Losartan Increases Bradykinin Levels in Hypertensive Humans

Duncan J. Campbell MBBS, PhD; Henry Krum, MBBS, PhD; Murray D. Esler, MBBS, PhD

Background—Studies in animals and humans indicate a role for kinins in the actions of angiotensin type 1 (AT1) receptor blockers. However, the effect of these compounds on kinin levels in humans is unknown.

Methods and Results—We measured angiotensin (Ang), bradykinin (BK), and kallidin peptides in subjects with essential hypertension administered placebo, losartan (50 mg OD), and eprosartan (600 mg OD) in randomized order in a double-blind, 3-period, 3-treatment, crossover trial. Peptides were measured in arterial blood using high-performance liquid chromatography–based radioimmunoassays. Losartan increased blood levels of BK-(1–9) and hydroxylated BK-(1–9) by 2-fold and reduced the BK-(1–7)/BK-(1–9) ratio by 55%. There was a trend for eprosartan to produce similar changes in bradykinin levels. There were no changes in blood kallidin levels. Both losartan and eprosartan increased plasma levels of Ang I, Ang II, and Ang-(2–8), and eprosartan increased Ang-(3–8) levels. Ang-(1–7) and Ang-(1–9) levels were unchanged. There was an associated 30% to 35% reduction in Ang II/Ang I ratio and 63% to 69% reduction in Ang-(1–7)/Ang I ratio. Plasma ACE activity was unchanged.

Conclusions—Losartan increases bradykinin levels. The reductions in BK-(1–7)/BK-(1–9), Ang II/Ang I, and Ang-(1–7)/Ang I ratios suggest that the increased bradykinin levels were the result of reduced metabolism by ACE and neutral endopeptidase. Increased bradykinin levels may represent a class effect of AT1 receptor blockers that contributes to their therapeutic actions and may also contribute to the angioedema that may accompany this therapy. (Circulation. 2005;111:315-320.)

Key Words: angiotensin ■ bradykinin ■ kallidin ■ enzymes ■ neutral endopeptidase

ACE inhibitors and angiotensin type 1 (AT1) receptor blockers are valuable therapies for hypertension, cardiac failure, and renal disease. Both reduced angiotensin (Ang) II levels and increased bradykinin levels may participate in the benefits of ACE inhibitor therapy.1–7 The AT1 receptor blocker losartan has been reported not to increase blood bradykinin levels in rats.8 However, animal and human studies implicate kinin peptides in the actions of AT1 receptor blockers, possibly mediated by the stimulation of AT2 receptors by the increased Ang II levels that accompany AT1 receptor antagonism.9–18 We therefore examined the effects of the AT1 receptor blockers losartan and eprosartan on kinin and angiotensin levels in hypertensive subjects.

In humans, plasma kallikrein forms bradykinin [BK-(1–9)] from high-molecular-weight kininogen, whereas tissue kallikrein forms kallidin [Lys0-BK-(1–9), KBK-(1–9)] from high- and low-molecular-weight kininogens (Table 1). Bradykinin peptides may also be generated by aminopeptidase-mediated cleavage of kallidin peptides. A proportion of kininogens is hydroxylated on the third proline (Hyp3) of the BK-(1–9) sequence, leading to the formation of hydroxylated kinin peptides that have similar biological activity to nonhydroxylated kinins.19 Kinins act via 2 types of kinin receptor, the type 1 (B1) and the type 2 (B2). BK-(1–9) and KBK-(1–9) are more potent at the B2 receptor, whereas their carboxypeptidase (kininase I) metabolites BK-(1–8) and KBK-(1–8), respectively, are also bioactive and more potent on B1 receptors. The ACE and neutral endopeptidase 24.11 (NEP) metabolites BK-(1–7) and KBK-(1–7) are inactive. Using high-performance liquid chromatography (HPLC)–based radioimmunoassays, we measured nonhydroxylated and hydroxylated bradykinin and kallidin peptides in arterial blood and calculated the BK-(1–7)/BK-(1–9) ratio because it provides an index of the rate of metabolism of BK-(1–9) to BK-(1–7). We also measured the levels of Ang I, Ang II, Ang-(2–8), Ang-(3–8), Ang-(1–7), and Ang-(1–9) in arterial plasma. Ang II, Ang-(2–8), Ang-(3–8), and Ang-(1–7) are bioactive and may contribute to the effects of AT1 receptor blocker therapy.20 Ang I is metabolized by ACE to Ang II and by NEP to Ang-(1–7), and we calculated the Ang II/Ang I and Ang-(1–7)/Ang I ratios because they provide indices of ACE and NEP activity, respectively. In addition, Ang II is

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metabolized to Ang-(1–7) by carboxypeptidases such as prolylcarboxypeptidase and ACE-related carboxypeptidase (ACE2).21–23 The sequences of the peptides measured are shown in Table 1.

