Kinins in humans

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Duncan, Ann-Maree, Athena Kladis, Garry L. Jennings, Anthony M. Dart, Murray Esler, and Duncan J. Campbell. Kinins in humans. Am J Physiol Regulatory Integrative Comp Physiol 278: R897–R904, 2000.—The kinin peptide system in humans is complex. Whereas plasma kallikrein generates bradykinin peptides, glandular kallikrein generates kallidin peptides. Moreover, a proportion of bradykinin peptides is hydroxylated on proline3 of the bradykinin sequence. We established HPLC-based radioimmunoassays for nonhydroxylated and hydroxylated bradykinin and kallidin peptides and their metabolites in blood and urine. Both nonhydroxylated and hydroxylated bradykinin and kallidin peptides were identified in human blood and urine, although the levels in blood were often below the assay detection limit. Whereas kallidin peptides were more abundant than bradykinin peptides in urine, bradykinin peptides were more abundant in blood. Bradykinin and kallidin peptide levels were higher in venous than arterial blood. Angiotensin-converting enzyme inhibition increased blood levels of bradykinin, but not kallidin, peptides. Reactive hyperemia had no effect on antecubital venous levels of bradykinin or kallidin peptide levels. These studies demonstrate differential regulation of the bradykinin and kallidin peptide systems, and indicate that blood levels of bradykinin peptides are more responsive to angiotensin-converting enzyme inhibition than blood levels of kallidin peptides. bradykinin; kallidin; angiotensin-converting enzyme inhibition; cardiac failure; reactive hyperemia

KININS ACT VIA TWO TYPES of kinin receptor, the type 1 (B1) and the type 2 (B2) receptors. The B2 receptor normally predominates, whereas B1 receptors are induced by tissue injury, such as that which occurs after myocardial ischemia (10) and inflammation (29). A complex variety of kinin peptides acts through these receptors. Plasma kallikrein forms bradykinin (BK)-(1–9) from high molecular weight kininogen, whereas in tissues, glandular kallikrein forms kallidin [Lys0-BK-(1–9), KBK-(1–9)] from low and high molecular weight kininogens (1). Moreover, bradykinin peptides may be generated by aminopeptidase-mediated cleavage of kallidin peptides. These peptides are more potent at the B2 receptor (27). A proportion of kinins is hydroxylated on proline3 (Hyp3) of the BK-(1–9) sequence (16, 17), and hydroxylated kinins have similar biological activity to nonhydroxylated kinins (27). These kinins are metabolized by enzymes collectively called kininases. The carboxypeptidase (kininase I) metabolites of BK-(1–9) and KBK-(1–9) are BK-(1–8) and Lys0-BK-(1–8) [KBK-(1–8)], respectively, which are also bioactive, but more potent on B1 receptors (27), whereas the angiotensin-converting enzyme (ACE; kininase II) and neutral endopeptidase 24.11 (NEP) metabolites BK-(1–7) [BK-(1–7)] and KBK-(1–7) are inactive.

Kinin peptides have important actions on blood vessels, the heart, and the kidney. An important hemodynamic effect of kinin peptides 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 (8, 9, 22, 34). Additional renal actions of kinin peptides include the production of a diuresis and natriuresis (12, 18, 23, 36). Kinins are also potent mediators of inflammation (1). Recent studies in humans indicate a role for endogenous kinin peptides in the regulation of coronary vascular tone (13) and in mediating the hypotensive effects of ACE inhibition (11). Moreover, animal studies suggest that kinins participate in the effects of type 1 ANG II antagonists (21) and NEP inhibitors (26, 37).

Despite the importance of kinins in health and disease, very little information is available concerning the identity of kinin peptides in humans, the levels of individual kinin peptides in blood and urine, and the regulation of kinin peptide levels. To better understand the relative contribution of plasma and glandular kallikrein to kinin peptide formation and the relative roles of different kinin peptides in mediating the effects of the kallikrein kinin system, we developed HPLC-based RIAs to measure nonhydroxylated and hydroxylated BK-(1–9) and KBK-(1–9) and their metabolites in blood and urine. We applied these RIAs to the identification and quantification of individual kinin peptides in arterial and venous blood and in urine of humans. We compared the levels of kinin peptides in arterial blood with levels in coronary sinus and antecubital, jugular, and renal venous blood.

We also investigated two aspects of regulation of kinin peptide levels in blood. Many different peptidases have the potential to contribute to kinin peptide metabolism (5). To evaluate the role of ACE in kinin peptide metabolism, we studied the effects of ACE inhibition on kinin peptide levels in arterial and venous blood. Given that kinins are potent vasodilators, we also investigated whether the marked vasodilatation associated
with reactive hyperemia is associated with increased blood levels of kinin peptides.

