Bradykinin B<sub>2</sub> Receptor Antagonism Attenuates Blood Pressure Response to Acute Angiotensin-Converting Enzyme Inhibition in Normal Men

Iain B. Squire, Kevin P.J. O’Kane, Niall Anderson, John L. Reid

Abstract—The physiological effects of angiotensin-converting enzyme (ACE) inhibition may be in part mediated by bradykinin. We investigated the effect of coadministration of the specific bradykinin B<sub>2</sub> receptor antagonist icatibant on hemodynamic and neurohormonal responses to acute intravenous ACE inhibition in normal men on a normal sodium diet. We performed a 4-phase, double-blind, double-dummy, placebo-controlled study in 12 male volunteers. The bradykinin antagonist icatibant (10 mg IV) was coadministered over the first 15 minutes of a 2-hour infusion of the ACE inhibitor perindoprilat (1.5 mg IV). Perindoprilat inhibited ACE activity and elicited the expected changes in active renin concentration and angiotensin peptides. Over the 3 hours after the start of drug infusion, perindoprilat lowered and icatibant increased mean arterial blood pressure (each \( P<0.0005 \) versus placebo). Coadministration of icatibant attenuated the mean arterial blood pressure response to perindoprilat (\( P<0.0005 \)) but had no effect on neurohormonal responses to perindoprilat. Our study indicates that the bradykinin B<sub>2</sub> receptor antagonist icatibant attenuates the short-term blood pressure–lowering effect of acute ACE inhibition in normal men on a normal sodium diet. Bradykinin B<sub>2</sub> receptor antagonism alone increases resting blood pressure. Bradykinin may be involved in the control of blood pressure in the resting state in humans. (Hypertension. 2000;36:132-136.)

Key Words: blood pressure ■ angiotensin-converting enzyme ■ bradykinin ■ icatibant

Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I (Ang I) to angiotensin II (Ang II) and the breakdown of bradykinin (BK), a potent vasodilator.\(^1,2\) The pharmacological effects of ACE inhibitors may be in part mediated via BK accumulation rather than reduced Ang II formation.\(^3\) There are at least 2 BK receptor subtypes, B<sub>1</sub> and B<sub>2</sub>; the known biological effect of BK is mediated via the B<sub>2</sub> receptor,\(^4\) the stimulation of which leads to the formation of NO\(^5\) and vasodilator prostaglandins.\(^6\)

Icatibant (Hoe140) is a specific B<sub>2</sub> receptor antagonist that inhibits with high potency a variety of B<sub>2</sub>-mediated effects.\(^7\) Icatibant attenuates the hypotensive response to ACE inhibition in the dog\(^8\) and rat\(^9,10\) and inhibits BK-induced vasodilation in a dose-dependent manner in human vascular beds in vivo.\(^11\) The drug has a long duration of action: its half-life of protection against BK-induced hypotension in rats is \(>5\) hours.\(^7,10\) A possible noncompetitive component to the action of the drug is suggested by flattening of the BK log–dose response curve at high doses.\(^11,12\)

The purpose of the present study was to investigate the effect of coadministration of icatibant on the hemodynamic and neurohormonal responses to acute ACE inhibition in normal men. We also sought to assess the possible role of BK in basal blood pressure (BP) control in this situation. We used a protocol of constant-rate intravenous infusion of ACE inhibitor previously used by our group.\(^13-15\) By so doing, we sought to achieve rapid, long-lasting, and profound inhibition of plasma ACE. By concomitant infusion of icatibant during the first part of ACE inhibitor administration, we aimed to ensure temporal overlap of ACE inhibition and BK receptor blockade.

Methods

Study Design
We performed a randomized, 4-phase, double-blind, double-dummy, placebo-controlled study in 12 healthy male volunteers (22.9±1.9 years). The study was approved by the local Ethical and Research Committee, and subjects gave written informed consent. Prestudy ECG, plasma cholesterol, and renal and hepatic function were normal in all subjects. None had previously been exposed to either study drug nor was taking any prescribed or over-the-counter medication. Subjects avoided high-salt foods, alcohol, tobacco, and caffeine for 48 hours before and during study days, which were at least 14 days apart.

