We determined the effect of chronic administration of the angiotensin converting enzyme (ACE) inhibitor, enalapril, on the in vivo pulmonary inactivation of bradykinin (BK) and conversion of angiotensin I (Ang I). In addition we assessed whether chronic ACE inhibition influenced the activity of prolylendopeptidase (PEP), which metabolizes Ang I to generate angiotensin-(1-7) (Ang-[1-7]) and inactivates BK. Male Wistar rats were treated orally with enalapril (10 mg/kg once a day) for 7 to 15 days (n = 20) and 21 to 30 days (n = 11). Vehicle-treated rats (7 to 30 days, n = 11) were used as controls. Pulmonary inactivation of BK and conversion of Ang I were determined in conscious enalapril- or vehicle-treated rats before and after intravenous administration of the ACE inhibitor enalaprilat (MK-422, 10 mg/kg).

Pulmonary inactivation of BK (%) was determined by comparing equipotent doses of BK injected by the intravenous and intraaortic routes, and Ang I conversion (%) by comparing the pressor effect of Ang I and Ang II injected intravenously. PEP-like activity in plasma and lung homogenates was determined fluorometrically using the synthetic substrate Suc-Gly-Pro-MCA. In control rats, pulmonary BK inactivation averaged 97.6% ± 0.54%. Acute ACE inhibition with MK-422 reduced BK inactivation to 42.0% ± 2.7%. However, in rats treated chronically with enalapril, BK inactivation was increased as compared with acute ACE inhibition, averaging 58.8% ± 3.7% at 7 to 15 days and 58.8% ± 4.5% at 21 to 30 days of treatment.

Intravenous administration of MK-422 to the enalapril-treated rats did not return the increased BK inactivation to the level observed during acute ACE inhibition. In contrast, Ang I conversion was significantly reduced from 46.7% ± 6.5% to 0.9% ± 0.2% by MK-422, and this inhibition remained essentially unchanged during chronic treatment. PEP-like activity in plasma and lung homogenates of control rats was 4.4 ± 0.3 nmol MCA/min/mL and 11.4 ± 0.9 nmol MCA/min/mg protein, respectively. After chronic treatment with enalapril there was a progressive increase of PEP-like activity in both plasma and lung, which after 21 to 30 days of treatment averaged 10.7 ± 1.7 nmol MCA/min/mL and 29.2 ± 2.8 nmol MCA/min/mg protein, respectively. These data indicate that chronic ACE blockade induces alternative BK-inactivating mechanisms and increases Ang-(1-7)- generating mechanisms. Am J Hypertens 1999; 12:1021–1029 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Angiotensin conversion, bradykinin inactivation, converting enzyme inhibitors, prolylendopeptidase, angiotensin-(1-7), post-proline cleaving enzymes.

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**ANGIOTENSIN CONVERTING ENZYME** (ACE) inhibitors act on both the renin-angiotensin (RAS) and kallikrein-kinin systems (KKS), releasing the hypertensive peptide angiotensin II (Ang II) and inactivating the hypotensive peptide bradykinin (BK). Therefore, the acute effect of ACE inhibitors (CEI) on humans and on hypertensive animals has been attributed to the decrease of Ang II formation and to the interference with kinin metabolism. However, the role of the RAS and kinins in the mechanism of the effects produced by chronic treatment with CEI is not yet clear. The plasma levels of Ang II, for example, have been reported to be normal after chronic treatment with CEI. In addition, the biologically active heptapeptide angiotensin-(1-7) (Ang-[1-7]), which can be formed directly from Ang I by an ACE-independent pathway, increases markedly in patients or rats chronically treated with CEI. The increase in plasma Ang-(1-7) during ACE inhibition is probably attributable to the changes in the metabolism of Ang I, whose plasma levels were increased in this situation, and to the decreased metabolism of Ang-(1-7) by ACE. Ang-(1-7) could contribute to the pharmacologic effect of CEI by potentiating the effects of BK or by contributing to vasodilator tone through the release of NO or prostaglandins.

