Effects of aminopeptidase P inhibition on kinin-mediated vasodepressor responses

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Kitamura, Shin-ichi, Luis A. Carbiní, William H. Simmons, and A. Guillermo Sciclón. Effects of aminopeptidase P inhibition on kinin-mediated vasodepressor responses. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1664–H1671, 1999.—We studied in anesthetized rats whether aminopeptidase P (AMP) may be involved in bradykinin (BK) metabolism and responses. For this we inhibited AMP with the specific inhibitor apstatin (Aps). Studies were done with Aps alone or together with the angiotensin-converting enzyme inhibitor lisinopril (Lis). Aps increased the vasodepressor response to an intravenous bolus of BK (400 ng/kg): vehicle, −3.0 ± 0.7 mmHg; Aps, −7.8 ± 0.7 mmHg (P < 0.01 vs. vehicle); Lis, −23.8 ± 1.8 mmHg; Aps + Lis, −37.5 ± 1.9 mmHg (P < 0.01 vs. Lis). Aps did not affect the vasodepressor response to BK given into the descending aorta. Plasma BK increased only in Aps + Lis-treated rats (in pg/ml): control, 51.9 ± 2.5; Aps, 83.5 ± 20.5; Lis, 725 ± 225; Aps + Lis, 1,668 ± 318 (P < 0.05, Aps vs. control and Lis vs. Aps + Lis). In rats with aortic coarctation hypertension, the acute antihypertensive effects of Aps plus Lis were greater than Lis alone (P < 0.01). Hoe-140, a BK B1-receptor antagonist, abolished the difference. We concluded that in the rat AMP contributes to regulation of BK metabolism and responses.

bradykinin; angiotensin-converting enzyme; kininases; blood pressure

Bradykinin is a potent vasoactive peptide that is known to elicit a number of biological responses. Kinins play a role in regulation of blood pressure (BP), renal function, and cardiac function as ascertained by biochemical and pharmacological criteria as well as by the effects of specific kinin receptor antagonists (22, 23, 25, 30, 33, 35). A common method of assessing the role of kinins has been to inhibit their metabolism by kininases. In particular, there are a number of data suggesting that part of the cardiovascular effect of angiotensin-converting enzyme (ACE) inhibitors is due to potentiation of the effects of kinins (13, 22, 30, 33). This suggests that responses to endogenous kinins are modulated by peptidases. A number of peptidases other than ACE, which possess kininase activity in vitro or in vivo, have been identified. There are data suggesting that, in some situations, neutral endopeptidase (NEP) 24.11 and carboxypeptidase N (kininase I) are as important as kininas (9–11, 19, 28, 34, 37); however, it is not certain whether these two peptidases play a significant role in metabolizing systemic kinins. Neither NEP 24.11 nor carboxypeptidase N inhibitors consistently magnify the BP response to kinins (1, 32). Plasma bradykinin was still rapidly degraded in rats treated with an ACE inhibitor. A cocktail of ACE, NEP 24.11, carboxypeptidase N, and aminopeptidases A and M inhibitors did not protect plasma bradykinin from degradation any better than an ACE inhibitor alone, suggesting that other kininases are responsible for bradykinin degradation in the presence of an ACE inhibitor (17, 18). Other peptidases whose roles are potentially important are NEP 24.15 and aminopeptidase P. Although an NEP 24.15 inhibitor has been reported to potentiate kinins and decrease BP, subsequent work revealed that these systemic effects were likely due to metabolism of the NEP 24.15 inhibitor to an ACE inhibitor (6, 14, 39).

