Role of Kinins in the Acute Antihypertensive Effect of the Converting Enzyme Inhibitor, Captopril

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SUMMARY The role of kinins in the acute antihypertensive effect of a converting enzyme inhibitor (CEI) was studied in sodium-depleted normotensive and in two-kidney, one clip chronically hypertensive rats (2K-1C). The 2K-1C were on a normal sodium diet. The acute vasodepressor effect of the CEI was determined in these two groups either after administration of normal rabbit globulins or antikinin globulins. The amount of kinin antibodies administered completely blocked the hypotensive effects of bradykinin, 400 ng/kg, and urinary kallikrein, 4 μg/kg. After administration of CEI in the sodium-depleted rats there was no significant difference (p > 0.05) in the acute changes in mean blood pressure (BP) between the group pretreated with normal rabbit globulins (ABP —32.3 ± 3.9 mm Hg) and the group pretreated with antikinin globulins (ABP —25 ± 2.5 mm Hg). In the 2K-1C pretreated with normal rabbit globulins, the CEI produced a decrease in BP of —21 ± 4.5 mm Hg. This decrease was almost completely blocked in the group pretreated with the antikinin globulins (ABP —4 ± 4.1 mm Hg). These differences in the changes in BP were significant (p < 0.02). These results suggest that the acute antihypertensive effect of the CEI in the sodium-depleted rats is probably due to inhibition of the conversion of angiotensin I to II while in the 2K-1C it is due, in part, to an increase in kinin concentrations secondary to the inhibition of kininase II. (Hypertension 3: 18-22, 1981)

KEY WORDS • bradykinin • kallikrein • anti-kinin globulins • sodium depletion • kininase II • angiotensin

ANGIOTENSIN I converting enzyme, or kininase II (EC 3.4.15.1), is a peptidyl dipeptidase that catalyzes the hydrolytic removal of carboxyl-terminal dipeptide from angiotensin I, kinins (bradykinin and lys-bradykinin), and other polypeptide substrates.1 Thus, this enzyme may participate in blood pressure (BP) regulation by the conversion of inactive angiotensin I to active vasopressor angiotensin II and/or by inactivation of the potent vasodepressors, kinins.

There have been converting enzyme inhibitors (CEI) developed that have been shown effective in reducing the BP in experimental and human hypertension.2-4 Theoretically, the antihypertensive effect of CEI can be caused by inhibition of the conversion of angiotensin I to angiotensin II and/or by inhibition of the destruction of kinins. There is evidence that the antihypertensive effect of the CEI is due, in part, to a blockade of the renin-angiotensin-aldosterone system.6-8 Although participation of the kallikrein-kinin system in the antihypertensive effect of CEI has been suggested,9,10 no direct evidence has been reported.

The purpose of this study was to assess the role of kinins in the acute antihypertensive effect of the new orally effective CEI (Captopril, D-3-mercapto-2-methylpropanoyl-L-proline)11 in two different experimental situations: normotensive rats on a low sodium diet and two-kidney, one clip hypertensive rats (2K-1C). For this, the acute vasodepressor effect of the CEI was determined in these two groups after the administration of semipurified normal rabbit globulins or semipurified kinin antibodies (antikinin globulins).

Materials and Methods

Antikinin Globulins

Kinin antibodies were produced in rabbits as previously described.13 The titer of the antiserum used for the preparation of the antikinin globulins was measured by radioimmunoassay. A dilution of 1:360,000 of this serum produced 40% binding of approximately
10 pg of [125I]-tyr-bradykinin. The serum was harvested and heated at 56°C for 30 minutes in a water bath and then diluted with an equal volume of distilled water. The globulins were precipitated at 4°C with 2 M (NH4)2SO4, dissolved, and extensively dialyzed in the cold against 5% dextrose or Ringer solutions. The volume was adjusted to one-fourth of the starting volume of the antisera. The resultant solution was stored frozen at −20°C. The final concentration of proteins, as determined by the method of Bradford,13 was 106 mg/ml. Normal rabbit globulins were prepared according to the same procedure, and the final protein concentration was 103 mg/ml. The preparations dialyzed against 5% dextrose were used in the rats on low sodium diets to avoid administering sodium to the sodium-depleted rats.