Methods

Patients
Patients with essential hypertension were enrolled in a double-blind, randomized, 3-period, 3-treatment, crossover study. Inclusion criteria were newly diagnosed essential hypertension or those previously treated from whom antihypertensive treatment could be safely withdrawn, 18 to 70 years old, with sitting diastolic blood pressure ≥90 mm Hg and sitting systolic blood pressure ≥140 mm Hg. Exclusion criteria included cardiovascular or other disease, secondary hypertension, diabetes, cardiac arrhythmia, autonomic abnormality, body mass index ≥35 kg/m², pregnancy, serious allergy or abnormal drug reaction, and alcohol or drug abuse. No other antihypertensive medication or drugs that affect autonomic function were allowed during the study. The study comprised 3 sequential periods of 6 weeks’ duration. The first 2 weeks of each 6-week period was a placebo washout period; during the last 4 weeks, patients received either placebo, losartan 50 mg OD, or eprosartan 600 mg OD, in randomized order. At the end of the 4-week treatment period, patients returned to the study center in the morning after a light breakfast. Patients were asked to avoid nicotine and caffeine products for 12 hours and alcohol and strenuous exercise for 24 hours before each assessment. The subjects rested supine and took their medication at the study center before a 5-cm 3F arterial catheter was inserted into the brachial or radial artery. Supine blood pressure was measured 3 times with a Dinamap device (GE Healthcare). Of 32 subjects recruited to the study, 24 were randomized, and 19 completed all 3 phases of the study. These were 9 men and 10 women of mean age 56 years (range, 45 to 69 years). Angiotensin and bradykinin peptide levels are reported for all 19 subjects. Kallidin peptide levels are reported for 18 subjects, because data were not available for 1 subject. The Human Research Ethics Committee of the Alfred Hospital approved the study, and all subjects gave written, informed consent.

Collection of Blood
Blood was collected into syringes from a 3-way tap close to the arterial catheter, 20 to 60 minutes after administration of the study medication. Blood for measurement of bradykinin peptides was immediately added to 4 mol/L guanidine thiocyanate (2 mL blood/10 mL guanidine thiocyanate) on ice. Blood for measurement of kallidin peptides was immediately added to 1 mol/L HCl (10 mL blood/20 mL HCl) on ice. Blood (10 mL) for measurement of angiotensin peptides was immediately added to heparinized tubes on ice containing 0.5 mL of an enzyme inhibitor cocktail (125 mmol/L EDTA, 2 μmol CGP 38560A (renin inhibitor), 50 mmol/L 1,10-phenanthroline, 1 g/L neomycin sulfate, and 2% ethanol in water), mixed, and immediately centrifuged at 4°C for 10 minutes, and the plasma was snap-frozen on dry ice and stored at −80°C. Blood for measurement of plasma ACE was added to heparinized tubes on ice and immediately centrifuged at 4°C for 10 minutes, and the plasma was snap-frozen on dry ice and stored at −80°C.

Biochemical Analyses
Samples for measurement of bradykinin and kallidin peptides were extracted with C18 Sep-Pak cartridges (Waters Chromatography Division) within 1 hour of collection and assayed by use of HPLC-based radioimmunoassay.24 Plasma for angiotensin peptide measurement was stored at −80°C until extracted with C18 Sep-Pak cartridges and assayed for Ang II, Ang I, Ang-(1–7), Ang-(1–9), and Ang-(2–8) by use of HPLC-based radioimmunoassay with amino-
terminal-directed antisera.25 Ang-(3–8) was measured with a carboxy-terminal–directed antiserum (A30)25 and corrected for cross-reactivity of 58% and recovery of 70%. Plasma ACE was measured by enzymatic assay by use of 3-(2-furylacryloyl)-L-phenylalanlyglycyl-glycine as substrate.26 All assays were performed without knowledge of the treatment status of the subjects.

Statistical Analysis
Data were analyzed by repeated-measures ANOVA, and comparisons with placebo were made by use of the Dunnett test. Because their distribution was skewed, peptide levels and peptide ratios were analyzed after logarithmic transformation and are presented as geometric mean and 95% CI. A probability value of \( P<0.05 \) was considered to be statistically significant.

Results
Losartan and eprosartan produced similar reductions in systolic blood pressure (placebo, 151±2 mm Hg; losartan, 142±3 mm Hg, \( P<0.05 \); eprosartan, 142±3 mm Hg, \( P<0.05 \)) but did not influence diastolic blood pressure (placebo, 85±2 mm Hg; losartan, 82±2 mm Hg; eprosartan, 82±2 mm Hg).