Studies were performed with the subjects in a supine position. Venous cannulas were inserted for drug administration and blood sampling. Supine BP and heart rate were measured in duplicate by use of automatic sphygmomanometry (Critikon, Dynamap, Johnson & Johnson) at 2-minute intervals for 1 hour before and 3 hours after...
the start of drug infusion and thereafter at 30-minute intervals for up to 12 hours.

Drugs were formulated on each day by the hospital pharmacy and were administered by constant-rate intravenous infusion (Braun Secura E, Melsungen AG). Perindoprilat (1.5 mg, Institut de Recherches Internationales Servier) or matching placebo (0.9% NaCl, Boots PLC) was infused over 120 minutes. Icatibant (10 mg, Hoechst Marion Roussel) or matching placebo (0.9% NaCl) was infused concomitantly over the first 15 minutes of ACE inhibitor infusion. In each phase, volunteers received perindoprilat + placebo, icatibant + placebo, perindoprilat + icatibant, or placebo + placebo according to a randomized-order Latin square design to avoid any systematic carryover effect.

Blood was drawn at fixed intervals for the determination of plasma ACE activity, perindoprilat concentration, active renin concentration (ARC), and Ang I and Ang II concentrations. Samples were collected into chilled tubes, placed on ice, processed immediately, and stored at −70°C until assay.

Laboratory Analyses
Plasma perindoprilat concentration and ACE activity were measured by high-performance liquid chromatography. The lower limit of detection is 0.44 nmol/L, and the interassay coefficient of variation is 8% at 12 nmol/L and 4% at 45 nmol/L. ACE activity was also estimated by use of the plasma [Ang II]/[Ang I] ratio. Established assays for angiotensin peptide concentrations were used. Intra-assay and interassay coefficients of variation were <10%.

Statistical Analyses
Studies of novel pharmacological agents in normal subjects may be hampered because the absolute magnitude of the response under investigation is limited, particularly in healthy volunteer subjects. Thus, we chose to apply robust statistical analysis to the present study. For each individual in each treatment arm, we studied (1) the profile of placebo and baseline-corrected BP and heart rate (mixed model ANOVA), (2) the area under the BP/time curve (ANOVA), and (3) the mean maximal change in BP and heart rate irrespective of the time course of each (ANOVA).

Mean arterial blood pressure (MAP) was calculated from the following: MAP = diastolic BP + (systolic BP – diastolic BP)/3. We prospectively decided to analyze data from the first 3 hours after the start of drug infusion and over the full 12-hour period. A mixed-model ANOVA was fitted to all data by use of Program 3V in the BMDP package. The model used fixed-effect terms for treatment, study phase, and time within phase, assuming between-phase carryover effects to be negligible. Random-effect terms were used to model interindividual variability. Baseline BPs or heart rates, estimated from the mean of duplicate measurements taken at 2-minute intervals over the 20 minutes preceding the start of infusion, were included as additional fixed-effect covariates. This model allowed simultaneous estimation of both within- and between-individual factors, as well as interactions between factors. Because of extensive control for confounding factors, the model had the power to investigate minor differences between treatments.

Hemodynamic Response
Placebo and baseline-corrected profiles of MAP with perindoprilat alone, icatibant alone, and the combination of the 2 treatments over the first 3 and 12 hours after the start of drug infusion are shown in Figures 1 and 2, respectively. As expected, there was wide interindividual variation in response to each treatment.

Profile of MAP Response
Over the first 3 hours, the maximum average change in MAP in response to perindoprilat alone was a fall of −3.5 ± 3.5 mm Hg at 130 minutes (Figure 1). In contrast, the maximum average change in MAP in response to icatibant alone was a rise of +2 ± 3.5 mm Hg, again seen at 130 minutes.