Aminopeptidase P is an aminocarboxylyl proline aminopeptidase specific for NH2-terminal Xaa-proline bonds. It is present in the lung, kidney, brain, intestine, and plasma in both rats and humans (38). In vitro data suggest that aminopeptidase P metabolizes bradykinin in the lung, heart, liver, and plasma (1, 8, 12, 15). Using substrate inhibition of aminopeptidase P as a tool, Ryan et al. (31) were the first to suggest that aminopeptidase P may be an important kininase in vivo. Recently, Simmons and co-workers (29) developed an aminopeptidase P inhibitor called apstatin [(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl-l-prolyl-l-prolyl-l-alaniamide]. Apstatin is a specific inhibitor of aminopeptidase P. It does not inhibit other enzymes known to degrade bradykinin, such as ACE, NEP 24.11, and NEP 24.15. Our preliminary work using this inhibitor suggested that aminopeptidase P is an important kininase in vivo, regulating systemic bradykinin levels, and that this role is revealed better when ACE is inhibited (21). However, more information is required before the role of aminopeptidase P in regulating bradykinin is affirmed. Because aminopeptidase P concentrations are quite high in the rat lung (26), it may be that apstatin is more effective in potentiating venous than arterial bradykinin. It is not clear whether blood levels of bradykinin are affected by apstatin alone or whether kinins in blood are increased more by a combination of apstatin and an ACE inhibitor compared with the ACE inhibitor alone, and it is not known whether the antihypertensive effects of ACE inhibitors are increased by combin-
ing them with the aminopeptidase P inhibitor apstatin. The present rat studies were conducted to examine whether 1) BP responses to both intravenous and intra-arterial bradykinin are altered by pretreatment with apstatin and 2) plasma kinin concentrations are altered during apstatin treatment. Studies were performed under basal conditions and during bradykinin infusion. In addition, we hypothesized that combined inhibition of ACE and aminopeptidase P enhances the antihypertensive effect of ACE inhibitor because of further potentiation of endogenous bradykinin. We previously reported that, in aortic coarctation hypertension, kinins mediate part of the acute antihypertensive effect of an ACE inhibitor (4). Thus we selected this model of hypertension to compare the antihypertensive effect of a combination of an ACE inhibitor, lisinopril and apstatin with lisinopril alone, and the role of kinins in this antihypertensive effect.

MATERIALS AND METHODS

Bradykinin and ACh were purchased from Sigma (St. Louis, MO). Lisinopril was a gift from Merck Sharp & Dohme (Rahway, N.J.). ANG I was purchased from Peninsula (Torrance, CA). Apstatin was custom-synthesized by BioMol Research Laboratories (Plymouth Meeting, PA).

Experimental Protocol

Male Wistar rats weighing 200–250 g (Charles River, Wilmington, MA) were given Purina Rat chow containing 0.4% NaCl and tap water ad libitum. On the day of the experiment, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and indwelling polyethylene catheters (PE-10 fused to PE-50 or PE-50) filled with heparinized saline were inserted into 1) the abdominal aorta via the left femoral artery for direct BP measurements, 2) the inferior vena cava via the left femoral vein for intravenous injections, and 3) the descending aorta via the left common carotid artery for blood sampling and intra-arterial injections. Arterial pressure was measured with a Statham pressure transducer (Gould, Cleveland, OH). All drugs were dissolved in 0.9% NaCl, and the volume of each bolus was 100 µl, followed by 100 µl of 0.9% NaCl. Bradykinin was infused with a model 355 syringe pump (Orion Research, Cambridge, MA) at a rate of 100 µl/min.

Effect of Apstatin, Lisinopril, and Apstatin Plus Lisinopril on the BP Response to an Intravenous Bolus of Bradykinin, ANG I, and ACh

This protocol was performed to ascertain the specificity and validate the dose of apstatin we would use in subsequent protocols. The apstatin obtained from BioMol was weaker than the previously used apstatin (21), which was a gift from Pharmacia-Uppjohn (Kalamazoo, MI). The dose was determined in preliminary experiments as that providing potentiation of the BP responses to intravenous bradykinin similar to those previously observed with Upjohn’s apstatin. Further increases (2- to 3-fold) in the dose of apstatin had no additional effects on the BP response to intravenous bradykinin.

