Sodium-Restricted Rats in Absence of \( \text{AT}_1 \) Receptor Blockade

As shown in Figure 6, CGP decreased SBP from a control of \( 102 \pm 3 \) to a nadir of \( 78 \pm 3 \) 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 \( \text{AT}_2 \) receptor decreases systemic arterial pressure in the normal rat. In the adult rat, \( \text{AT}_2 \) receptors are present only in low copy, and Ang II increases BP by an action at \( \text{AT}_1 \) receptors; therefore we reasoned that an \( \text{AT}_2 \) receptor action on BP would best be magnified by stimulating the \( \text{AT}_2 \) receptor during functional absence of the \( \text{AT}_1 \) receptor (\( \text{AT}_1 \) receptor blockade). In both acute studies in anesthetized animals and chronic studies in conscious animals, Ang II decreased BP in the presence of \( \text{AT}_1 \) receptor blockade. This action of Ang II was due to stimulation of \( \text{AT}_2 \) receptors because it was blocked completely by the specific \( \text{AT}_2 \) receptor antagonist PD. The conclusion that \( \text{AT}_2 \) receptor stimulation is vasodilatory was corroborated by additional observations showing that the selective \( \text{AT}_2 \) receptor agonist CGP also decreased BP in \( \text{AT}_1 \) receptor-blocked animals and that this action of CGP was also blocked by PD. Further evidence supporting the vasodilator action of the \( \text{AT}_2 \) receptor included the CGP-mediated decrease in BP in conscious animals in the absence of \( \text{AT}_1 \) receptor blockade and its prevention by PD. Taken altogether, these data confirm and extend previous observations\(^4\text{–}^{15}\) that \( \text{AT}_2 \) 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 \( \text{AT}_2 \) receptor may stimulate a vasodilator pathway, which is counterregulatory to the pressor action of Ang II at the \( \text{AT}_1 \) receptor. In the absence of cardiac output measurements, it remains possible that at least part of the depressor response to \( \text{AT}_2 \) receptor stimulation could...
be due to a depressive effect of cardiac AT2 receptors. However, this is unlikely because the depressor response to CGP was blocked completely by inhibition of NO synthase, implying that the AT2 receptor mediates a vasodilator pathway.

It was possible that AT2 receptor–stimulated NO and cyclic GMP induce vasodilation and hypotension in the AT1 receptor–blocked rat on normal sodium intake, as is the case in sodium-restricted rats. We performed additional experiments to determine whether the AT2 receptor–mediated chronic vasodilator effect could be blocked with inhibition of NO synthase. L-NAME completely blocked the depressor action of CGP, an AT2 receptor agonist, in sodium-restricted rats, indicating that NO mediates the vasodilator pathway through the AT2 receptor.

Several studies have failed to demonstrate AT2 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 hypertensive response to Ang II even at 1000 pmol/kg per minute in the presence of AT1 receptor blockade with irbesartan. Because these studies used a 100-fold–lower infusion rate of a different AT1 receptor antagonist than used in the present study, it is possible that the AT1 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, AT1 and AT2 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 AT1 receptor blocker increases renin release and stimulates Ang II generation. Therefore animals given LOS or VAL should have high circulating Ang II, which can stimulate unblocked AT2 receptors. In our chronic studies in conscious animals, high circulating Ang II should have been present for 3 days of AT1 receptor blockade before initiating the Ang II or CGP. Increased circulating Ang II has been observed after AT1 receptor blockade in several studies. The further reduction in BP when Ang II or CGP was added indicates that despite high circulating Ang II, AT2 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 AT1 receptor blockade is attributable to AT2 receptor stimulation. In addition, the sustained reduction in BP when the AT1 receptor is stimulated in the presence of AT2 receptor blockade implies that combination therapy, if and when available, may be superior to AT1 receptor blockade alone.

References
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