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Bradykinin and des-Arg⁹-bradykinin metabolic pathways and kinetics of activation of human plasma

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Cyr, Mélanie, Yves Lepage, Charles Blais, Jr., Nicole Gervais, Massimo Cugno, Jean-Lucien Rouleau, and Albert Adam. Bradykinin and des-Arg⁹-bradykinin metabolic pathways and kinetics of activation of human plasma. *Am J Physiol Heart Circ Physiol* 281: H275–H283, 2001.—In the serum of 116 healthy individuals, exogenous bradykinin (BK) half-life (27 ± 10 s) was lower than that of des-Arg⁹-BK (643 ± 436 s) and was statistically different in men compared with women. The potentiating effect of an angiotensin-converting enzyme (ACE) inhibitor was, however, more extensive for BK (9.0-fold) than for des-Arg⁹-BK (2.2-fold). The activities of ACE, aminopeptidase P (APP), and kininase I were respectively 44 ± 12 , 22 ± 9 , and 62 ± 10 nmol·min⁻¹·ml⁻¹. A mathematical model ($y = kt^\alpha e^{-\beta t}$, $t > 0$), applied to the BK kinetically released from endogenous high-molecular-weight kininogen (HK) during plasma activation in the presence of an ACE inhibitor, revealed a significant difference in the rate of formation of BK between men and women. For des-Arg⁹-BK, the active metabolite of BK, the rate of degradation was higher in women compared with men, correlating significantly with serum APP activity ($r^2 = 0.6485$, $P < 0.001$). In conclusion, these results constitute a basis for future pathophysiological studies of inflammatory processes where activation of the contact system of plasma and the kinins is involved.

kinins; angiotensin-converting enzyme inhibitors; contact system activation

BRADYKININ (BK) is a nonapeptide released from high-molecular-weight kininogen (HK) under hydrolysis by plasma kallikrein. Plasma kallikrein originates from prekallikrein during the activation of the contact system of plasma by a negatively charged surface (3). This activation has been considered for a long time as the unique mechanism leading to BK release. Recently, however, three different laboratories (10, 23, 35) have described HK activation by plasma kallikrein at the endothelial level.

BK exerts its pharmacological effects, mainly vasodilation, by activating constitutively expressed B₂ receptors. However, the peptide is short-lived in various

biological environments due to proteolytic cleavage. The different purified peptidases capable of degrading BK have been reviewed extensively (15). The importance of their participation in BK metabolism depends, however, on the nature of the biological milieu as well as the pathophysiological or experimental conditions (5, 15). Previously, the laboratory of Adam (13) developed an analytic approach that allowed the metabolism of exogenous BK to be measured in a limited number of human serum samples using the substrate at a nanomolar concentration. Adam and co-workers showed that BK is metabolized by three metallopeptidases. Angiotensin-converting enzyme (ACE) constitutes the main degradation pathway that transforms BK into its final inactive metabolite BK(1–5) (13, 15). The next most important enzyme is aminopeptidase P (APP), which transforms BK into the inactive peptide BK(2–9) (6). This metabolite is further degraded by dipeptidyl peptidase IV into BK(4–9) (6). Finally, kininase I, a generic name for different carboxypeptidases that transform BK into des-Arg⁹-BK (15), constitutes a minor metabolic pathway unless ACE is inhibited. Des-Arg⁹-BK, the active metabolite of BK, stimulates B₁ receptors when expressed during experimental inflammatory conditions (4, 25, 26). In its turn, des-Arg⁹-BK is metabolized by ACE and APP, already involved in BK inactivation. However, in the case of des-Arg⁹-BK, APP represents the main metabolic pathway (6).

Because ACE has a higher affinity for BK than for angiotensin I (21, 22), it is now considered as a kininase rather than an angiotensinase (9, 21, 22). These kinetic characteristics and numerous experimental pieces of evidence, mainly of a pharmacological nature, plead for a role of BK in the cardiovascular and metabolic effects of ACE inhibitors (18, 24, 40). Besides these beneficial effects, ACE inhibitors are also endowed with acute and chronic side effects, among which are angioedema (AE), hypersensitivity reactions (HSR) during hemodialysis, severe hypotensive reaction (SHR) associated with some blood product trans-

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fusions, and cough (16, 20, 42). Although BK has been claimed to be responsible for these side effects, its participation has been scarcely documented, and only poor experimental evidence, if any, suggests such a pathophysiological role for the B₂ agonist. To try to elucidate a potential role of kinins in the pathophysiology and symptoms of these acute and chronic side effects of ACE inhibitors, we (6, 7, 11) investigated BK metabolism in the serum of a limited number of patients who presented such side effects. Patients with a history of an HSR or a SHR associated with an ACE inhibitor exhibited, at the serum level, an increased half-life ($t_{1/2}$) of des-Arg⁹-BK, this kinetic parameter correlating negatively with the APP activity.

The fact that the decreased activity of APP was not attributed to a circulating inhibitor but rather to an enzyme defect (6) required definition of the metabolism of kinins in normal individuals before extending these preliminary studies to larger groups of patients and their relatives who developed ACE inhibitor complications such as AE, HSR, or SHR.