METHODS

Subjects and sample collection. Four groups of experimental subjects were studied. 1) Antecubital venous blood and urine were obtained from 12 healthy male laboratory personnel aged 30-40 years. 2) Arterial and regional venous blood were obtained from subjects taking part in studies of regional sympathetic activity (33, 35); these included obese normotensive subjects (n = 9, all male, age 39-51 yr, body mass index (BMI) 28-33), subjects with panic disorder (n = 7, 4 males, 3 females, age 29-53 yr, BMI 17-26), and normal control subjects (n = 6, 3 males, 3 females, age 27-55 yr, BMI 21-25). 3) Arterial and coronary sinus blood were obtained from 12 subjects (10 males, 2 females, age 35-67 yr) undergoing assessment for severe cardiac failure, all of whom were receiving ACE inhibitor therapy. 4) Arterial blood was obtained from subjects in whom arterial cannulas were inserted immediately before anesthesia for cardiac surgery (n = 23, age 44-77 yr), 12 of whom were receiving ACE inhibitor therapy; most of the cardiac surgery subjects were to undergo coronary artery bypass surgery. All subjects were Caucasian. These protocols were approved by the Human Research Ethics Committees of St. Vincent's and Alfred Hospitals, and all subjects gave informed consent.

Sample collection. Whereas laboratory personnel were ambulant, all other subjects were supine. Arterial and antecubital venous blood were collected through short plastic catheters. Coronary sinus, jugular venous, and renal venous blood were collected through a central venous catheter, as described previously (33, 35). Blood was collected into plastic syringes. For measurement of bradykinin peptides, 2 ml blood was immediately transferred to a tube containing 10 ml 4 M guanidine thiocyanate and 1% (vol/vol) trisfluoroacetic acid (GTC-TFA) and mixed thoroughly. For measurement of kallidin peptides, 10 ml blood was immediately transferred to a tube containing 20 ml 1 M HCl. Blood samples (2 ml) were also collected into 10 ml GTC-TFA for measurement of angiotensin peptides, as described elsewhere (7). For measurement of the concentration of kinin peptides in urine, male subjects were asked to drink 500 ml water, to empty their bladder, and then to provide the next 10-50 ml that collected in their bladder. One milliliter of this freshly voided urine was immediately transferred to a tube containing 10 ml GTC-TFA.

The effects of ACE inhibition on antecubital venous levels of kinin peptides were studied in normal laboratory personnel, from whom blood was obtained before and after administration of the ACE inhibitor perindopril. Perindopril (8 mg) was administered 16 and 4 hr before blood sampling. The effects of ACE inhibition on arterial and coronary sinus levels of kinin peptides were examined by comparing control (pooled obese, panic disorder, and normal) subjects of the regional sympathetic activity studies with ACE inhibitor-treated cardiac failure subjects and by comparing cardiac surgery subjects receiving ACE inhibitor therapy with those not receiving such therapy.

The effects of reactive hyperemia were studied in normal laboratory personnel. A Teflon catheter was inserted into the antecubital vein, blood was collected for measurement of kinin peptides, and the catheter was flushed with heparinized saline (10 IU/ml). A blood pressure cuff was inflated to 50 mmHg above systolic pressure to occlude the brachial artery. After 5 min, the cuff was deflated and antecubital venous blood was collected from the catheter during reactive hyperemia, ~30 s after release of the cuff.

Extraction and HPLC of kinin and kallidin peptides. The HCl/blood samples for measurement of kallidin peptides were centrifuged and the plasma was decanted, frozen in dry ice, then thawed, recentrifuged to sediment protein precipitate, and the supernatant extracted with C18 Sep-Paks (Waters Chromatography Division, Milford, MA). The GTC-TFA blood and GTC-TFA urine samples were extracted with C18 Sep-Paks after centrifugation. The Sep-Pak cartridges were prewashed sequentially with 3 ml methanol, 10 ml 0.1% TFA in water, 10 ml methanol-TFA-water (80:1:19, vol/vol/vol), then 10 ml 1% TFA in water. After sample loading, the Sep-Pak cartridge was washed with 10 ml 0.5% sodium chloride-0.5% TFA in water, then 2 ml 1% TFA in water, and eluted with 6 ml methanol-TFA-water (80:1:19) into siliconized borosilicate glass tubes. The sample was taken to dryness in a vacuum centrifuge before further purification by cation exchange chromatography and a second C18 Sep-Pak extraction.