The changes in the plasma profile of angiotensins after chronic treatment with ACE inhibitors appears to be produced not only by the decreased ACE activity but also by modification of non-ACE metabolism of Ang I in plasma or tissues. There are no data, however, regarding the effect of chronic ACE inhibition on BK metabolism in vivo.

In addition to ACE, the enzyme prolylendopeptidase (PEP, EC 3.4.21.26) can influence both the RAS and the KKS. Prolylendopeptidase can generate Ang-(1-7) directly from Ang I or from Ang II by cleavage of the Pro7–Phe8 bond of these peptides and can hydrolyze BK by cleaving the Pro3–Gly4 and Pro7–Phe8 bonds.

In the present study we attempted to evaluate the effect of chronic ACE inhibition with enalapril treatment on the metabolism of BK and Ang I by determining the inactivation of BK and conversion of Ang I to Ang II in the pulmonary vascular bed using an in vivo technique. Measurements of PEP-like activity were also made in the plasma and lung of rats chronically treated with the ACE inhibitor enalapril to evaluate the possible role of changes to this enzymatic activity in the formation of Ang (1-7) or BK metabolism.

**METHODS**

The peptides Hip-His-Leu, His-Leu, Ang I, Ang II, and BK were synthesized in the laboratory of Profs. L. Juliano-Netto and A.C.M. Paiva, Escola Paulista de Medicina, Sao Paulo. MK 421 and MK 422 were provided by Merck (Merck Sharp & Dohme, Brazil). Z-Pro-Prolinal (ZPP) was a gift from Dr. S. Wilk, Mount Sinai Hospital, New York, NY. CBZ-2-Gly-Pro-MCA was purchased from Bachem (Torrance, California) and MCA, α-phthalaldehyde, DTT, and Tris were purchased from Sigma (St. Louis, Missouri). Other solvents and chemicals were reagent grade or equivalent.

**Animals** Male Wistar rats weighing 100 to 250 g depending on the phase of the study were raised in the animal house of the Department of Physiology and Biophysics, ICB, UFMG, Belo Horizonte, MG.

**General Procedures** Catheters were implanted under ether anesthesia 24 h before the experiments. Blood pressure was measured with a cannula inserted into the abdominal aorta through the femoral artery and connected to a pressure transducer (HP 1280-C; Hewlett-Packard, Boston, Massachusetts), fed into a four-channel polygraph (HP 7754 A; Hewlett-Packard, Boston, Massachusetts). Pulsatile arterial pressure (systolic and diastolic) was recorded directly and mean arterial pressure (MAP) was recorded using an expanded scale (0.5 mm deflection/mm Hg). The arterial cannula used to administer peptides was introduced into the thoracic aorta through the left carotid artery, whereas intravenous administration was carried out through a cannula inserted into the inferior vena cava through the femoral vein. Blood for enzyme determinations was collected through a cannula implanted into the abdominal aorta. The cannulas were exteriorized on the dorsum through subcutaneous tissue to permit free movement of the conscious animals. The cannulas were always kept filled with physiologic saline and closed at the tip with a metal pin. The animals were maintained in individual cages with water and ration ad libitum for 24 h after implantation of the cannulas and before the beginning of the measurements.

**Administration of ACE Inhibitors** Rats were treated chronically with 10 mg/kg MK 421 (Enalapril; Renitec, Merck Sharp & Dohme, Brazil) administered by gavage as a single dose between 8:00 and 10:00 AM. Different groups of rats were treated for 7 to 15 or 21 to 30 days. Control rats for the chronic treatment received 0.5 mL of water by gavage at the same time each day and for the same number of days as the treated animals. Because there was no difference in the parameters measured in the study among the members of the control groups, the data were combined and are presented as a single control group. The inhibition of plasma ACE activity was 98.9% 30 min immediately after MK421 administration and 80% to 90% after 3 h.
Acute treatment with an ACE inhibitor was carried out with enalaprilat (MK-422, 10 mg/kg) administered intravenously as a bolus to animals previously chronically treated with MK 421 and to control animals. Rats received MK 422 5 min after the first series of measurements of Ang I conversion and BK inactivation in vivo (denoted chronic enalapril MK 421 in Tables 1 and 2).