After surgery and a 30-min stabilization period, BP responses to an intravenous bolus of bradykinin (50, 100, 200, and 400 ng/kg in random order), ANG I (400 ng/kg), and ACh (400 ng/kg) were monitored. After a 60-min stabilization period, rats were divided into four groups. Group 1 received vehicle (saline), group 2 received apstatin (800 µg/kg), group 3 received lisinopril (40 µg/kg), and group 4 received both lisinopril and apstatin. Five minutes after the last pretreatment, BP responses to bradykinin, ANG I, and ACh were repeated in groups 1 and 2. Groups 3 and 4 received bradykinin alone. In control and apstatin rats, bradykinin was also given at 1,600 ng/kg; this dose was not used in rats receiving lisinopril because of the excessive drop in BP (>50 mmHg). For each dose, the maximum change in mean BP (MBP) and the area under the curve (AUC) were determined. (AUC incorporates both BP and the duration of action, i.e., time needed to return to baseline.) Area measurements were determined directly from the records using Sigma Scan (Jandel Scientific, Corte Madera, CA). Lisinopril was selected as an ACE inhibitor because of reports that millimolar concentrations failed to inhibit pig aminopeptidase P in vitro (29). In preliminary experiments (n = 3), pretreatment with 40 µg/kg lisinopril inhibited the acute pressor response to intravenous 400 ng/kg ANG I by 90%.

Effect of Apstatin on the BP Response to an Intravenous Bolus of Bradykinin

To determine whether the BP response to intra-arterial bradykinin injection is also potentiated after treatment with apstatin, alternate intra-arterial and intravenous injections of bradykinin were conducted. After completion of surgery and a 30-min stabilization period, BP responses to bolus intravenous injection of bradykinin (400 ng/kg) and intraarterial injection of bradykinin (100 ng/kg) were monitored. The rats then received apstatin (800 µg/kg) or vehicle. Five minutes after that, BP responses to bolus intra-arterial and intravenous injections of bradykinin were repeated. For each injection, maximum changes in MBP were determined.

Time Course of the Effect of Apstatin on the BP Response to an Intravenous Bolus of Bradykinin, ANG I, and ACh

Apstatin is a peptide analog, and therefore, its inhibitory activity may be short-lived due to rapid degradation. To determine whether the effects of apstatin are short lasting, periodical administrations of bradykinin, ANG I, and ACh were conducted after a single bolus of a solution containing apstatin. After completion of surgery and a 30-min stabilization period, BP responses to bradykinin (400 ng/kg), ANG I (400 ng/kg), and ACh (400 ng/kg) were monitored. After 60-min stabilization, rats received 1) vehicle or 2) apstatin (800 µg/kg). At 5, 60, and 240 min after treatment, BP responses to the different agonists were repeated.

Effect of Apstatin, Lisinopril, and Apstatin Plus Lisinopril on Plasma Kinin Concentration and BP Changes During Bradykinin Infusion

Collection of blood and extraction and measurement of bradykinin peptides from blood. To study the effect of lisinopril and apstatin plus lisinopril on endogenous kinins, three groups of rats were studied, a control group (n = 8) pretreated with vehicle and experimental groups pretreated with lisinopril (40 µg/kg) or apstatin (800 µg/kg) plus lisinopril. After treatment, rats were infused with saline (100 µl/min), and 5 min later, blood was sampled from the aortic catheter.

To study the effect of apstatin, lisinopril, and apstatin plus lisinopril on plasma kinin concentrations and BP changes during bradykinin infusion, four groups of rats were studied after surgery and a 30-min stabilization period. Group 1 received vehicle (saline), group 2 received apstatin (800 µg/kg), group 3 received lisinopril (40 µg/kg), and group 4 received both apstatin (800 µg/kg) and lisinopril (40 µg/kg). Five minutes after pretreatment, bradykinin infusion was...
started at a rate of 100 ng/min and continued for 5.5 min. Before and during bradykinin infusion, MBP was recorded continuously. Five minutes after we began the bradykinin infusion, blood was sampled.

