Angiotensin Type 2 Receptor Mediates Valsartan-Induced Hypotension in Conscious Rats

Helmy M. Siragy, Marc de Gasparo, Robert M. Carey

Abstract—Inhibition of the renin-angiotensin system is associated with vasodilation and reduction in blood pressure. We hypothesized that angiotensin type 1 (AT) receptor (AT,R) blockade is associated with increased production of renal nitric oxide (NO) mediated by release of bradykinin (BK). By use of a microdialysis technique, changes in renal interstitial fluid (RIF) BK, NO end products nitrite and nitrate (NOX), and cGMP were monitored in response to intravenous infusion of the AT,R blocker valsartan (10 mg/kg), the angiotensin type 2 (AT) receptor (AT,R) blocker PD123319 (50 μg · kg⁻¹ · min⁻¹), and the BK B₂ receptor blocker icatibant (10 μg · kg⁻¹ · min⁻¹) in conscious rats (n = 10) during low sodium intake. RIF BK, NOX, and cGMP significantly increased during valsartan treatment, whereas AT,R blockade caused a significant decrease in these autacoids. During icatibant infusion, RIF NOX and cGMP decreased by 64% and 40%, respectively, whereas BK increased. Combined administration of valsartan and icatibant, of valsartan and PD123319, or of valsartan, PD123319, and icatibant prevented the increase in RIF cGMP and NOX in response to valsartan alone. These data demonstrate that AT,R blockade with valsartan is associated with release of renal BK, which in turn mediates NO production. The results suggest that increased angiotensin II, in response to sodium restriction and valsartan infusion, stimulates AT,R, which mediates a BK and NO cascade. (Hypertension. 2000;35:1074-1077.)

Key Words: receptors, angiotensin II ■ bradykinin ■ nitric oxide ■ cyclic GMP ■ valsartan

All components of the renin-angiotensin system are present within the kidney. Angiotensin II (Ang II) is the most active component of this system. The tissue production of Ang II in proximity to its receptors on the target cells constitutes a paracrine function for this hormone. Ang II plays a major role in regulating body fluid and electrolyte homeostasis and blood pressure. The majority of the effects of Ang II are mediated by stimulation of the angiotensin type-1 (AT₁) receptor. Inhibition of the AT₁ receptor results in decreased renin secretion, resulting in a subsequent increase in Ang II production, a decrease in blood pressure, diuresis, and natriuresis. The interaction between Ang II and other vasoactive peptides contributes to the preservation of these functions. One of the important peptides that is produced in the kidney is bradykinin (BK). Mainly by inducing diuresis and natriuresis, BK has been shown to regulate renal functions in a manner opposite that induced by Ang II.

In the present study, we evaluated the effect of AT₁ receptor blockade on renal production of BK and its mediators, nitric oxide (NO) and cGMP. We hypothesized that AT₁ receptor blockade is associated with increased production of renal interstitial fluid (RIF) BK. We used a novel microdialysis technique to monitor changes in RIF BK, the NO end products nitrite and nitrate (NOX), and cGMP during AT₁, angiotensin type-2 (AT₂), or BK B₂ receptor blockade.
Effects of AT1, AT2, and BK B2 Receptor Blockade Individually or Combined

Animals (n=10) were placed in metabolic cages. Each animal served as its own control, and different treatments were carried out in the same group of animals. One day before surgery, while rats were consuming a normal sodium diet (0.28% NaCl), baseline body weight, PRA, and SBP were measured, and a 24-hour urine sample was collected for calculation of urinary volume and sodium excretion (UNaV). After surgery, the animals served as its own control, and different treatments were carried out in response to low sodium intake.

Analytical Methods

Urinary sodium concentrations were measured by using a NOVA Biomedical analyzer. PRA was measured by radioimmunoassay. SBP was measured at 30-minute intervals in the tail, and recorded values were averaged for each study period. RIF BK levels were measured by ELISA. The sensitivity of this assay is 1 pg/mL and is 100% specific for BK. It does not react with any other peptides. RIF NOX and cGMP levels in dialysate samples were measured by using an enzyme immunoassay kit. The sensitivity is 2.5 μmol/L and 0.11 pmol/mL for NOX and cGMP, respectively, and the specificity is 100% for both. The intra-assay and interassay cross-reactivities with other cyclic nucleotides were <0.01%.

Statistical Analysis

Comparisons among pharmacological agents and controls were examined by ANOVA, including a repeated measure term, by use of the general linear models procedure of the Statistical Analysis System. Multiple comparisons of individual pairs of effect means were conducted by using the least squares method of pooled variance. Data are expressed as mean±SE. Statistical significance was identified at P<0.05.