**Determination of Ang I Conversion and BK Inactivation In Vivo** Angiotensin I conversion and BK inactivation were measured in vivo in conscious unrestrained rats according to a technique described by Salgado and Krieger in which Ang I and Ang II are given intravenously and BK is given intravenously and intraarterially. The only modification was that percent conversion was calculated by constructing dose–response curves for Ang I and Ang II rather than by quantitating by matching to a standard pressure change. Different amounts of the peptide in 0.1 mL of saline were injected intravenously as a bolus to produce pressure effects of 5 to 25 mm Hg. BK inactivation was measured in a similar manner by comparing the dose hypotensive response curves (log dose vs. response) of BK injected intravenously and intraarterially in 0.1 mL as a bolus. The fraction of BK metabolized during intravenous passage through the lungs was calculated and is reported as percent inactivation.

**Determination of Plasma ACE and PEP Activity** Plasma was prepared from 0.2 mL of blood obtained with a tuberculin syringe previously washed with 50 IU/mL heparin connected to the abdominal aorta catheter. Blood was transferred to polypropylene tubes and centrifuged at 800 g for 20 min at room temperature. Plasma was maintained on ice until the enzyme assays.

Plasma ACE activity was measured by the fluorometric method described by Santos et al. using Hip-His-Leu as substrate, within 2 to 3 h after blood collection. Duplicate aliquots of plasma (10 μL) were incubated with 500 μL 5 mmol/L Hip-His-Leu in 0.5 mol/L sodium borate buffer, pH 8.3, containing 0.9 mol/L NaCl for 15 min at 37°C. The reaction was stopped by the addition of 1.2 mL of 0.34 mol/L NaOH, 100 μL orthophthaldehyde (20 mg/mL in methanol) was added and 200 μL 3 N HCl was added after 10 min at room temperature. After centrifugation at 800 g for 5 min, the fluorescence of the supernatant solution (365 nm excitation and 495 nm emission) was measured against water. Blanks were prepared by inverting the order of addition of enzyme and NaOH. A standard curve of 0.5 to 20 nmol His-Leu/tube was prepared for each assay. Enzyme activity is reported as nanomoles of His-Leu per minute per milliliter. Assays were carried out under conditions that provided constant velocity and constant enzyme-specific activity. The specificity of the assay was demonstrated by 98% inhibition with 5 μmol/L of MK 422.

Plasma PEP activity was measured using 0.25 mmol/L CBZ-Gly-Pro-MCA in 20 mmol/L Tris-HCl buffer, pH 8.3, containing 1 mmol/L EDTA and 1 mmol/L DTT. Duplicate aliquots of plasma, (10 μL) were added to 200 μL substrate (37°C) and incubated for 15 min at 37°C. The reaction was stopped by the addition of 1 mL of 1 mol/L sodium acetate buffer, pH 4.2, and the fluorescent product (MCA) was measured against water at 380 nm for excitation and at 455 nm for emission. Blanks were prepared by inverting the order of addition of plasma and sodium acetate buffer. A standard curve for the product was prepared using 2, 4, 20, and 100 pmol/tube. Enzyme activity is reported as nanomoles of MCA per minute per milliliter.

As was the case for ACE, assay conditions were selected to provide constant velocity and constant enzyme specific activity for the data reported here. Z-Pro-Prolinal (1 μmol/L) inhibited the hydrolysis of CBZ-Gly-Pro-MCA by rat plasma by 64%.

**Determination of Pulmonary ACE Activity** The animals were heparinized intravenously (200 U/100 g), and the heart and lungs were exposed under ether anesthesia. The lungs were perfused in situ through a needle syringe introduced into the right ventricle and the ascending aorta was cut to provide an exit path. The perfusion consisted of 20 mL of saline at 25°C followed by 20 mL of 0.32 mol/L in sucrose in 50 mmol/L sodium borate buffer, pH 7.4, at 4°C. The lungs were washed after being submitted to these maneuvers to reduce contamination by blood.

