

Angiotensin-Converting Enzyme Inhibitor-Associated Angioedema Is Characterized by a Slower Degradation of des-Arginine⁹-Bradykinin

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ABSTRACT

Angioedema (AE) is a rare but potentially life-threatening side effect of therapy with inhibitors of angiotensin-converting enzyme (ACE), the main bradykinin (BK)-inactivating metalloproteinase in humans. The pathogenesis of ACE inhibitor (ACEi)-associated AE (AE+) is presently unknown, although there is increasing evidence of a kinin role. We analyzed the metabolism of endogenous BK (B₂ receptor agonist) and its active metabolite, des-Arg⁹-BK (B₁ receptor agonist), in the presence of an ACEi during in vitro contact activation of plasma from hypertensive patients (*n* = 39) who presented AE+. Kinetic parameters were compared with those measured in a control group (AE-) of hypertensive patients (*n* = 39) who never manifested any acute or chronic side effects while treated with an

ACEi. The different kinetic parameters were analyzed using a mathematical model ($y = k t^{\alpha} e^{-\beta t}$) previously applied to a normal, healthy population. The slope of BK degradation, but not its formation from high-molecular-weight kininogen, was lower in AE+ patients when compared with the AE- controls. des-Arg⁹-BK accumulation during the kinetic measurements was significantly higher in AE+ plasma. This accumulation of the B₁ agonist in AE+ patients paralleled its half-life of degradation. In conclusion, our results show, for the first time, that an abnormality of endogenous des-Arg⁹-BK degradation exists in the plasma of patients with ACEi-associated AE, suggesting that its pathogenetic mechanism lies in the catabolic site of kinin metabolism.

Angiotensin-converting enzyme inhibitors (ACEi) have been used successfully for 20 years in the treatment of different cardiovascular and metabolic diseases (Unger and Gohlke, 1994). Despite their clinical effectiveness, ACEi have acute side effects, the symptoms of which vary according to the clinical context (Blais et al., 2000). Although rare, these side effects are potentially life-threatening. Anaphylactoid reactions (ARs) in patients treated with ACEi have been reported during hemodialysis with a negatively charged membrane (Verresen et al., 1990), and severe hypotensive reactions have been associated with blood product transfusions or plasma and low-density lipoprotein apheresis (Owen and Brecher, 1994; Fried et al., 1996; Cyr et al., 2001a). Angioedema (AE), another side effect occurring in patients treated with ACEi for hypertension and heart failure, consists of recurrent self-limiting local swellings involving subcutaneous tissues and mucosal layers of the upper airways and bowel. Its reported frequency is apparently similar

to that of AR and severe hypotensive reactions (Israïli and Hall, 1992), although the recent OCTAVE study, involving over 25,000 hypertensive patients, has reported an overall AE incidence higher than that currently admitted. In fact, 0.68% of patients treated with enalapril exhibited an AE episode (Black, 2002). More recently, cases of AE were reported among stroke victims treated with recombinant tissue-type plasminogen activator while concomitantly medicated with an ACEi (Hill et al., 2000).

The clinical symptoms of AE have been attributed to bradykinin (BK) (Israïli and Hall, 1992; Nussberger et al., 1998). BK is a nonapeptide, the prototype of a family of vasodilator peptides, the kinins, released from high-molecular-weight kininogen (HK) during activation of the plasma contact system (Bhoola et al., 1992). BK exerts its pharmacological activities by binding to its B₂ receptor before being metabolized by different peptidases (Hall, 1992). The nature of these peptidases depends on the biological milieu and the pathophysiological background (Decarie et al., 1996; Erdős and Skidgel, 1997). In human plasma, we have shown that BK is

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ABBREVIATIONS: ACEi, angiotensin-converting enzyme inhibitor(s); AR, anaphylactoid reaction; AE, angioedema; AE+, ACEi-associated angioedema; AE-, no ACEi-associated angioedema; BK, bradykinin; HK, high-molecular-weight kininogen; ACE, angiotensin-converting enzyme; APP, aminopeptidase P; AUC, area under the curve; CPN, carboxypeptidase N; des-Arg⁹-BK, des-arginine⁹-bradykinin.