In the present paper, we defined the metabolic profile of BK and its active metabolite, des-Arg⁹-BK, in a large number of healthy individuals (men and women) to obtain reference values. To accomplish these goals, we first applied to these samples the experimental approach we developed previously for a small number of men ($n = 7$) (13). The calculated kinetic parameters were verified by the measurement of metallopeptidase activities (ACE, APP, and kininase I) responsible for the serum metabolism of kinins. In a second part, the kinetics of generation of endogenous BK and des-Arg⁹-BK from HK were assessed when the contact system was activated by glass beads in the presence of an ACE inhibitor. Finally, comparisons were made between the results obtained by the different analytical approaches.

MATERIALS AND METHODS

Blood Samples

Twenty milliliters of blood were obtained by venipuncture from the forearm of 116 healthy Caucasian volunteers (70 women and 46 men: 78 with were <40 yr old and 38 were ≥40 yr old) who were not under any medication (mean age: 33 ± 14 yr, range 19–77 yr). Total blood was collected half into dry tubes and the other half into tubes containing 0.1 mol/l sodium citrate as anticoagulant (1 volume of sodium citrate to 9 volumes of blood). After centrifugation (22°C, 15 min, 2,500 g), the serum and plasma samples were decanted and stored at –20°C until biochemical investigation.

This study was reviewed and approved by the ethics committee for research on human subjects of the Université de Montréal.

Drugs, Peptides, and Reagents

BK and des-Arg⁹-BK were obtained from Peninsula Laboratories (Belmont, CA). The ACE inhibitor enalaprilat was from Merck Frosst Canada (Kirkland, QC, Canada). L-Arginine, dansyl-Ala, and *o*-phthalaldehyde were from Sigma-Aldrich (Oakville, ON, Canada). Arg-Pro-Pro was from Bachem (Torrance, CA). Dansyl-Ala-Arg was a generous gift

from Dr. R. A. Skidgel (Dept. of Pharmacology, University of Illinois, College of Medicine, Chicago, IL). β-Mercaptoethanol and chloroform were purchased from Fisher Scientific (Montréal, QC, Canada). HPLC grade ethanol was procured from American Chemicals (Montréal, QC, Canada).

Metabolism of Exogenous BK and Des-Arg⁹-BK

Incubation procedure. A first set of incubations was performed to assess the metabolic profile of exogenous BK in the absence of in vitro ACE inhibition. Synthetic BK was added to the serum samples (final concentration of the peptide = 471 nM) (13). After various incubation periods at 37°C, ranging from 30 s to 120 min, the reaction was stopped by adding cold anhydrous ethanol at a final concentration of 80% vol/vol. The samples were then incubated at 4°C for 1 h and centrifuged (4°C, 15 min, 3,000 g) for the complete precipitation of kinin precursors. The supernatant was decanted and evaporated to dryness in a Speed Vac Concentrator (Savant; Farmingdale, NY). The residues were stored at –20°C until quantification of the immunoreactive peptides BK and des-Arg⁹-BK was performed.

A second set of incubations was undertaken to define the influence of complete in vitro inhibition of ACE on BK metabolism. Serum was preincubated at 37°C for 20 min with enalaprilat (final concentration = 130 nM) before the addition of exogenous BK. This solution was then incubated and treated in the same manner as described above.

Quantification of BK and des-Arg⁹-BK. The residues of the evaporated ethanolic extracts were resuspended in 50 mM Tris·HCl buffer, pH 7.4, containing 100 mM NaCl and 0.05% Tween 20. After resuspension, residual BK and formed des-Arg⁹-BK were quantified by two specific competitive chemiluminescent enzyme immunoassays, as previously described (12, 32). To detect and quantify immune complexes, both assays used highly specific polyclonal rabbit immunoglobulins raised against the carboxy-terminal end of BK and des-Arg¹⁰-kallidin and the digoxigenin-anti-digoxigenin system (Boehringer Mannheim; Laval, QC, Canada). These methods have been validated, and their analytic performances were reported (4, 8, 12, 32).

Kinetic parameters. The kinin (*S*) hydrolysis rate constant (*k*) was obtained with a first-order equation $S = S_0 \times e^{-kt}$. $t_{1/2}$ was calculated as $t_{1/2} = \ln(2)/k$ (28).

The hydrolysis constant rate without [*k*(–)] or with [*k*(+)] enalaprilat served to estimate ACE relative activity (ACE%) = $100 \times [1 - k(+)/k(-)]$. The relative participation of kininase I in BK metabolism in the absence or the presence of an ACE inhibitor was expressed as the percentage of added BK transformed into des-Arg⁹-BK.

Serum Metallopeptidase Activity Measurement

Serum ACE activity. ACE activity in serum samples was determined by Buhlmann ACE radioenzymatic assay (Angiotensin-Converting Enzyme ³H-REA, ALPCO; Windham, NH) according to the manufacturer's instructions. This assay measured the hydrolysis of the synthetic substrate [³H]hippuryl-glycyl-glycine into [³H]hippuric acid and a dipeptide, solvent extraction separated tritiated hippuric acid from the unreacted substrate, and radioactivity was measured in a beta counter. For each sample, tests were performed in duplicate. ACE activity was expressed as ACE units, as described by the manufacturer (1 unit ACE = 1 nmol of formed [³H]hippuric acid·min⁻¹·ml⁻¹).

