Angiotensin-Converting Enzyme Inhibition Potentiates Angiotensin II Type 1 Receptor Effects on Renal Bradykinin and cGMP

Helmy M. Siragy, Marc de Gasparo, Mohamed El-Kersh, Robert M. Carey

Abstract—Angiotensin (Ang) receptor blockers (ARBs) increase bradykinin (BK) by antagonizing Ang II at its type 1 (AT₁) receptors and diverting Ang II to its counterregulatory type 2 (AT₂) receptors. Because the effect of ARBs on BK is constrained by the short half-life of BK and because ACE inhibitors block the degradation of BK, this study was designed to test the hypothesis that an ACE inhibitor can potentiate ARB-induced increases in renal interstitial fluid (RIF) BK levels. We used a microdialysis technique to recover BK and cGMP in vivo from the RIF of sodium-depleted, conscious Sprague-Dawley rats infused for 60 minutes with the AT₁ receptor blocker valsartan (0.17 mg/kg per minute), with the active metabolite of the ACE inhibitor benazepril (benazeprilate, 0.05 mg/kg per minute), or with the specific AT₂ receptor blocker PD 123,319 (50 \( \mu g/kg \) per minute) alone or combined. Each animal served as its own control. RIF BK and cGMP levels increased significantly over 1 hour in response to valsartan, benazeprilate, or both but not to a vehicle control (\( P<0.01 \)). The combined benazeprilate-valsartan effect was greater than the sum of their individual effects, suggesting potentiation rather than addition, and was abolished by PD 123,319. We demonstrate for the first time that an ACE inhibitor (benazepril) and an ARB (valsartan) potentiate each other, and we postulate that such combinations may be beneficial in clinical states marked by Ang II elevation, such as chronic heart failure, postinfarction left ventricular dysfunction, and hypertension. (Hypertension. 2001;38:183-186.)

Key Words: angiotensin-converting enzyme inhibitors \( \blacklozenge \) receptors, angiotensin II \( \blacklozenge \) bradykinin \( \blacklozenge \) rats \( \blacklozenge \) cyclic GMP

The renin-angiotensin system (RAS) exerts paracrine and autocrine effects on the heart, vasculature, adrenal glands, kidneys, and brain in addition to its endocrine role in maintaining systemic perfusion and renal function.\(^1\) Prolonged RAS activation in conjunction with other growth promoters may contribute to cardiovascular hypertrophy and hyperplasia. Angiotensin (Ang) II, the principal effector of the RAS, produces both physiological and detrimental effects via its ubiquitously distributed type 1 (AT₁) receptors.\(^2\) Although the functions of the less expressed type 2 (AT₂) receptors have not been fully elucidated, initial studies have suggested that these receptors mediate counterregulatory homeostatic effects (eg, vasodilation, inhibition of cell proliferation, increased apoptosis, and differentiation) in a yin-yang relationship with the AT₁ receptors.\(^3,4\)

Studies from our laboratory have indicated that AT₂ receptor stimulation is associated with increased bradykinin (BK),\(^5\) NO,\(^6,7\) and cGMP levels in renal interstitial fluid (RIF).\(^8\) BK is a vasoactive peptide hormone that serves as an intermediate in a cascade mediated by AT₂ receptors in opposition to actions initiated at AT₁ receptors.\(^7\) Intrarenal generation of BK modulates NO production,\(^9\) which in turn leads to the activation of cGMP,\(^10\) the final link in the biochemical cascade set in motion by AT₂ receptor stimulation. A murine strain with a disrupted BK-B₂ gene displays exaggerated vasopressor responses to Ang II, indicating a normally functioning B₂ maintenance.\(^11\) BK contributes to the regulation of renal fluid, and electrolyte homeostasis by B₂ receptor is essential for the cardiovascular homeostatic increase in urinary flow rate and sodium excretion.\(^12-14\) BK also dilates human coronary arteries\(^15\) and decreases experimentally induced infarct size in dogs.\(^16\) Its absence leads to dilated and failing cardiomyopathy in BK-B₂ receptor gene knockout mice.\(^17\) Finally, endogenous BK may help preserve cardiac function in the failing heart.\(^18\)