Results

Changes in PRA, 24-Hour Urinary Volume, UNaV, and Blood Pressure Responses to Low Sodium Intake

PRA during normal sodium intake was 1.6±1 ng · mL⁻¹ · h⁻¹, and increased to 13.8±3.1 ng · mL⁻¹ · h⁻¹ (P<0.0001) by day 6 of low sodium intake. In contrast, 24-hour urinary volume and UNaV decreased significantly from 22.3±2.0 mL/d and 1150±30 μmol/d to 3.1±0.8 mL/d and 173±8 μmol/d (P<0.0001), respectively, in response to low sodium intake. SBP was 118±4 mm Hg during normal sodium intake and did not change in response to low sodium intake.

Figure 1. RIF BK, NOX, and cGMP increased in response to low sodium intake. Values are mean±SE (n=10). *P<0.0001 vs normal.

RIF BK, NOX, and cGMP Response to Low Sodium Intake

During normal sodium intake, RIF BK, NOX, and cGMP recoveries were 53±12 pg/min, 0.1±0.02 μmol/min, and 0.12±0.02 μmol/min, respectively. By day 6 of low sodium intake, there were significant increases (Figure 1) in recovery of RIF BK, NOX, and cGMP to 360±20 pg/min, 0.28±0.01 μmol/min, and 0.9±0.01 μmol/min (P<0.0001), respectively.

Changes in SBP in Response to AT1 and BK B2 Receptor Blockade During Low Sodium Intake

There were no changes in SBP in response to vehicle (5% dextrose) administration. Administration of valsartan (the AT1 receptor blocker) caused a significant decrease in SBP (Figure 2) from 119±3 to 110±2 mm Hg (P<0.05). In contrast, icatibant (the BK B2 receptor antagonist) increased SBP from 118±2 to 124±3 mm Hg (P<0.05). There was no change in SBP during PD administration. Combined administration of valsartan and icatibant, of valsartan and PD, or of valsartan, PD, and icatibant completely prevented (to similar levels) the decrease in SBP that was observed with valsartan alone (Figure 2).

Changes in RIF BK, NOX, and cGMP in Response to AT1 and BK B2 Receptor Blockade

There were no changes in RIF BK, NOX, and cGMP during time-control vehicle administration. RIF BK, NOX, and...
cGMP increased \((P<0.001)\) during valsartan treatment (Figure 3). Similarly, RIF BK increased during icatibant treatment or combined valsartan and icatibant treatment \((P<0.001)\). However, in contrast to valsartan, icatibant decreased RIF NOX and cGMP by 64\% \((P<0.0001)\) and 40\% \((P<0.001)\), respectively (Figure 3). PD alone or combined with valsartan decreased RIF BK, NOX, and cGMP to those levels observed during normal sodium intake \((P<0.0001)\). Combined administration of valsartan and icatibant or of valsartan, PD, and icatibant prevented the increase in RIF cGMP and NOX. Similarly, combined treatment with valsartan, PD, and icatibant reduced RIF BK \((P<0.0001)\) to levels similar to those observed during treatment with PD alone or PD and valsartan.

**Discussion**

The present study demonstrates that AT\(_1\) receptor blockade is associated with an increase in renal tissue levels of BK, NO, and cGMP. Our results suggest that the vasodilator response to AT\(_1\) receptor blockade is mediated, at least in part, by the concomitant increase in tissue BK, which is stimulated via the AT\(_2\) receptor. Further vasodilator action is obtained via BK stimulation of NO in this pathway. The observed levels of RIF BK, NOX, and cGMP are not due to changes in renal hemodynamics induced by the use of various drugs, according to our previous studies\(^6,7\) which have demonstrated the following: (1) Losartan, a renal vasodilator agent, and Ang II, a renal vasoconstrictor hormone, increase RIF BK. (2) Ang II increases RIF cGMP, whereas \(N\)-nitro-L-arginine methyl ester, an NO synthase inhibitor and a renal vasoconstrictor, decreases RIF cGMP. These data demonstrate that changes in recovered RIF substances are specific to each treatment regardless of the general effects of treatments on renal hemodynamics.

Sodium depletion is associated with an increase in RIF Ang II and BK concentrations.\(^10,11\) It is likely that the increase in RIF BK is secondary to the increase in RIF Ang II levels. This thesis is strengthened by the observations that exogenous Ang II increases RIF BK during normal sodium intake.\(^7\) BK increases NO production,\(^12\) which activates soluble guanylyl cyclase, releasing cGMP into the RIF. This response is mediated at the BK B\(_2\) receptor, because it can be blocked by icatibant, a specific B\(_2\) receptor antagonist. In the present and previous studies\(^6,7\) the RIF BK, NOX, and cGMP levels during time-control studies were stable and did not change from day to day. However, we should caution that these studies were not designed to determine the absolute levels of recovered substances.