Serum APP activity. APP activity was measured in serum samples by a modified version of the assay described earlier by Blais et al. (6) and Simmons and Orawski (36). Briefly, 20

μl of serum were incubated with Arg-Pro-Pro (final concentration = 0.5 mM) in 0.1 M HEPES buffer, pH 7, for a final volume of 200 μl . After an incubation period of 6 h at 37°C, the reaction was stopped by adding cold anhydrous ethanol (800 μl). The samples were then centrifuged (4°C, 15 min, 3,000 g), and the supernatant was decanted and incubated at room temperature (between 10 and 30 min) with 3 ml of the revelation buffer (0.05 M borate buffer, pH 9.5, containing 150 mg/l *o*-phthalaldehyde and 50 $\mu\text{l/l}$ β -mercaptoethanol). Fluorescence emission was read on an Aminco-Bowman Luminescence Spectrometer (Rochester, NY). The excitation and emission wavelengths were 310 and 455 nm, respectively. The emission intensity of a calibration curve with L-arginine (0 to 5 mM) was obtained under the same conditions. The results are expressed as nanomoles of arginine released per minute per milliliter of serum sample.

Serum kininase I activity. Kininase I activity was measured by a modification of the method described by R. A. Skidgel (37). Briefly, 20 μl of serum were incubated at 37°C, for 70 min, with dansyl-Ala-Arg (final concentration: 0.2 mM) in 0.1 M HEPES buffer, pH 7 (total volume: 250 μl). After the incubation period, the reaction was stopped by adding citric acid 1 M (150 μl). Dansyl-Ala was extracted (15 s) by 3 ml of chloroform. After centrifugation (4°C, 10 min, 1,000 g) and removal of the aqueous phase, the fluorescence of the organic phase was measured, as described for APP, using 340 and 495 nm, respectively, as excitation and emission wavelengths. The emission intensity of a calibration curve (dansyl-Ala, 0 to 1 mM) was determined under the same conditions. The results are expressed as nanomoles of dansyl-Ala released per minute per milliliter of serum sample.

Kinetics of Release of Endogenous BK and Des-Arg⁹-BK During Plasma Activation

Contact system activation. After 1 ml of plasma was preincubated with enalaprilat (final concentration = 130 nM) for 20 min at 37°C in polypropylene tubes, the contact system was activated by incubating the plasma with glass beads (37°C, with agitation), using a modification of the method of Renaux et al. (33). The reaction was stopped after various incubation periods (0, 1, 2, 3, 4, 6, 12, 24, 36, 60 min for BK and 0, 3, 6, 12, 18, 24, 36, 48, 60, 120 min for des-Arg⁹-BK) by adding cold anhydrous ethanol at a final concentration of 80% vol/vol. The samples were then treated as described above for the metabolism of exogenous kinins before the quantification of endogenous BK and des-Arg⁹-BK.

Mathematical treatment. For the endogenous measurements obtained at different times t , the mathematical model $y = kt^\alpha e^{-\beta t}$, $t > 0$, was fitted to each subjects. This model with three parameters k , α , and β ($k > 0$, $\alpha \geq 0$, $\beta \geq 0$) corresponds to a form similar to gamma distribution (34). Parameter k represents a scale parameter: large (small) k values are for large (small) values of the endogenous measurements. When α is small (large), the speed in obtaining the maximum is fast (slow), whereas for small (large) values of β , the speed at which the curve decreases to 0 is slow (fast). Parameter α is therefore related to the shape of the first part of the curve (the part that corresponds to formation), whereas parameter β is related to the second part of the curve, corresponding to the degradation.

To study the various curves, different functions of the parameters were calculated. 1) Time of the maximum: the value of time t for which the maximum of the curve was obtained $t = \alpha/\beta$; 2) maximum: the value of the maximum of the curve that corresponds to the value of the curve for $t = \alpha/\beta$; 3) the area: area under the curve that is mathematically

given by $k\Gamma(\alpha + 1)/\beta^{\alpha + 1}$, where $\Gamma(\alpha + 1)$ is the gamma function; 4) half-life of formation (t_f): the value t_f in the interval $0 = \alpha/\beta$ for which $t^\alpha e^{-\beta t} = (0.5)(\alpha/\beta)^\alpha e^{-\alpha}$; 5) half-life of degradation (t_d): the value t_d in the interval α/β to ∞ for which $t^\alpha e^{-\beta t} = (0.5)(\alpha/\beta)^\alpha e^{-\alpha}$; 6) slope of the half-life of formation: the value of the slope of the curve at half-life formation = $ke^{-\beta t} t_f^{\alpha-1}(\alpha - \beta t_f)$; and 7) slope of the half-life of degradation: the value of the slope of the curve at half-life degradation: $ke^{-\beta t} t_d^{\alpha-1}(\alpha - \beta t_d)$.

Statistical Analysis

All data are expressed as means \pm SD. Two-way analysis of variance was performed to study the effect of sex (men and women) and age (<40 yr and \geq 40 yr) on the variables and the parameters of the mathematical model (29). When significant results were observed, a Satterwaite-Welch approach (30) was used to test the possible effect of the heterogeneity of variances on the P value. A P value < 0.05 was considered significant. Reference limits were determined nonparametrically by using the conventional empirical 2.5 and 97.5 percentiles. Pearson correlation and simple linear regression were used to study the linear relationship between certain variables (31).