Losartan increased blood BK-(1–9) and Hyp3-BK-(1–9) levels by \( \approx 2 \)-fold and reduced the BK-(1–7)/BK-(1–9) ratio by 55% (Figure; Table 2). There were trends for similar changes with eprosartan therapy that did not achieve statistical significance. Neither drug affected BK-(1–7), BK-(1–8), Hyp3-BK-(1–7), Hyp3-BK-(1–8), or kallidin peptide levels.

Both losartan and eprosartan increased plasma levels of Ang II, Ang I, and Ang-(2–8), and eprosartan increased plasma Ang-(3–8) levels (Figure; Table 2). Neither drug affected plasma levels of Ang-(1–7) or Ang-(1–9). Whereas Ang II levels increased by 2- to 2.5-fold, Ang I levels increased by 3- to 3.7-fold; consequently, there was a 30% to 35% reduction in Ang II/Ang I ratio with drug administration. Moreover, the Ang-(1–7)/Ang I ratio was reduced by 63% to 69% with drug administration.

Neither losartan nor eprosartan affected plasma ACE activity (placebo, 66±6 U/L; losartan, 68±6 U/L; eprosartan, 68±6 U/L).

Discussion
Our finding that losartan increased BK-(1–9) and Hyp3-BK-(1–9) levels in arterial blood of hypertensive subjects provides support for a role for bradykinin in mediating the effects of losartan therapy. The \( \approx 2 \)-fold increase in arterial blood bradykinin levels with losartan administration was similar to the increase in arterial blood bradykinin levels we measured for ACE inhibitor therapy.1,2 Eprosartan produced similar changes in kinin peptide levels that did not achieve statistical significance but suggest that increased bradykinin levels are a class effect of AT1 receptor blockers. The modest increases in Ang II levels in response to losartan and eprosartan were similar to previous reports and consistent with incomplete blockade of AT1 receptors at the doses used.27,28 Our finding of reduced BK-(1–7)/BK-(1–9) ratio with losartan therapy suggested that the increased bradykinin levels were the consequence of reduced metabolism. Because we measured peptide levels in arterial blood, these levels largely reflect the consequences of their metabolism by the extensive peptidase activity of the pulmonary vasculature.29

Our measurements of angiotensin peptides support the proposal that losartan caused reduced bradykinin metabolism. Both ACE and NEP metabolize BK-(1–9) to BK-(1–7).10 The 30% to 35% reduction in plasma Ang II/Ang I ratio was consistent with reduced ACE activity. The lack of change in plasma ACE activity suggests that the fall in plasma Ang II/Ang I ratio was a result of an alteration in pulmonary metabolism of Ang I without change in plasma ACE levels. The 63% to 69% reduction in plasma Ang-(1–7)/Ang I ratio was greater than the reduction in plasma Ang II/Ang I ratio and suggests that reduction in pulmonary NEP activity made a greater contribution to the increase in bradykinin levels than reduction in pulmonary ACE activity.

There are a number of possible mechanisms by which AT1 receptor blockers may influence pulmonary ACE and NEP activity. The AT1 receptor exerts a tonic negative influence on ACE activity,30 and AT1 receptor blockers may thereby reduce ACE (and possibly NEP) activity by stimulation of
AT2 receptors by the increased Ang II levels that accompany AT1 receptor blockade. Losartan and eprosartan increased the levels of bioactive angiotensin peptides additional to Ang II, including Ang-(2–8) and Ang-(3–8), which may also contribute to the effects of these compounds. In addition, losartan and its metabolites have AT1 receptor–independent actions.31

Our previous studies in rats showed that losartan administration in high doses (10 mg/kg) for 8 days did not modify circulating bradykinin levels and decreased bradykinin levels in kidney.8 We also found that losartan increased plasma ACE levels, similar to the effects of ACE inhibition32 and consistent with tonic negative feedback regulation of ACE by Ang II.8 The different results we obtained in humans may represent a species difference or the longer duration of administration and lower doses of losartan used in the present study. Acute administration of losartan does not affect bradykinin-induced vasodilatation in human forearm vasculature33 and suggests that the changes in bradykinin levels we observed in the present study require chronic administration.