Immediately after dissection, 500 mg of tissue was homogenized at 4°C with 5 mL of 0.32 mol/L sucrose containing 50 mmol/L sodium borate buffer, pH 7.4. Homogenization was carried out with a Potter-Elvehjem Teflon pestle using 15 incursions at 11,000 rotations/min. The homogenate was centrifuged at 800 g for 10 min at 4°C. ACE activity was determined in the supernatant solution within 2 to 3 h after tissue homogenization. Protein was measured by the method of Lowry et al. using bovine serum albumin as the standard.

ACE activity of the lung extract was measured as described above for plasma ACE. Blanks and other control experiments related to linearity of the enzyme assay, specificity, and dipeptidase activity were the same as described for the measurement of the plasma enzyme. MK-422 (5 μmol/L) inhibited the hydrolysis of Hip-His-Leu by the soluble fraction of an untreated rat by 99.9%.

**Determination of Pulmonary PEP Activity** The preparation of the lung extract was the same as described for ACE activity except that the tissue was...
homogenized in 20 mmol/L Tris-HCl buffer, pH 8.3, containing 1 mmol/L EDTA (1:10 wt/vol). Pulmonary PEP activity was measured as described for the plasma enzyme using the same procedures for blanks and controls. Z-Pro-Prolinal (1 μmol/L) inhibited the hydrolysis of CBZ-Gly-Pro-MCA by the rat lung soluble fraction by 83%.

Statistically significant differences between groups were determined by ANOVA followed by the Newmann-Keuls test or Student’s t test, with P, .05 taken as the level of significance.

RESULTS

In vivo Pulmonary Conversion of Ang I and Inactivation of BK In control rats, pulmonary BK inactivation was 97.6% ± 0.57%, whereas Ang I conversion, which averaged 46.7% ± 6.46%, was more variable (range 16% to 76%). As shown in Table 1, acute administration of enalaprilat produced, as expected, a marked reduction of Ang I conversion (0.9% ± 0.20%), whereas BK inactivation was reduced by about 57% (42.0% ± 2.70%). The equipotent doses of Ang I and Ang II needed to produce a 20 mm Hg increase of MAP before enalaprilat were 7.1 ± 0.60 pmol and 3.3 ± 0.50 pmol, respectively (Figure 1). After acute treatment the equipotent doses were 4.1 ± 0.70 pmol and 551.0 ± 64.00 pmol for Ang II and Ang I, respectively. For BK the equipotent doses needed to produce a 20 mm Hg decrease in MAP before enalaprilat were 1632.0 ± 526.00 pmol (intravenously) and 26.9 ± 9.90 pmol (intraarterially) and decreased to 19.2 ± 3.80 pmol (intravenously) and 11.5 ± 2.50 pmol (intraarterially) after treatment (Figure 2). Acute blockade of ACE also produced a slight decrease in MAP (Table 1).

Chronic treatment with enalapril produced a 20% increase in BK metabolism as compared with acute treatment during all periods studied (P < .05; Table 1). Acute administration of enalapril to chronically enalapril-treated rats produced only minor changes in BK metabolism (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before Enalapril</th>
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<tr>
<td></td>
<td>n</td>
<td>MAP (mm Hg)</td>
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<td>Vehicle</td>
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<tr>
<td>Enalapril-treated</td>
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<td>(7–15 d)</td>
<td>20</td>
<td>103 ± 2</td>
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<tr>
<td>Enalapril-treated</td>
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<tr>
<td>(21–30 d)</td>
<td>11</td>
<td>106 ± 1</td>
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* P < .05 compared to vehicle (ANOVA followed by the Newmann-Keuls test). † P < .05 compared to before enalaprilat (Student’s paired t test).

MAP = mean arterial pressure; Vehicle = tap water; enalaprilat, 10 mg/kg, IV.

