SPECIAL REPORT

Potentiation by aminopeptidase P of blood pressure response to bradykinin

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We examined whether a specific aminopeptidase P (APP) inhibitor, apstatin, increases vasodepressor responses to bradykinin in anaesthetized rats, and whether it would augment blood pressure responses further after treatment with the angiotensin-converting enzyme inhibitor (ACEi), lisinopril. Apstatin doubled the maximum blood pressure response to bradykinin. The area under the curve (AUC), which incorporates both peak blood pressure changes and duration of response, was doubled in apstatin-treated rats *vs* controls and in the apstatin + lisinopril group *vs* lisinopril alone. These data demonstrate that APP is an important kininase *in vivo*.

Keywords: Bradykinin; peptidases; kininases; aminopeptidase P; angiotensin-converting enzyme inhibitors; blood pressure

Introduction Bradykinin is believed to play a role in the regulation of blood pressure and renal and cardiac function. Inhibition of bradykinin metabolism by kininases is usually needed to uncover the effects of kinins (Carretero & Scicli 1990), suggesting that responses to endogenous kinins are modulated by peptidases. The only kininase inhibitors known to potentiate responses to exogenous bradykinin significantly are ACEi, and some of the effects of ACEi are mediated by kinins (Carretero & Scicli, 1990; Wiemer *et al.*, 1994). After inhibition of ACE *in vivo* the half life of plasma kinins remains unchanged, suggesting that other peptidases are also important (Ishida *et al.*, 1989).

Aminopeptidase P (EC 3.4.11.9) is known to metabolize bradykinin *in vitro* (Ward *et al.*, 1991; Simmons & Orawski, 1992), but it is not clear whether it is an important kininase *in vivo*. Simmons' group (Prechel *et al.*, 1994) developed an inhibitor of APP called apstatin [(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl-L-prolyl-L-prolyl-L-alaninamide]. Apstatin inhibits rat lung membrane-bound APP ($K_i = 2.6 \mu\text{M}$) but fails to inhibit other enzymes known to degrade bradykinin, such as ACE, neutral endopeptidases 24.11 and 24.15, and prolol endopeptidase. We examined whether blood pressure responses to bradykinin in rats treated with lisinopril are potentiated further by the addition of apstatin.

Methods Male Wistar rats (200–250 g, Charles River, Wilmington, MA) were given a standard diet and water *ad libitum* for 2 days before the experiment. After sodium pentobarbitone anaesthesia (50 mg kg⁻¹, i.p.), heparinized indwelling catheters (PE10 fused to PE50) were inserted into the abdominal aorta *via* the left femoral artery for direct blood pressure measurements, and into the inferior vena cava *via* the left femoral vein for i.v. injections. All drugs were dissolved in 0.9% NaCl and given as a bolus (100 μl , followed by 100 μl 0.9% NaCl).

After a 30 min stabilization period, blood pressure responses to bradykinin (50, 100, 200 and 400 ng kg⁻¹ in random order), angiotensin I (400 ng kg⁻¹), and acetylcholine (400 ng kg⁻¹) were monitored. Sixty minutes later, rats were divided into four groups as follows: G1: saline; G2: apstatin (80 μg kg⁻¹); G3: lisinopril (400 μg kg⁻¹); G4: lisinopril + apstatin. After 5 min, blood pressure responses to bradykinin, angiotensin I and acetylcholine were repeated in G1

and G2. G3 and G4 received bradykinin alone. For each dose, the maximum change in mean blood pressure (ΔBP) and the area under the curve (AUC) were determined. The ACEi lisinopril was selected because of reports that nm concentrations failed to inhibit pig APP *in vitro* (Hooper *et al.*, 1992).

Lisinopril was a gift from Merck (Rahway, NJ) and apstatin was provided by Dr R.J. Shebuski of Upjohn (Kalamazoo, MI, U.S.A.).

Univariate repeated-measures ANOVA with the Greenhouse-Geisser sphericity correction was used to evaluate ΔBP responses across both dose and treatment. ANOVA was used to test for changes across the four bradykinin doses within each treatment, and paired *t* tests were used to test for changes across the two treatment categories at each dose.