mainly metabolized by three metallopeptidases. Angiotensin-converting enzyme (ACE) and X-Pro aminopeptidase (aminopeptidase P; APP) are, respectively, the first and second inactivating metallopeptidases in importance (Blais et al., 1999, 2000; Cyr et al., 2001b). A third enzyme, carboxypeptidase N (CPN), represents a minor metabolic pathway in the absence of ACE inhibition. It is responsible for the transformation of BK into its active metabolite, des-arginine⁹-bradykinin (des-Arg⁹-BK). This metabolite has a poor affinity for B₂ receptors but interacts with B₁ receptors, the synthesis of which is dramatically increased in experimental models of inflammation (Marceau et al., 1998). The pharmacological activities of des-Arg⁹-BK, similar to those of BK, are short-lived because of its breakdown by two metallopeptidases already involved in the inactivation of BK: ACE and APP. In this case, however, APP represents the main inactivating pathway in plasma (Cyr et al., 2001b).

Although an increase of plasma BK concentrations during the acute phase of ACEi-induced AE was reported recently (Nussberger et al., 1998), the metabolism of endogenous kinins has yet to be documented in these patients.

The objective of the present study was to define the metabolism of endogenous BK and its active metabolite, des-Arg⁹-BK, in the plasma of hypertensive patients who presented with ACEi-associated AE (AE+). For this purpose, we applied to these samples an analytical approach that we developed recently for a large population of normal, healthy people (Cyr et al., 2001b). The calculated kinetic parameters characterizing this metabolism have been compared with those measured for the plasma of patients who never showed any acute or chronic ACEi side effects (AE-).

Materials and Methods

Patients

Venous blood was obtained from 39 hypertensive patients (20 men, 19 women) who presented with clinically documented AE+. These patients were from the University of Milan (Milan, Italy), the Academische Ziekenhuis (Leuven, Belgium), and Hôpital du Sacré-Coeur (Montréal, QC, Canada). All the patients were white; their age ranged from 40 to 78 years; and they received enalapril, quinapril, ramipril, fosinopril, or captopril to treat systemic hypertension. AE occurred between 1 day and 8 years after the initiation of therapy and affected the tongue, lips, face, eyelids, and buccal mucosa. At the time of AE, patients were treated with histamine receptor H₁ and H₂ blockers, epinephrine, and steroids. At the time of blood sampling (between 1 and 70 months after AE episodes), ACEi therapy had been discontinued except in 5 patients who presented recurrent (two to six) episodes before ACEi therapy was discontinued.

Control plasma was obtained from 39 white hypertensive patients (with similar mean age and sex proportion) who never developed any acute (AE, AR, severe hypotensive reactions) or chronic (cough, diarrhea or gastrointestinal disturbance, peripheral edema) side effects while treated with an ACEi. AE- patients were from the University of Milan, the Academische Ziekenhuis, and the Centre Hospitalier de l'Université de Montréal (Montréal, QC, Canada). At the time of blood sampling, these patients were still treated with an ACEi. All patients from the different centers were selected according to the same questionnaire.

Blood Samples

This study was reviewed and approved by the ethics committee for research on human subjects from the teaching hospitals of the Uni-

versities of Montréal, Milan, and Leuven, and informed consent was obtained from all patients.

Twenty milliliters of blood were sampled by venipuncture from the forearm 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, 2500g), the plasma samples were decanted and stored at -80°C until biochemical investigation.

Drugs, Peptides, and Reagents.

BK and des-Arg⁹-BK were acquired from Peninsula Laboratories (Belmont, CA). The ACEi enalaprilat was obtained from Merck Frosst Canada (Kirkland, QC, Canada). High-pressure liquid chromatography-grade ethanol was obtained from American Chemicals (Montréal, QC, Canada).