![FIGURE 1](https://example.com/fig1.png) Intravenous doses of Ang I and Ang II required to produce changes of 20 mm Hg in MAP in conscious rats before and 10 min after administration of enalaprilat (10 mg/kg IV). (Top) Vehicle-treated rats (n = 11). (Middle) Rats treated orally with 10 mg/kg enalapril for 7 to 15 days (n = 20). (Lower) Rats treated orally with 10 mg/kg enalapril for 21 to 30 days (n = 11). Data are reported as means ± SEM. Data before enalaprilat were obtained 30 to 60 min after administration of the last dose of enalapril. (P < .05 compared to the dose used before enalaprilat; Student’s t test.)
metabolism (Table 1). The vascular reactivity to BK evaluated by the comparison of the doses necessary to produce a 20 mm Hg decrease in MAP was not significantly changed by chronic treatment with enalapril (Figure 2). Chronic enalapril treatment for 21 to 30 days produced an increase in the Ang I to Ang II conversion (6.9% ± 2.90% v 0.9% ± 0.20% during acute ACE inhibition; Table 1). However, after administration of a supplementary dose of enalapril, Ang I conversion decreased to a value that did not differ from that observed during acute ACE inhibition (1.6% ± 0.50%; Table 1). Chronic treatment with enalapril did not change MAP significantly.

Effect of ACE Inhibition on ACE and ProlylEndopeptidase-Like Activity

**Acute Treatment**  
Acute administration of enalaprilat reduced plasma ACE by about 99% (93.4 ± 8.10 to 1.02 ± 0.10 nmol His-Leu/min/mL; Table 2). No significant changes in plasma PEP-like activity were observed (4.41 ± 0.30 v 4.1 ± 0.90 nmol MCA/min/mL after treatment).

**Chronic Treatment**  
Plasma ACE activity in the chronically enalapril-treated groups was significantly inhibited, but the enzymatic activity was higher when compared with the level observed in the acute inhibition group (Table 2). Administration of an additional intravenous dose of ACE inhibitor further reduced plasma ACE activity in chronically enalapril-treated animals by 80% to 90% (Table 2). Lung ACE activity was significantly reduced in the rats treated with enalapril (155.7 ± 14.80 to 93.4 ± 8.10 nmol His-Leu/min/mg protein; Table 2) for 7 to 15 days. After this initial inhibition there was an increase in lung ACE activity, which was similar to that of the control group after 21 to 30 days of treatment (179.4 ± 18.90 nmol His-Leu/min/mg protein; Table 2).

As shown in Figure 3, the PEP-like activity in plasma increased progressively during chronic enalapril treatment (4.4 ± 0.30 nmol MCA/min/mL in the control group compared with 12.3 ± 3.70 nmol MCA/min/mL after 21 to 30 days of enalapril treatment). After an additional intravenous dose of enalaprilat, plasma PEP-like activity did not change significantly (7 to 15 days: 6.0 ± 0.70 nmol MCA/min/mL before enalaprilat v 7.9 ± 1.20 nmol MCA/min/mL after enalaprilat; 21 to 30 days: 10.7 ± 1.70 nmol MCA/min/mL v 10.7 ± 0.70 nmol MCA/min/mL after enalaprilat). Lung PEP activity also increased during
chronic ACE inhibition, being significantly augmented after 7 to 15 or 21 to 30 days of enalapril treatment (17.1 ± 2.30 nmol MCA/min/mg protein and 29.1 ± 2.80 nmol MCA/min/mg protein in the control group; Figure 3).

**DISCUSSION**

In the present study we have shown by an in vivo method that chronic treatment with the ACE inhibitor enalapril produced a significant increase in BK degradation. Moreover, PEP-like activity also increased in plasma and lung homogenate of enalapril-treated rats.

It is well known that, in addition to ACE, other peptidases can participate in BK metabolism. These include aminopeptidase P, carboxypeptidase M, carboxypeptidase N, neutral endopeptidase 24.11, and PEP. Aminopeptidase P cleaves the Arg¹-Pro² bond of BK, whereas neutral-endopeptidase 24.11 and PEP cleaves the Pro⁷-Phe⁸ peptide bond. Prolylendopeptidase can also cleave the Pro⁵-Gly⁴ bond and endopeptidase 24.11 the Gly⁴-Phe⁵ bond of BK. Endopeptidase 24.15 cleaves the Phe⁵-Ser⁶ bond of the nonapeptide. In contrast, carboxypeptidases M and N cleave the Phe⁸.Arg⁹ peptide bond. The finding that BK-(1-9) and BK-(1-7) are the predominant BK peptides in rat plasma and tissues suggests that the major pathway for BK metabolism is the hydrolysis of the Pro⁷-Phe⁸ bond. In situ perfused rat lungs, Pesquero et al. have observed that BK-(1-7), BK-(1-5), and BK-(4-9) were the predominant fragments recovered in the perfusate after BK administration. The formation of BK-(1-7) and BK-(1-5) was abolished by enalapril but not by ZPP, N[1(RS)-carboxy-3-phenylpropyl]-Ala-Ala-Phe.p-aminobenzoate (CPP-Ala-Ala-Phe.pAB) or N[1(RS)-carboxy-3-phenylpropyl]-Phe.p-aminobenzoate (CPP-Phe.pAB), indicating that ACE but not PEP, endopeptidase 24.11, or endopeptidase 24.15 was responsible for the formation of these fragments. In vivo, however, ACE inhibition did not prevent the formation of BK-(1-7), suggesting a role for endopeptidase 24.11 or PEP-like enzymes in its generation.

