Inhibition of Aminopeptidase P Potentiates Wheal Response to Bradykinin in Angiotensin-Converting Enzyme Inhibitor-Treated Humans

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ABSTRACT

Bradykinin is a nonapeptide that contributes to the cardioprotective effects of angiotensin-converting enzyme (ACE) inhibitors. During ACE inhibition, an increased proportion of bradykinin is degraded through non-ACE pathways. Studies in animals suggest that aminopeptidase P (EC 3.4.11.9) may contribute to the metabolism of bradykinin. The purpose of the present study was to determine the contribution of aminopeptidase P to the degradation of bradykinin in humans in the presence and absence of ACE inhibition. To do this, we measured the wheal response to intradermal injection of bradykinin (0, 1, or 10 μg) in the presence or absence of intradermal administration of the specific aminopeptidase P inhibitor apstatin (5 or 10 μg) and oral administration of the ACE inhibitor quinapril (10 mg) in six healthy subjects. Both bradykinin (ANOVA; F = 101.18, P < .001) and apstatin alone (F = 7.01, P = .049) caused a wheal of dose-dependent size. There was no significant interaction between apstatin and bradykinin (F = 4.94, P = .175). Pretreatment with 10 mg of quinapril significantly shifted the dose-response curve for bradykinin to the left (effect of quinapril; F = 77.96, P < .001) and there was significant interaction between quinapril and bradykinin (F = 7.82, P = .041). The effect of quinapril was significantly potentiated by coinjection of 10 μg of apstatin (effect of apstatin; F = 21.60, P = .006), such that there was significant interactive effect of quinapril and apstatin (F = 20.83, P = .006) on the wheal response to bradykinin. Collectively, these data suggest that aminopeptidase P plays a minor role in the degradation of bradykinin in human skin in the absence of ACE inhibition but contributes significantly to the degradation of bradykinin in the presence of ACE inhibition.

Abbreviation: ACE, angiotensin-converting enzyme.

Bradykinin is a nonapeptide produced locally in the heart and kidney that exhibits potent cardioprotective effects through its effects on nitric oxide, prostaglandins, and endothelium-derived hyperpolarizing factor (Vanhoutte, 1989). Studies in intact animals and in animal and human sera suggest that angiotensin-converting enzyme (ACE) accounts for 50 to 85% of bradykinin-degrading activity, depending on the species studied (Sheikh and Kaplan, 1989; Decarie et al., 1996; Ersahin and Simmons, 1997). Drugs that inhibit ACE are widely used in the treatment of hypertension, diabetic nephropathy, congestive heart failure, and postmyocardial infarction, and bradykinin contributes significantly to their vascular effects (Brown and Vaughan, 1998). During ACE inhibition, an increased proportion of bradykinin is degraded through non-ACE pathways (Ishida et al., 1989). One such enzyme involved in the degradation of bradykinin, aminopeptidase P (X-Pro aminopeptidase; EC 3.4.11.9), inactivates bradykinin by hydrolyzing the N-terminal Arg1-Pro2 bond (Ryan et al., 1968; Yaron and Naider, 1993).

Recently, the role of aminopeptidase P in the degradation of bradykinin has been evaluated in intact rats (Kitamura et al., 1995, 1999) and isolated tissue preparations (Ryan et al., 1994; Prechel et al., 1995; Ersahin and Simmons, 1997) with the specific aminopeptidase P inhibitor apstatin. Apstatin inhibits rat lung membrane-bound aminopeptidase P with an IC50 value of 4.1 μM and human membrane-bound aminopeptidase P with an IC50 value of 2.9 μM and fails to inhibit a variety of membrane-bound peptidases or bradykinin-degrading enzymes. The IC50 values for aminopeptidase M and dipeptidyl-peptidase IV are 600 and 1100 μM, respectively. The IC50 values are >800 μM for aminopeptidase A, ACE, dipeptidyl-peptidase I-like activity, bestatin-sensitive/amastatin-insensitive membrane dipeptidase, microsomal dipeptidase, neutral endopeptidases 24.11 and 24.15, and prolyl oligopeptidase (Prechel et al., 1995). Studies with apstatin suggest that aminopeptidase P accounts for 30% of degradation of bradykinin in both the pulmonary (Prechel et al., 1995) and coronary (Ersahin and Simmons, 1997) circu-
lation of the rat. Similarly, apstatin potentiates the blood pressure response to infused bradykinin in intact rats, contributes to blood pressure reduction in hypertensive rats, and protects against ischemia/reperfusion injury in the isolated rat heart (Kitamura et al., 1995, 1999; Ershahin et al., 1999).

