Role of Angiotensin Converting Enzyme and Other Peptidases in In Vivo Metabolism of Kinins

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Arterial plasma kinins and mean arterial pressure were measured in intact and bilaterally nephrectomized rats infused with vehicle or bradykinin to study the role of 1) angiotensin converting enzyme (ACE) and other peptidases and 2) the kidney (a kininase-rich organ) in the metabolism of kinins in vivo. Before the infusion, rats were pretreated with vehicle, enalaprilat (an ACE inhibitor), or a cocktail of kininase inhibitors containing 1) enalaprilat, 2) dl-2-mercaptomethyl-3-guanidinoethyl-thiopropanoic acid (MGTA), a carboxypeptidase N inhibitor, 3) phosphoramidon, a neutral endopeptidase 24.11 inhibitor, and 4) bestatin, an aminopeptidase B inhibitor. In the rats with vehicle (n=8), the cocktail did not significantly increase endogenous kinins (from 31±6 to 41±9 pg/ml, p=0.94). In the rats infused with bradykinin (peptidase substrate), plasma kinins increased threefold in the group pretreated with the vehicle, 21-fold in the enalaprilat group, and 22-fold in the cocktail group. These increases were doubled by nephrectomy but were not affected by ureteral ligation. In the groups pretreated with the cocktail or enalaprilat, the hypotensive effect of bradykinin was correlated with plasma kinin concentration (r=0.75, p<0.001). After bradykinin infusion was stopped, plasma kinins decreased by half in 10-12 seconds in the rats pretreated with vehicle, enalaprilat, or cocktail. We concluded that ACE and the kidney are important to the metabolism of circulating kinins while carboxypeptidase N, neutral endopeptidase 24.11 and aminopeptidase B are not. We also concluded that other tissue peptidases, not affected by either the above inhibitors or nephrectomy, play an important role in kinin metabolism. (Hypertension 1989;14:322-327)
tap water ad libitum. The day before the experiments, the rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.), and polyethylene catheters (PE; Clay-Adams, Parsippany, New Jersey) were inserted into the abdominal aorta via the right femoral artery (PE-10) for direct blood pressure measurements, into the inferior vena cava via the left femoral vein (PE-10) for injection of kininase inhibitors or continuous infusion of bradykinin (Peninsula Labs, Inc., Belmont, California), and into the descending thoracic aorta via the left carotid artery (PE-50) to collect blood. All catheters were passed beneath the skin and brought out at the scapular region. Blood samples (0.5-1.0 ml) were obtained for plasma kinin determination according to Shimamoto’s method 10 using a syringe containing 0.05-0.1 ml of the following kininogenase and kininase inhibitors: 1) $1 \times 10^7$ kunitz inhibitor units/ml aprotinin (a gift from Bayer AG Wuppertal-Ellerfeld, Leverkusen, FRG), 2) 0.8 g/l soybean trypsin inhibitor (SBTI) (Sigma Chemical Co., St. Louis, Missouri), 3) 4 g/l polybrene (Aldrich, Milwaukee, Wisconsin), 4) 20 g/l EDTA, and 5) 10 g/l 1,10-phenanthroline (both from Fisher Scientific Co., Fairlawn, New Jersey). To avoid activation of plasma kallikrein by the catheters or surgical procedures, SBTI (2 mg/0.5 ml i.v.) was injected before the experiments. Blood withdrawn was immediately replaced with samples obtained from a donor rat nephrectomized 24 hours earlier. Bilateral nephrectomy and ureteral ligation were performed via a flank incision 24 hours before the experiments with the rats under ether anesthesia. During the experiments, the rats were kept semi-restrained in cylindrical plastic containers. Arterial pressure was measured with a Statham transducer (Gould, Cleveland, Ohio). Five minutes before the infusion of vehicle or bradykinin, the rats were pretreated with vehicle (1 ml saline/kg), enalaprilat (0.2 mg/kg), or a cocktail of kininase inhibitors (1 ml/kg) containing 1) 0.2 mg/ml enalaprilat (a gift from Merck Sharp & Dohme, Rahway, New Jersey), 2) 10 mg/ml MGTA (Calbiochem-Behring Corp., La Jolla, California), 3) 4 mg/ml phosphoramidon (Pep- tide Institute Protein Research Foundation, Osaka, Japan), and 4) 3 mg/ml bestatin (Sigma Chemical Co., St. Louis, Missouri). Vehicle or bradykinin was infused with a Harvard 990 pump (Dover, Massachusetts) at a rate of 100 $\mu$l/min. All drugs were dissolved in saline (0.9% NaCl).