We have found that the pulmonary inactivation of BK in vivo is 97.6% ± 0.57%, which is similar to the percent inactivation reported by Salgado and Krieger and by Pesquero et al. After enalaprilat administration, BK inactivation was reduced to 42% ± 2.7%. The larger percent of the remaining activity found by Pesquero et al. in enalaprilat-treated rats (69%) probably reflects a difference in the dose of the ACE inhibitor (10 mg/kg in our study and 0.5 mg/kg in their study). The efficacy of the dose of enalapril used in our study was demonstrated by the dramatic reduction of Ang I to Ang II conversion, which was reduced by 98% (46.7% ± 6.46% to 0.9% ± 0.20%). A similar extent of inhibition (98.9%) was observed for plasma ACE (93.4 ± 8.10 to 1.02 ± 0.10 nmol His-Leu/min/mL). These findings indicate that the remaining inactivation of BK after acute enalaprilat administration was not attributable to incomplete blockade of ACE. These data obtained for perfused rat lungs are consistent with observations in vivo.

Chronic treatment with enalapril (21 to 30 days) produced a significant increase in pulmonary BK inactivation measured 30 min after the last administration (from 42% ± 2.70% to 58.7% ± 4.50%, P < .05). The increased inactivation of BK was evident even after administration of a supplementary dose of enalapril (55.1% ± 3.90%). In parallel with the increased BK inactivation, Ang I to Ang II conversion increased from 0.9% ± 0.20% to 6.9% ± 2.90% after chronic treatment with enalapril. However, the increased Ang I conversion was reversed by administration of a supplementary dose of enalapril, suggesting that this was attributable to a residual ACE-like activity. The similar findings obtained for plasma ACE agree with this interpretation. The findings regarding Ang I conversion and plasma ACE activity are in accordance with the findings of other studies in this area.
with several studies that have shown that chronic ACE inhibition leads to a marked increase in ACE synthesis, which is usually masked by the presence of the ACE inhibitor.29–32

Taken together, the results obtained with chronic treatment with enalapril regarding Ang I to Ang II conversion clearly indicate the need for caution when attempting to interpret data obtained by measurement of angiotensin peptides in animals chronically treated with ACE inhibitors. We would expect Ang I level in plasma or tissue to be within the normal range within a few hours after administration of a similar dose of the ACE inhibitor during chronic treatment with enalapril. This does not necessarily mean that the levels would be normal or above those expected due to the contribution of alternative enzymatic pathways.7,23,34 Actually no evidence was found in our study or in other studies7,27,35 that non-ACE pathways could play a biologically significant role in the Ang I to Ang II conversion in vivo. Whether our finding with enalapril can be extended to other ACE inhibitors remains to be established.

We have observed that ACE activity in lung homogenate was not reduced as effectively as the Ang I to Ang II conversion evaluated in vivo. This difference suggests that the ACE inhibitor did not affect an important fraction of ACE, probably extravascular. Another likely explanation is the inactivation of the ACE inhibitor during the preparation of the lung homogenate.

We have no direct data to explain the increase in BK inactivation after chronic treatment with ACE inhibitors. In addition to an adaptive response to chronically increased BK levels, one possible explanation is that BK or other peptides cleaved by ACE participate in the regulation of the expression of BK-inactivating peptidases. The increase in PEP-like activity observed in plasma and lung homogenates of enalapril-treated rats supports this view.