Sodium depletion was produced by feeding female Wistar rats weighing 130–150 g a low sodium diet (0.05% sodium, Nutritional Biochemicals Company, Cleveland, Ohio) and deionized water for 3 weeks. In addition, on the day of the experiment these rats received an intraperitoneal injection of furosemide (10 mg/rat). Two-kidney, one clip hypertension was induced in female Wistar rats weighing 150–200 g by placing a U-shaped silver clip with an internal gap of 0.2 mm around the left renal artery. The contralateral kidney was left untouched. Systolic BP was measured by the tail-cuff method in unanesthetized rats, and 4–7 weeks later those rats with a systolic BP of 150 mm Hg or higher were selected for this study.

The 2K-1C were fed a normal sodium diet (0.45% sodium, Ralston Purina Company, Richmond, Indiana) and deionized water ad libitum. Then 1 or 2 days before the experiment PE 10 catheters were chronically implanted into the ascending aorta, abdominal aorta, and inferior vena cava through the right carotid artery, femoral artery, and femoral vein respectively, and led subcutaneously to the scapular region of the rat as previously described.14 Direct mean BP was recorded by a pressure transducer (Micron MP 15) and a four-channel recorder (Brush 440). During the experiment, unanesthetized rats were kept semirestrained in cylindrical plastic containers, and mean BP was recorded continuously. After recording e BP for 60 to 90 minutes, 400 ng/kg of angiotensin I (1-Asp-5-I13-Ang I) and angiotensin II (Hypertensin, CIBA), 800 ng/kg of 1-norepinephrine (Levophed, Winthrop), 400 ng/kg of bradykinin (Bachem Inc., Torrance, California), 4 μg/kg of purified urinary rat kallikrein, and 28 μg/kg of sodium nitroprusside (Nipride, Roche, Nutley, New Jersey) were administered as separate bolus injections via the right carotid catheter into the ascending aorta. All injections were given in a volume of 0.1 ml followed by 0.2 ml of 5% dextrose. After the BP had returned to basal levels, 0.6 to 0.7 ml/100 g of either antikinin globulins or normal rabbit globulins were injected slowly via the venous catheter. Subsequently, BP had returned to control levels, either 100 mg/kg of CEI (Captopril, Squibb and Sons, Inc., Princeton, New Jersey) dissolved in 2 ml of 5% dextrose or 2 ml/kg of 5% dextrose was injected intravenously. Blood pressure was recorded for 90 minutes after administration of CEI or 5% dextrose. The BP responses to angiotensin I and II, 1-norepinephrine, bradykinin, kallikrein, and sodium nitroprusside were then determined again. The urinary rat kallikrein used for the BP response was purified as previously described.15

The sodium-depleted group was subdivided into the following two subgroups: 1) nine rats injected with normal rabbit globulins and CEI; and 2) seven rats injected with antikinin globulins and CEI.

The 2K-1C group was subdivided into the following four subgroups: 1) 10 rats injected with normal rabbit globulins and CEI; 2) 10 rats injected with antikinin globulins and CEI; 3) six rats injected with normal rabbit globulins and 5% dextrose; and 4) six rats injected with antikinin globulins and 5% dextrose.

All results are expressed as a mean ± SE. To determine if the difference between the BP of these groups of rats was significant, the Hotelling's t-square test was used.16 p > 0.05 was considered not significant (n.s.).

