Type 2 Bradykinin-Receptor Antagonism Does Not Modify Kinin or Angiotensin Peptide Levels

Duncan J. Campbell, Athena Kladis, Todd A. Briscoe, Jialong Zhuo

Abstract—Type 2 bradykinin (B₂)-receptor antagonists have been used to define the role of endogenous kinin peptides. However, interpretation of the effects of B₂-receptor antagonists has been limited by lack of information concerning the effects of these antagonists on endogenous kinin and angiotensin peptide levels. If kinin levels were subject to short-loop-feedback regulation mediated through B₂ receptors, then a reactive increase in kinin levels might blunt the effects of B₂-receptor antagonism and stimulate type 1 bradykinin receptors. Moreover, kinins have been implicated in the control of renin secretion. We investigated whether endogenous kinin levels are subject to short-loop-feedback regulation mediated by the B₂ receptor and whether endogenous kinins acting through the B₂ receptor influence plasma renin levels and circulating and tissue angiotensin peptide levels. The B₂-receptor antagonist icatibant (1 mg/kg) was administered to rats by intraperitoneal injection, and circulating and tissue levels of angiotensin and kinin peptides were measured after 4 hours. Icatibant produced 75% occupancy of B₂ receptors in the inner stripe of the renal medulla. Icatibant did not influence plasma levels of renin, angiotensinogen, angiotensin-converting enzyme, neutral endopeptidase, or circulating or tissue levels of angiotensin and bradykinin peptides. This study demonstrated that kinin levels are not subject to short-loop-feedback regulation mediated through B₂ receptors and that endogenous kinin levels acting through the B₂ receptor do not modulate the renin-angiotensin system. (Hypertension. 1999;33:1233-1236.)

Key Words: bradykinin || receptors, bradykinin || renin || angiotensinogen

The nonapeptide bradykinin [BK(1–9)] has important actions on blood vessels, heart, and kidney. There are 2 types of kinin receptor, the type 1 (B₁) and the type 2 (B₂) receptors. By far the most important hemodynamic effect of BK(1–9) in vivo is the hypotensive vasodilatation produced by stimulation of endothelial B₂ receptors of arteries and arterioles, with subsequent endothelial release of nitric oxide and prostaglandins. Additional renal actions of BK(1–9) include the production of diuresis and natriuresis. Whereas the diuretic effect of BK(1–9) administered by the renal artery is mediated by B₂ receptors, both B₁ and B₂ receptors may participate in BK(1–9)–induced natriuresis and increase in renal blood flow. B₂ receptors are induced by tissue injury, such as that which occurs after myocardial ischemia and inflammation. The role of endogenous kinins has been determined mainly by study of the effects of kinin antagonists, most often the B₂-receptor antagonist icatibant (D-Arg[[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]]-bradykinin). However, interpretation of the effects of B₂-receptor antagonists has been limited by lack of information concerning the effects of these antagonists on endogenous kinin levels. If kinin levels were subject to short-loop-feedback regulation mediated by B₂ receptors, then a reactive increase in kinin levels may blunt the effects of B₂-receptor antagonism and stimulate B₁ receptors. In support of a feedback regulation of kinin levels mediated by the B₂ receptor, icatibant administration for 7 days is reported to increase kallikrein activity, but not kallikrein mRNA levels, in kidney of adult rats. Moreover, Siragy et al report that icatibant increases renal interstitial BK(1–9) levels in dogs in low-sodium balance.

One consequence of B₂-receptor antagonism may be a change in angiotensin II (Ang II) levels. Kinin administration increases renin secretion, possibly mediated by increased nitric oxide formation, and icatibant is reported to decrease plasma renin levels in anesthetized rabbits, which suggests that endogenous kinins may tonically stimulate renin secretion. Moreover, the location of B₂ receptors in the kidney is predominantly in the renal tubules, vascular endothelium, and renomedullary interstitial cells of the renal medulla, locations appropriate for the modification of renin secretion, possibly by the modification of sodium delivery to the macula densa.

The purpose of this study was to determine whether endogenous kinin levels are subject to short-loop-feedback regulation through the B₂ receptor, and whether endogenous kinins acting through the B₂ receptor influence plasma renin levels and circulating and tissue angiotensin peptide levels.

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Methods

Animals
Male Sprague-Dawley rats (~300 g) were allowed free access to tap water and standard rat chow that contained 0.25% sodium and 0.76% potassium (GR2, Clarke-King & Co). This study was performed in accordance with the guidelines of the Animal Experimentation Ethics Committee of St Vincent’s Hospital.

