Inhibition of Tissue Angiotensin Converting Enzyme
Quantitation by Autoradiography

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SUMMARY Inhibition of angiotensin converting enzyme (ACE) in serum and tissues of rats was studied after administration of lisinopril, an ACE inhibitor. Tissue ACE was assessed by quantitative in vitro autoradiography using the ACE inhibitor [125I]351A, as a ligand, and serum ACE was measured by a fluorimetric method. Following oral administration of lisinopril (10 mg/kg), serum ACE activity was acutely reduced but recovered gradually over 24 hours. Four hours after lisinopril administration, ACE activity was markedly inhibited in kidney (11% of control level), adrenal (8%), duodenum (8%), and lung (33%; p < 0.05). In contrast, ACE in testis was little altered by lisinopril (96%). In brain, ACE activity was markedly reduced 4 hours after lisinopril administration in the circumventricular organs, including the subfornical organ (16-22%) and organum vasculosum of the lamina terminalis (7%; p < 0.05). In other areas of the brain, including the choroid plexus and caudate putamen, ACE activity was unchanged. Twenty-four hours after administration, ACE activity in peripheral tissues and the circumventricular organs of the brain had only partially recovered toward control levels, as it was still below 50% of control activity levels. These results establish that lisinopril has differential effects on inhibiting ACE in different tissues and suggest that the prolonged tissue ACE inhibition after a single oral dose of lisinopril may reflect targets involved in the hypotensive action of ACE inhibitors. (Hypertension 11: 230-238, 1988)

KEY WORDS • angiotensin converting enzyme inhibitors • hypotensive action • lisinopril • blood-brain barrier • renin

ANGIOTENSIN converting enzyme (ACE; EC3.4.15.1) is a peptidyl dipeptidase that is responsible for the conversion of angiotensin I to angiotensin II. High concentrations of ACE are found in vascular endothelial cells, renal proximal tubules, intestinal mucosa, the male reproductive system, and some specific areas of the brain.

Recently, ACE inhibitors such as captopril and enalapril have been found to be effective for the treatment of hypertension and congestive heart failure. The mechanism of the hypotensive action of these drugs is unclear. Although the circulating renin-angiotensin system was initially considered the primary target for ACE inhibitors, this possibility must be reevaluated because ACE inhibitors are effective in low or normal renin states. Furthermore, the blood pressure fall after administration of ACE inhibitors can be temporally dissociated from serum ACE inhibition. An alternative explanation for the hypotensive effect of ACE inhibitors is inhibition of localized tissue ACE.

In the present study, we visualized ACE in the different tissues of rat by quantitative in vitro autoradiography and compared the activity and distribution of ACE in different tissues with those seen after oral administration of lisinopril, a new, potent ACE inhibitor.
Materials and Methods

Radioligand

351A, a tyrosyl derivative of enalaprilic acid (kindly provided by Dr. C. Sweet, Merck Institute of Therapeutic Research, West Point, PA, USA), was radiiodinated with 125I by the chloramine-T method and purified by SP-C25 Sephadex chromatography. The binding properties of this ligand have been published previously. In Vitro Autoradiographic Measurement of ACE

Adult male Sprague-Dawley rats (Biological Research Laboratories, Austin Hospital, Victoria, Australia; weight, 200–250 g) were gavage-fed lisinopril (supplied by Dr. D. Jeremy, Merck Sharp & Dohme, Australia), 10 mg/kg, and killed 0, 4, and 24 hours later.

Brain, kidney, testis, adrenal, lung, and duodenum were removed quickly, snap-frozen in isopentane at −40°C, and used for autoradiographic localization of ACE. Frozen sections (20 μm) of the tissues were cut in a cryostat maintained at −20°C. The sections were thaw-mounted onto gelatin-coated slides, dried in a desiccator for 2 hours at 4°C, and then incubated in 10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl and bovine serum albumin, 2 g/L (Buffer A), with [125I]351A, 0.3 μCi/ml, for 1 hour at 20°C. Non specific binding was determined in parallel incubations containing 1 mM EDTA. After incubation, the sections were transferred through four successive 1-minute washes of Buffer A at 0°C, dried under a stream of cold air, placed in x-ray cassettes, and exposed to AgfaScopix CR3 x-ray film (Agfa Gevaert, Australia) for 1 to 3 days.