Results

Since the effect of CEI and antikinin globulins on the BP response to vasoactive drugs in the groups studied were similar, they were combined and expressed as one group in figure 1. The doses of CEI

![Figure 1. Pressor response to angiotensin I, angiotensin II, and norepinephrine (top) and depressor response to bradykinin, kallikrein, and sodium nitroprusside (bottom). White columns indicate untreated group (control); dashed columns indicate group treated with normal rabbit globulins (NR-GL) plus converting enzyme inhibitor (CEI); and black columns indicate group treated with antikinin globulins (Kinin-Ab) plus CEI.](image-url)
used almost completely blocked the pressor effect of angiotensin I while the vasodepressor effect of bradykinin and kallikrein was greatly potentiated. The doses of antikinin globulin used completely blocked the depressor effect by both bradykinin and kallikrein. The pressor response to angiotensin II and 1-norepinephrine and the vasodepressor response to nitroprusside were not significantly affected by these two treatments.

In the normotensive sodium-depleted rats treated with either normal rabbit globulins or antikinin globulins, the CEI produced a significant decrease ($p < 0.01$) in BP. The decrease in BP in these two subgroups was similar ($p > 0.05$). Figure 2 shows the changes in BP for these two subgroups.

In the 2K-1C rats treated with normal rabbit globulins and CEI (Subgroup 1), 90 minutes after CEI the BP decreased $21 \pm 4.5$ mm Hg ($p < 0.01$). In Subgroup 2 treated with antikinin globulins and CEI, 90 minutes after CEI the BP decreased only $4.4 \pm 4.1$ mm Hg (n.s.). The difference in the decrease in BP between these two subgroups was significant ($p < 0.02$). In Subgroup 3 treated with normal rabbit globulins and 5% dextrose, and in Subgroup 4 treated with antikinin globulins and 5% dextrose, the BP had a tendency to increase; however, these changes were not significant. Figure 3 shows the changes in BP for these four subgroups.

**Discussion**

The antihypertensive effect of CEI may be due either to a blockade of the conversion of angiotensin I to angiotensin II or to inhibition of the destruction of endogenous kinins, which are potent vasodilators. In this study, we have tested the possibility that part of the acute vasodepressor effect of the orally active CEI, Captopril, is due to an increase in endogenous kinin concentrations. For this purpose, we tried to block the acute antihypertensive effect of high doses of the CEI.
with antibodies against kinins in normotensive rats on a low sodium diet and in 2K-1C on a regular sodium diet. Although the antikinin globulins used were developed against lys-bradykinin, they also cross-reacted with bradykinin and met-lys-bradykinin, which are the three naturally occurring kinins in mammals.44 The doses of antikinin globulins used completely blocked the depressor effect of injected bradykinin. The injections of this peptide were done in the ascending aorta of the rat since the vasodepressor effect of kinins is more marked when it is injected into the arterial rather than venous circulation. This is due to the fact that over 80% of the kinins are destroyed by one passage through the pulmonary circulation.17

It could be argued that bradykinin may not be the kinin formed by the endogenous kininogenases (kinin-generating enzyme) in the rat. However, the depressor effect of the injection of purified urinary rat kallikrein was also completely blocked by antikinin globulins, indicating that the antibodies used were effective in blocking the depressor effect of kinins generated from the endogenous kininogen (kallikrein substrate) in the circulation of the rat. The antikinin globulins did not alter the vasodepressor effect of nitroprusside, thus indicating that the inhibition of the vasodepressor effect of the bradykinin and urinary rat kallikrein was specific.

The antikinin globulins did not alter the acute hypotensive effect of the CEI in the sodium-depleted rats. This suggests that during sodium depletion the antihypertensive effect of the CEI is due to the blockade of the conversion of angiotensin I to II and not to an increase in the concentration of kinins.

In this study, PRA was not measured; however, during sodium depletion, its increase has repeatedly been reported. Thus, it is reasonable to assume that renin was increased in the sodium-depleted rats and that this increase, as in other species, played an important role in the homeostasis of BP.18 This model was selected for study since in humans it has been reported that blood kinins during sodium depletion are increased.19,20 Accordingly, we expected that an inhibition of kinin destruction by the CEI could be an important part of the acute antihypertensive mechanism of this drug. However, this clearly is not the case, at least in the sodium-depleted rat. McCaa,21 using a different approach, also concluded that the hypertensive actions of CEI are due to the decrease in circulating angiotensin II and not to the accumulation of circulating kinins in the sodium-depleted dogs.