Samples for bradykinin assay were resuspended in 2 ml TFA-acetoni-trile-water (0.1:2.97:9.7), 2 ml TFA-acetoni-trile-water (0.1:2.97:9.7) in preparation for further purification on a cation exchange Isolute propylsulphonyl (PRS) cartridge (International Sorbent Technology, Mid Glamorgan, UK). The PRS cartridge was pretreated with 2 ml acetoni-trile, 10 ml TFA-acetoni-trile-water (0.1:2.97:9.7), 5 ml TFA-water (98% water), and the supernatant extracted with 1 M sodium chloride, and 10 ml TFA-acetoni-trile-water (0.1:2.97:9.7). After sample loading, the PRS cartridge was washed with 5 ml TFA-acetoni-trile-water (0.1:2.97:9.7), then eluted with 5 ml 1 M sodium chloride. A second C18 Sep-Pak procedure was required to remove sodium chloride from the sample. The sample was eluted from the PRS cartridge directly onto a C18 Sep-Pak cartridge pretreated as described above, then washed with 10 ml 0.1% TFA in water, eluted with 6 ml methanol-TFA-water (80:1:1.199.9) and taken to dryness.

Samples for kallidin peptide assay were resuspended in 5 ml acetoni-trile-20 mM sodium borate in water, pH 9.5 (2.98), in preparation for further purification on a cation exchange carboxymethyl (CM) Sep-Pak cartridge (Waters Chromatography Division). The CM cartridge was pretreated with 2 ml acetoni-trile, 10 ml acetoni-trile-20 mM sodium borate (2.98), 5 ml 1 M sodium chloride in water, and 10 ml acetoni-trile-20 mM sodium borate (2.98). After sample loading, the CM cartridge was washed with 4 ml acetoni-trile-20 mM sodium borate (2.98), then eluted with 5 ml 1 M sodium chloride directly onto a C18 Sep-Pak cartridge pretreated as described above, then washed with 10 ml 0.1% TFA in water, eluted with 6 ml methanol-TFA-water (80:1:1.199.9) and taken to dryness.

Urine samples were further purified on Isolute PRS cartridges as described above for GTC-TFA blood extracts. After evaporation to dryness, all extracts were acetylated and treated with piperidine as described elsewhere (7) before HPLC.

All peptides were separated on a 100 × 4.6 mm Brownlee RP-18 Spheri-5 column preceded by a 15 × 3.2 mm RP-18 guard column (Applied Biosystems, Foster City, CA). Solvent A was 0.1% TFA and 0.15 M sodium chloride in water; solvent B was 0.1% TFA and 90% acetoni-trile in water. Peptides were eluted with a linearly increasing gradient of 18-38% solvent B over 30 min. The flow rate was 1 ml/min, and 0.5-min fractions were collected into 10 × 75 mm borosilicate glass tubes containing 50 µl of 5 mg/ml protease-free bovine serum albumin (Miles, Diagnostics Division, Kankakee, IL) in water. HPLC fractions were evaporated to dryness before RIA.

Peptide RIA. Acetylated kinin peptides were measured with two different NH2 terminal-directed antisera. Acetylated bradykinin peptides were measured with antibody B24, previously described (5, 7), which enabled the measurement of the
acetylated forms of BK-(1—7), BK-(1—8), and BK-(1—9). Antibody B24 cross-reacted with Hyp 3-bradykinin peptides, allowing measurement of both hydroxylated and nonhydroxylated bradykinin peptides (Table 1). Kallidin peptides were measured with antibody K10, raised in a rabbit immunized with α,ε-diacyt-Lys 3-Hyp 3-Lys 9-BK-(1—9) conjugated via the COOH terminal lysine residue to bovine thyroglobulin with glutaraldehyde. This antibody enabled the measurement of the acetylated forms of KKB-(1—7), KKB-(1—8), and KKB-(1—9), and the corresponding hydroxylated peptides. Antibody K10 had similar cross-reactivities for kallidin and Hyp 3-kallidin peptides (Table 1). All RIA were performed in duplicate. Data were corrected for antibody cross-reactivity and peptide recovery.