RESULTS

Metabolism of Exogenous BK and Des-Arg⁹-BK

Half-life of BK and des-Arg⁹-BK. The $t_{1/2}$ of BK and des-Arg⁹-BK, in the absence and presence of enalaprilat, in 116 healthy individuals is presented in Table 1. In the absence of ACE inhibition, BK was rapidly degraded ($t_{1/2} = 27 \pm 10$ s). This value contrasted with the $t_{1/2}$ of des-Arg⁹-BK, which was 24 times higher (mean value 643 ± 436 s). When serum samples were preincubated with enalaprilat at a concentration that completely inhibited ACE activity, the $t_{1/2}$ of BK and des-Arg⁹-BK was increased (244 ± 83 s and $1,410 \pm 1,149$ s, respectively). The potentiating effect of enalaprilat was more pronounced in the case of BK, $t_{1/2}$ being increased by 9.0-fold compared with 2.2-fold for des-Arg⁹-BK. The mean values for des-Arg⁹-BK $t_{1/2}$ (in the absence and presence of enalaprilat) presented elevated SD due to the wide ranges of $t_{1/2}$ without (196 to 2,878 s) and with (330 to 6,704 s) enalaprilat.

For the $t_{1/2}$ of BK and des-Arg⁹-BK without and with enalaprilat, no significant interaction between sex and age was observed and the factor age was not significant. For the factor sex, only the $t_{1/2}$ of BK (in the absence of enalaprilat) was significantly lower in men compared with women (24 ± 8 vs. 30 ± 11 s, $P = 0.0030$).

Relative participation of ACE and kininase I in the metabolism of kinins. The relative contributions of the various metallopeptidases in the degradation of BK and des-Arg⁹-BK in human serum are shown in Table 1. ACE constituted the main enzyme responsible for the BK inactivation accounting for $88 \pm 6\%$ of total BK metabolic transformation. Also, this relative participation of ACE in BK metabolism showed no significant sex-to-age interaction and age effect but was statistically higher in men than in women ($90 \pm 3\%$, $n = 46$ vs. $87 \pm 7\%$, $n = 70$, $P = 0.027$). Kininase I participation

Table 1. Half-life of BK and des-Arg⁹-BK with or without enalaprilat and the relative participation of ACE and kininase I in the metabolism of kinins in the sera of 116 healthy individuals

| | | | | | | | | <i>t</i> _{1/2} , s | |
|-------------------------------------------------|----------|------------------|----------|----------------------------|----------------------|------------------|---------------|-----------------------------|--|
| BK | | | | Des-Arg ⁹ -BK | | | | | |
| Without enalaprilat | | With enalaprilat | | Without enalaprilat | | With enalaprilat | | | |
| 27 ± 10 | | 244 ± 83 | | 643 ± 436 | | 1,410 ± 1,149 | | | |
| Men | Women | Men | Women | Men | Women | Men | Women | | |
| 24 ± 8* | 30 ± 11* | 240 ± 80 | 246 ± 86 | 732 ± 417 | 584 ± 442 | 1,600 ± 1,182 | 1,285 ± 1,118 | | |
| <40 y | ≥40 y | <40 y | ≥40 y | <40 y | ≥40 y | <40 y | ≥40 y | | |
| 27 ± 9 | 28 ± 12 | 238 ± 85 | 255 ± 79 | 700 ± 478 | 525 ± 308 | 1,564 ± 1,280 | 1,093 ± 737 | | |
| Relative Participation of ACE and Kininase I, % | | | | | | | | | |
| BK | | | | Des-Arg ⁹ -BK | | | | | |
| % ACE | | % Kininase I | | % Kininase I + enalaprilat | | % ACE | | | |
| 88 ± 6 | | 11 ± 5 | | 46 ± 23 ^a | | 50 ± 12 | | | |
| Men | Women | Men | Women | Men | Women | Men | Women | | |
| 90 ± 3† | 87 ± 7† | 11 ± 5 | 12 ± 5 | 49 ± 22 ^b | 45 ± 23 ^c | 50 ± 13 | 51 ± 12 | | |
| <40 y | ≥40 y | <40 y | ≥40 y | <40 y | ≥40 y | <40 y | ≥40 y | | |
| 88 ± 5 | 88 ± 7 | 12 ± 5 | 10 ± 5 | 49 ± 23 ^d | 42 ± 22 ^e | 51 ± 22 | 50 ± 12 | | |

Values are means ± SD (men, *n* = 46; women, *n* = 70; <40 y, *n* = 78; ≥40 y, *n* = 38; ^a*n* = 110; ^b*n* = 43; ^c*n* = 67; ^d*n* = 75; ^e*n* = 35 individuals). BK, bradykinin; ACE, angiotensin-converting enzyme; *t*_{1/2}, half-life. **P* = 0.0030; †*P* = 0.0266.

in BK metabolism is minor (11 ± 5%) but became more pronounced (46 ± 23%) when ACE was inhibited. In the case of des-Arg⁹-BK, ACE represented only 50 ± 12% of total degrading activity. The relative participation of both metallopeptidases in the serum metabolism of kinins showed no significant sex-to-age interaction and was not statistically different between both sexes and both categories of age.

Serum metallopeptidase activities. The values obtained for serum ACE, APP, and kininase I are presented in Table 2. For each of these serum activities, no significant interaction sex-to-age was observed, and the factor age was not significant. The difference in ACE activity was highly significant between men (*n* = 46) and women (*n* = 70) (49 ± 13 vs. 41 ± 11 nmol·min⁻¹·ml⁻¹, *P* = 0.0097). Similarly, significantly higher serum APP activity was measured in women (24 ± 9 nmol·min⁻¹·ml⁻¹, *n* = 70) compared with men (19 ± 7 nmol·min⁻¹·ml⁻¹, *n* = 46) (*P* = 0.0292). In contrast, kininase I exhibited similar values in both

groups of men and women (60 ± 10 vs. 63 ± 11 nmol·min⁻¹·ml⁻¹ for men and women, respectively).