Metabolism of Endogenous BK and des-Arg⁹-BK

Contact System Activation. Plasma was activated as described earlier for normal healthy people (Cyr et al., 2001b). Briefly, 1 ml of plasma was preincubated with enalaprilat for 20 min at 37°C in polypropylene tubes at a concentration (130 nM) that totally inhibits ACE activity. The contact system was then activated by incubation of the plasma with glass beads (37°C, with agitation). The reaction was stopped after various incubation periods (0–60 min for BK, and 0–120 min for des-Arg⁹-BK) by adding cold anhydrous ethanol at a final concentration of 80% (v/v). The samples were then incubated at 4°C for 1 h and centrifuged (4°C, 15 min, 3000g) for the complete precipitation of kinin precursors. The supernatant was decanted and evaporated to dryness in a SpeedVac concentrator (Thermo Savant, Holbrook, NY). The residues were stored at -80°C until quantification of the immunoreactive peptides BK and des-Arg⁹-BK.

Quantification of BK and des-Arg⁹-BK

The residues of 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 enzyme immunoassays, as described previously (Decarie et al., 1994; Raymond et al., 1995). These methods have been validated and their analytical performances reported (Blais et al., 2000).

Mathematical Treatment

The following mathematical model, $y = k t^\alpha e^{-\beta t}$, $t > 0$, was fitted to the concentrations of endogenous BK and des-Arg⁹-BK measured at different times (t) for each AE+ and AE- subject. This three-parameter (k , α , and β ; $k > 0$, α and $\beta \geq 0$) model corresponds to a form similar to gamma distribution (Rice, 1995) and has been described and validated earlier (Cyr et al., 2001b). The α and β parameters are, respectively, related to the shape of the first and the second part of the curve corresponding to the formation and the degradation of each peptide. These α and β parameters allow the calculation of other kinetic parameters: time of the maximum, the value of t for which the maximum of the curve was obtained $t = \alpha/\beta$; maximum, the value of the maximum of the curve, which corresponds to the value of the curve for $t = \alpha/\beta$; AUC, the area under the curve, which is mathematically given by $k\Gamma(\alpha + 1)/\beta^{\alpha+1}$, where $\Gamma(\alpha + 1)$ is the gamma function; half-life of formation (t_f), the value t_f in the interval 0 to α/β for which $t^\alpha e^{-\beta t} = (0.5)(\alpha/\beta)^\alpha e^{-\alpha}$; half-life of degradation (t_d), the value t_d in the interval α/β to ∞ for which $t^\alpha e^{-\beta t} = (0.5)(\alpha/\beta)^\alpha e^{-\alpha}$; slope of the half-life of formation, the value of the slope of the curve at half-life formation $= ke^{-\beta t_f} t_f^{\alpha-1}(\alpha - \beta t_f)$; and slope of the half-life of degradation, the value of the slope of the curve at half-life degradation: $ke^{-\beta t_d} t_d^{\alpha-1}(\alpha - \beta t_d)$.

Statistical Analysis

The means of the parameters of the two groups (AE+ and AE-) were compared, using a t test with the Satterwaite-Welch approach and taking into account the possible heterogeneity of variances

(Neter et al., 1996). p values less than 0.05 were considered statistically significant.

Results

Plasma Metabolism of Endogenous BK and des-Arg⁹-BK from AE+ and AE- Patients. Figure 1 illustrates the comparative profiles of the patient means for the synthesis and degradation of BK and its active metabolite, des-Arg⁹-BK, measured during the activation of AE+ and AE- plasma in the presence of an ACEi, with the mean reference population profile published earlier (Cyr et al., 2001b). The mathematical model parallels the actual measured concentrations and illustrates a clear difference in the kinetic profiles of the B₁ receptor agonist, des-Arg⁹-BK, between AE+ and AE- patients.