For the kallidin RIA with antibody K10, 125I-labeled acetyl-Lys 3-Hyp 3-Tyr 8-BK-(1—9) was used as tracer for the RIA; Lys 3-Hyp 3-Tyr 8-BK-(1—9) was iodinated with 125I using the chloramine-T procedure (15), then acetylated (7), and the monoiodinated 125I-acetyl-Lys 3-Hyp 3-Tyr 8-BK-(1—9) was purified by HPLC. Tracer (~4,000 cpm), standard [α,ε-diacyt-Lys 3-Hyp 3-Lys 9-BK-(1—9)], and antiserum were diluted in assay buffer (100 mM sodium phosphate, 10 mM EDTA, 154 mM sodium chloride, 1 g/l sodium azide, 1 g/l casen, pH 7.0). The total volume was 250 µl per assay tube, and each assay was incubated at 4°C for 48 h before separation of free from bound radioactivity with albumin-dextran-coated charcoal (4). For antiserum K10 at a dilution of 1/417,000, 50% displacement was obtained with 8 fmol/tube (4). For antiserum K10 at a dilution of 1/417,000, 50% displacement was obtained with 8 fmol/tube (4). For antiserum K10 at a dilution of 1/417,000, 50% displacement was obtained with 8 fmol/tube (4). For antiserum K10 at a dilution of 1/417,000, 50% displacement was obtained with 8 fmol/tube (4). For antiserum K10 at a dilution of 1/417,000, 50% displacement was obtained with 8 fmol/tube (4). The detection limit was ~0.2 fmol/tube, and the RIA had a within-assay coefficient of variation of 10% and a between-assay coefficient of variation of 17%.

The blank for each assay was assessed by extraction of 10 ml GTC-TFA or 10 ml water in 20 ml HCl, and these blank extracts (n = 4 for each assay) were processed as described above and then subjected to HPLC before RIA. Blank extracts contained no detectable immunoreactivity.

Recoveries of bradykinin peptides from blood were determined by collecting duplicate 2-ml blood samples from normal laboratory personnel; to one of the duplicate GTC-TFA blood samples was added either 20 fmol bradykinin peptides or 100 fmol Hyp 3-bradykinin peptides, and the samples were processed as described above. Recoveries of kallidin peptides from blood were determined by collecting duplicate 10-ml blood samples from normal laboratory personnel; to one of the duplicate HCl blood samples was added 100 fmol kallidin peptides, and the samples were processed as described above. Recoveries of kinin peptides from urine were determined by preparing replicate GTC-TFA urine samples, to one of which was added either 500 fmol bradykinin peptides, 2,500 fmol Hyp 3-bradykinin peptides, or 50,000 fmol kallidin peptides.

BK-(1—9), BK-(1—8), BK-(1—7), BK-(1—6), BK-(1—5), Hyp 3-BK-(1—7), Hyp 3-BK-(1—8), Hyp 3-BK-(1—9), Hyp 3-Tyr 8-BK-(1—9) were obtained from Bachem, Torrance, CA; α,ε-diacyt-Lys 3-Hyp 3-Lys 9-BK-(1—9), KBK-(1—9) and Hyp 3-Tyr 8-BK-(1—9) were obtained from Auspep, Parkville, Australia. Hyp 3-BK-(1—8), KBK-(1—4), KBK-(1—5), KBK-(1—6), BK-(1—7), BK-(1—8), Hyp 3-BK-(1—4), Hyp 3-BK-(1—5), Hyp 3-BK-(1—6), Hyp 3-BK-(1—7), and Hyp 3-BK-(1—8) were obtained from Chiron Mimotopes Peptide Systems, Clayton, Australia. All peptide concentrations were determined by amino acid analysis using stocks of ~1 mg/ml in 20% acetic acid, which were stored at ~30°C. Working solutions of RIA standards (1 µmol/l in 1 mg/ml lysozyme, 10 mM acetic acid) were stored at ~30°C and were discarded after thawing once.

**RESULTS**

Antibody B24 recognized both nonhydroxylated and hydroxylated bradykinin peptides, and antibody K10 recognized both nonhydroxylated and hydroxylated kallidin peptides (Table 1). The elution positions of kinin peptides were determined by HPLC and RIA of standard acetylated kinin peptides. HPLC achieved satisfactory separation of nonhydroxylated and hydroxylated bradykinin and kallidin peptides and their metabolites (Fig. 1). Both nonhydroxylated and hydroxylated bradykinin and kallidin peptides were identified in blood and urine, although the levels in blood were often below the detection limit (Fig. 1, Tables 2 and 3). Whereas kallidin peptides were more abundant than bradykinin peptides in urine, bradykinin peptides were more abundant in blood. Although not presented here, we also measured bradykinin peptides in blood samples collected from the central venous catheter; these bradykinin peptide levels were very variable, with frequent high values suggesting that artifactual activation of plasma kallikrein had occurred with generation of bradykinin peptides during sampling. In these samples,
hydroxylated bradykinin peptides were present in amounts similar to those of nonhydroxylated bradykinin peptides. Collection of blood from the central venous catheter did not appear to modify kallidin peptide levels, in comparison with antecubital venous kallidin peptide levels (Table 4). Nonhydroxylated and hydroxylated peptides were summed to simplify presentation of kinin peptide levels in Tables 4 and 5.