The contribution of aminopeptidase P to the degradation of bradykinin in humans is not known. Human aortic endothelial cells express aminopeptidase P (Ryan et al., 1996). Determining if aminopeptidase P contributes to the degradation of bradykinin in humans may have important clinical implications. For example, a defect in a non-ACE pathway of bradykinin degradation could account for the development of ACE inhibitor-associated angioedema in some patients. In addition, non-ACE enzymes involved in the degradation of bradykinin represent potential targets for cardioprotective drugs. The purpose of the present study was to determine whether aminopeptidase P contributes to degradation of bradykinin in humans. To do this, we measured the wheal response to intradermal injection of bradykinin in the presence or absence of the aminopeptidase P inhibitor apstatin. A similar strategy has been used previously to define the role of ACE in the degradation of bradykinin in humans.

Materials and Methods

Subjects. Six healthy subjects were studied. Subjects were asked to refrain from use of nonsteroidal anti-inflammatory drugs, vasoactive substances (e.g., over-the-counter sympathomimetics), or anti-histamines for at least 3 days before either study day. All subjects gave written informed consent, and the study was approved by the Institutional Review Board of Vanderbilt University.

Drugs. The trifluoroacetate salt of apstatin, N-[2S,3R]-3-aminoo-2-hydroxy-4-phenylbutanoyl-l-prolyl-l-prolyl-l-alaninamide (Sigma Chemical Co., St. Louis, MO), and bradykinin (Sigma Chemical Co.) were sterilized, lyophilized, and tested for pyrogenicity in the Vanderbilt University Pharmacy. On the morning of each study day, apstatin and bradykinin were dissolved in 0.9% sterile normal saline to yield concentrations twice the desired final study concentration. Equal volumes of the apstatin and bradykinin solutions were then mixed to yield the final solution. One hundred microliters of this solution was injected intradermally at three different sites, separately with a tuberculin syringe and a 27.5-gauge needle. Quinapril 10-mg tablets (Parke Davis, Morris Plains, NJ) were obtained from the Vanderbilt University Pharmacy.

Protocol. All subjects participated in three study days, separated by at least 1 day. The doses of bradykinin and apstatin administered in random order on these first two study days were as follows: vehicle alone, 1 μg of bradykinin + vehicle, 10 μg of bradykinin + vehicle, 5 μg of apstatin + vehicle, 1 μg of bradykinin + 5 μg of apstatin, 10 μg of bradykinin + 5 μg of apstatin + vehicle, 1 μg of bradykinin + 10 μg of apstatin, and 10 μg of bradykinin + 10 μg of apstatin. Each dose was administered in a total volume of 100 μl. Doses were administered intradermally at three different sites, separated by at least 3 cm, on the volar aspect of each forearm. On the third study day, apstatin and bradykinin were administered intradermally 1 h after oral administration of the ACE inhibitor quinapril (10 mg). The 1-h time interval was chosen so that plasma quinapril concentrations would be maximal at the time of wheal measurement (Olson et al., 1989). The doses of bradykinin and apstatin administered in random order on the third study day were as follows: vehicle alone, 1 μg of bradykinin + vehicle, 10 μg of bradykinin + vehicle, 10 μg of apstatin + vehicle, 1 μg of bradykinin + 10 μg of apstatin, and 10 μg of bradykinin + 10 μg of apstatin.

Following intradermal injection of drugs on each study day, wheal size was measured at 15 and 20 min. Pilot studies revealed that wheal size peaked at 15 min and that there was no effect of treatment on time-to-peak wheal size or on the duration of the wheal response. The edge of the wheal was demarcated with a ball-point pen and this outline was transferred to a piece of paper with acetate tape. Wheal area was taken as the average of three measurements made with a Jandel Scientific Graphic Digitizer (model 2210; Jandel Scientific Corp., San Rafael, CA) and Sigma-Scan (version 3.90; Jandel Scientific Corp.).

Statistical Analysis. Data are presented as means ± S.E. The effect of apstatin on the wheal response to bradykinin was compared with repeated-measures ANOVA in which within-subject factors were bradykinin dose, apstatin dose, and the presence or absence of quinapril. Post hoc comparisons were made with paired t tests, as appropriate. A two-sided P value <.05 was considered significant.