Reference intervals. Reference intervals for all variables (*t*_{1/2} of BK with and without enalaprilat, *t*_{1/2} of des-Arg⁹-BK in the presence or absence of enalaprilat, relative participation of ACE and kininase I in the serum metabolism of BK and des-Arg⁹-BK and serum ACE, APP, and kininase I activities) were determined in 116 healthy individuals and are shown in Table 3. When significant differences between men and women

Table 2. Enzyme activities of ACE, APP, and kininase I in the serum of 116 healthy individuals

| ACE, nmol·min ⁻¹ ·ml ⁻¹ | | APP, nmol·min ⁻¹ ·ml ⁻¹ | | Kininase I, nmol·min ⁻¹ ·ml ⁻¹ | |
|--------------------------------------------------|----------|--------------------------------------------------|---------|---------------------------------------------------------|---------|
| 44 ± 12 | | 22 ± 9 | | 62 ± 10 | |
| Men | Women | Men | Women | Men | Women |
| 49 ± 13* | 41 ± 11* | 19 ± 7† | 24 ± 9† | 60 ± 10 | 63 ± 11 |
| <40 y | ≥40 y | <40 y | ≥40 y | <40 y | ≥40 y |
| 44 ± 11 | 44 ± 14 | 21 ± 9 | 23 ± 10 | 61 ± 11 | 62 ± 10 |

Values are means ± SD (men, *n* = 46; women, *n* = 70; <40 y, *n* = 78; ≥40 y, *n* = 38). APP, aminopeptidase. **P* = 0.0006; †*P* = 0.0033.

Table 3. Distribution of variables in a healthy population

| Variable | Total Population (<i>n</i> = 116) | Men (<i>n</i> = 46) | Women (<i>n</i> = 70) |
|------------------------------------------------------------|------------------------------------------|-------------------------|---------------------------|
| <i>t</i> _{1/2} BK, s | | | |
| Without enalaprilat | 15–53 | 15–41 | 15–54 |
| With enalaprilat | 123–478 | | |
| <i>t</i> _{1/2} des-Arg ⁹ -BK, s | | | |
| Without enalaprilat | 236–1,901 | | |
| With enalaprilat | 468–4,473 | | |
| Relative participation of ACE and kininase I for BK | | | |
| % ACE | 68–95 | 83–94 | 68–95 |
| % kininase I | 4–22 | | |
| % kininase I + enalaprilat for des-Arg ⁹ -BK | 9–91 | | |
| % ACE | 27–72 | | |
| Serum enzymatic activities | | | |
| ACE, nmol·min ⁻¹ ·ml ⁻¹ | 23–69 | 26–72 | 23–63 |
| APP, nmol·min ⁻¹ ·ml ⁻¹ | 7–39 | 7–30 | 7–39 |
| Kininase I, nmol·min ⁻¹ ·ml ⁻¹ | 38–80 | | |

Data are reference intervals (2.5–97.5 percentiles of each distribution).

were observed, that is, for the variables $t_{1/2}$ of BK in the absence of enalaprilat, the relative participation of ACE in the metabolism of BK, ACE, and APP activities (see Tables 1 and 2). Separate reference intervals for men and women are presented in Table 3.

Correlation between kinetic parameters and enzyme activities. Table 4 shows the correlation between serum enzymatic activities (ACE and APP) and the $t_{1/2}$ of BK and des-Arg⁹-BK in the absence and presence of enalaprilat. As expected, a significant inverse relationship could be calculated between the measured $t_{1/2}$ of BK in the absence of ACE inhibition and ACE activity ($r = -0.6677$, $P = 0.0001$). Also, significant correlations could be calculated between serum APP activity and the calculated $t_{1/2}$ of BK in the presence and absence of enalaprilat ($r = -0.5206$, $P = 0.0001$ and $r = -0.2433$, $P = 0.0085$, respectively) and the $t_{1/2}$ of des-Arg⁹-BK in the absence ($r = -0.6924$, $P = 0.0001$) and presence ($r = -0.6840$, $P = 0.0001$) of enalaprilat. In addition, the relationship between serum APP activity and the relative contribution of ACE in BK metabolism was also significant ($r = -0.6403$, $P = 0.0001$) (data not shown).

Metabolism of Endogenous BK and Des-Arg⁹-BK

Analysis of the influence of the factors sex and age on the different parameters characterizing the gamma model fitted to endogenous measures in time are showed in Table 5 with means \pm SD. We observe no significant interaction between sex and age. No significant result was obtained for the factor age. Some significant results were obtained between men and women for the α parameter ($P = 0.0005$), the constant k ($P = 0.0001$), time of the maximum ($P = 0.0065$), and slope of formation ($P = 0.0002$) for BK, whereas for des-Arg⁹-BK, the difference in the means between men and women was significant only for the constant k ($P = 0.0080$).

Table 6 represents the reference intervals calculated for the different kinetic parameters. In this case to, no influence of age could be calculated for the synthesis or the degradation of BK and des-Arg⁹-BK. Figure 1 shows the mean kinetic profiles of formation and degradation of bradykinin and des-Arg⁹-bradykinin for men and women after activation of the contact system, in the presence of enalaprilat, with glass beads in normal human plasma.