Arterial and venous kinin peptide levels were similar for normotensive obese subjects, subjects with panic disorder, and the normal subjects of the regional sympathetic nervous activity studies, and kinin peptide levels were pooled for these three groups of subjects, shown as control subjects in Tables 4 and 5. All samples were not collected from every subject, and arterial and venous kinin peptide levels were compared within each subject by paired t-test. Antecubital venous bradykinin peptide levels were higher than arterial levels (Table 4). Moreover, kallidin peptide levels were higher in coronary

Table 3. Recoveries, detection limits, and endogenous levels of kinin peptides in urine

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Recovery, %</th>
<th>Detection Limit, fmol/ml</th>
<th>Endogenous Levels, fmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK-(1–7)</td>
<td>85 ± 8</td>
<td>1</td>
<td>70 ± 48</td>
</tr>
<tr>
<td>BK-(1–8)</td>
<td>63 ± 8</td>
<td>1</td>
<td>39 ± 27</td>
</tr>
<tr>
<td>BK-(1–9)</td>
<td>32 ± 8</td>
<td>2</td>
<td>264 ± 193</td>
</tr>
<tr>
<td>Hyp3-BK-(1–7)</td>
<td>64 ± 10</td>
<td>4</td>
<td>111 ± 92</td>
</tr>
<tr>
<td>Hyp3-BK-(1–8)</td>
<td>53 ± 14</td>
<td>3</td>
<td>134 ± 105</td>
</tr>
<tr>
<td>Hyp3-BK-(1–9)</td>
<td>58 ± 14</td>
<td>4</td>
<td>438 ± 388</td>
</tr>
<tr>
<td>KBK-(1–7)</td>
<td>61 ± 11</td>
<td>2</td>
<td>176 ± 119</td>
</tr>
<tr>
<td>KBK-(1–8)</td>
<td>42 ± 10</td>
<td>3</td>
<td>402 ± 216</td>
</tr>
<tr>
<td>KBK-(1–9)</td>
<td>23 ± 6</td>
<td>3</td>
<td>1210 ± 1029</td>
</tr>
<tr>
<td>Hyp3-KBK-(1–7)</td>
<td>60 ± 7</td>
<td>2</td>
<td>180 ± 131</td>
</tr>
<tr>
<td>Hyp3-KBK-(1–8)</td>
<td>63 ± 9</td>
<td>2</td>
<td>676 ± 513</td>
</tr>
<tr>
<td>Hyp3-KBK-(1–9)</td>
<td>39 ± 8</td>
<td>3</td>
<td>3740 ± 4493</td>
</tr>
</tbody>
</table>

For recoveries and endogenous levels, data shown as means ± SD, n = 6 or 7. Detection limits refer to measurement of bradykinin peptides using antibody B24 and kallidin peptides using antibody K10 in 1 ml urine.

Table 4. Comparison of arterial and venous levels of kinin peptides

<table>
<thead>
<tr>
<th>Kinin Peptide, fmol/ml</th>
<th>Number</th>
<th>Regional study: control subjects</th>
<th>Arterial</th>
<th>Antecubital vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyp3-BK + BK</td>
<td>16</td>
<td>2.7 ± 0.3</td>
<td>&lt;0.7</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Kallidin (Hyp3-KBK + KBK)</td>
<td>16</td>
<td>3.9 ± 0.9</td>
<td>1.3 ± 0.2*</td>
<td>2.7 ± 0.9*</td>
</tr>
</tbody>
</table>

Regional study: control subjects

<table>
<thead>
<tr>
<th>Arterial</th>
<th>Coronary sinus</th>
<th>Jugal vein</th>
<th>Arterial</th>
<th>Renal vein</th>
<th>Cardiac failure subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 ± 0.2</td>
<td>1.8 ± 1.0</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>1.4 ± 0.7</td>
<td>0.7 ± 0.1†</td>
</tr>
</tbody>
</table>

Cardiac failure subjects receiving ACE inhibitor therapy

Arterial | Coronary sinus |
---------|---------------|
<0.6    | <0.6          |
<0.8    | <0.8          |
<0.6    | <0.6          |

Data shown as means ± SE. Nonhydroxylated and hydroxylated peptides were summed to simplify presentation of kinin peptide levels. *P < 0.01, †P < 0.05. Data analyzed by paired t-test.
Table 5. Effects of ACE inhibition on kinin peptides in blood