**Experimental Groups**

Unless otherwise indicated, blood for plasma kinin determination was withdrawn immediately before pretreatment, 5 minutes after the vehicle or bradykinin infusion was begun, and 15 and 30 minutes after the infusion. **Effect of cocktail on endogenous plasma kinins and plasma kinin concentrations during bradykinin infusion.** To study the effect of kininase inhibitors on endogenous kinins, two groups of rats were studied, a control group ($n=8$) pretreated with vehicle and an experimental group pretreated with the cocktail ($n=8$). Five minutes after pretreatment, both groups were infused with saline for 6 minutes (0.1 ml/min).

To study the effect of kininase inhibitors on plasma kinin concentrations during bradykinin infusion, two groups of rats were studied, a control group pretreated with vehicle and an experimental group given the cocktail. Each group was subdivided into three subgroups that were infused with bradykinin 5 minutes after pretreatment at a rate of 100 ($n=10$ vehicle and 10 cocktail), 500 ($n=5$ and 4), or 1,000 ng/min/rat ($n=10$ each). In the rats given 500 ng bradykinin, blood was withdrawn 3 and 5 minutes after the start of the infusion to determine whether plasma kinin concentration had stabilized. Since no significant differences were found, plasma kinins were determined only at 5 minutes into the infusion thereafter. An additional group of rats ($n=10$) pretreated with enalaprilat alone (0.2 mg/kg) and infused with bradykinin (100 ng/min) was also examined.

**Effect of nephrectomy and ureteral ligation on endogenous plasma kinins and plasma kinin concentrations during bradykinin infusion.** To investigate the role of the kidney in kinin metabolism, nephrectomized rats were pretreated with vehicle ($n=10$), cocktail ($n=10$), or enalaprilat ($n=10$). Five minutes later they were infused with bradykinin (100 ng/min).

To determine whether the effect of nephrectomy (if any) was due to elimination of renal excretory function, we also studied a group of rats ($n=5$) whose ureters had been ligated 24 hours earlier. They were pretreated with cocktail and infused with bradykinin (100 ng/min).

**Effect of kininase inhibitors and nephrectomy or ureteral ligation on plasma kininase activity.** To estimate the effect of in vivo administration of the above kininase inhibitors on plasma kininase activity, rats were injected with vehicle ($n=6$), enalaprilat ($n=5$), cocktail ($n=6$), MGTA ($n=5$) or phosphoramidon ($n=5$). Blood was collected before and 10 minutes after the injection.

To examine the effect of nephrectomy or ureteral ligation on plasma kininase activity, we compared intact ($n=12$), nephrectomized ($n=12$), and ligated rats ($n=11$). Blood was withdrawn from the abdominal aorta 24 hours after surgery in a syringe containing 200 units heparin/ml blood.

**Analytical Techniques**

Plasma kinins were extracted with chilled ethanol as described by Shimamoto et al., 10 and concentrations were determined by radioimmunoassay. 11 (The antibody used does not cross-react with [des-Arg$^2$]- or [des-Phe$^6$-Arg$^2$]-bradykinin.) Plasma kininase activity was determined as a function of bradykinin hydrolysis (Ishida et al, unpublished data). Briefly, a solution of 0.1 M Tris buffer (pH 7.4) containing a...
final concentration of 5 μg/ml bradykinin and 1% plasma (vol/vol) was incubated at 37° C for 20–30 minutes. Kininase activity was expressed as the decrease in bradykinin during the incubation period as measured by radioimmunoassay.