As pointed out, enalapril-treated rats presented an increase of PEP-like activity in plasma and lung. The PEP inhibitor ZPP (1 μmol/L) inhibited the plasma activity by 64% and the lung homogenate activity by 83%. Because the K_i of ZPP for PEP is 14 nmol/L,19 these data suggest that the enzymatic activity cannot be attributed only to this enzyme, especially in plasma. In agreement with our data, a ZPP-insensitive PEP-like enzyme has been recently described by Cunningham and O’Connor36 in serum. The biological significance of our findings regarding PEP-like activity remains to be established. However, the observation that ZPP was able to partially block Ang-(1-7) formation in human umbilical vein endothelial cells in culture (up to 40%)37 and the recent demonstration of membrane-bound forms of PEP-like activity in the brain,38,39 the relevance of prolylendopeptidase in the metabolism of hormones such as thyrotropin-releasing hormone19 and gonadotropin-releasing hormone,38 and the important role of this enzyme in the generation of Ang-(1-7) in the same tissues40 illustrate the potential biological importance of this enzyme. Whether the increased PEP-like activity effectively contributes to the increased BK metabolism or Ang-(1-7) formation in plasma or tissues during chronic ACE inhibition remains to be investigated. It should be pointed out that NEP 24,11, the other enzyme that could be responsible for the cleavage of the Pro7-Phe8 bond of BK or Ang I in vivo, has been reported to be decreased by chronic enalapril treatment.18

Chronic ACE inhibition leads to a 25-fold increase in Ang-(1-7) in plasma and a four- to fivefold increase in Ang-(1-7) levels has been reported in lung.7 The increase in Ang-(1-7) concentration has been interpreted to be the result of the increased levels of Ang I and the decreased Ang-(1-7) breakdown by ACE,10,11,12,41 Indeed, the potentiation of the Ang-(1-7) effect at the rostral ventrolateral medulla by the ACE inhibitor ramiprilat42 suggests that the inactivation of Ang-(1-7) by ACE is functionally relevant. In this study we have obtained preliminary evidence that, in addition to these mechanisms, an increase in other enzymatic activities, such as PEP-like activity, can also play a role in the changes of plasma or tissue Ang-(1-7) concentration during chronic ACE blockade. In the circulation, Yamamoto et al43 and Iyer et al41 have presented evidence for a major role of NEP in Ang-(1-7) formation. In addition, as deduced from the data presented in the figures of the study by Yamamoto et al,43 rats given ZPP and enalaprilat presented a reduction in the rate of disappearance of Ang I, suggesting that ZPP-sensitive enzymes contributed to the metabolism of the decapeptide. A decrease in the peak levels of Ang-(1-7) after intravenous injection of Ang I was also observed in rats given ZPP and enalaprilat as compared to enalaprilat-treated rats. However, the effects of ZPP on basal conditions were not reported.43 Taken together the observations in specific tissues and in the circulation suggest that the enzymatic pathways involved in the formation of Ang-(1-7) and probably in BK metabolism are tissue specific. The increase of Ang-(1-7) produced by chronic ACE inhibitor administration may contribute to the cardiovascular effects of these drugs by several mechanisms including facilitation of baroreflex44 and potentiation of the vasodilator effect of BK,10,13,45 or direct vascular effects.41

In summary, we have provided evidence that chronic ACE blockade in rats induces an increase in non-ACE enzymatic activities involved in the pulmonary inactivation of BK. The Ang I to Ang II conversion was apparently increased in chronically enalapril-treated rats, suggesting the induction of alternative pathways for Ang II formation. However, administration of enalaprilat to the chronically enalapril-treated rats reduced Ang I conver-
sion to levels observed with acute ACE blockade. This finding illustrates the importance of the care when collecting blood samples from ACE inhibitor-treated animals to avoid erroneous conclusions regarding the importance of alternative pathways for Ang I conversion in this condition. The observation that PEP-like activity was increased in enalapril-treated rats suggests that post-proline cleaving enzymes could contribute to both the increased BK inactivation and Ang-(1-7) formation observed in these animals.

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