Rats (n=9 to 10 per group) were given 0.3 mg icatibant (1 mg/kg) in 0.5 mL 0.15 mol/L sodium chloride, or 0.5 mL vehicle, by intraperitoneal injection. After 4 hours, the rats were killed by decapitation, and trunk blood was collected for the measurement of plasma levels of angiotensin-converting enzyme (ACE), neutral endopeptidase 24.11 (NEP), renin, angiotensinogen, and angiotensin peptides. The left kidney, heart (cardiac ventricles), lung, and aorta were rapidly removed, weighed, and immediately homogenized in 4 mol/L guanidine thiocyanate, 1% trichloroacetic acid (GTC/TFA) for the measurement of tissue levels of angiotensin and bradykinin peptides. The right kidney was frozen in isopentane cooled to the temperature of dry ice for in vitro autoradiography. Blood bradykinin peptides were measured in separate groups of rats (n=9 to 10 per group) given icatibant or vehicle by identical protocols. After 4 hours, these rats were anesthetized with diethyl ether, and 2 mL blood was collected from the inferior vena cava in syringes that contained 10 mL GTC/TFA for the measurement of bradykinin peptides. Icatibant was a generous gift from Hoechst AG, Frankfurt, Germany.

Extraction and Radioimmunoassay of Angiotensin and Bradykinin Peptides
Plasma levels of Ang II and angiotensin I (Ang I) were measured as described previously. Briefly, trunk blood (2 to 3 mL) was rapidly collected in tubes that contained 0.5 mL inhibitor solution (1 mmol/L renin inhibitor acetyl-His-Pro-Phe-Val-Sta-Leu-Phe-NH$_2$, 146 μmol/L pepstatin, 50 mmol/L 1,10-phenanthroline, 125 mmol/L EDTA, 2 g/l neomycin sulfate, 2% dimethyl sulfoxide, and 2% ethanol in water) at 4°C. The blood was centrifuged, and the plasma (1 to 2 mL) was immediately extracted with Sep-Pak C$_18$ cartridges (Waters Chromatography Division). Blood and tissues homogenized in GTC/TFA were processed as described previously and extracted with Sep-Pak C$_18$ cartridges. Peptides were acetylated and treated with iodomethane before high-performance liquid chromatography (HPLC) and assay of HPLC fractions by N-terminal directed radioimmunoassay. Data were corrected for recovery as reported elsewhere.

Measurement of ACE, NEP, Renin, and Angiotensinogen in Plasma
Trunk blood used for measurement of ACE, NEP, renin, and angiotensinogen was collected in heparinized tubes on ice, then centrifuged. The blood was rapidly frozen on dry ice and stored at −80°C. ACE activity was measured with the use of 3-(2-furylacryloyl)-l-phenylalanine-glycyl-glycine as substrate. NEP enzymatic activity was measured with succinyl-Ala-Ala-Phe-amidomethylcoumarin as substrate; further incubation with aminopeptidase M released free amidomethylcoumarin that was measured fluorometrically. The plasma concentrations of active aminopeptidase M released free amidomethylcoumarin that was measured fluorometrically. The plasma concentrations of active aminopeptidase M released free amidomethylcoumarin that was measured fluorometrically.

In Vitro Autoradiography
Cryostat sections of kidney (20 μm) were cut and mounted on gelatin–chrome alum–coated slides. In vitro autoradiography was performed as described by Dean et al. with the use of [125I]-HPP-icatibant (3,4-hydroxyphenyl-propionyl-D-Arg$_6$-[Hyp$_3$,THI$_2$,D-Tic$_1$,Oic$_1$]-bradykinin) as a tracer.

Statistical Analysis
Data are presented as mean±SEM. Comparisons with vehicle-treated rats were made with a 2-tailed t test. If more than half the samples of a mean had values less than the minimum detectable for that particular assay, then the sample mean is shown as less than the minimum detectable. If values were below the minimum detectable, then they were set at half the minimum detectable for statistical calculations. Logarithmic transformation of the data was performed when necessary to obtain similar variances between groups. All tests were 2-tailed. Differences were considered significant at P<0.05. Statistical analyses were performed with SuperANOVA (Abacus Concepts, Inc). Detectable differences were calculated with Sample-Power (SPPS).

Results

In vitro autoradiography of cryostat sections of kidney showed that in vivo administration of icatibant produced 75% inhibition of binding of [125I]-HPP-icatibant to the inner stripe of the renal medulla, the main site of B$_2$ receptors in kidney. However, icatibant administration did not modify circulating or tissue levels of kinin or angiotensin peptides or plasma levels of ACE, NEP, renin, or angiotensinogen (Tables 1 to 3).