Results BP was not altered by apstatin alone but was reduced from 109 ± 2 to 100 ± 2 mmHg by lisinopril and from 104 ± 2 to 96 ± 3 mmHg by lisinopril plus apstatin ($P < 0.05$).

Apstatin pretreatment roughly doubled responses to bradykinin. Treatment with lisinopril resulted in marked potentiation of the ΔBP response to bradykinin, which was significantly higher than that induced by apstatin ($P < 0.01$). Responses to bradykinin tended to be higher in rats given both lisinopril and apstatin than in those given lisinopril alone, but the differences were not statistically significant (Figure 1). ΔBP responses to angiotensin I and acetylcholine were not affected by apstatin: for angiotensin I, ΔBP was 35.3 ± 3.3 and 38.2 ± 3.4 mmHg ($n = 2$) for controls and apstatin, respectively (NS), while for acetylcholine the values were -26.2 ± 3.2 and -25.2 ± 2.8 ($n = 5$) (NS). The AUC was increased significantly by apstatin: $P < 0.01$ for apstatin *vs* controls and $P < 0.025$ for apstatin + lisinopril *vs* lisinopril alone (Figure 2).

Discussion Although plasma and tissue ACE and carboxypeptidase N play an important role in kinin degradation *in vivo*, other peptidases also contribute to kinin metabolism (Ishida *et al.*, 1989). One may be APP, which is widely distributed in the body (Holtzman *et al.*, 1987). Inhibition of APP with apstatin significantly potentiated the vasodepressor effect of bradykinin, heightening both the ΔBP and the duration of the effect. Potentiation of the response to bradykinin was not due to ACE inhibition, since responses to angiotensin I were not affected by apstatin; nor was it due to some nonspecific alteration in response to a vasodepressor,

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Figure 1 Changes in the maximum hypotensive response to an i.v. bolus of bradykinin in controls and rats pretreated with either apstatin ($80 \mu\text{g kg}^{-1}$), lisinopril ($400 \mu\text{g ml}^{-1}$) or both: (□) controls ($n = 6$); (■) apstatin ($n = 6$); (○) lisinopril ($n = 6$); (●) apstatin + lisinopril ($n = 8$). Values are mean $\pm$ s.e.mean. * $P < 0.025$, apstatin vs controls; ♦ $P < 0.05$ and ♦♦ $P < 0.001$, apstatin vs lisinopril.

since the reaction to acetylcholine was also unaffected. We gave bradykinin intravenously; if the primary effect of apstatin were diminished metabolism by the lung, more kinins should enter the arterial side, resulting in an increased response, which was the case for apstatin alone.

Potentialization of the vasodepressor response to bradykinin by apstatin was markedly less than that induced by lisinopril, indicating that APP plays a lesser role than ACE as a kininase. Vasodepressor responses to bradykinin in rats treated with both lisinopril and apstatin were 5 to 8 mmHg higher than in the group treated with lisinopril alone. The differences were not significant, probably due to the dispersion of the data and/or relatively small sample size. In contrast, the AUC was doubled by adding apstatin to the ACEi.

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Figure 2 Effect of treatment with apstatin, lisinopril or both on the area under the curve (AUC) representing the hypotensive response to bradykinin. (AUC includes both maximum response and duration): (□) controls ($n = 6$); (■) apstatin ($80 \mu\text{g kg}^{-1}$; $n = 6$); (○) lisinopril ($400 \mu\text{g kg}^{-1}$; $n = 6$); (●) apstatin + lisinopril ($n = 8$). Values are mean $\pm$ s.e.mean. * $P < 0.01$, apstatin vs controls; ♦ $P < 0.025$, lisinopril + apstatin vs lisinopril alone. AUC was significantly higher after lisinopril than after apstatin at all doses ($P < 0.01$).

Thus inhibition of APP added significantly to the reduction in bradykinin metabolism rate. This suggests that ACE is a more important kininase than APP in the lung; however, inhibition of APP in other organs may contribute to the prolonged response. The kidney is one candidate, since it plays an important role in bradykinin degradation (Ishida *et al.*, 1989) and is rich in APP (Holtzman *et al.*, 1987). We conclude that APP contributes to bradykinin degradation *in vivo*, suggesting that combining APP and ACE inhibitors may augment the bradykinin-mediated effects of ACEi.

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