Using a Grollman renal-wrap hypertension model, we also have shown that the Ang II receptor blocker (ARB) losartan normalizes systolic blood pressure and increases RIF levels of BK, NO, and cGMP in the contralateral kidney.\(^19\) These findings support the hypothesis that stimulation of AT₂ receptors introduces a counterregulatory effect encompassing vasodilation, antiproliferation, apoptosis, and differentiation.\(^3\)

ACE inhibitors increase the short half-life of BK because ACE is a nonspecific kininase that degrades BK in addition to...
cleaving the Ang I decapeptide. This suggests that ACE inhibitors and ARBs may potentiate each other in increasing BK levels through independent pharmacological mechanisms. To test this hypothesis, we devised experiments in conscious rats during sodium restriction (a condition known to activate the RAS) to quantify the individual or combined effects of AT₁ receptor blockade with valsartan, ACE inhibition with benazeprilate, and specific AT₂ receptor blockade with PD 123,319 on RIF BK and cGMP.

Methods

Renal Microdialysis Technique
The microdialysis probe used for the determination of RIF BK has been described previously. Briefly, each end of a single hollow-fiber dialysis tube (5.0 mm long, 0.1-mm ID) with a 10,000-Da molecular mass cutoff (Hospal) was inserted into the manually dilated ends of two 300-mm-long (inflow and outflow) hollow polyethylene tubes with 0.12-mm ID and 0.65-mm OD. In vitro recoveries of cold and radiolabeled BK or cGMP by dialysis probes were 55% and 78%, respectively, for BK and were 59% and 70%, respectively, for cGMP. Negligible amounts of these peptides stick to the polyethylene tube of the dialysis probes, as demonstrated by recovery of >99.8% of these substances in the perfusate.

This method offers several advantages over measurements of BK and cGMP in blood or urine. It permits sampling from small animals in vivo without causing undesirable hemodynamic changes and allows in vivo autacoid monitoring at almost any site in an organ or tissue, exposing local changes that may not be detected by circulatory measurements. Additionally, the concentration of hormones and autacoids measured in the interstitial space, which is closer to target receptors, may be more representative than that in the circulation. Because autacoids (eg, kinins) can form and be degraded in urine, urinary measurements may not reflect target organ concentrations accurately. Finally, the 10,000-Da molecular cutoff of the microdialysis membrane excludes undesirable substances (eg, enzymes and carrier proteins). The isolation of unbound materials can facilitate bioanalytic measurement in a small volume without the need for additional extraction procedures.

Animal Preparation
The experiments were approved by the University of Virginia Animal Research Committee, conducted in accordance with institutional guidelines, and performed in ten 4-week-old conscious Sprague-Dawley rats (Harlan Teklad, Madison, Wis). The rats were placed under general anesthesia with ketamine (80 mg/kg IM) and xylazine (8 mg/kg IM), and the right and left kidneys of each rat were exposed via a midline abdominal incision. Each renal capsule was penetrated with a 31-gauge needle tunneled into the outer cortex 1 mm from the outer surface for 5 mm before exiting the capsule. The needle tip was inserted into 1 end of the dialysis probe and pulled until the dialysis fiber was situated in the renal cortex. The dialysis probe inflow and outflow tubes were tunneled subcutaneously through a bevel-tipped stainless-steel tube and emerged near the interscapular region. To gain vascular access, a heparinized polyethylene tube was inserted into the right jugular vein, flushed daily with 10% heparin in 5% dextrose aqueous solution, and capped with a small piece of copper wire. The exterior ends of the tubes were secured by suturing them to the skin at the exit site and covering them with a stainless-steel spring to protect them from rat-inflicted damage.

The rats were housed under controlled conditions (temperature, 21 ± 1°C; humidity, 60 ± 10%; and lighting, 8 hr/20 hr) and were allowed 7 days to recover and become acclimatized to the laboratory. Experiments were started promptly at 8:00 AM daily to avoid diurnal variation in experimental parameters. For collection of RIF, the inflow tube was connected to a gas-tight syringe filled with lactated Ringer’s solution, which was perfused at a rate of 3 μL/min. The effluent was collected from the outflow tube for 60-minute sample periods.