Application of the mixed-model ANOVA to the profile of BP response revealed differences among treatments. Over the first 3 hours, perindopril reduced MAP (P < 0.0005 versus

Results
All treatments were well tolerated with no adverse events. Perindoprilat concentrations were as expected from previous studies. Icatibant did not affect the peak concentration of perindoprilat (104 ± 33 nmol/L perindoprilat and 95 ± 29 nmol/L perindoprilat + icatibant, P = 0.54) or the area under the concentration/time curve for up to 12 hours (250 ± 2 nmol/L per hour perindoprilat and 250 ± 1.6 nmol/L per hour perindoprilat + icatibant, P = 0.92).

Figure 1. Baseline and placebo-corrected changes in MAP in the first 3 hours after 1 mg IV perindoprilat ( ), 10 mg IV icatibant ( ), or the combination of perindoprilat + icatibant ( ) in healthy male volunteers. Arrows indicate duration of drug infusions.

Figure 2. Baseline and placebo-corrected changes in MAP over 12 hours after 1 mg IV perindoprilat ( ), 10 mg IV icatibant ( ), or the combination of perindoprilat + icatibant ( ) in healthy male volunteers. Arrows indicate duration of drug infusions.
placebo), primarily reflecting reduced diastolic BP (DBP, \( P<0.0005 \)). The fall in systolic BP (SBP) failed to reach statistical significance (\( P=0.084 \)). In contrast, infusion of icatibant alone was associated with a rise in MAP (\( P=0.001 \) versus placebo), SBP (\( P<0.0005 \)), and DBP (\( P=0.002 \)). Coadministration of icatibant attenuated the fall in MAP to perindoprilat (\( P=0.001 \) versus perindoprilat). Similar patterns were observed for the full 12-hour period. Perindoprilat reduced and icatibant increased MAP; each effect was significant (\( P<0.0005 \) versus placebo). Over 12 hours, the attenuation by icatibant of the effect on MAP of perindoprilat alone just failed to reach significance (\( P=0.072 \)).

Over the first 3 hours, treatment with perindoprilat alone was associated with a modest increase (3 bpm, \( P<0.0005 \) versus placebo), and icatibant alone was associated with a modest reduction (\(-5 \) bpm, \( P<0.0005 \) versus placebo) in heart rate. Coadministration of icatibant did not alter the heart rate response to perindoprilat (\( P=0.059 \)). Over 12 hours, perindoprilat alone was associated with an increase in heart rate (5 bpm, \( P<0.0005 \) versus placebo) with no effect of icatibant alone (\( P=0.313 \) versus placebo) or in combination with perindoprilat (\( P=0.190 \)).

**Area Under the MAP/Time Curve**

Comparison of areas under the MAP/time curves (AUCs) to 3 hours (AUC\(_3\)) indicated differences between treatments in keeping with the mixed model ANOVA (mean AUC; perindoprilat \(-36 \) mm Hg \( \cdot \) h, icatibant \(+5.5 \) mm Hg \( \cdot \) h; and perindoprilat+icatibant \(-12.9 \) mm Hg \( \cdot \) h; \( P<0.05 \)). Analysis of the AUC to 12 hours (AUC\(_{12}\)) revealed differences that did not achieve significance (perindoprilat \(-52 \) mm Hg \( \cdot \) h, icatibant \(-7.8 \) mm Hg \( \cdot \) h, and perindoprilat+icatibant \(-20.2 \) mm Hg \( \cdot \) h; \( P=0.058 \)).

**Mean Maximum Hemodynamic Changes**

Analysis of the mean maximum changes in MAP and heart rate has disadvantages in not correcting for time and period, as in the ANOVA model, and in being sensitive to outlying values. However, analysis of the individual mean maximum changes in BP (as opposed to the maximum group mean as in the MAP response) over 12 hours produced results similar to those in MAP and AUC.