In each blood sampling, 2 ml of blood needed for plasma kinin determination were collected immediately in a chilled 3-ml polypropylene syringe containing 200 µl of the following kininogenase and kinase inhibitors: 1.5 gli aprotinin (a gift from Bayer Wuppertal-Eberfeld, Leverkusen, Germany), 0.8 g/l soybean trypsin inhibitor (Sigma), 4 g/l polybrene (Aldrich, Milwaukee, WI), 20 g/l EDTA, and 10 g/l 1,10-phenanthroline (both from Fisher, Fair Lawn, NJ).

Collected blood was transferred to a chilled 5-ml plastic tube, plasma was separated by centrifugation for 15 min at 2,000 g, and then 0.8 ml of plasma was mixed with 1.6 ml of cold ethanol and centrifuged again for 15 min. The supernatant was transferred quantitatively into 5-ml polypropylene tubes and evaporated to dryness using a Savant Speed Vac concentrator. The dry residue was dissolved in 1 ml of saline and extracted with a Bond Elute C18 cartridge (Analytichem, Harbor City, CA) as described by Campbell et al. (3). The eluate was collected in a 5-ml polypropylene tube and again evaporated to dryness. As described previously, the dry residue was reconstituted in buffer, and bradykinin concentrations were measured by RIA (5).

**Effect of Apstatin on the Acute Antihypertensive Response to Lisinopril in Rats With Aortic Coarctation Hypertension**

Male Wistar rats (215–230 g, Charles River) were housed in a constant-temperature room with a 12:12-h light-dark cycle and given a standard diet and water ad libitum. Hypertension was induced by complete aortic ligation between the renal arteries. Seven days later, experiments were conducted under Inactin anesthesia (125 µg/kg ip). Heparinized indwelling catheters (PE-50) or temperature probes were inserted into the aorta via the right common carotid artery for measurements of direct BP, collection of blood samples, or measurement of cardiac output. Another heparinized indwelling catheter (PE-50) was inserted into the superior vena cava via the right external jugular vein for bolus administration of drugs and cold saline. BP was measured with a Statham transducer (Gould, Oxford, CA) and recorded on a Brush recorder (Gould, Cleveland, OH). Cardiac output was measured with a Cardiotherm computer (model 500, Columbus Instruments, Columbus, OH) as described by the manufacturer. Total peripheral resistance was calculated from the equation

\[ \text{Mean blood pressure (mmHg)} = \text{cardiac output (ml·min}^{-1}·	ext{kg}^{-1}) \times \text{peripheral resistance} \]

Changes in systemic peripheral resistance induced by the different treatments in each individual rat were expressed as a percentage of the peripheral resistance value before treatment, which was taken as 100%.

**Experiment 1.** Twenty-eight hypertensive rats were used to determine whether combined inhibition of ACE and aminopeptidase P has a more potent antihypertensive effect than ACE inhibition alone. After a 30-min stabilization period, rats were given either vehicle or Hoe-140 (500 µg/kg). Five minutes after pretreatment, the rats in the vehicle pretreatment group received lisinopril (50 µg/kg) or apstatin (800 µg/kg) plus lisinopril, whereas those in the Hoe-140 pretreatment group received apstatin plus lisinopril. MBP was recorded continuously through the carotid catheter during the experiments.

**Experiment 2.** Sixteen hypertensive rats (4/group) were used to determine whether combined inhibition of ACE and aminopeptidase P has a more potent antihypertensive effect than ACE inhibition alone by inducing a greater decrease in peripheral resistance. After a 30-min stabilization period, mean basal BP and cardiac output were measured. Rats were pretreated with either vehicle (saline) or Hoe-140 (500 µg/kg). Five minutes after vehicle injection, rats received either lisinopril (50 µg/kg) or apstatin (800 µg/kg) plus lisinopril. Rats pretreated with Hoe-140 received apstatin plus lisinopril. Hoe-140 alone induced no hemodynamic changes. Sixty minutes after treatment, a second measurement of MBP and cardiac output was conducted.

**Statistical Analysis**

Univariate repeated-measures ANOVA with the Greenhouse-Geisser sphericity correction was used to evaluate BP 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. P < 0.05 was regarded as significant.