The system was calibrated for enzyme activity using a set of enzyme standards that were prepared by serial dilutions of membrane-rich fractions prepared from the caudate putamen of brain, outer medulla of kidney, or testis in 5% gelatin solution in Buffer A. Then, 5-μl aliquots of these enzyme standards were applied to slides, dried at 4°C in a desiccator, and subjected to the same incubation procedure used for the tissue sections. Samples of the membrane suspensions were also assayed for ACE and protein.

The x-ray films were processed in a Kodak RP X-OMAT automatic developer (Rochester, NY, USA), and optical density was quantitated using an EyeCom Model 850 image analysis system (Spatial Data Systems, Springfield, VA, USA) coupled to a DEC 11/23 LSI computer (Digital Equipment Corp., Maynard, MA, USA). The optical density of the autoradiographs was calibrated in terms of the ACE activity (pmol/min/mg protein) by fitting calibration curves with the computer using the enzyme standards as reference points. The sections used for autoradiography were stained with thionin (brain) or hematoxylin and eosin for histological localization of the autoradiographs.

Four rats were studied at each time point. Serial sections were taken from brain regions of interest, and six sections were taken in series from each of the other tissues. These sections from different time points were incubated in parallel with the controls and subjected to film exposure, development, and computer analysis in parallel. ACE activity in the area of interest was measured and expressed as a percentage of ACE activity of the same area from sections of the control.

Serum ACE and Lisinopril Measurement

Trunk blood collected after decapitation was used to measure serum ACE activity by a fluorimetric method, and lisinopril concentration was measured by radioimmunoassay.

Statistics

Values are expressed as means ± SD. Comparisons between groups were by two-way analysis of variance.

Results

Computer-processed images of tissue autoradiographs are presented in Figures 1 and 4. Nonspecific binding, measured in the presence of 1 mM EDTA, was completely undetectable and produced no visible image on the x-ray films. The autoradiographs shown in Figures 1 and 4, therefore, represent specific binding only.

Regional Inhibition of Brain ACE

Localization of brain ACE by quantitative in vitro autoradiography before and after lisinopril treatment is shown in Figure 1. A very high density of ACE was observed in the ventricular choroid plexus, subformical organ (SFO), and organum vasculosum of the lamina terminalis (OVLT), and a high density was seen in the basal ganglia of the control brains, as previously described (see Figure 1, A and D). Following lisinopril treatment, ACE activity in the SFO and OVLT was markedly reduced at 4 hours (see Figure 1, B and E) and had partially recovered toward control levels at 24 hours (see Figure 1, C and F). On the other hand, ACE in the choroid plexus, caudate putamen, and globus pallidus was not affected significantly after lisinopril administration. Figure 2 shows the quantitative data obtained by computerized image processing. Since ACE activity in the SFO increased from the rostral to caudal, the SFO was divided into three groups for analysis. At 4 hours, ACE activity in the SFO and OVLT was markedly reduced (rostral SFO, 22 ± 4%; mid SFO, 19 ± 4%; caudal SFO, 16 ± 9%; OVLT, 7 ± 4% of the activity of control rats; p < 0.05 for rostral SFO, p < 0.01 for others; see Figure 2). At 24 hours, these values had partially recovered (rostral, 48 ± 15%; mid SFO, 37 ± 9%; caudal SFO, 31 ± 1%; OVLT, 11 ± 4% of the activity of control rats; p < 0.05 for rostral and mid SFO, p < 0.01 for caudal SFO and OVLT; see Figure 2). Quantitative ACE values in the choroid plexus and two regions of the basal ganglia—caudate putamen and globus pallidus—were unchanged at 4 hours (choroid plexus, 99 ± 13%; caudate putamen, 95 ± 16%; globus pallidus, 102 ± 26% of control; NS for each) and 24 hours (choroid plexus, 94 ± 10%; caudate putamen,
FIGURE 1. Autoradiographic localization of ACE in rat forebrain before (A, D) and 4 (B, E) and 24 hours after lisinopril (C, F), 10 mg/kg p.o., administration. Coronal sections at levels of A–C and D–F are 7.7 and 9.0 mm, respectively, from the interaural line. Nonspecific binding in the presence of 1 mM EDTA completely abolished these patterns of binding. ACE activity in the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) was inhibited markedly 4 hours after administration (B, E) and partially recovered by 24 hours (C, F) without any changes in other regions of the brain. CPu = caudate putamen; ChP = choroid plexus; GP = globus pallidus. The color code is as follows: red = very high ACE activity; yellow = high; green = moderate; light blue = low; deep blue = undetectable. The white scale at the bottom of A and D is 5 mm long.
Localization and Inhibition of ACE in Peripheral Organs