Table 4. Correlations among APP, ACE activities, and $t_{1/2}$ BK and des-Arg⁹-BK in serum of 116 healthy individuals with and without enalaprilat

| | BK | | Des-Arg ⁹ -BK | |
|-----|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Without enalaprilat | With enalaprilat | Without enalaprilat | With enalaprilat |
| APP | -0.2433 ($P = 0.0085$) | -0.5206 ($P = 0.0001$) | -0.6924 ($P = 0.0001$) | -0.6840 ($P = 0.0001$) |
| ACE | -0.6677 ($P = 0.0001$) | | | |

Data are Pearson correlation coefficient.

Table 5. Parameters characterizing gamma model fitted to endogenous measures

| Parameter | n | BK | Des-Arg ⁹ -BK |
|--------------------------------------------------------|-----|-----------------------|--------------------------|
| α | | | |
| Total | 109 | 0.73 \pm 0.20 | 0.82 \pm 0.21 |
| Men | 40 | 0.82 \pm 0.18* | 0.82 \pm 0.20 |
| Women | 69 | 0.66 \pm 0.19* | 0.82 \pm 0.22 |
| <40 y | 72 | 0.72 \pm 0.21 | 0.80 \pm 0.22 |
| \geq 40 y | 37 | 0.75 \pm 0.20 | 0.86 \pm 0.21 |
| β | | | |
| Total | 109 | 0.18 \pm 0.05 | 0.07 \pm 0.04 |
| Men | 40 | 0.18 \pm 0.05 | 0.06 \pm 0.03 |
| Women | 69 | 0.17 \pm 0.06 | 0.08 \pm 0.05 |
| <40 y | 72 | 0.17 \pm 0.05 | 0.06 \pm 0.04 |
| \geq 40 y | 37 | 0.18 \pm 0.06 | 0.08 \pm 0.04 |
| k | | | |
| Total | 109 | 39,853 \pm 11,335 | 3,825 \pm 2,216 |
| Men | 40 | 33,323 \pm 9,276† | 2,943 \pm 1,265§ |
| Women | 69 | 43,638 \pm 10,729† | 4,336 \pm 2,485§ |
| <40 y | 72 | 39,755 \pm 12,066 | 3,940 \pm 2,217 |
| \geq 40 y | 37 | 40,042 \pm 9,913 | 3,600 \pm 2,234 |
| Time of maximum, min | | | |
| Total | 109 | 4 \pm 1 | 16 \pm 9 |
| Men | 40 | 5 \pm 1 | 18 \pm 8 |
| Women | 69 | 4 \pm 1 | 15 \pm 9 |
| <40 y | 72 | 4 \pm 1 | 17 \pm 10 |
| \geq 40 y | 37 | 4 \pm 1 | 15 \pm 8 |
| Maximum, pg/100 μ l | | | |
| Total | 109 | 54,161 \pm 11,945 | 13,825 \pm 5,687 |
| Men | 40 | 51,243 \pm 13,537 | 13,130 \pm 5,787 |
| Women | 69 | 55,853 \pm 10,657 | 14,227 \pm 5,631 |
| <40 y | 72 | 53,544 \pm 11,597 | 14,250 \pm 5,760 |
| \geq 40 y | 37 | 55,362 \pm 12,672 | 12,997 \pm 5,525 |
| Area, min \cdot pg \cdot 100 μ l ⁻¹ | | | |
| Total | 109 | 783,949 \pm 289,160 | 789,638 \pm 810,010 |
| Men | 40 | 747,440 \pm 249,324 | 815,377 \pm 590,987 |
| Women | 69 | 805,113 \pm 309,689 | 774,716 \pm 917,135 |
| <40 y | 72 | 775,059 \pm 267,087 | 840,793 \pm 857,563 |
| \geq 40 y | 37 | 801,247 \pm 331,188 | 690,092 \pm 708,894 |
| Slope of $t_{1/2}$ of Formation | | | |
| Total | 109 | 25,971 \pm 10,612 | 2,389 \pm 5,604 |
| Men | 40 | 19,886 \pm 5,418‡ | 1,386 \pm 631 |
| Women | 69 | 29,498 \pm 11,297‡ | 2,961 \pm 6,980 |
| <40 y | 72 | 25,890 \pm 10,566 | 1,955 \pm 1,211 |
| \geq 40 y | 37 | 26,126 \pm 10,395 | 3,217 \pm 9,499 |
| Slope of $t_{1/2}$ of degradation | | | |
| Total | 109 | -3,200 \pm 1,108 | -274 \pm 146 |
| Men | 40 | -2,999 \pm 982 | -212 \pm 102 |
| Women | 69 | -3,316 \pm 1,166 | -309 \pm 155 |
| <40 y | 72 | -3,134 \pm 1,143 | -273 \pm 164 |
| \geq 40 y | 37 | -3,328 \pm 1,040 | -275 \pm 102 |

Values are means \pm SD. * $P = 0.0002$; † $P = 0.0018$; ‡ $P = 0.0002$; § $P = 0.0080$.