**RESULTS**

Effects of Apstatin on the MBP Response to Bolus Intravenous and Intra-Arterial Administration of Bradykinin

A small and transient dose-dependent decrease in MBP was observed after bolus intravenous administration of bradykinin in both vehicle- and apstatin-treated rats. Pretreatment with apstatin approximately doubled the vasodepressor response to an intravenous bolus of bradykinin (P < 0.01). This moderate effect of apstatin was maintained for >4 h in the time course study (Fig. 1). To test whether apstatin alters responses to both intravenous and intra-arterial bradykinin, we used doses of bradykinin that decreased BP to about the same extent. Apstatin did not affect the vasodepressor response to an intra-arterial bolus of bradykinin.
As expected, treatment with lisinopril resulted in marked potentiation of the BP response to intravenous bradykinin, which was significantly higher than that induced by apstatin (P < 0.001). The vasodepressor response to bradykinin in the rats treated with both apstatin and lisinopril was significantly higher than in rats treated with lisinopril alone (P < 0.01) (Fig. 2A). BP responses to 400 ng/kg ANG I and ACh were not affected by apstatin. For ANG I, MBP was 39.0 ± 3.2 and 43.7 ± 3.0 mmHg (n = 6) for controls and apstatin, respectively (P = NS), whereas for ACh, the values were −30.0 ± 1.5 and −29.8 ± 1.3 mmHg (n = 5; P = NS). No changes were observed in the responses to either ANG I or ACh were observed during the 4 h of the time course experiments (data not shown).

We used the responses to intravenous bradykinin to measure the AUC as a means of representing both the magnitude and duration of the response. The AUC in the rats treated with apstatin alone was significantly greater than in the vehicle group but smaller than that observed in lisinopril-treated rats. The AUC of the rats treated with both apstatin and lisinopril was also significantly greater than that of those treated with lisinopril alone (P < 0.03) (Fig. 2B).

In the bradykinin infusion studies (100 ng/min iv for 5 min), the maximum BP response to bradykinin infusion was significantly higher in the rats treated with apstatin plus lisinopril compared with lisinopril alone (P < 0.04). Furthermore, after the maximum decrease in BP was attained, it tended to return to baseline in the rats treated with lisinopril alone, whereas it remained low in those rats treated with both apstatin and lisinopril (P < 0.001) (Fig. 3).

Effects of Apstatin on Basal Plasma Kinin Concentrations

Lisinopril did not affect basal endogenous kinin levels (control, 48.0 ± 1.4 pg/ml; lisinopril, 57.5 ± 7.6 pg/ml; P = NS); however, apstatin plus lisinopril significantly increased endogenous kinin levels (121.8 ± 30.6 pg/ml; P < 0.05 vs. control or lisinopril).

In the intravenous bradykinin infusion study, bradykinin in arterial plasma was no different than the noninfused rats, a demonstration of the known role of lung peptidases in bradykinin metabolism. Treatment with apstatin alone significantly increased plasma kinins compared with control (apstatin, 83.5 ± 20.5 pg/ml; control, 51.9 ± 2.5 pg/ml; P < 0.04). Plasma kinin concentrations in the rats treated with both apstatin and lisinopril were significantly higher than in those treated with lisinopril alone (apstatin + lisinopril, 1,668 ± 318 pg/ml; lisinopril, 725 ± 225 pg/ml; P < 0.02) (Fig. 4).

Effects of Apstatin on MBP in Rats With Hypertension Secondary to Aortic Coarctation

In rats with aortic coarctation hypertension, MBP was significantly decreased by acute administration of lisinopril, from 152 ± 4 to 129 ± 6 mmHg (P < 0.02). The decrease induced by apstatin plus lisinopril was...
significantly higher, from 158 ± 4 to 98 ± 4 mmHg (P < 0.01, lisinopril vs. apstatin + lisinopril). Pretreatment with Hoe-140 abolished this difference; MBP decreased from 154 ± 6 to 132 ± 9 mmHg (P = NS vs. lisinopril alone) (Fig. 5).