Analytical Methods
ELISA was used to measure BK and cGMP recovered in vivo from RIF. These assay sensitivities are 1 pg/mL for BK and 0.11 pmol/mL for cGMP, with 100% specificity for both substances.

Effects of Sodium Depletion, ACE Inhibition, AT₁ Receptor Blockade, and AT₂ Receptor Blockade, Individually or Combined
Rats were placed in metabolic cages. Each experimental animal served as its own control, and different treatments were administered to the same group of animals on different days. Rats were tested while being given a normal-sodium diet (0.28% NaCl) before being subjected to a low-sodium diet (0.05% NaCl) for 13 days to stimulate Ang II synthesis. On days 7 to 13, the animals received a 60-minute intravenous infusion of vehicle (5% dextrose in water), which served as a control, followed by another 60 minutes of intravenous treatment with either valsartan (AT₁ receptor blocker) at a rate of 0.17 mg/kg per minute, benazeprilate (active metabolite of the ACE inhibitor benazepril) at 0.05 mg/kg per minute, or PD 123,319 (specific AT₂ receptor blocker, Parke-Davis) at 50 μg/kg per minute, individually or in combination. RIF samples were collected from both kidneys during control and treatment periods. Samples from both kidneys for each period were pooled together for measurements of BK and cGMP.

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

Results

RIF BK and cGMP Responses to Low-Sodium Diet, Valsartan, and Benazepril, Individually and Combined
Baseline RIF BK and cGMP recoveries during normal sodium intake were 10.9 ± 0.98 pg/min and 0.38 ± 0.12 pmol/min, respectively, and increased 3- and 5-fold to 31.7 ± 0.87 pg/min (P < 0.0001) and 2.1 ± 0.08 pmol/min (P < 0.0001), respectively, after 7 days of low sodium intake. Benazepril increased RIF BK and cGMP by >50% from 32.0 ± 0.71 pg/min and 2.04 ± 0.06 pmol/min, respectively, to 49.4 ± 1.2 pg/min (P < 0.0001), BK increment 17.4 ± 1.4 pmol/min (P < 0.0001), and 3.3 ± 0.06 pmol/min (P < 0.0001, cGMP increment 1.3 ± 0.10 pmol/min), respectively (Figure 1). Similarly, valsartan increased RIF BK and cGMP from 32.5 ± 0.56 pg/min and 1.99 ± 0.06 pmol/min, respectively, to 41.2 ± 1.7 pg/min (P < 0.0001) and 2.86 ± 0.05 pmol/min (P < 0.0001, increment 8.7 ± 1.9 pg/min for BK and 0.87 ± 0.04 pmol/min for cGMP), respectively (Figure 1). Combined administration of benazepril and valsartan increased both RIF BK and cGMP 2-fold, from 31.9 ± 0.7 pg/min and 2.1 ± 0.05 pmol/min, respectively, to 65.3 ± 1.2 pg/min (P < 0.0001) and 4.4 ± 0.1 pmol/min (P < 0.0001, increment of 33.4 ± 0.8 pg/min for BK and 2.31 ± 0.07 pmol/min for cGMP), respectively. The increases in RIF BK and cGMP during combined benazepril and valsartan treatment were greater than their increments during individual administration (P < 0.0001). Furthermore,
combined administration of benazeprilate and valsartan was associated with increases in RIF BK and cGMP significantly greater than \( P<0.01 \) the sum of the responses to benazeprilate and valsartan monotherapy.

**RIF BK and cGMP Responses to AT\(_2\) Receptor Blocker PD 123,319, Alone or Combined With Benazeprilatation and/or Valsartan**

AT\(_2\) receptor blockade with PD decreased RIF BK (Figure 2A) and cGMP (Figure 2B) from 32.5 ± 0.43 pg/min and 2.05 ± 0.05 pmol/min, respectively, to 18.3 ± 0.91 pg/min \( P<0.0001 \) and 0.91 ± 0.07 pmol/min \( P<0.0001 \), respectively. Similarly, PD 123,319 administration prevented the increase in RIF BK and cGMP associated with benazeprilatation \( P<0.0001 \), valsartan \( P<0.0001 \), or the combination of benazeprilatation and valsartan \( P<0.0001 \).