<table>
<thead>
<tr>
<th>Kinin Peptide, fmol/ml</th>
<th>Number</th>
<th>ANG II/ANG I Ratio, mol/mol</th>
<th>(1—7)</th>
<th>(1—8)</th>
<th>(1—9)</th>
<th>(1—7)/(1—9) Ratio, mol/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin (Hyp3-BK + BK) peptides</td>
<td>Cubital venous (normal laboratory personnel)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.74 ± 0.08</td>
<td>2.1 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>&lt;1.0</td>
<td>ND</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>6</td>
<td>0.08 ± 0.02*</td>
<td>3.2 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Arterial (regional study; comparison of control subjects with cardiac failure subjects)</td>
<td>Control</td>
<td>21</td>
<td>3.9 ± 0.8</td>
<td>2.9 ± 0.3</td>
<td>&lt;0.7</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>9</td>
<td>0.4 ± 0.2*</td>
<td>4.9 ± 0.9</td>
<td>1.5 ± 0.3*</td>
<td>2.0 ± 0.5†</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>Arterial (cardiac surgery subjects)</td>
<td>Control</td>
<td>18</td>
<td>1.06 ± 0.31</td>
<td>2.0 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>5</td>
<td>0.04 ± 0.02*</td>
<td>4.0 ± 1.8</td>
<td>1.6 ± 0.5</td>
<td>7.0 ± 1.7†</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Kallidin (Hyp3-KBK + KBK) peptides</td>
<td>Cubital venous (normal laboratory personnel)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.74 ± 0.08</td>
<td>0.8 ± 0.2</td>
<td>&lt;0.8</td>
<td>&lt;0.6</td>
<td>ND</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>6</td>
<td>0.08 ± 0.02*</td>
<td>0.6 ± 0.1</td>
<td>&lt;0.8</td>
<td>&lt;0.6</td>
<td>ND</td>
</tr>
<tr>
<td>Arterial (regional study; comparison of control subjects with cardiac failure subjects)</td>
<td>Control</td>
<td>18</td>
<td>4.2 ± 0.9</td>
<td>0.9 ± 0.2</td>
<td>1.9 ± 0.5</td>
<td>0.65 ± 0.07</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>7</td>
<td>0.5 ± 0.3*</td>
<td>&lt;0.6</td>
<td>&lt;0.8†</td>
<td>&lt;0.6</td>
<td>ND</td>
</tr>
<tr>
<td>Coronary sinus (regional study; comparison of control subjects with cardiac failure subjects)</td>
<td>Control</td>
<td>10</td>
<td>3.3 ± 1.0</td>
<td>1.7 ± 0.5</td>
<td>2.1 ± 0.6</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>7</td>
<td>0.2 ± 0.1*</td>
<td>&lt;0.6</td>
<td>&lt;0.8†</td>
<td>&lt;0.6</td>
<td>ND</td>
</tr>
<tr>
<td>Arterial (cardiac surgery subjects)</td>
<td>Control</td>
<td>18</td>
<td>1.06 ± 0.31</td>
<td>0.72 ± 0.06</td>
<td>0.98 ± 0.07</td>
<td>0.72 ± 0.12</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>5</td>
<td>0.04 ± 0.02*</td>
<td>0.84 ± 0.14</td>
<td>1.32 ± 0.30</td>
<td>0.94 ± 0.18</td>
<td>0.98 ± 0.23</td>
</tr>
</tbody>
</table>

Data shown as means ± SE. Data for angiotensin converting enzyme (ACE) inhibition for cardiac surgery subjects refers to those subjects with ANG II/ANG I ratio <0.15 mol/mol (see RESULTS). Nonhydroxylated and hydroxylated peptides were summed to simplify presentation of kinin peptide levels. Cubital venous data were compared by paired t-test. P < 0.01, †P < 0.05, ACE inhibition compared with control.

The use of HPLC and specific RIA in the present study enabled the precise identification and quantification of individual kinin peptides and their metabolites in sinus, jugular venous, and renal venous than arterial blood of control subjects (Table 4). However, kallidin peptide levels in cardiac failure subjects were below the assay detection limit, with no apparent differences in levels between arterial and coronary sinus blood.

ACE inhibition suppressed the ANG II/ANG I ratio by ~90% in normal laboratory personnel and in cardiac failure subjects. However, for the 12 cardiac surgery subjects receiving ACE inhibitor therapy, only 5 had suppressed ANG II/ANG I ratio (<0.15 mol/mol). This presumably represents the varying dosage and timing of the last dose of ACE inhibitor received by these subjects. For those subjects receiving ACE inhibitor therapy who did not have suppressed ANG II/ANG I ratio (1.3 ± 0.6 mol/mol, means ± SE, n = 7), the ratio was similar to that of subjects not receiving ACE inhibitor therapy (0.9 ± 0.3 mol/mol, n = 11), and kinin data from these 18 subjects were pooled. ACE inhibition did not influence antecubital venous levels of bradykinin peptides (Table 5). However, arterial bradykinin peptide levels were higher in ACE inhibitor-treated cardiac failure subjects than in control subjects of the regional sympathetic nervous activity studies (Table 5). Moreover, ACE inhibition increased bradykinin peptide levels in cardiac surgery subjects (Table 5). It was of note that Hyp3-BK + BK-(1—7) peptide levels were not suppressed and the Hyp3-BK + BK-(1—7)/(1—9) ratio was not modified by ACE inhibition. In contrast to its effects on bradykinin peptide levels, ACE inhibition did not increase kallidin peptide levels in either arterial, antecubital venous, or coronary sinus blood. Moreover, kallidin peptide levels were lower in arterial and coronary sinus blood of ACE inhibitor-treated cardiac failure subjects than control subjects (Table 5).