Treatment with lisinopril or apstatin plus lisinopril significantly decreased systemic peripheral resistance [peripheral resistance = BP/cardiac output index (ml·min⁻¹·100 g body wt⁻¹)], with the decrease being more pronounced in the apstatin plus lisinopril group. Hoe-140 attenuated these changes: lisinopril, 27.8 ± 1.9%; apstatin + lisinopril, 44.0 ± 0.7% (P < 0.01 vs. lisinopril); Hoe-140 + apstatin + lisinopril, 18.3 ± 2% (P < 0.01 vs. apstatin + lisinopril; P < 0.05 vs. lisinopril) (Fig. 6). In each group, the cardiac output indexes did not change significantly with treatment. The values were as follows (in ml·min⁻¹·100 g body wt⁻¹): before lisinopril, 40.1 ± 2.5; after lisinopril, 43.1 ± 1.3; before apstatin + lisinopril, 47.1 ± 6; after apstatin + lisinopril, 52.8 ± 6.3; before Hoe-140 + apstatin + lisinopril, 40 ± 1.2; after Hoe-140 + apstatin + lisinopril, 40.0 ± 3.0.

DISCUSSION

Bradykinin is believed to be an important regulator of renal and cardiovascular function as well as a mediator of inflammatory reactions. Degradation by kininases is known to play an important role in regulating bradykinin levels and thus bradykinin-mediated biological responses (2, 20, 22, 23, 30, 35, 36). In vitro bradykinin is susceptible to degradation by a number of endo- and exopeptidases (10, 36). Although many of these peptidases are widely distributed in various tissues and cells of the body, only a few have been identified as participating in the regulation of systemic bradykinin in vivo. An important kininase in vivo is ACE (or kininase II) (11, 17, 18, 36). The availability of ACE inhibitors was pivotal to the present understanding of its role as a main kininase. We present data suggesting that aminopeptidase P is also important in the degradation of bradykinin in vivo. Until now, the effect of aminopeptidase P on kinin metabolism in vivo was not studied directly because there were no potent and specific inhibitors. However, Ryan et al. (31) using the aminopeptidase P substrate Arg-Pro-Pro-benzylamide as a competitive inhibitor of aminopeptidase P presented evidence that aminopeptidase P plays an important role in kinin degradation and the BP response to intravenous bradykinin. Recently, a specific inhibitor of aminopeptidase P was developed and named apstatin (29). For the first time, we were able to directly study whether inhibition of aminopeptidase P with a specific inhibitor modulates the vasodepressor activity of bradykinin in vivo. For this we studied how apstatin alone or in combination with an ACE inhibitor, lisinopril, altered 1) the vasodepressor response to exogenously administered bradykinin, 2) plasma kinin concentration, and 3) BP in a model of severe hypertension induced by aortic ligation between the renal arteries. We have confirmed our own
preliminary data (21) showing that apstatin induced a small but significant potentiation of the acute hypertensive response induced by intravenous bradykinin both in terms of magnitude and duration of the response. These effects were not due to a nonspecific interaction between apstatin and ACE, since the hypertensive response to ANG I was not affected, and the potentiation by Aps of the bradykinin-induced decrease in BP was not due to a nonspecific effect of apstatin on endothelium-dependent vasodilation, since the hypertensive effect of ACh was not affected either. In two rats, we studied whether apstatin would affect the hypertensive response to sodium nitroprusside and found that the response was not altered (data not shown). We can exclude the possibility that the effect of apstatin was caused by metabolism of the aminopeptidase P inhibitor into an ACE inhibitor, since potentiation of the hypertensive response to bradykinin and the lack of effect on the response to ANG I remained unaltered for more than 4 h after administration of apstatin. The doubling of the hypertensive response to bradykinin was only observed if it was given intravenously, not intrarterially. This suggests that aminopeptidase P activity located in the lung was primarily responsible for the doubling of the bradykinin vasodepressor responses in the presence of apstatin.