Autoradiographic visualization of ACE in testis, adrenal, kidney, lung, and duodenum is shown in Figure 4. A high density of ACE was demonstrated in adrenal medulla, deep renal proximal tubules, lung parenchyma, duodenal mucosa, and vascular wall of each organ (see Figure 4, D, G, J, and M). ACE activity in these tissues was markedly inhibited by lisinopril at 4 hours (see Figure 4, E, H, K, and N) and recovered partially at 24 hours (see Figure 4, F, I, L, and O). Quantitative values obtained from computer analysis are shown in Figures 5 and 6. Marked inhibition of ACE was seen 4 hours after lisinopril administration in adrenal (8 ± 1% of the activity of control rats; p<0.01), kidney (11 ± 2% of control; p<0.01), and duodenum (8 ± 5% of control; p<0.01). The degree of inhibition in lung (33 ± 8% of control; p<0.01) was less than that in the other tissues (see Figures 5 and 6). Twenty-four hours after lisinopril administration, ACE activity in adrenal (27 ± 3% of control; p<0.01), kidney (38 ± 5% of control; p<0.01), lung (42 ± 10% of control; p<0.01), and duodenum (48 ± 20% of control; p<0.05) had recovered partially toward control levels.

In testis, a high density of ACE was visualized in seminiferous tubules (see Figure 4A). This level was not altered after lisinopril treatment (4 hours: 96 ± 6%; 24 hours: 100 ± 12% of control levels; NS for both; see Figure 4, B and C, and Figure 5).

Serum ACE and Serum Lisinopril

Serum ACE activity and serum lisinopril concentration following the 10 mg/kg dose are shown in Figure 7. Four hours after lisinopril administration, serum lisinopril was 198 ± 52 ng/ml and serum ACE activity was inhibited by 96% (4 ± 1% of control levels; p<0.01). By 24 hours, most of the lisinopril in serum had been eliminated (5 ± 3 ng/ml) and serum ACE had completely recovered to control levels (103 ± 4% of control levels).

Discussion

Clinical and experimental experience with ACE inhibitors indicates that inhibition of the circulating renin-angiotensin system alone cannot explain their hypotensive action.9,10 Thus, in this study, inhibition of ACE in specific tissues of physiological importance, such as brain, kidney, adrenal, lung, and intestine, was assessed before and after lisinopril treatment. Lisinopril was chosen for this study because it is a very stable drug that is not metabolized further, largely being excreted unchanged in the urine.19 Also, because of the drug’s high affinity, it has a very slow dissociation rate from the enzyme,13 which allows monitoring of in vivo occupancy of the enzyme active site in subsequent in vitro autoradiographic analysis.