Comparison Between Exogenous and Endogenous BK Metabolism

Correlations were calculated between the metabolic parameters obtained from different experimental approaches. A highly significant correlation could be measured between the $t_{1/2}$ of des-Arg⁹-BK generated from

both exogenous (x) and endogenous (y) BK in the presence of ACE inhibition ($y = 1004.20 + 1.27x$, $r^2 = 0.8269$, $P < 0.001$). Moreover, a highly significant correlation could also be assessed between the $t_{1/2}$ of endogenous des-Arg⁹-BK (in the presence of enalaprilat) (y) and APP activity (x) ($y = 6005.46 - 146.43x$, $r^2 = 0.6485$, $P < 0.001$).

DISCUSSION

In this paper, we present for the first time a global view of kinetic activation of the contact system of

Table 6. *Distribution of parameters of the model*

| | <i>n</i> | BK | des-Arg ⁹ -BK |
|-------------------------------------|----------|-------------------|--------------------------|
| α | | | |
| Total | 109 | 0.41–1.12 | 0.50–1.27 |
| Men | 40 | 0.51–1.23 | |
| Women | 69 | 0.38–1.06 | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| β | | | |
| Total | 109 | 0.102–0.306 | 0.017–0.166 |
| Men | 40 | | |
| Women | 69 | | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| <i>k</i> | | | |
| Total | 109 | 18,321–63,796 | 1,342–8,876 |
| Men | 40 | 12,931–50,506 | |
| Women | 69 | 23,838–66,836 | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| Time of maximum, min | | | |
| Total | 109 | 2.71–6.86 | 4.52–40.42 |
| Men | 40 | 3.52–6.87 | |
| Women | 69 | 2.67–6.57 | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| Maximum | | | |
| Total | 109 | 30,518–77,727 | 601–30,649 |
| Men | 40 | | |
| Women | 69 | | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| Area, min·pg·100 μl^{-1} | | | |
| Total | 109 | 360,760–1,454,347 | 121,004–3,414,760 |
| Men | 40 | | |
| Women | 69 | | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| Slope formation | | | |
| Total | 109 | 10,346–58,304 | 745–4,757 |
| Men | 40 | 9,835–28,990 | |
| Women | 69 | 14,773–60,147 | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |
| Slope degradation | | | |
| Total | 109 | –539 to –1,495 | –528 to –106 |
| Men | 40 | | |
| Women | 69 | | |
| <40 y | 72 | | |
| ≥40 y | 37 | | |

Data are reference intervals (2.5–97.5% of each distribution).

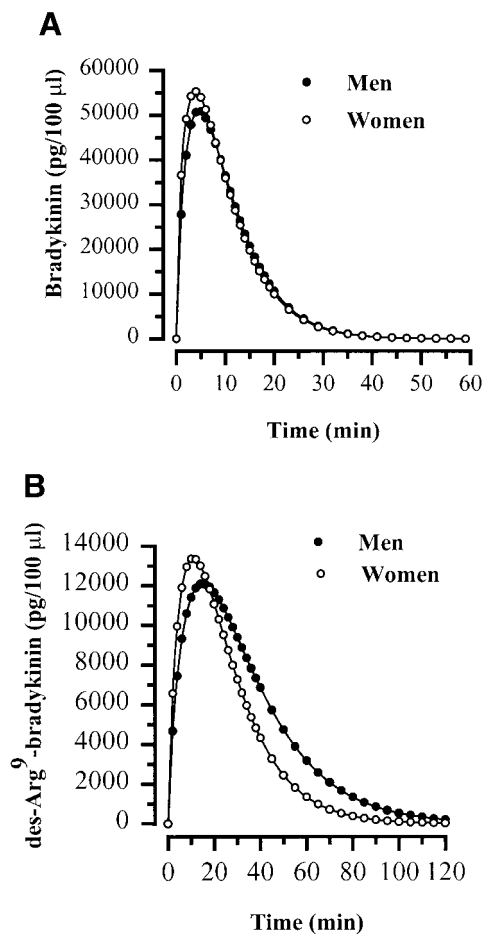


Fig. 1. Mean kinetic profiles of formation and degradation of bradykinin (A) and des-Arg⁹-bradykinin (B) for men and women after activation of the contact system, in the presence of enalaprilat, with glass beads in normal human plasma. Kinetic profile for bradykinin shows a difference between men ($n = 40$) and women ($n = 69$) in the formation part of the curve, whereas for des-Arg⁹-bradykinin a difference is observed at the degradation level.

plasma and the characteristics of both BK and des-Arg⁹-BK metabolic pathways in the blood of a large number of healthy people. We used three different analytic approaches. First, we determined the $t_{1/2}$ of BK and des-Arg⁹-BK in the absence and presence of an ACE inhibitor when BK was added to serum (13). Second, we measured the enzyme activities of metalloproteinases responsible for the BK metabolism in serum (13). Third, we defined the kinetics of release and degradation of endogenous BK and des-Arg⁹-BK when the contact system of plasma was activated with a negatively charged surface (23, 33). Although sex influences the values of several kinetic parameters, no effect of age could be evidenced.