Results

Subject Characteristics. Table 1 provides the characteristics of the subjects who participated in the study. Mean arterial pressure measured 1 h after oral administration of 10 mg of quinapril was significantly lower compared with baseline (P < .05). Following intradermal injection of bradykinin and/or apstatin during treatment with quinapril, three subjects reported facial flushing. One of these subjects developed tachycardia lasting several minutes. Heart rate increased from 56 beats/min to 135 beats/min without significant change in blood pressure.

Effect of Aminopeptidase P Inhibitor and ACE Inhibitor on Wheal Response to Bradykinin. Figure 1 shows the effect of apstatin on the wheal response to bradykinin at 15-min postinjection. Bradykinin (ANOVA; F = 101.18, P < .001) caused a dose-dependent wheal. Apstatin increased wheal size in an additive fashion (F = 7.013, P = .049). However, there was no significant synergistic interaction between apstatin and bradykinin (F = 4.94, P = .175). Figure 2 shows the effect of apstatin, quinapril, or both on the dose-response curve for bradykinin. Pretreatment with 10 mg of quinapril significantly shifted the dose-response curve for bradykinin to the left (effect of quinapril; F = 77.96, P < .001) and there was significant interaction between quinapril and bradykinin (F = 7.82, P = .041). This effect of quinapril was significantly potentiated by injection of 10 μg of apstatin (effect of apstatin; F = 21.60, P = .006), such that there was significant interaction between quinapril and apstatin (F = 20.83, P = .006).

Discussion

Aminopeptidase P is a membrane-bound metalloendopeptidase that cleaves the N-terminal amino acid from peptides with a prolyl residue in the second position and a small side chain amino acid in the third position (Yaron and Naider, 1993; Yoshimoto et al., 1994). Studies with isolated perfused organ models suggest that aminopeptidase P contributes to the
bradykinin, then apstatin may have produced a wheal by blocking the degradation of endogenous bradykinin. Testing this hypothesis would require the coadministration of apstatin and a bradykinin antagonist.

As reported by numerous investigators (Ferner et al., 1989; McAlpine and Thomson, 1989), ACE inhibition significantly potentiated the wheal response to bradykinin. In the absence of ACE inhibitor, the wheal responses to apstatin and bradykinin were additive. However, apstatin significantly potentiated the effect of ACE inhibition on the wheal response to bradykinin. The lack of a synergistic effect of apstatin on the wheal response to exogenous bradykinin in the absence of an ACE inhibitor is consistent with data from Damas et al. (1996) who reported that apstatin potentiated bradykinin-induced swelling in rat paws in the presence but not absence of the ACE inhibitor lisinopril. Similarly, Kitamura et al. (1995) reported that the potentiation of the vasodepressor response to bradykinin in rats by apstatin was markedly less than that of the ACE inhibitor lisinopril. Collectively, these data suggest that aminopeptidase P plays a minor role in the degradation of bradykinin in human skin in the absence of ACE inhibition but contributes significantly to the degradation of bradykinin in the presence of acute ACE inhibition. Further studies are needed to determine the contribution of aminopeptidase P to the degradation of bradykinin during chronic ACE inhibition.

Finally, coinjection of 10 μg/100 μl apstatin (1.7 × 10^{-4} M) was required to potentiate the wheal response to intradermal bradykinin in ACE pretreated subjects. The high dose of apstatin required to shift the dose-response curve for bradykinin to the left suggests that apstatin is a relatively weak inhibitor of aminopeptidase P. An IC_{50} of apstatin of 2.9 μM for membrane-bound human aminopeptidase P has been reported (Prechel et al., 1995). The 100-fold higher concentration required to see an effect in the present study may reflect dilution at the site of injection. Although the current study did not address the stability of apstatin, studies in the rat suggests that apstatin remains stable for at least 5 h (Kita-mura et al., 1999).

In conclusion, the present study demonstrates that aminopeptidase P contributes to the degradation of bradykinin in human skin in the presence of ACE inhibition. If confirmed in the human peripheral vasculature, the results would suggest that development of drugs that inhibit aminopeptidase P in combination with ACE may enhance the effects of endoge- nous bradykinin and thereby offer cardiovascular benefit.

References


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