The AT\(_1\) receptor blocker valsartan and the AT\(_2\) receptor blocker PD were used to evaluate whether the AT\(_1\) or AT\(_2\) receptor was involved in the process of increasing renal BK levels. RIF BK increased during sodium depletion. RIF BK levels further increased during valsartan treatment and decreased during PD administration. These responses suggest that the AT\(_2\) receptor is responsible for the increase in RIF BK and that during sodium depletion, BK formation is tonically stimulated by Ang II at the AT\(_2\) receptor. In the presence of AT\(_1\) receptor blockade at the level of renal juxtaglomerular cells, increased circulating Ang II\(^{13}\) enhances BK production through stimulation of the unblocked AT\(_2\) receptor.

Our previous studies\(^5\) demonstrated that under conditions of increased Ang II levels, AT\(_1\) receptor stimulation is associated with an increase in RIF cGMP, the production of which is mediated by AT\(_2\) receptor stimulation of NO.\(^6\) In the
present study, AT₂ receptor stimulation of BK production offers clarification of the mechanisms leading to NO release. Whether the changes in RIF BK stimulated by Ang II contribute to the regulation of renal hemodynamic and excretory function awaits further investigation.

In the present study, it was technically impossible to measure U_{Na+} or renal blood flow in conscious rats during the different experimental manipulations. However, changes in blood pressure suggest that the hypertensive effect of AT₁ receptor blockade is at least partially mediated by BK release through stimulation of the AT₂ receptor. In addition, present knowledge suggests that the renal kallikrein-kinin system is involved in the regulation of sodium and water excretion and may participate in blood pressure control. BK is known to release NO from vascular endothelial, renal interstitial, or epithelial cells. At a portion of the renal effects of kinins appears to be mediated by NO, because inhibition of NO synthesis reduces the renal vasodilator response to BK.

Previously, we have shown that AT₂ receptor stimulation mediates NO release. Furthermore, acute administration of icatibant reduced NO to levels similar to those observed during AT₂ receptor blockade. The present study clearly indicates that partial renal NO production is mediated by the AT₂ receptor via increased production of BK. AT₁ receptor blockade shifts Ang II toward stimulation of the unblocked AT₂ receptor to release BK. Consequently, AT₂ receptor blockade reduces renal BK and NO.

The mechanism whereby AT₂ receptor stimulation releases BK is unclear. However, our data suggest that the increase in kinin production under such conditions counteracts the vasoconstrictor mechanisms activated in response to increased Ang II. The observed increase in blood pressure during icatibant treatment confirms the role of bradykinin in blood pressure regulation. In the present study, BK₂ receptor blockade completely prevented the blood pressure–reducing effects of valsartan. The specific contribution of AT₂ receptor–mediated BK to the hypertensive effects of valsartan is supported by the fact that combined valsartan and PD prevented the valsartan hypertensive response to the same magnitude produced by combined valsartan and icatibant. Combined administration of valsartan, PD, and icatibant did not have any potentiation or synergistic effects on blood pressure or RIF BK; this finding suggests that PD and icatibant are influencing the same pathway. These data support our previous finding that BK partially mediates the hypertensive effects of AT₁ receptor blockade. Additionally, our results are supported by a recent finding that in mice overexpressing the AT₂ receptor, the AT₁ receptor–mediated vasodilation was caused by the effect of BK, leading to activation of endothelial NO/cGMP. Combined administration of valsartan and PD caused a greater decrease in renal tissue levels of NO and cGMP than did combined treatment with valsartan and icatibant. These results suggest that the AT₂ receptor can directly stimulate NO in addition to its effect through kinin release. Thus, AT₂ receptor–mediated BK release and the subsequent generation of NO via BK₂ receptor stimulation directly contribute to the blood pressure–lowering effects of valsartan. Because RIF cGMP levels parallel changes of RIF NOX during AT₁, AT₂, and BK₂ receptor blockade, it is likely that cGMP may be important in vasodilator signal transduction.

In conclusion, AT₂ receptor blockade with valsartan is associated with hypotension and increased production of renal BK, NO, and cGMP. AT₂ receptor blockade with PD inhibited both the hypotension and renal autacoid responses to valsartan, confirming that during AT₁ receptor blockade, there is concomitant stimulation of the AT₂ receptor. The increase in renal NO and cGMP during AT₂ receptor stimulation is mediated by BK because BK₂ receptor blockade inhibited this response.

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References
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