DISCUSSION

The use of HPLC and specific RIA in the present study enabled the precise identification and quantification of individual kinin peptides and their metabolites in normal laboratory personnel (Table 6).
in blood and urine. A critical aspect of methodologies for the measurement of kinin peptides is the avoidance of activation of kallikrein with consequent artifactual generation of kinin peptides. This is of particular concern for the measurement of bradykinin peptides in blood, where plasma kallikrein can be readily activated. For measurement of bradykinin peptides in blood, we collected blood into GTC-TFA, which we previously showed to effectively prevent bradykinin generation and degradation (5). In separate studies, we showed that similar bradykinin peptide levels were obtained for blood collected into plastic syringes and immediately added to tubes containing GTC-TFA and for blood collected through a short catheter into syringes containing GTC-TFA (data not shown). The kallidin RIA was less sensitive than the bradykinin RIA, and we required a larger volume of blood to measure kallidin peptides. Our collection of 10 ml blood into 20 ml 1 M HCl was based on the methodology of Böänner et al. (2). Antecubital venous and arterial blood were collected through short (~5 cm) plastic or Teflon cannulas. We found that blood collection through a central venous catheter resulted in elevated bradykinin but not kallidin peptide levels, suggestive of activation of plasma kallikrein during sample collection through the central venous catheter. Moreover, we found that collection of antecubital venous blood through steel needles may occasionally result in elevated bradykinin peptide levels (data not shown), which were not observed when blood was collected through short plastic cannula.

We found hydroxylated kinins to be an important component of the kallikrein kinin system in humans and to be of approximately equal abundance to that of nonhydroxylated kinins. The ratio of hydroxylated to nonhydroxylated bradykinin and kallidin peptides varied widely between subjects (data not shown), most likely because of a similar variation in extent of hydroxylation of kininogen in these subjects (16).

Most previous studies of circulating kinins in humans have measured total kinin immunoreactivity, without attempt to separate bradykinin peptides from kallidin peptides, to separate nonhydroxylated and hydroxylated kinin peptides, and to separate cross-reacting metabolites (2, 25, 30, 32). Nussberger (24) recently reported an HPLC-based RIA for bradykinin and showed that the measured levels were lower than when total Immunoreactivity was measured (25). Using HPLC-based RIA, Hilgenfeldt et al. (14) reported plasma bradykinin peptide levels of ~2 pg/ml (~2 fmoI/ml) in normal subjects, in agreement with the present study. However, these authors reported plasma kallidin levels of 81 pg/ml (~68 fmoI/ml), a level that is much higher than the levels found in this study and also much higher than previous reports of total plasma kinin immunoreactivity using antisera with 100% cross-reactivity with bradykinin and kallidin (32).

In previous studies in rats, we showed that tissue kinin peptide levels are much higher than those of blood (5, 6), indicative of kinin production in tissues. Our present finding that venous bradykinin and kallidin peptide levels were higher than arterial levels in control subjects is also consistent with local tissue production of kinins. Given that blood kinin levels were low, we were interested to discover a manipulation that might increase these levels. The failure of reactive hyperemia to influence venous kinin levels suggests that kinins do not participate in this phenomenon. However, it is also possible that the high blood flow may have diluted any increase in kinin release during reactive hyperemia.

We previously showed that ACE inhibition increases circulating kinin levels in the rat (6). Moreover, studies in humans have shown increased plasma immunoreactive kinin levels after ACE inhibition (25, 31, 32). We found no effect of ACE inhibition on bradykinin peptide levels in antecubital venous blood of normal laboratory personnel. However, arterial bradykinin peptide levels were higher in ACE inhibitor-treated cardiac failure subjects than in control subjects of the regional sympathetic nervous activity studies. These data raised the question whether these increased bradykinin peptide levels were due to ACE inhibition, cardiac failure, or their combination. Therefore, we reexamined this question in cardiac surgery subjects with arterial cannulas. The degree of ACE inhibition, as determined by measurement of the arterial ANG II/ANG I ratio was very variable in cardiac surgery subjects receiving ACE inhibitor therapy. However, increased arterial bradykinin peptide levels were seen in cardiac surgery patients with suppressed ANG II/ANG I ratio. The failure of ACE inhibition to increase Hyp^3-BK + BK-(1—9) ratio most likely indicates the role of other enzymes, such as NEP, in bradykinin peptide metabolism.