Statistics

All results are expressed as mean±SEM. Analysis of variance (ANOVA) was done at each time point to test for possible differences among groups. If the ANOVA detected a difference among the groups, Tukey's pairwise comparison test was performed to test for differences between pairs of groups. A two-sample t test was used to compare 1) plasma kinin concentrations and mean arterial pressure during bradykinin infusion at a rate of 100 ng/min, 2) half-life of bradykinin, and 3) basal kinin concentrations and kininase activity (intact, nephrectomized, and ureteral ligated rats). A paired t test was used to evaluate the effect of kininase inhibitors on plasma kininase activity and the difference of kinin concentrations obtained at 3 and 5 minutes after the start of infusions. Pearson's correlation coefficients were calculated between kinin concentrations and arterial pressure falls. p<0.05 was considered significant.

Results

Effect of Cocktail on Endogenous Plasma Kinins and Plasma Kinin Concentrations During Bradykinin Infusion

In the rats infused with vehicle, pretreatment with the cocktail tended to increase endogenous kinin concentrations (from 31±6 to 41±9 pg/ml; p=0.94); however, the difference was not significant (Figure 1) nor was the decrease in mean arterial pressure caused by the cocktail significantly greater than that caused by the vehicle (6.3±2.9 vs. −0.3±1.6 mm Hg, p=0.07).

Mean arterial pressure and plasma kinin concentrations in the rats pretreated with vehicle or cocktail and infused with bradykinin at different rates are shown in Figure 1. During the infusion of bradykinin in the cocktail-pretreated groups, plasma kinin concentrations were almost 10 times higher than in the groups pretreated with vehicle. Mean arterial pressure did not change in the vehicle-pretreated groups but decreased in the cocktail-pretreated groups.

There was no difference in plasma kinin concentration between the groups pretreated with enalaprilat or cocktail and infused with bradykinin (100 ng/min) (697±31 vs. 883±112 pg/ml, NS). Plasma kinin concentrations at 3 and 5 minutes after the start of bradykinin infusion (500 ng/min) were 358±14 and 426±52 pg/ml (NS) in the rats pretreated with vehicle and 4,344±488 and 4,278±403 pg/ml (NS) in the rats pretreated with the cocktail. Thus, 5 minutes of bradykinin infusion was sufficient to cause a steady state of plasma kinin concentration. (Mean arterial pressure was not recorded in these groups.)

Effect of Nephrectomy and Ureteral Ligation on Endogenous Plasma Kinins and Plasma Kinin Concentrations During Bradykinin Infusion

Plasma kinin concentration was 34.9±2.5 pg/ml in the nonnephrectomized rats (n=66), 28.8±3.2 pg/ml in the nephrectomized rats (n=30), and 30.1±2.1 pg/ml in the ligated rats (n=5). There were no significant differences among these groups.

In the nephrectomized rats pretreated with vehicle and infused with bradykinin (100 ng/min), plasma kinin concentration was higher than in the intact rats (235±20 vs. 110±15 pg/ml); however, the difference was not significant (p>0.05) (Figure 2).

In the nephrectomized rats pretreated with cocktail and infused with bradykinin (100 ng/min), plasma kinin concentration was almost doubled compared with either the intact or ligated rats (1,600±187 vs. 805±91 and 883±112 pg/ml) (p<0.05).

In the nephrectomized rats pretreated with enalaprilat or cocktail, plasma kinins were similar (1,762±162 and 1,600±187 pg/ml, respectively, NS).

Figure 3 demonstrates the fall in mean arterial pressure in the nephrectomized rats pretreated with vehicle and infused with bradykinin (100 ng/min), which was correlated with the increase in plasma kinin concentration (r=0.75, p<0.001).
FIGURE 2. Effect of kininase inhibitors, nephrectomy, and ureteral ligation on plasma kinin concentration during bradykinin infusion (100 ng/min). Similar responses were seen in rats pretreated with enalaprilat and cocktail. Vc, vehicle; CEI, enalaprilat; Cktl, cocktail; Nx, nephrectomy; Uret Lig, ureteral ligation. *p<0.05 compared with intact or ligated rats pretreated with inhibitors. tp<0.05 compared with intact rats pretreated with vehicle. (Vertical bars represent SEM.)