For BK, no difference between AE+ and AE- plasma could be calculated for the different kinetic parameters (α , t_b , slope) characterizing the ascending part of the curve. The latter represents the kinetics of generation of this peptide from HK (Ta-

ble 1). The β parameter and its slope, which reflects the catabolism of the B₂ agonist by APP and kinase I in the presence of ACE inhibition, were lower ($p = 0.022$ and $p = 0.016$, respectively) in AE+ samples compared with the AE- control group. However, no difference could be detected for the maximal concentration and total amount of BK formed, as reflected by a similar AUC during the 60-min observation period.

The main metabolic differences between AE+ and AE- patients were calculated for des-Arg⁹-BK. These results (Table 1) are illustrated in Figs. 1 and 2. The AUC, reflecting des-Arg⁹-BK accumulation during the 120-min incubation period, was significantly higher in the AE+ group ($p = 0.005$). Similarly, a higher maximal concentration of the peptide ($p = 0.001$), which was also delayed in time ($p = 0.030$), was observed for these patients. These anomalies are related to an important difference affecting the degradation of the B₁ receptor agonist, as reflected in a lower β value ($p < 0.001$) and a higher half-life of degradation, t_d ($p = 0.001$), in the AE+ group. Although the α value was lower for AE+ sam-

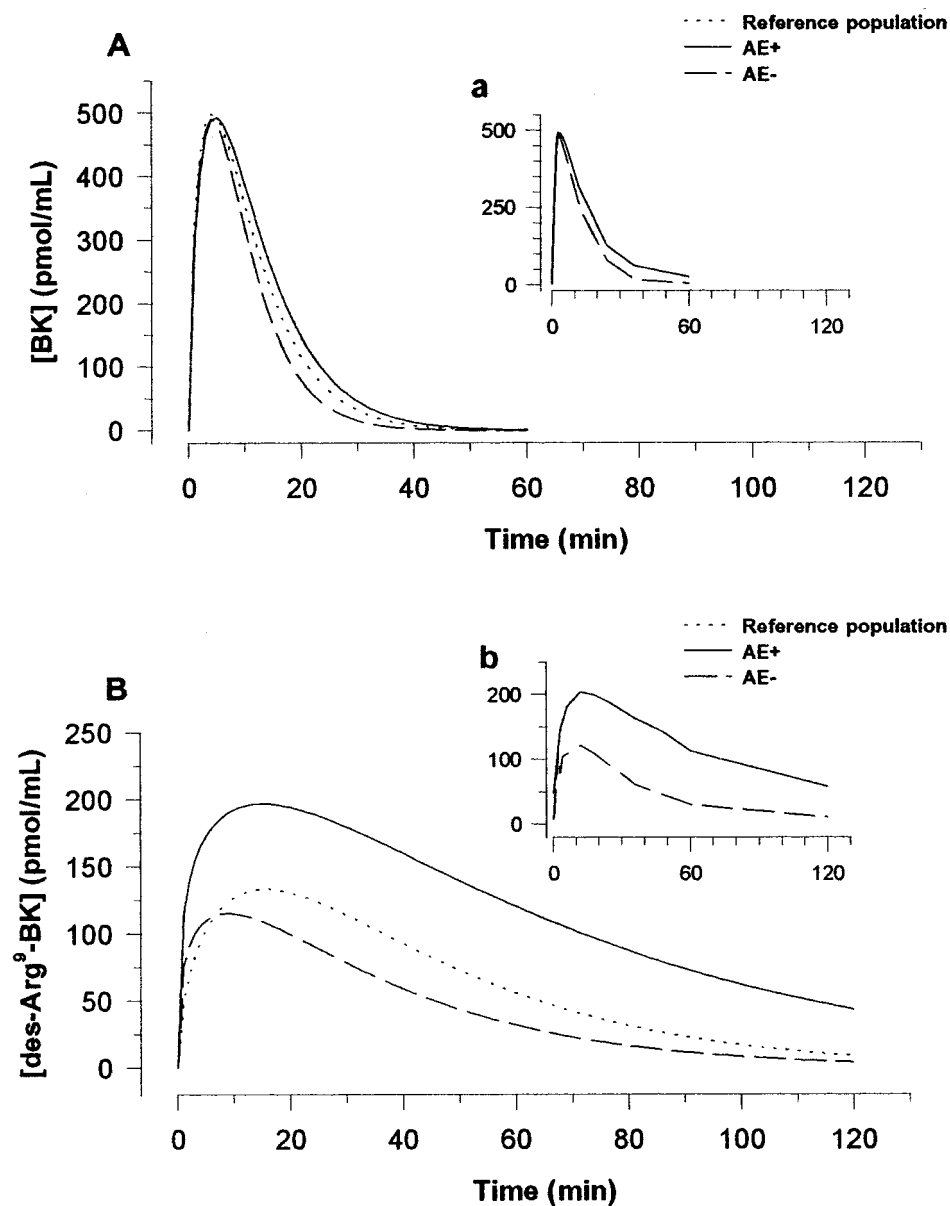