In the 2K-1C rat, a significant acute antihypertensive effect was observed after the administration of the CEI. This effect was almost completely blocked by the antikinin globulins. This result was unexpected, since in the 2K-1C model renin seems to be an important pathogenetic factor.44 It could be that the blockade of the antihypertensive effect by the antikinin globulins was due to an expansion in plasma volume as a consequence of the administration of serum proteins (~150 mg). This is unlikely, however, since a similar expansion was produced in a 2K-1C group injected with normal rabbit globulins, yet in this group the CEI produced a significant acute antihypertensive effect. Thus, a significant part of the acute antihypertensive effect of the CEI seemed to be mediated by kinins.

The 2K-1C group injected with normal rabbit globulins and the group injected with antikinin globulins plus 5% dextrose instead of CEI were used as time controls. In these groups a tendency for the BP to increase was observed. This increase may be due, in part, to a small increase in plasma volume and cardiac output produced by the administration of proteins. The BP increase produced by the antikinin globulins in the 2K-1C model was no greater than that produced by the normal rabbit globulins. These results suggest that, in this model, blood kinins under basal conditions (no CEI treatment) do not play a significant role as vasodepressors. However, kinins seem to play an important role as naturally occurring vasodepressors when their destruction is blocked by the converting enzyme inhibitor. This suggests that the formation and destruction of these vasodilator peptides occur in an equilibrium situation, and that when this steady state is altered, kinins become important in the regulation of BP. In the sodium-depleted rats, the effect of the antikinin globulins in the 2K-1C model was no greater than that produced by the normal rabbit globulins. Thus, we felt that in this model the possibility of circulating kinins in basal conditions (no CEI) playing an important role as vasodepressors was low, since kinins did not appear to be very significant as circulating vasodepressors when their destruction was inhibited. Therefore, we did not feel it was justifiable to use significant amounts of this scarce and valuable antiserum.

This study does not support or exclude the possibility that kinins, as local hormones, play an important role in the antihypertensive effect of the converting enzyme inhibitor. For example, in the kidney, kinins have been reported to be formed in the lumen of the distal nephron22 where they may play a role in the regulation of water and electrolyte excretion and/or in the regulation of the local blood flow to this part of the nephron.23 Yet, the antikinin globulins will not reach this part of the nephron, since due to their high molecular weight they will not be filtered by the glomeruli. However, CEI has been reported to increase the concentration of kinins in this area of the nephron.24 Similarly, the antikinin globulins will not reach the central nervous system where kinins have been reported to be present.24 Thus, these results should be interpreted in light of the possibility that the antikinin globulins probably block only circulating kinins and not tissue kinins.

The BP was measured for only 90 minutes since we found that the blockade of the depressor effect of kinin and kallikrein by antikinin globulins started to dissipate 2 hours after its administration, and after 4 to 6 hours had almost completely disappeared (unpublished results). This may be due, in part, to the fact that antibodies against kinins have a small, but significant cross-reaction with plasma kininogen (kallikrein substrate).45 The kininogens and the antibodies form a
high molecular weight complex which is probably removed by the reticular endothelial system. Results obtained from using antibodies for the blockade of vasoactive naturally occurring substances should be interpreted with caution since the antigen-antibody complex may cause anaphylactoid reactions, release of histamine, and activation of the plasma prekallikrein and complement systems, which could decrease the BP independent of the blockade of vasoactive hormones. In this study, this could only decrease the BP independent of the blockade of CEI. Thus, the inhibition of the hypotensive effect of the CEI. In conclusion, these results suggest that the acute antihypertensive effect of the CEI in the sodium-depleted rats is probably due to inhibition of the conversion of angiotensin I to II, while in the chronic 2K-IC it is probably due in part to an increase in kinin concentrations secondary to the inhibition of kininase II.

References

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doi: 10.1161/01.HYP.3.1.18

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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