We tested whether the combination of an ACE inhibitor and apstatin would potentiate the vasodepressor response to bradykinin more than the ACE inhibitor alone. The dose of the ACE inhibitor (lisinopril) we used blocked the hypertensive response to ANG I by ~90%, indicating that it inhibited ACE. Treatment with a combination of apstatin and lisinopril resulted in the largest hypertensive response to both acute injections and infusions of bradykinin. When we compared the vasodepressor responses to bolus administration of bradykinin in rats treated with lisinopril either alone or combined with apstatin, a significantly higher response was observed in the combined treatment group. More revealing results were obtained when we analyzed the area under the vasodepressor curve, which incorporates both changes in BP and duration of the response. This area was doubled by the addition of apstatin to lisinopril, suggesting that aminopeptidase P contributes to degradation of bradykinin and that combined inhibition of aminopeptidase P and ACE augments more bradykinin-mediated effects than the ACE inhibitor alone.

The further potentiation of the hypertensive response to bradykinin by combined inhibition of aminopeptidase P and ACE compared with ACE inhibition alone was especially evident during bradykinin infusion. In control conditions, an initial hypertensive response was observed at the beginning of the infusion, but BP returned to baseline even though bradykinin was infused continuously. This may be due to receptor desensitization, compensatory reflexes, or rapid degradation by kininases. Potentiation of the vasodepressor response to bradykinin infusion by apstatin was only a fraction of that observed with the ACE inhibitor lisinopril. Even with lisinopril treatment, BP tended to return to baseline before the end of the bradykinin infusion, whereas combined administration of lisinopril and apstatin resulted in a prolonged hypertensive response. This suggests that one of the reasons for the rapid return to baseline in the control group was kinin degradation mediated by both ACE and aminopeptidase P. The data suggest that, when ACE was inhibited, another peptidase(s) became important as a kinin-degrading enzyme and that this peptidase is largely aminopeptidase P.

From these observations, we wanted to determine whether plasma kinin concentration was affected by inhibition of aminopeptidase P in a manner consistent with the pharmacological data. Measurement of differences in arterial kinin levels during treatment with the different kininase inhibitors also indicated that, in the rat, aminopeptidase P helps regulate circulating kinin levels if ACE is inhibited. As we have observed before (4) and using similar methodology, during treatment with an ACE inhibitor endogenous plasma kinins tend to increase, although not to a statistically significant degree. In contrast, plasma kinin concentrations increased significantly during combined inhibition of aminopeptidase P and ACE. The contribution of these two peptidases to regulation of circulating kinins was again clearly revealed during the experiments involving bradykinin infusion. Plasma kinins increased significantly with apstatin alone by ~60%. As expected (17, 18), inhibition of ACE with lisinopril resulted in a >10-fold increase in plasma kinins, indicating that ACE is the foremost important kininase, at least in the rat. Combined treatment with apstatin and lisinopril doubled the concentration of plasma kinins compared with lisinopril alone. Thus both the BP responses to bolus injections of bradykinin and particularly bradykinin infusion, as well as the changes in circulating kinins, suggest that aminopeptidase P plays a role, albeit a lesser one than ACE, in regulating the concentration of bradykinin that passes from the venous to the arterial circulation. The present data suggest that if ACE is inhibited, aminopeptidase P activity in the lung becomes an important kininase.

Bradykinin has been shown to be extensively degraded during a single pass through the rat lung by enzymes located on the plasma membrane of vascular endothelial cells (9). Pesquero et al. (27) showed that one of these kininases may be aminopeptidase P. These authors further reported that, in the isolated perfused rat lung, degradation of bradykinin by aminopeptidase P results in des-Arg-bradykinin, a good substrate for dipeptidyl peptidase IV, which in turn would release Pro-Pro-bradykinin from des-Arg-bradykinin. Thus aminopeptidase P and dipeptidyl peptidase IV may act in sequence to metabolize bradykinin. Our data do suggest that aminopeptidase P is a very important kininase in vivo.