**Discussion**

A major finding of the present study is that renal interstitial BK and cGMP levels are increased with both the ACE inhibitor benazeprilatation and the AT\(_1\) receptor blocker valsartan. The concurrent administration of valsartan and benazeprilatation increased BK and cGMP levels to a greater extent than when either agent was administered alone. RIF BK and cGMP during combined benazeprilatation-valsartan treatment were greater than the sums of their individual responses, suggesting potentiation rather that additive effects. The specific AT\(_2\) receptor blocker PD 123,319 abolished RIF BK and cGMP responses to both valsartan and benazeprilatation. These findings suggest that AT\(_2\) receptor blockade increases renal BK and cGMP levels via the AT\(_2\) receptor, an effect that is further potentiated with ACE inhibition. In the present study, it is not clear why AT\(_2\) receptor blockade with PD 123,319 reduced BK responses to benazeprilatation. A possible explanation may be related to increased activity of renal ACE, which may minimize the action of benazeprilatation at the given dose. Hunley et al\(^22\) reported that mice lacking the AT\(_2\) receptor have increased levels of tissue ACE and suggested that the AT\(_2\) receptor may regulate this enzyme expression and activity.

The present study supports and advances the results of our previous work demonstrating that Ang II stimulation of the AT\(_2\) receptor leads to increases in RIF BK, NO, and cGMP in sodium-depleted rats treated with valsartan.\(^{21}\) These data are supported by a recent report \(^{23}\) demonstrating that AT\(_1\) receptor stimulation increases aortic kininogen activity, leading to increased synthesis of bradykinin. This mechanism appears to be mediated by the ability of the AT\(_2\) receptor to induce intracellular acidosis through inhibition of the amiloride-sensitive Na\(^+\)-H\(^+\) exchanger activity in vascular smooth muscle cells. The present results also corroborate the experiments of Liu et al.\(^{24}\) who used the BK-B\(_2\) receptor antagonist icatibant to demonstrate that BK mediated the therapeutic cardiovascular effects of both ACE inhibition and AT\(_1\) receptor blockade in rat models of heart failure and that the BK effects associated with this blockade could be inhibited by antagonizing the AT\(_2\) receptor.

Work in other laboratories has concentrated on examining the combined use of ACE inhibitors and AT\(_1\) receptor blockade. Studies in conscious spontaneously hypertensive rats have demonstrated the synergistic effects of combined benazeprilatation (converting-enzyme inhibitor) and valsartan (AT\(_1\) receptor blocker) on blood pressure.\(^{25}\) Beneficial results were also obtained in a series of studies by Spinale and colleagues,\(^ {26,27} \) who used the rapid pacing model of heart failure in pigs. Although early experiments have used animal models, evidence is increasing indicating that both AT\(_1\) and AT\(_2\) receptors are present in humans.\(^4,28,29\) Moreover, the beneficial hemodynamic effects of combined ACE inhibition and AT\(_1\) receptor blocker have been demonstrated in patients.
with heart failure. Preliminary studies in patients with heart failure who were treated with combined administration of the ACE inhibitor lisinopril and valsartan produced improvement in those individuals compared with individuals treated with the ACE inhibitor alone. More data on this combination therapy are forthcoming from a recently completed Heart Failure Trial.

The present study demonstrates, for the first time, the increase in tissue BK with ACE inhibition and also confirms our results from previous studies demonstrating BK production with AT1 receptor stimulation. Furthermore, our results show that BK produced during AT1 receptor blockade with valsartan can be significantly potentiated with benazepril. These findings imply potential clinical applications for the combination of ACE inhibitors and AT1 receptor blockers in the treatment of cardiovascular diseases. They also suggest that the underlying pharmacological basis for any benefits of such dual therapy is related at least in part to increased BK levels as well as more complete blockade of the RAS.

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