Fig. 1. Kinetic gamma model-fitted profiles of formation and degradation of BK (A) and des-Arg⁹-BK (B) for mean AE+ (solid line) and AE- (dashed line) patients after activation of the contact system, in the presence of enalaprilat, with glass beads. Dotted lines are values for reference population. Superscript graphs (a and b) are actual measured value profiles.

TABLE 1

Parameters characterizing the gamma model fitted to endogenous measurements of kinins
Data represent means \pm S.D.

Parameter	BK (<i>n</i> = 39)		des-Arg ⁹ -BK (<i>n</i> = 39)	
	AE-	AE+	AE-	AE+
α	0.94 \pm 0.53	0.75 \pm 0.31	0.85 \pm 0.35	0.62 \pm 0.33
	<i>p</i> = 0.055		<i>p</i> = 0.004	
Slope of the $t_{1/2}$ of formation	22,992 \pm 7,572	27,105 \pm 31,838	2.65 E10 \pm 1.65 E11	5.48 E7 \pm 3.17 E8
	<i>p</i> = 0.435		<i>p</i> = 0.325	
$t_{1/2}$ of formation (min)	0.86 \pm 0.29	0.82 \pm 0.49	2.32 \pm 1.26	2.61 \pm 2.47
	<i>p</i> = 0.725		<i>p</i> = 0.526	
Maximum (nM)	500 \pm 138	510 \pm 207	131 \pm 56	231 \pm 165
	<i>p</i> = 0.809		<i>p</i> = 0.001	
Time of maximum (min)	4.2 \pm 0.8	6.1 \pm 8.1	12.1 \pm 6.6	16.9 \pm 11.3
	<i>p</i> = 0.152		<i>p</i> = 0.030	
β	0.24 \pm 0.17	0.16 \pm 0.08	0.08 \pm 0.04	0.05 \pm 0.03
	<i>p</i> = 0.022		<i>p</i> < 0.001	
Slope of the $t_{1/2}$ of degradation	-3,877 \pm 2,117	-2,825 \pm 1,604	-293 \pm 143	-309 \pm 298
	<i>p</i> = 0.016		<i>p</i> = 0.755	
$t_{1/2}$ of degradation (min)	12.2 \pm 2.7	25.2 \pm 61.8	36.9 \pm 23.5	59.6 \pm 34.7
	<i>p</i> = 0.191		<i>p</i> = 0.001	
AUC (pmol/ml·60 min or 120 min)	6,305 \pm 2,247	16,175 \pm 48,333	5,660 \pm 5,214	1.64 E6 \pm 2.26 E6
	<i>p</i> = 0.207		<i>p</i> = 0.005	

ples, no significant differences could be measured for the $t_{1/2}$ of formation and its slope.