A number of the cardiovascular and renal effects of ACE inhibitors are affected by kinin receptor antagonists (2, 13, 20, 22, 24, 30, 33, 36), suggesting that kinins may contribute to the cardiovascular effects of ACE inhibitors. Thus the present findings suggesting...
that concomitant inhibition of ACE and aminopeptidase P further decreases kinin degradation may have potential applications, since kinin-mediated effects of ACE inhibitors might be augmented further by the addition of an aminopeptidase inhibitor. We hypothesize that the kinin-dependent antihypertensive effect of combined treatment with apstatin and lisinopril would be greater than that of lisinopril alone. To address this hypothesis, we used aortic coarctation hypertension as a model because it has been reported that part of the acute antihypertensive effects of ACE inhibitors in this model is mediated by kinins (4). Apstatin alone had no significant effect on BP (n = 2; data not shown). We found that lisinopril alone rapidly lowered BP in these hypertensive rats, but the decrease was significantly greater in the rats treated with both apstatin and lisinopril. This difference was abolished by a bradykinin B2-receptor antagonist, suggesting that simultaneous inhibition of ACE and aminopeptidase P had greater antihypertensive effects than just ACE inhibition alone and that this greater effect was mediated by endogenous kinins. The change in BP induced by either lisinopril or apstatin plus lisinopril was due to vasodilation as indicated by the marked decrease in systemic peripheral resistance. Interestingly, the decrease in peripheral resistance was lowest in the group treated with the kinin antagonist, consistent with kinins mediating part of the change in peripheral resistance induced by both lisinopril and apstatin plus lisinopril.

These data demonstrate that, in the rat, inhibition of aminopeptidase P can further augment the kinin-mediated decrease in BP induced by treatment with an ACE inhibitor. As a word of caution, because there are large interspecies variations in aminopeptidase P activities of plasma, lung, and kidney (7), these results may not be automatically extrapolated to other species, including humans.

It has been proposed that some of the effects of ACE inhibitors are mediated by discrete increases in the local (tissue) concentration of kinins (3, 22, 24, 33). Aminopeptidase P is widely distributed in the body (38). Thus it is conceivable that effects due to a localized increase of kinins in discrete tissues could be further augmented by combined treatment with an ACE inhibitor and an aminopeptidase P inhibitor. Systemic peripheral resistance is regulated at the levels of the arterioles, and apstatin had a kinin-mediated increase of the changes induced in this parameter by lisinopril. This suggests that aminopeptidase P regulates vascular kinin concentration when ACE is inhibited. It would be of interest to determine whether in vivo aminopeptidase P is an important kininase in discrete tissues other than the lung, such as the vasculature, heart, and kidney, all known targets of ACE inhibitors.

In summary, we tested whether apstatin, a specific inhibitor of aminopeptidase P, increased the vasodepressor response to bradykinin and affected its plasma concentration. Apstatin increased both the bradykinin-induced decrease in BP and duration of the hypotensive response. Simultaneous treatment with lisinopril and apstatin resulted in more marked prolongation of the hypotensive response to bradykinin as well as a higher concentration of plasma kinins compared with an ACE inhibitor alone. In a model of severe hypertension induced by complete ligation of the aorta between the renal arteries, the antihypertensive effect of combined treatment with apstatin and lisinopril was greater than that induced by lisinopril alone. The drop in BP was due to a decrease in peripheral resistance, and the difference between apstatin plus lisinopril and lisinopril alone was abrogated by a kinin receptor antagonist, suggesting that it was due to endogenous kinins. These data demonstrate that combined inhibition of ACE and aminopeptidase P is more effective than an ACE inhibitor alone in slowing down the rate at which bradykinin is degraded in plasma as well as further augmenting bradykinin-mediated effects. We conclude that in the rat, aminopeptidase P contributes to bradykinin degradation. Combined administration of aminopeptidase P and ACE inhibitors may be a valuable tool to selectively augment the kinin-mediated effects of ACE inhibitors in vivo.

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