Perindoprilat reduced MAP (9.5±3.2 mm Hg, \( P<0.005 \) versus placebo), SBP (12.8±10.3 mm Hg, \( P<0.05 \)), and DBP (9.0±3.5 mm Hg, \( P<0.005 \)). Icatibant alone increased BP (mean maximum increase in MAP 17.4±10.3 mm Hg, \( P<0.005 \); SBP 21.3±23.3 mm Hg, \( P<0.05 \); and DBP 17.3±14.1 mm Hg, \( P<0.005 \); all versus placebo). Coadministration of icatibant with perindoprilat (mean maximum reduction in MAP 9±7.2 mm Hg, SBP 18.6±16.1 mm Hg, and DBP 7.4±5.8 mm Hg) had no effect on the mean maximal response to perindoprilat alone (\( P=0.220 \)). In keeping with the results from the ANOVA model, perindoprilat alone was associated with an increase in HR (\(+3.8±3.5 \) bpm, \( P<0.0005 \) versus placebo). Icatibant alone did not affect HR (14±7.1 bpm, \( P=0.313 \) versus placebo). Coadministration of icatibant did not alter the HR response to perindoprilat (\( P=0.111 \)).

**Neurohormonal Parameters**

Baseline plasma ACE activity (perindoprilat 25.8±6.8 IU/mL, icatibant 25.5±6.7 IU/mL, perindoprilat+icatibant 26.0±6.4 IU/mL, and placebo 25.8±8.4 IU/mL; \( P=0.95 \)), baseline AR (perindoprilat 14.8±6.6 \( \mu \)U/mL, icatibant 14.6±14.3 \( \mu \)U/mL, perindoprilat+icatibant 13.8±6.3 \( \mu \)U/mL, and placebo 16.1±10 \( \mu \)U/mL, \( P=0.91 \)), Ang I (perindoprilat 17.4±3.9 pmol/L, icatibant 16±6.6 pmol/L, perindoprilat+icatibant 17.8±5.6 pmol/L, and placebo 17.9±6.2 pmol/L; \( P=0.83 \)), and Ang II (perindoprilat 7.5±3.3 pmol/L, icatibant 8.1±9.5 pmol/L, perindoprilat+icatibant 8.3±6.3 pmol/L, and placebo 6.5±2.7 pmol/L; \( P=0.89 \)) concentrations did not differ among the study phases. Inhibition of ACE activity was profound and nearly identical with perindoprilat (mean maximum inhibition 95.4±3.7\%\) and perindoprilat+icatibant (mean maximum inhibition 94.7±3.3\%\) (Figure 3). Similarly, perindoprilat lowered the [Ang II]/[Ang I] ratio, with the maximum change being at 2 hours and being unaffected by coadministration of icatibant (\( P=0.899 \)) (Table). Icatibant alone had no effect on plasma ACE activity (\( P=0.833 \) versus placebo) or [Ang II]/[Ang I+Ang II] ratio (\( P=0.236 \) versus placebo). Neither placebo nor icatibant had any effect on ARC (Figure 4). Coadministration of icatibant did not alter the ARC response to perindoprilat.

**Discussion**

We have demonstrated direct attenuation of the BP-lowering effects of acute ACE inhibition by the specific B\(_2\) receptor antagonist icatibant in normal men taking a normal sodium diet. Icatibant alone elicited a significant increase in MAP, suggesting a role for BK in the basal control of BP in this situation. Icatibant had no effect on neurohormonal responses.

**Change in [Ang II]/[Ang I+II] Ratio**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Placebo</th>
<th>Perindoprilat</th>
<th>Icatibant</th>
<th>Perindoprilat+Icatibant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>117±21%</td>
<td>7.7±5.2%*</td>
<td>118±16%</td>
<td>16±14%*</td>
</tr>
</tbody>
</table>

*Activity at 2 hours is expressed as percentage of baseline activity. *\( P<0.005 \) vs placebo.
to ACE inhibition. Our results provide the first evidence of a role for BK in BP homeostasis in normal men and in the response to acute ACE inhibition.

The extent of contribution of BK accumulation to the hemodynamic effects of ACE inhibition has long been debated. In models of renovascular hypertension, B2 receptor antagonism attenuates the BP-lowering effects of ACE inhibition11 but, in contrast, has no effect on the hemodynamic response to ACE inhibition in kinin-deficient22 or genetically hypertensive23 animals. Such studies have yielded limited support for the theory that endogenous BK contributes to BP control in the resting state in renovascular hypertension and in normotensive rats.24,25 In the rat, ACE inhibition at doses without effect on BP prevents26 and reverses27 left ventricular hypertrophy.