Comparison of des-Arg⁹-BK Metabolism in AE+ Patients, AE- Patients, and a Reference Population. The kinetic parameters of des-Arg⁹-BK metabolism were different for AE+ and AE- plasma and have been compared with the mean values calculated earlier for the reference population. As illustrated in Fig. 2, significantly higher values in AE+ samples were calculated for the AUC (*p* = 0.030), for the maximal concentration of peptide generated (*p* = 0.008), and the t_d half-life of degradation (*p* = 0.036). The β value was significantly lower in AE+ samples (*p* = 0.004). For the same parameters in AE- patients, significantly lower values were calculated for the AUC (*p* = 0.012) and the half-life of degradation t_d (*p* = 0.032). The β value was significantly higher (*p* = 0.039). No significant difference with the reference population could be calculated for the various kinetic parameters characterizing des-Arg⁹-BK formation in the AE+ and AE- groups.

Influence of the Time of AE Occurrence and the Time of Blood Sampling. The influence of the time interval between the start of medication and the AE episode on the kinetic parameters characterizing des-Arg⁹-BK metabolism in the AE+ group was considered at three levels: 1 month or less, 1 year or less, and more than 1 year. One-way analysis of variance did not allow the measurement of significant differences in β values among these three time intervals. Similarly, we could not measure a significant effect of the time between AE and blood sampling (1 year or less, 2 years or less, or more than 2 years).

Discussion

In this article, we provide evidence, for the first time, of an anomaly affecting the degradation of endogenous kinins, mainly of des-Arg⁹-BK, in the plasma of patients who presented ACEi-associated AE. These results must be discussed

in light of our previous observations on the same patients, showing a significant decrease of APP activity, but no difference of CPN activity in AE+ patients when compared with the AE- control group and the reference population (Adam et al., 2002).

The experimental approach used in this paper has been developed recently in our laboratory and validated by application to a large number of normal, healthy people (Cyr et al., 2001b). It uses glass beads, a well known activator of the plasma contact system (Kaplan et al., 1998). The kinetic studies in AE+ and AE- groups of plasma were performed in the presence of an ACEi to mimic what would happen in vivo in the plasma of patients treated with such a drug. ACEi also increases the transformation of BK into des-Arg⁹-BK, which otherwise represents a minor metabolic pathway in humans (Decarie et al., 1996; Cyr et al., 2001b). We assessed the pharmacokinetic characteristics of this activation. The release and the degradation of endogenous BK and des-Arg⁹-BK formed during the activation process have been measured, using highly sensitive and specific immunoassays developed in our laboratory (Decarie et al., 1994; Raymond et al., 1995). These assays employ highly specific antibodies to the carboxy-terminal end of both peptides, which is responsible for B₂ (BK) and B₁ (des-Arg⁹-BK) pharmacological activity, respectively.

Under our experimental conditions, we did not find evidence of any abnormality in the formation of BK from HK. In fact, the α , t_r , and slope of the t_r parameters, the values of which are related to formation of the peptide, were similar in both AE+ and AE- plasma. These observations argue against a quantitative or qualitative defect in one of the constituents of the contact system (Factor XII, prokallikrein, or HK). Also unlikely is a defect of antiproteases responsible for the control of this system, even though quantitative or qualitative defects of C1 esterase inhibitor have been associated with hereditary angio-neurotic edema (Agostoni and Cicardi, 1992).