Many studies show a link between AT2 receptors and B2 receptors that may contribute to the effects of AT1 receptor blockade.9–18 Furthermore, Tsutsumi et al34 reported increased kinin-forming activity in the vasculature of transgenic mice with AT2 receptor overexpression in vascular smooth muscle cells. AT2 receptor overexpression prevented the pressor effect of Ang II, which was restored by either AT2 or B2 receptor antagonism.34

We previously showed that ACE inhibition increases bradykinin and kallidin levels in the arterial blood of human subjects.1,2 In contrast to the effects of ACE inhibition, losartan did not increase kallidin levels. The different response of bradykinin and kallidin peptides to the administration of losartan may represent the different compartments in which these peptides are formed. Whereas bradykinin peptides are produced by plasma kallikrein, kallidin peptides are generated by tissue kallikrein. An important site of bradykinin peptide formation is likely to be the endothelial surface,35,36 whereas kallidin peptides are likely to be produced at extravascular sites. Bradykinin and kallidin peptides may also be metabolized by different enzymes. Kokkonen et al37 report that kallidin metabolism by cardiac membranes is via aminopeptidase M–like activity, whereas bradykinin is metabolized by NEP.

ACE inhibitor therapy is associated with an incidence of angioedema of 0.4% to 0.7%,38–40 whereas the incidence of angioedema with AT1 receptor blocker therapy is 0.1% to 0.4%.41,42 Increased levels of bradykinin may contribute to angioedema during ACE inhibitor therapy,43 and our results suggest that bradykinin may also contribute to the angioedema that may accompany losartan therapy. The lower

### TABLE 2. Plasma Angiotensin and Blood Kinin Peptide Levels During Treatment With Placebo, Losartan, or Eprosartan

<table>
<thead>
<tr>
<th>Peptide, fmol/mL, or Peptide Ratio, mol/mol</th>
<th>Placebo</th>
<th>Losartan</th>
<th>Eprosartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang-(1–7)</td>
<td>1.5 (0.3–8.3)</td>
<td>1.8 (0.5–6.2)</td>
<td>1.8 (0.7–4.5)</td>
</tr>
<tr>
<td>Ang II</td>
<td>3.1 (1.0–9.5)</td>
<td>7.6 (1.3–44.5)*</td>
<td>6.5 (2.0–21.5)*</td>
</tr>
<tr>
<td>Ang-(1–9)</td>
<td>0.13 (0.02–0.75)</td>
<td>0.18 (0.03–1.19)</td>
<td>0.10 (0.03–0.43)</td>
</tr>
<tr>
<td>Ang I</td>
<td>1.8 (0.2–15.7)</td>
<td>6.6 (0.9–46.6)*</td>
<td>5.5 (0.9–34.4)*</td>
</tr>
<tr>
<td>Ang-(2–8)</td>
<td>0.24 (0.05–1.09)</td>
<td>0.53 (0.07–4.00)*</td>
<td>0.67 (0.09–4.94)*</td>
</tr>
<tr>
<td>Ang-(3–8)</td>
<td>0.34 (0.07–1.76)</td>
<td>0.58 (0.07–4.56)</td>
<td>0.76 (0.16–3.78)*</td>
</tr>
<tr>
<td>Ang II/Ang I ratio</td>
<td>1.7 (0.3–10.7)</td>
<td>1.1 (0.6–2.2)*</td>
<td>1.2 (0.5–3.0)*</td>
</tr>
<tr>
<td>Ang-(1–7)/Ang I ratio</td>
<td>0.86 (0.09–7.91)</td>
<td>0.27 (0.04–1.70)*</td>
<td>0.32 (0.07–1.54)*</td>
</tr>
<tr>
<td>BK–(1–7)</td>
<td>1.4 (0.5–4.3)</td>
<td>1.4 (0.4–5.2)</td>
<td>1.6 (0.5–5.8)</td>
</tr>
<tr>
<td>BK–(1–8)</td>
<td>0.08 (0.02–0.41)</td>
<td>0.10 (0.02–0.47)</td>
<td>0.07 (0.01–0.36)</td>
</tr>
<tr>
<td>BK–(1–9)</td>
<td>0.18 (0.02–1.90)</td>
<td>0.41 (0.04–4.39)*</td>
<td>0.31 (0.05–2.01)</td>
</tr>
<tr>
<td>BK–(1–7)/BK–(1–9) ratio</td>
<td>7.8 (0.9–65.3)</td>
<td>3.5 (0.6–20.1)*</td>
<td>5.3 (0.8–36.6)</td>
</tr>
<tr>
<td>Hyp3-BK–(1–7)</td>
<td>&lt;0.14</td>
<td>&lt;0.14</td>
<td>&lt;0.14</td>
</tr>
<tr>
<td>Hyp3-BK–(1–8)</td>
<td>0.53 (0.12–2.37)</td>
<td>0.37 (0.07–1.85)</td>
<td>0.51 (0.09–2.89)</td>
</tr>
<tr>
<td>Hyp3-BK–(1–9)</td>
<td>0.18 (0.04–0.91)</td>
<td>0.32 (0.05–2.23)*</td>
<td>0.27 (0.05–1.60)</td>
</tr>
<tr>
<td>KBK–(1–7)</td>
<td>0.46 (0.07–3.33)</td>
<td>0.28 (0.02–3.51)</td>
<td>0.29 (0.03–3.32)</td>
</tr>
<tr>
<td>KBK–(1–8)</td>
<td>0.10 (0.01–1.35)</td>
<td>0.05 (0.01–0.28)</td>
<td>0.05 (0.01–0.41)</td>
</tr>
<tr>
<td>KBK–(1–9)</td>
<td>0.22 (0.02–2.70)</td>
<td>0.12 (0.01–2.86)</td>
<td>0.17 (0.01–2.05)</td>
</tr>
<tr>
<td>Hyp3-KBK–(1–7)</td>
<td>0.04 (0.01–0.49)</td>
<td>0.03 (0.01–0.26)</td>
<td>0.02 (0.01–0.11)</td>
</tr>
<tr>
<td>Hyp3-KBK–(1–8)</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Hyp3-KBK–(1–9)</td>
<td>0.04 (0.01–0.26)</td>
<td>0.03 (0.01–0.16)</td>
<td>0.02 (0.01–0.11)</td>
</tr>
<tr>
<td>KBK–(1–7)/KBK–(1–9) ratio</td>
<td>2.1 (0.6–7.1)</td>
<td>2.2 (0.2–27.2)</td>
<td>1.7 (0.6–4.7)</td>
</tr>
</tbody>
</table>