Previous experimental studies have implicated the brain as a possible site of action for ACE inhibitors.20-22 All components of the renin-angiotensin system have been identified in brain.20 Captopril, when administered centrally, lowers blood pressure in the spontaneously hypertensive rat (SHR).21 The hypotensive effect...
FIGURE 4. Autoradiographic localization of ACE in rat testis (A–C), adrenal (D–F), kidney (G–I), lung (J–L), and duodenum (M–O). A, D, G, J, and M represent control period; B, E, H, K, and N were taken 4 hours after lisinopril administration; C, F, I, L, and O were taken 24 hours after lisinopril administration. The white scale at the bottom of A, D, G, J, and M is 5 mm long.
lasts for a longer period than when captopril is given peripherally.21 Thus, it has been suggested that a central action of captopril could reduce blood pressure in the absence of peripheral ACE inhibition in the SHR.21,22 It is still controversial whether captopril or enalapril can cross the blood-brain barrier and suppress central ACE.10,11,21,22 However, most previous studies have used total brain homogenates to assess ACE activity,10,11 and ACE is now known to be highly localized in specialized areas of brain.5,6,12 Examples of such areas are the circumventricular organs, including the SFO and OVLT, which are rich sites of ACE5,12 and angiotensin II receptors23 and are known to be involved in volume and blood pressure control.24 These areas are regarded as outside the blood-brain barrier.

In the present study, brain ACE was visualized and quantitated by in vitro autoradiography, which allows precise and discrete anatomical areas to be analyzed. We have shown that ACE activity was markedly inhibited after oral administration of lisinopril in the SFO and OVLT, the circumventricular organs. In contrast, in other brain regions, including the choroid plexus, ACE activity was not changed following ACE inhibitor treatment. The choroid plexus is a part of the blood-brain barrier and consists of an epithelial layer, directed toward the ventricular cavities, and an endothelial layer, consisting of capillaries separated by a basement membrane.25 Previous immunocytochemical studies have revealed that ACE is abundant on the brush border of epithelial cells of the choroid plexus.26,27 Thus, ACE in the choroid plexus is largely inside the blood-brain barrier. Our finding indicates that lisinopril does not readily cross the blood-brain barrier acutely. This finding is consistent with a previous study that showed that captopril did not cross the blood-brain barrier in Wistar rats.30 Unger et al.31 have reported significant ACE inhibition in peripheral tissues and in brain following chronic oral treatment with two different ACE inhibitors in SHR. Marked reduction of ACE activity occurred in the cerebral cortex and hypophysis, whereas the inhibition in the hypothalamus and the medulla oblongata was only moderate. This differential reduction of ACE in the regions of brain supports the importance of the blood-brain barrier. The results of the study are similar to our results, though there are minor discrepancies, which might be due to differences in animal strain, drugs, and duration of the treatment. Whether after chronic therapy or in hypertension lisinopril can diffuse into other brain regions needs to be studied. The pattern of ACE inhibition in the SFO and OVLT was similar to that in kidney, adrenal, and lung, which may be additional sites of action for ACE inhibitors. Which of these sites is responsible for the blood pressure–lowering effect of lisinopril is not known.

Cohen and Kurz11 demonstrated that captopril inhibited ACE in brain cortex homogenates of the SHR, where activity is relatively low.5,6 However, inhibition was not demonstrated with enalapril treatment. They concluded that central inhibition of ACE was not a requirement for acute blood pressure reduction by ACE inhibitors, because captopril, but not enalapril, inhibited brain ACE, yet both reduced blood pressure.11 This study does not take into account the possibility of important regional differences in the permeability of various brain regions to circulating ACE inhibitors. It is also possible that compounds that do not pass through the blood-brain barrier in normal rats might do so in the SHR because of increased blood-
brain permeability in hypertension. Differences in lipid solubility of ACE inhibitors might also affect the blood-brain barrier permeability. Further investigations are necessary to distinguish between these possibilities.

Other organs that may be functionally important are the kidney, adrenal, and lung. The pronounced inhibition of ACE in the kidney, adrenal, and to a lesser degree, lung suggests good penetration of the drug into these tissues. This observation is consistent with the kidney being the primary route of excretion of the ACE inhibitor. It is not clear why the ACE in lung was less affected than the ACE activity in other tissues.