The first experimental approach extends the results obtained previously on a limited number of healthy male individuals ($n = 7$) (13). The kinetics of exogenous kinins studied were performed in serum rather than in plasma. In fact, we have shown previously that the $t_{1/2}$ of BK, measured in plasma anticoagulated with citrate, does not follow first-order kinetics, although the

$t_{1/2}$ of BK and des-Arg⁹-BK is similar in both conditions. With this approach (13), we demonstrated that BK $t_{1/2}$ is significantly higher in women than in men. When sera were preincubated with enalaprilat, this difference was not further observed. In the case of des-Arg⁹-BK, the $t_{1/2}$ was much higher than that of BK. The preincubation of serum with an ACE inhibitor also increased des-Arg⁹-BK $t_{1/2}$, but the potentiating effect was lower than for BK. These results confirm our previous observations showing that ACE, the main degrading pathway of BK, is a secondary pathway for des-Arg⁹-BK (13). Under the same conditions, kininase I is a minor pathway, but its importance increases when ACE is inhibited.

We have previously used this approach to define the metabolism of exogenous kinins in the sera of patients who presented an acute side effect when treated with an ACE inhibitor. In patients who manifested HSR while on an ACE inhibitor and hemodialysis with a negatively charged membrane, we found a significantly higher $t_{1/2}$ of des-Arg⁹-BK but not of BK compared with patients who had never developed such an acute side effect while dialyzed under the same conditions (6). This difference affecting des-Arg⁹-BK degradation was exaggerated when serum samples were preincubated with an ACE inhibitor. A similar anomaly affecting des-Arg⁹-BK metabolism could be observed in patients who presented SHR while treated with an ACE inhibitor and transfused with platelets or red blood cells that had been depleted of leukocytes by passage through a negatively charged membrane (11). When applied to the sera of patients who manifested AE associated with an ACE inhibitor, we could not, however, find a difference compared with healthy subjects (7).

We have reported previously that BK is mainly metabolized in serum by three metallopeptidases, ACE, APP, and kininase I, although des-Arg⁹-BK in its turn is degraded by only ACE and APP. In this study, we observed lower ACE activity in women. This finding must be related to the BK $t_{1/2}$ and the calculated relative participation of ACE. Recently, it was reported that estrogen treatment modulates ACE mRNA concentration in rat tissues, with a resultant attenuation of tissue and serum ACE activity (17). Moreover, a decreased plasma level of ACE activity with a concomitant increase of plasma BK was observed in both hypertensive and normotensive postmenopausal women under hormone replacement therapy (39).

We have previously shown that APP is significantly lower in patients with a history of HSR (HSR+) dialyzed with a negatively charged membrane when compared with patients who did not present such reactions (HSR-), these activities being negatively correlated with des-Arg⁹-BK $t_{1/2}$ calculated in the presence of an ACE inhibitor (6). In this study, we confirmed the highly significant correlation between both parameters. Moreover, the higher activity of APP in women can be related to the fact that this metallopeptidase is encoded by chromosome X (38, 41).

Activation of plasma by its contact with a negatively charged surface is classically used to trigger the intrinsic coagulation pathway (23). An early study measured the kinetic release and degradation of BK (19) but did not consider des-Arg⁹-BK metabolism. This new approach, which reflects the activation of HK by plasma kallikrein, is important for at least two reasons. First, it allows a more physiological understanding of the plasma metabolism of BK and des-Arg⁹-BK than that obtained by spiking serum with exogenous BK, even at a nanomolar concentration. Second, it could allow new insights into the role of *in vivo* activation of the contact system in different pathophysiological conditions where its involvement is suspected (14, 27). To do that we activated the plasma with glass beads, a process that has been reported to be efficient in triggering the contact system (23). This activation is obtained in the presence of an ACE inhibitor to increase the importance of the BK/des-Arg⁹-BK metabolic pathway. The development of a mathematical model permits the analysis of different parameters characterizing the genesis of both endogenous B₁ and B₂ agonists and their degradation. We could observe, for the first time, that men and women differ in their formation of BK, whereas a difference is seen in the degradation of des-Arg⁹-BK. Further studies are necessary to explore the sex difference in the generation of endogenous BK. The difference cannot be attributed to the concentration of HK. In fact, in a precedent study (1), Adam et al. could find no evident difference between men and women for the precursor of BK, and they could not detect a significant difference of plasma prekallikrein activity when assessed by a chromogenic substrate in men and women (2).

In comparison to the results obtained for the exogenous peptide, the $t_{1/2}$ of endogenous des-Arg⁹-BK could be correlated not only with the $t_{1/2}$ of des-Arg⁹-BK generated from exogenous BK but also with APP activity. These observations show for the first time that this metallopeptidase and its sex-related differences are physiologically relevant in the degradation of the endogenous B₁ agonist.

The results presented in this study constitute a new basis for further investigations not only of the BK and des-Arg⁹-BK metabolic pathways but also of the contact system of plasma. First, studies will be needed in the future to examine the pharmacogenetic aspect of side effects of ACE inhibitors. In fact, because APP has been shown to be encoded by chromosome X (38, 41) and because we have previously measured a decreased activity of that enzyme in HSR+ patients (6), we are working on defining the genetic aspects of this side effect. These investigations will also be extended to SHR. For ACE inhibitor-associated AE, in which we previously failed to evidence an anomaly of the exogenous BK/des-Arg⁹-BK metabolic pathways, we cannot exclude a dissociation between the metabolism of exogenous and that of endogenous BK, BK being generated and degraded locally. The kinetic measurement of endogenous BK and of des-Arg⁹-BK during plasma activation will be particularly interesting in this latter

case. Finally, the kinetic release of BK from endogenous HK and its metabolism to des-Arg⁹-BK could open a new area of research to reassess the role of endogenous kinins in different pathological states (14, 27), where activation of the contact system of plasma has been documented.

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