We found that ACE inhibition increased bradykinin peptide levels in arterial, but not in antecubital venous, blood. This may reflect the relative contribution of ACE to kinin metabolism in different vascular beds. Whereas ACE may be a major pathway of kinin metabolism in the lung, such that ACE inhibition increases arterial bradykinin peptide levels, other kininases may predominate in the vascular beds drained by the antecubital vein. The failure of ACE inhibition to modify kinin levels in antecubital venous blood in this study is at variance with previous reports (25, 32).

Despite increased bradykinin peptide levels, ACE inhibition did not increase kallidin peptide levels in arterial blood. We consider it unlikely that the increase in bradykinin peptide levels was due to artifactual generation of bradykinin peptides by plasma kallikrein during sample collection, because ACE inhibition would be expected to protect bradykinin and kallidin peptides to the same extent. A more likely explanation is that bradykinin and kallidin peptides may be formed in different compartments, where ACE may make a greater or lesser contribution to kinin metabolism. Thus if ACE were a major kininase in the compartment where bradykinin was formed, one would expect ACE inhibition to increase kinin levels. By contrast, if non-ACE kininas were predominant in the compartment where kallidin was formed, ACE inhibition might not affect
kallidin peptide levels. Further studies are required to identify the compartments where kinin peptides are formed and the mechanisms of kinin peptide formation and metabolism. We recently characterized kinin peptides in human atrial tissue (3). Although ACE inhibition suppressed the ANG II/ANG I ratio in human atrial tissue, ACE inhibition did not modify bradykinin or kallidin peptide levels in this tissue, a finding consistent with the recent report that NEP may be the major pathway of kinin peptide metabolism in human heart (19).

Arterial levels of bradykinin peptides were higher in the control cardiac surgery patients (3.6 ± 0.6 fmol/ml) than in the control subjects from the regional study (< 1.0 fmol/ml; Table 5). We are cautious in our interpretation of this difference because this was not a planned comparison and these two groups of subjects were studied at different sites at different times. However, given that most of the cardiac surgery patients had coronary artery disease and probable generalized vascular disease, these data are consistent with a possible role for the altered vascular wall of atherosclerotic vessels in the increased bradykinin peptide levels in arterial blood of cardiac surgery subjects. It is also of interest that arterial and coronary sinus levels of kallidin peptides were lower in ACE inhibitor-treated cardiac failure subjects than in control subjects. This suppression of kallidin peptide levels was not seen in arterial blood of cardiac surgery subjects, suggesting that the activity of the glandular kallikrein kinin system may be suppressed in severe cardiac failure.

Kallidin peptides were more abundant than bradykinin peptides in urine. We previously reported higher bradykinin but not kallidin peptide levels in urine of subjects with interstitial cystitis than in subjects with stress incontinence (28). Moreover, we found bradykinin peptides to be more abundant than kallidin peptides in human atrial tissue (3). These studies demonstrate the importance of separate measurement of bradykinin and kallidin peptides to define the role of these peptides in health and disease. Many aspects of the formation of kinin peptides remain to be defined. In addition to the generation of bradykinin and kallidin peptides by plasma and glandular kallikrein, respectively, neutrophil elastase may participate with either plasma kallikrein or mast cell tryptase in the generation of bradykinin peptides, particularly at sites of inflammation (1, 20).

In conclusion, we applied HPLC-based RIA to the specific measurement of nonhydroxylated and hydroxylated bradykinin and kallidin peptides and their metabolites in blood and urine. These studies demonstrate differential regulation of the bradykinin and kallidin peptide systems and indicate that blood levels of bradykinin peptides are more responsive to ACE inhibition than blood levels of kallidin peptides.

Perspectives
Quantification of individual bradykinin and kallidin peptides and their metabolites provides information essential for analysis of the pathways of kinin formation. These new methodologies can be applied to study of the differential roles of bradykinin and kallidin peptides in health and disease states. There is a need to identify the mechanisms of kinin formation in different tissue compartments. Our evidence for differential regulation of bradykinin and kallidin peptide levels and their differential modification by ACE inhibition raises the possibility for specific manipulation of endogenous kinin levels to promote the beneficial effects of these peptides while avoiding their harmful effects.

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REFERENCES