The degradation of BK in the presence of an ACEi, how-

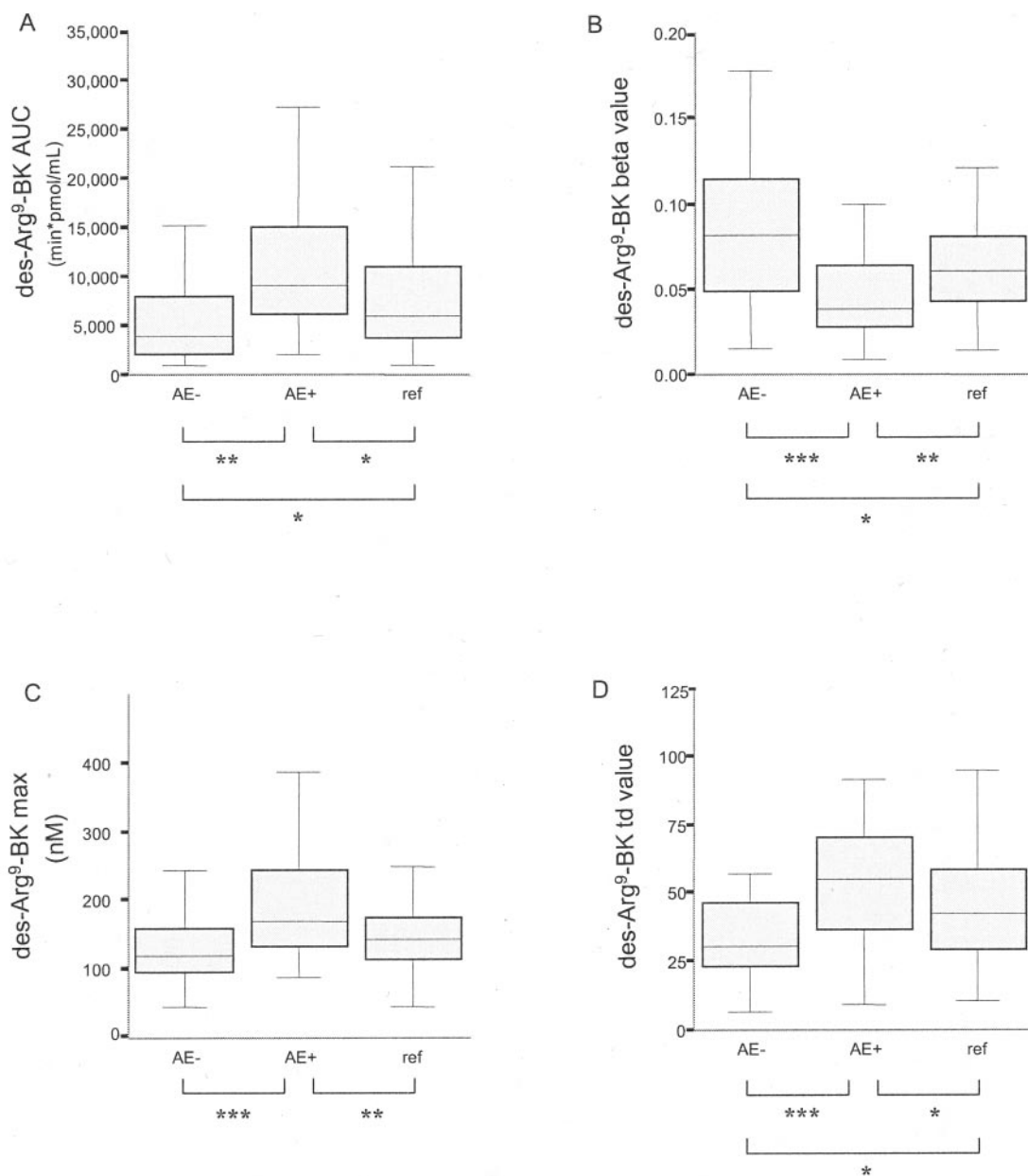


Fig. 2. Panels representing des-Arg⁹-BK area under the curve (panel A), des-Arg⁹-BK β values (panel B), des-Arg⁹-BK maximal concentration (panel C), and des-Arg⁹-BK t_d value (panel D). Values measured in AE+ and AE- patients ($n = 39$), both compared with the reference population value ($n = 109$), are shown. Box plots display summary statistics for the distribution. The lower boundary of the box is the 25th percentile and the upper boundary is the 75th percentile. The line in the box represents the median. The largest and smallest values that are not outliers are also shown. Lines are drawn from the ends of the box to these values. *, $p < 0.05$; **, $p < 0.01$; ***, $p \leq 0.001$.

ever, is statistically slower in AE+ than in AE- control patients. Despite significant p values, these differences are not sufficient to lead to an increased concentration of BK during the activation period. Our observations may be explained by the fact that even in the presence of a decreased activity of APP and ACE inhibition, BK is still transformed into des-Arg⁹-BK by CPN.