To evaluate the effect of kininase inhibitors on the half-life of circulating kinins, three groups that exhibited roughly the same kinin concentration during bradykinin infusion were selected (Figure 4), namely, rats 1) pretreated with vehicle and infused with bradykinin (1,000 ng/min), 2) pretreated with enalaprilat, or 3) pretreated with cocktail and infused with bradykinin (100 ng/min). The half-life of bradykinin \([\text{BK}^{1/2}]\) was taken as the time needed to lower the bradykinin concentration by 50% after the infusion was stopped. In these three groups of rats \([\text{BK}^{1/2}]\) was 11.4±0.8, 10.4±0.5, and 10.2±1.1 seconds, respectively. There were no significant differences among them.

FIGURE 3. Correlation between plasma kinin concentration and decrease in mean arterial pressure in rats infused with bradykinin (100 ng/min) and pretreated with vehicle or kininase inhibitors. Correlation was significant \((r=0.75, p<0.001)\). Uret Lig, ureteral ligation; Nx, nephrectomy. (Vertical and horizontal bars represent SEM.)

Effect of Kininase Inhibitors and Nephrectomy or Ureteral Ligation on Plasma Kininase Activity

Plasma kininase activity decreased by a similar amount after injection of enalaprilat or cocktail, falling by 48.5±3.3% \((p<0.001)\) and 48.6±5.5% \((p<0.001)\), respectively. No significant decreases were seen after injection of vehicle, MGTA, or phosphoramidon (Table 1).

There were no significant differences in plasma kininase activity among intact \((11.2±0.4 \mu g \text{ bradykinin hydrolyzed/ml plasma/min incubation time})\), nephrectomized \((11.4±1.1)\), or ligated rats \((12.1±0.5)\).

Discussion

We found that the concentration of endogenous kinins did not significantly increase after injection of a cocktail of kininase inhibitors; however, infusion of different doses of bradykinin increased plasma kinin concentrations in a dose-dependent manner, becoming 10 times higher in the rats pretreated with the cocktail than in the vehicle group (Figure 1). The effect of the cocktail on plasma kinins was similar to the ACE inhibitor enalaprilat alone (Figure 2). Nephrectomy did not affect endogenous plasma kinins; however, when rats were infused with bradykinin, nephrectomy nearly doubled plasma kinin concentrations (Figure 2). The fall in blood pressure was correlated with the increase in plasma kinin concentration \((r=0.75, p<0.001)\) (Figure 3). In the rats pretreated with vehicle, bradykinin infusion \((1,000 \text{ ng/min})\) resulted in a plasma kinin concentration of 757 pg/ml, which was not enough to decrease blood pressure. This is in agreement with Salgado et al.,\(^\text{12}\) who found that plasma kinin concentration needs to be greater than 1,000 pg/ml to decrease blood pressure. In contrast, in the group pretreated with the cocktail, blood pressure fell by 14 mm Hg when the kinin concen-
Plasma kininase activity was determined before and after injection of various kininase inhibitors. Values are expressed as mean±SEM.

*Value significantly different from vehicle, p<0.001.

We know of two studies regarding the role of carboxypeptidase N on metabolism of circulating kinins. Ryan et al\textsuperscript{13} observed that carboxypeptidase N bound to the pulmonary endothelium was far more potent than that in serum and also noted that this enzymatic activity was completely suppressed by MGTA in rats, which failed to potentiate the hypotensive action of exogenous IV bradykinin. Conversely, Salgado et al\textsuperscript{22} observed that MGTA did potentiate the hypotensive response to intra-arterial bradykinin; however, this may not be due to inactivation of kininase I, since it also potentiated sodium nitroprusside, angiotensin I and II, and vasopressin.

Plasma kininase activity was determined before and after injection of various kininase inhibitors. Only enalaprilat had a significant effect on rat plasma kininase activity, although some authors have suggested that carboxypeptidase N is partially responsible for kinin degradation in human plasma\textsuperscript{6,23,24} so such activity could be species dependent. In vitro studies of rat plasma have shown that the contribution of carboxypeptidase N to total kininase activity is only 11% (Ishida et al, unpublished data), which may explain why MGTA did not alter plasma kininase activity in vivo.