The testis contains high concentrations of ACE, which suggests that the enzyme may play a physiological role in this tissue. In the present study, while the serum and organ ACE were markedly inhibited, ACE in testis was not altered significantly. Soffer and colleagues have shown that the terminal amino acid sequence and messenger RNA for the testicular enzyme differ from those of lung ACE. This difference could not, however, account for differences in drug effects between these organs. [125I]351A binds strongly to ACE of testicular origin. Although there has been some reported variation in the equilibrium dissociation constant for [125I]351A binding to ACE from various tissues, the differences were small and insufficient to account for the large difference in drug effect shown in this study between testis and other organs. It is likely that a blood-testis barrier limits access of the drug to testis.

We have also shown changes in intestinal ACE following lisinopril treatment. The role of ACE in the intestinal mucosa is unknown, but it could be involved in the hydrolysis of dietary oligopeptides to dipeptides. Binding of the inhibitor to the intestinal ACE may affect this function or possibly its absorption.

The present study has shown that the pattern and degree of tissue ACE inhibition by lisinopril vary among different peripheral tissues and among regions in the brain. These observations suggest that prolonged tissue, but not serum, ACE inhibition after a single oral dose of lisinopril may reflect targets involved in the hypotensive action of the drug. This pattern may reflect access of the drug to various tissue sites. The selective effect of lisinopril in brain ACE suggests that the blood-brain barrier plays an important role in the access of this ACE inhibitor to the central nervous system. Finally, these observations indicate that quantitative in vitro autoradiography is a powerful method for studying the access of tightly binding inhibitor drugs to tissues.

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BK was purchased from Peninsula Laboratories; enalaprilat was a gift from Merck, Sharp & Dohme; sodium nitroprusside was purchased from Abbott; and kinin antagonist was synthesized as previously described. All drugs were dissolved with 0.9% NaCl, and the volume of each bolus injection was 0.1 ml, followed by 0.2 ml of 0.9% NaCl solution. Male Sprague-Dawley rats (weight, 230–260 g) were used for these experiments (age, 8–9 weeks; Charles River, Wilmington, MA, USA), and they were cared for according to established principles. The rats were housed in a constant-temperature room with a 12-hour light-dark cycle and had free access to tap water and rat chow. All surgical maneuvers were done with the rats under ether anesthesia. Hypertension was induced by complete aortic ligation between both renal arteries. One day before the experiments, two PE-10 catheters (Clay-Adams, Parsippany, NJ, USA) were inserted into the ascending aorta through the right carotid artery and exteriorized in the scapular region, as previously described. In the normotensive rats, one additional PE-50 catheter was inserted into the abdominal aorta through the left external iliac artery. During the experiments the rats were kept semirestrained in cylindrical plastic containers. Constant infusions were made by a Harvard pump (Model 990, Millis, MA, USA) delivering 30 μl/min of either the kinin antagonist (40 μg/kg/min) or vehicle (0.9% NaCl). BP was measured with a Statham transducer (Gould, Oxford, CA, USA) and recorded on a Brush recorder (Gould, Cleveland, OH, USA).

**Group 1**

Eight normotensive rats were used to determine whether the kinin antagonist D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-DPhe-Thi-Arg-TFA can block the vasodepressor effect of exogenous BK. On the day of the experiments, mean BP was recorded continuously through the abdominal aorta catheter. After 30 to 60 minutes of stabilization, a saline infusion was started and three bolus injections of BK (100, 200, and 400 ng/kg) were administered through the ascending aortic catheters at 5-minute intervals. Then, a kinin antagonist infusion was started through one of the ascending aortic catheters, and after 5 minutes the BK bolus injections were repeated. Thirty minutes later, the procedure was repeated but the rats were pretreated with enalaprilat (60 μg/kg). In five of these rats, three different bolus injections of sodium nitroprusside (4, 8, and 16 μg/kg) were given before and during kinin antagonist infusion.

**Groups 2 and 3**

Fifteen hypertensive rats were used to determine whether kinins play a role in the acute antihypertensive effect of the CEI. On the day of the experiment, one of the catheters in the ascending aorta was connected to a three-way stopcock for direct BP recording and infusion delivery. The other catheter was used for the CEI injection. After 60 minutes of stabilization, the rats were infused for 15 minutes with either saline (Group 2, n = 7) or kinin antagonist (Group 3, n = 8). Five minutes after the beginning of the infusion, a bolus injection of enalaprilat (60 μg/kg) was given. The BP was recorded for 30 minutes after the CEI administration.