The accumulation of des-Arg⁹-BK, as assessed by the AUC, is significantly higher in AE+ when compared with AE- plasmas. As CPN activities are similar in both groups of samples, this increase of B₁ agonist concentration is a consequence of a decrease of its metabolism by APP, a pivotal degrading enzyme in the presence of an ACEi (Adam et al., 2002). Contrary to our observations with BK, the differences

affecting inactivation of the B₁ agonist are strongly significant, as reflected by the p values near and below 0.001 for β and the half-life of degradation (t_d). These strong differences explain the much more pronounced accumulation of des-Arg⁹-BK during the activation of AE+ plasma.

Our in vitro observations are physiologically relevant. In fact, we have previously evidenced in vivo that an accumulation of immunoreactive des-Arg⁹-BK parallels a proinflammatory effect mediated by the B₁ receptors (Blais et al., 1997). Although the pharmacological role of B₁ agonist and its receptors has been characterized in different experimental models, such a role in human pathology has been poorly defined (Marceau et al., 1998). We have, however, recently described an anomaly in the degradation of exogenous des-Arg⁹-BK added to the plasma

of patients who presented an AR while treated with an ACEi and dialyzed with a negatively charged membrane (Blais et al., 1999). It is well known that these dialyzed patients are chronically inflamed and exhibit high concentrations of blood proinflammatory cytokines, well known to induce the B₁ receptor in animals (Pertosa et al., 2000). Although both B₁ and B₂ kinin receptor subtypes exhibit some structural homology, similar signaling pathways and similar pharmacological consequences, functional responses show two main differences (Faussner et al., 1999). On the one hand, B₁ receptors are inducible, whereas B₂ receptors are constitutively present. On the other hand, some evidence now exists for an agonist-induced temporary desensitization of B₂ receptors involving receptor phosphorylation and endocytosis (Blaukat et al., 1996; Faussner et al., 1999). Furthermore, some recent evidence suggests that chronic ACE inhibition itself induces functional vascular and renal B₁ receptor expression, possibly involving homologous up-regulation (Marin-Castano et al., 2002). Another group has also reported that enalaprilat and other ACEi could directly activate human B₁ receptors, even in the absence of an exogenous B₁ receptor agonist (Ignjatovic et al., 2002). In this case, however, the presence of endogenous kinin was not documented.

As plasma BK has been previously shown to be increased during the acute episode of AE (Nussberger et al., 1998), this peptide could initiate the inflammatory process via the B₂ receptor, thereafter relayed by des-Arg⁹-BK and stimulating its B₁ receptor. These findings do not mean that des-Arg⁹-BK is necessarily the only mediator of AE. In fact, some pharmacological evidence suggests that kinins could lead to the local release of neurokinins, particularly the sensory neuropeptide substance P (Ferreira et al., 2000). Interestingly, in this regard, a decrease in dipeptidyl peptidase IV activity, a substance P-degrading enzyme, was also reported in a limited number of hypertensive patients during ACEi-associated AE (Lefebvre et al., 2002). Thus, a multifactorial nature of ACEi-associated AE is expected and could explain its rarity. This side effect results from the gathering of at least three different factors: pharmacological (ACEi treatment), metabolic, and triggering factors. Our data clearly show an anomaly in the degradation of endogenous des-Arg⁹-BK in the plasma of patients with ACEi-associated AE, suggesting that its pathogenetic mechanism lies in the catabolic site of kinin metabolism. However, the triggering factors responsible for *in vivo* kinin release remain to be defined. In this regard, recently reported AE associated with recombinant tissue-type plasminogen activator used in stroke (Francis et al., 1991; Hill et al., 2000), with its capacity to activate the kinin-forming cascade *in vitro* (Molinario et al., 2002), could bring new insights.

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