Data shown as geometric mean (95% CI), n = 19 for angiotensin and bradykinin peptides; n = 18 for kallidin peptides. Data represent fmol/mL plasma for angiotensin peptides and fmol/mL blood for kinin peptides; peptide ratios are shown as mol/mol.

*P < 0.01, †P < 0.05 vs placebo.
incidence of angioedema with AT1 receptor blocker than with ACE inhibitor therapy may be because ACE inhibitors increase both bradykinin and kallidin, whereas AT1 receptor blockers increase only bradykinin.

BK-(1–5) is a major metabolite of BK-(1–9) that has antithrombin activity. Although we did not measure BK-(1–5), we did measure 12 other kinin peptides and 6 angiotensin peptides. ACE metabolizes BK-(1–7) to BK-(1–5). It is of note that BK-(1–7) levels were not altered by losartan, and it is therefore unlikely that BK-(1–5) levels were increased by losartan. Moreover, it is unlikely that endogenous BK-(1–5) levels inhibit thrombin in vivo, because plasma BK-(1–5) levels (30 to 40 pmol/L) are at least 6 orders of magnitude below the micromolar concentrations required to inhibit thrombin.

The increase in bradykinin levels with losartan therapy is one of many interactions between the renin-angiotensin and kallikrein-kinin systems. These 2 systems share many enzymes involved in peptide metabolism. Moreover, prolylcarboxypeptidase may participate in the activation of plasma prekallikrein, and kallikrein may participate in activation of prorenin.

Ang II induces expression of B2 receptors, an effect mediated by AT1 receptors. ACE inhibitors potentiate the effects of bradykinin not only by inhibition of bradykinin metabolism but also by a process dependent on crosstalk between ACE and the B2 receptor. Furthermore, in addition to a role for bradykinin in mediating the effects of AT1 receptor stimulation, AT1 and B2 receptors form heterodimers that enhance AT1 receptor signaling and may contribute to the increased responsiveness to Ang II shown by women with preeclampsia.

In conclusion, we showed that losartan increased bradykinin levels. The reductions in Ang II/Ang I, Ang-(1–7)/Ang I, and BK-(1–7)/BK-(1–9) ratios suggest that the increased bradykinin levels were the result of reduced metabolism by ACE and NEP. The similar effects of eprosartan on Ang II/Ang I and Ang-(1–7)/Ang I ratios and the trend toward similar changes in BK-(1–7)/BK-(1–9) ratio and bradykinin levels suggest that these represent a class effect of AT1 receptor blockers. Increased bradykinin levels may contribute to the therapeutic effects of AT1 receptor blockers and may also contribute to the angioedema that may accompany this therapy.

Disclosure

Dr. Campbell, Dr. Kladis, and Dr. Esler have received speaker’s honoraria and served as consultants to Solvay, the manufacturers of eprosartan, and Dr. Esler is Chair of the Australian Cardiovascular Board for Solvay.

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