**Group 4**

Nine hypertensive rats with a PE-50 catheter inserted into the ascending aorta were used to determine plasma kinin concentrations. On the day of the experiment, a 2-ml blood sample was drawn 10 minutes before and 10 minutes after the enalaprilat injection (60 μg/kg). An equivalent volume of blood obtained from 24-hour-nephrectomized donor rats was returned to the experimental rats after each blood withdrawal. The BP before and 10 minutes after CEI injection was recorded.

The dose of enalaprilat used inhibited more than 70% of the vasopressor action of angiotensin I (100 ng) in normotensive (from 36 ± 2 to 9 ± 1 mm Hg; n = 2) as well as in hypertensive (from 22 ± 3 to 6 ± 1 mm Hg; n = 3) animals. Plasma kinin concentrations were determined with a direct radioimmunoassay, as previously described. Blood samples were obtained with a syringe containing kininase and kininogenase inhibitors, as previously described. The extraction of kinins from plasma was performed by ethanol.

**Statistical Analysis**

The data are expressed as means ± SEM. Statistical analysis was done by multivariate repeated-measures analysis of variance. Differences between groups were determined using unpaired or paired *t* tests, and Bonferroni's adjustment was used.

**Results**

The mean BP of the Group 1 normotensive rats (*n* = 8) was 113 ± 3 mm Hg and was not significantly affected by kinin antagonist infusion (112 ± 3 mm Hg), CEI injection (109 ± 3 mm Hg), or kinin antagonist infusion after CEI injection (110 ± 3 mm Hg). The hypotensive responses to BK were significantly blunted when the animals were infused with BK antagonist (*p* < 0.001), whereas the hypotensive responses to sodium nitroprusside (4, 8, and 16 μg/kg) before and during kinin antagonist infusion were not affected (Figure 1). As expected, the vasodepressor effect of BK was significantly enhanced by CEI treatment (*p* < 0.001; see Figure 1).

Eight days after complete aortic ligation, the rats were hypertensive and there were no significant differences in mean BP among the three hypertensive groups (Group 2: 180 ± 7 mm Hg, *n* = 7; Group 3: 177 ± 3 mm Hg, *n* = 8; Group 4: 181 ± 4 mm Hg, *n* = 9). Following 5 minutes of kinin antagonist treatment, the BP of the hypertensive rats (Group 3) did not change.
CEI produced a significant decrease in mean BP in both groups; however, in Group 3 the acute antihypertensive effect of CEI was significantly decreased by the infusion of the kinin antagonist (p < 0.01; Figure 2).

The mean BP of the hypertensive rats used for plasma kinin determination (Group 4) responded to the CEI treatment in the same way as the hypertensive Group 2 rats infused with vehicle (change in mean BP: -42 ± 4 and -48 ± 6 mm Hg, respectively, 5 minutes after treatment). The plasma kinin concentrations were not increased significantly by CEI administration (41 ± 10 before vs 68 ± 20 pg/ml after CEI injection; Figure 3).

**Discussion**

Since the discovery that angiotensin converting enzyme (kininase II) has at least two main effects, converting the inactive angiotensin I to active angiotensin II and destroying kinins, many studies have been done to elucidate whether kinins have a role in mediating the hypotensive effect of CEI. However, most of the approaches were indirect, and the results obtained in these studies must be interpreted with caution.