Neutral endopeptidase 24.11, which hydrolyzes angiotensins, enkephalins, and kinins, has been found in various human tissues, primarily the kidney\textsuperscript{14} and lung,\textsuperscript{25} and is reportedly elevated in serum of patients with adult respiratory distress syndrome\textsuperscript{16} or sarcoidosis.\textsuperscript{26} Ura et al\textsuperscript{9} indicated that neutral endopeptidase 24.11 is a major kininase in rat urine and that administration of its inhibitor phosphoramidon caused urinary kinin excretion to increase, suggesting that it is important to metabolism of kinins in the nephron. However, our study showed that this kininase does not contribute significantly to metabolism of circulating kinins and indeed may not have access to them.

We know of only one study of the role of aminopeptidase in the control of blood pressure. Aoyagi et al\textsuperscript{20} reported that bestatin halted hypertension in young, spontaneously hypertensive rats, prompting further research into its role in kinin metabolism. Since the bradykinin antibody we used cannot differentiate [des-Arg\textsuperscript{1}]bradykinin from bradykinin, it is impossible to know the effect of bestatin on plasma kinin concentration. However, any cleavage at the N-terminal of bradykinin leads to significantly different among rats pretreated with vehicle or kininase inhibitors (Figure 4).

We found that nephrectomy doubled plasma kinin concentrations and increased the hypotensive effect of bradykinin infusion. Most likely this is not due to lack of filtration by the kidney with resulting accumulation of plasma kinins, since ureteral ligation had no effect (Figure 2). Nor does accumulation of kininase inhibitors due to acute renal failure seem likely, since plasma kininase activity in vitro was the same in intact and nephrectomized rats.

<table>
<thead>
<tr>
<th>Kininase inhibitor</th>
<th>Before treatment</th>
<th>10 minutes after treatment</th>
<th>Δ (%) decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10.6±1.0</td>
<td>10.6±0.8</td>
<td>-1.5±5.1</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>10.4±0.7</td>
<td>5.4±0.5</td>
<td>48.5±3.3*</td>
</tr>
<tr>
<td>Cocktail</td>
<td>10.1±0.4</td>
<td>5.4±0.5</td>
<td>46.8±5.5*</td>
</tr>
<tr>
<td>MGTA</td>
<td>9.3±0.8</td>
<td>8.8±0.5</td>
<td>3.7±7.3</td>
</tr>
<tr>
<td>Phosphoramidon</td>
<td>9.9±0.7</td>
<td>9.6±0.7</td>
<td>2.9±2.0</td>
</tr>
</tbody>
</table>

Plasma kininase activity is expressed in micrograms bradykinin hydrolyzed per 1 milliliter plasma per 1 minute incubation.
reported that plasma kininase activity was increased in patients with chronic renal failure. More likely, renal kininases contribute significantly to total kininase activity. Nasjletti et al. demonstrated that large amounts of kinins were inactivated by passage through the renal circulation. Our data further indicate that the kidney plays a very important role in the degradation of circulating kinins.

The present study suggests that kininases other than ACE participate in the degradation of circulating kinins. Indeed, Bakhle pointed out that most bradykinin hydrolysis in the rat lung is due to peptidases other than ACE despite conflicting data. Bakhle's observation is supported by our findings, in particular that 1) a cocktail of inhibitors did not increase endogenous kinin concentrations, 2) there was no significant difference in the rate of disappearance of kinins between vehicle- and inhibitor-treated rats, and 3) approximately half of the kininase activity in the plasma was not affected by the cocktail (Table 1). The peptidases responsible for the remaining kininase activity remain to be identified.

In conclusion, of the peptidases we tested, ACE proved to be the most potent in metabolizing circulating kinins, whereas the contribution of carboxypeptidase N, neutral endopeptidase 24.11, and amilating kinins, whereas the contribution of carboxypeptidases responsible for the remaining kininase activity remain to be identified.

References


Key Words: angiotensin converting enzyme • peptidases • kinins • kininase • blood pressure
Role of angiotensin converting enzyme and other peptidases in in vivo metabolism of kinins.
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Hypertension. 1989;14:322-327
doi: 10.1161/01.HYP.14.3.322

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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