When the data were analyzed for each peptide in each tissue, this study had 80% power (α=0.05, tails=2) to detect a difference in kinin or angiotensin peptide levels of 40% to 100% of control values. This is an underestimation of power when necessary to obtain similar variances between groups. All tests were 2-tailed. Differences were considered significant at P<0.05.
circulating or tissue angiotensin peptide levels. These data are essential for the interpretation of the effects of B2-receptor antagonism because the data exclude the possibility of a reactive rise in endogenous kinin levels that may blunt the effects of B2-receptor antagonism and stimulate B1 receptors and because the data exclude an effect of icatibant on mRNA levels for kallikrein, B2 receptor, and ACE in kidney. However, our results are not consistent with the report by Siragy et al of increased renal kallikrein, B2 receptor, and ACE in kidney. 5

We used in vitro autoradiography of 125I-HPP-icatibant binding to kidney sections to demonstrate effective blockade of B2 receptors by icatibant. Other studies have shown that similar or lower doses of icatibant block the depressor effects of BK1–9) in rats. 4,9

Little is known about the regulation of endogenous kinin levels, although there are interrelationships between the kinin and angiotensin systems. Whereas ACE inhibition increases endogenous kinin levels, presumably by inhibition of kinin metabolism, there is little information concerning the factors that may modulate kinin production. Both the kinin and angiotensin systems are involved in fluid and electrolyte homeostasis. Sodium depletion stimulates renin secretion and also increases kinin levels in microdialysate fluid from dog kidney, an effect inhibited by concomitant renin inhibition. Furthermore, Ang II stimulation of nitric oxide and cyclic GMP production in vasculature is dependent on the action of kinins on the B2 receptor. 25,26 Moreover, AT1-receptor antagonism reduces kinin levels in blood and kidney of Sprague-Dawley rats, whereas kinin levels are increased in lung and brown adipose tissue of the TGR(mRen-2)27 rat, a high-angiotensin model of hypertension. 28 These studies suggest that Ang II may be a tonic positive regulator of kinin levels. Despite evidence that suggests a role for kinins in the regulation of renin secretion, the results of the present study do not support a reciprocal relationship whereby endogenous kinins regulate angiotensin peptide levels. The present results are limited to the effect of B2-receptor antagonism of 4-hour duration. Longer periods of B2-receptor antagonism may have effects on kinin and angiotensin peptide levels, which may be mediated by longer-term changes in fluid and electrolyte homeostasis. However, evidence against this possibility includes the failure of icatibant administration for 7 days to modify renin mRNA levels in kidney of adult rats 11 and the normal plasma renin levels and normal renin and AT1 receptor mRNA levels in kidney of the B2-receptor gene knockout mouse. 29

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### References


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**TABLE 2. Effects of Icatibant on Circulating and Tissue Levels of Angiotensin Peptides**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ang II</th>
<th>Ang I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25±3</td>
<td>12±2</td>
</tr>
<tr>
<td>Icatibant</td>
<td>30±5</td>
<td>10±1</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>5 (−6.1, 16.1)</td>
<td>−2 (−6.6, 2.6)</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>216±17</td>
<td>30±3</td>
</tr>
<tr>
<td>Icatibant</td>
<td>209±16</td>
<td>25±3</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>−7 (−56, 42)</td>
<td>−5 (−13.9, 3.9)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10±1</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Icatibant</td>
<td>9±1</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>−1 (−3.8, 1.8)</td>
<td>0.2 (−1.1, 1.4)</td>
</tr>
<tr>
<td>Aorta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>48±7</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Icatibant</td>
<td>45±4</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>−3 (−19.9, 13.9)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>83±9</td>
<td>1.9±0.6</td>
</tr>
<tr>
<td>Icatibant</td>
<td>94±6</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>11 (10, 32)</td>
<td>0.9 (−0.1, 1.9)</td>
</tr>
</tbody>
</table>

Data are mean±SEM, n=9–10.

**TABLE 3. Plasma Levels of Renin, Angiotensinogen, ACE, and NEP**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Icatibant</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE, U/L</td>
<td>101±3</td>
<td>97±2</td>
<td>−4 (−12, 4)</td>
</tr>
<tr>
<td>NEP, nmol·mol⁻¹·min⁻¹</td>
<td>0.40±0.02</td>
<td>0.38±0.01</td>
<td>−0.02 (−0.06, 0.02)</td>
</tr>
<tr>
<td>Renin, nmol</td>
<td>18±4</td>
<td>17±2</td>
<td>−1 (−10, 8)</td>
</tr>
<tr>
<td>Ang I · L⁻¹·h⁻¹</td>
<td>593±33</td>
<td>591±39</td>
<td>−2 (−108, 104)</td>
</tr>
</tbody>
</table>

Data shown as mean±SEM, n=10.


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