Recently, a family of BK analogues having antagonistic properties have been synthesized. The antagonistic capacity appears to reside in the substitution of the proline 7 of BK with D-phenylalanine, whereas other substitutions increase the affinity for kinin receptors and the potency of the antagonist. In this study, we used one of the kinin antagonists, D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-DPhe-Thi-Arg-TFA, to determine the possibility that part of the acute vasodepressor effect of the CEI enalaprilat in rats with severe hypertension is due to the inhibition of endogenous kinin destruction. This kinin antagonist has been shown in our laboratory to be most effective in blocking the vasodilator effect of BK in the hindleg of the dog. At the dose used, the kinin antagonist produced a significant decrease in the hypotensive responses of exogenous BK in normal rats, even when the vasodepressor effect of BK was increased by CEI administration. The lack of change in the vasodepressor effect of sodium nitroprusside during treatment with the antagonist suggests its specificity on kinin receptors.

The aortic ligation model of hypertension was selected for the study since the rapid development of hypertension and its severity produce endothelial and vascular damage that may lead to activation of plasma kallikrein and increased formation of kinins. Accordingly, we expected that the acute antihypertensive mechanism of CEI could be due in part to inhibition of kinin destruction.
In the hypertensive rat, a significant, acute antihypertensive effect was observed after administration of the CEI. This effect was significantly blocked by the kinin antagonist. Similar inhibition of the antihypertensive action of CEI has been reported from our laboratory, using antibodies to kinins in the two-kidney, one clip renovascular hypertensive rat model. In the present study, we used a specific kinin receptor antagonist that has, at the doses used, no agonistic effect on the BP of the rat. The BP of normal rats, normal rats treated with CEI, and hypertensive rats not treated with CEI did not change during kinin antagonist infusion, suggesting that kinins do not play a major role in the regulation of BP in these conditions. However, in this model of hypertension kinins seem to play a major role in the acute antihypertensive effect of the CEI.

We noticed that the BP did not decrease in the hypertensive rats treated with CEI when the antagonist infusion was stopped. We do not have a good explanation for this observation, since the BP response to exogenous kinins returned after 10 minutes; however, because the antagonist and BK have very similar structures, the tissue half-life of the antagonist may have been increased by CEI, though other explanations cannot be excluded.

We measured the plasma kinin concentration in the hypertensive animals to see whether they increased after the inhibition of their destruction by CEI. We did not find any significant difference. Circulating kinin concentrations after CEI administration have been reported in both experimental animals and humans to increase, to remain the same, or even to decrease. These discrepancies may be due to methodological differences in measuring kinin concentration.

In the present study we used a very sensitive radioimmunoassay for measuring kinins and drew blood with a syringe containing high concentrations of inhibitors of both generating and degrading kinin enzymes. The lack of increased plasma kinin concentrations after CEI administration does not exclude the possibility that kinins play an important role as vasodepressors when their destruction is blocked by CEI in hypertensive rats. The kalikrein-kinin system has been regarded as a local hormonal system, and the existence of kinin-generating and kinin-degrading enzymes in the vascular tissue has been reported. The effect of the CEI may be due to an increase in tissue kinins, which could act locally on vascular resistance.

Recently, Benetos et al. have reported that the acute antihypertensive effects of CEI in two-kidney, one clip hypertensive rats could be partly reversed by a kinin antagonist. The kinin antagonist used by them and that used by us are very similar in structure and properties. These investigators concluded that endogenous kinins contribute to the acute antihypertensive effects of CEI in this renovascular hypertensive model. They also reported that high doses of the antagonist have a hypertensive effect in normotensive rats. Lower doses of the antagonist were effective in blocking the vasodepressor activity of exogenous kinins, but the BP was not affected. They concluded that kinins participate in BP regulation in the normotensive state and that high concentrations of the antagonist may be necessary to displace not only circulating but possibly tissue-bound kinins as well. On the other hand, increased local tissue concentrations of kinins, measured by the decrease of the number of tissue kinin receptors, without a change in kinin circulating concentrations has been described after captopril administration. Taken together, these studies suggest that circulating kinins may not reflect changes in the concentration of kinins in vascular tissue. Kinins formed within or in the vicinity of the resistance vessels may modulate vascular tone and change vascular resistance, regional blood flow distribution, and in some circumstances, BP. Thus, the present results are consistent with the hypothesis that kinins play a role in the acute antihypertensive effect of CEIs in severe hypertension.

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