In humans, BK contributes to ACE inhibitor–induced vasodilation in radial28 and coronary29 arteries. The attenuation of vascular endothelial dysfunction by ACE inhibition appears to result from BK potentiation.28 Local BK production may be involved in the control of BP in hypertensive subjects.30 Attenuation by aspirin31 and indomethacin32 of the vasodilator effects of ACE inhibition in heart failure suggests the involvement of BK. However, a recent study suggested that endogenous BK makes no contribution to the vasodilator effect of chronic ACE inhibition in heart failure.33

In a recent single-blind study, icatibant attenuated the acute hemodynamic effect of oral captopril by 50% in normotensive and hypertensive subjects on a low-salt diet.34 Our results suggest a role for endogenous BK in BP homeostasis in normal men. The physiological relevance of these findings, in particular with respect to the hemodynamic and therapeutic responses to ACE inhibitors in clinical practice, in terms of hemodynamic response and morbidity/mortality, is unclear. Further studies in patients in which the renin-angiotensin system is activated, in particular in the setting of heart failure and in the setting of chronic ACE inhibition, may help to

The present study may be criticized on the basis of our failure to address a possible nonspecific vasoconstrictor effect of icatibant. To our knowledge, icatibant has not previously been administered to normal subjects in the doses used in the present study. A previous study in human subjects showed no effect of up to 100 μg/kg icatibant on BP or heart rate.11 Noncompetitive antagonism of the B2 receptor by icatibant occurs in vitro,12 and a similar effect in vivo is suggested by flattening of the dose-response curve in human subjects.11 Gainer et al34 infused icatibant with the ACE inhibitor but did not administer icatibant alone. These authors also studied the effects on BP of losartan (an angiotensin receptor antagonist) administered orally but did not study the combination of losartan with icatibant. Thus, these authors failed to address the possibility of either a specific or nonspecific vasoconstrictor effect of icatibant. Our findings of an increase in BP and reduction in heart rate with icatibant are equally well explained by specific BK receptor blockade. Our finding of an increase in heart rate after acute ACE inhibition is at odds with findings from previous studies in our unit in patients with heart failure13,15 and in salt-depleted volunteers.35 There is no clear explanation for this observation.

It has been suggested that the B2 receptor has inherent activity in the absence of agonist and that icatibant may act as an inverse agonist, stabilizing and inactivating the receptor. To the best of our knowledge, inverse agonism has been demonstrated for icatibant in cultured rat myometrial cells38 but never in human tissue in vitro or in vivo. Moreover, such an effect would not explain the differing effects on BP of icatibant alone compared with icatibant given with ACE inhibitor. Finally, it may be suggested that acute dosing studies do not reflect chronic ACE inhibitor use in clinical practice. This (and other methodological differences) may explain similarities33 and differences34 between our study and others.

In summary, we have demonstrated attenuation by icatibant, the specific BK B2 receptor antagonist, of the BP-lowering effect of acute ACE inhibition in normal men. Our results suggest a role for endogenous BK in BP homeostasis in normal men. The physiological relevance of these findings, in particular with respect to the hemodynamic and therapeutic responses to ACE inhibitors in clinical practice, in terms of hemodynamic response and morbidity/mortality, is unclear.
clarify the role of BK in the pathophysiological effects of ACE inhibition.

Acknowledgments
This study was supported by a grant from Institut de Recherches Internationales Servier (IRIS), Courbevoie, France. Perindoprilat was a gift from IRIS. Hoe 140 was a gift from Hoechst Marion Roussel, Frankfurt, Germany.

References
Bradykinin B₂ Receptor Antagonism Attenuates Blood Pressure Response to Acute Angiotensin-Converting Enzyme Inhibition in Normal Men
Iain B. Squire, Kevin P. J. O'Kane, Niall Anderson and John L. Reid

doi: 10.1161/01.HYP.36.1.132

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/36/1/132

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/