Kinin Contribution to Renal Vasodilator Effect of Captopril in Rabbit

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This study was conducted to examine the role of bradykinin in the persistence of the renal vasodilator effect of captopril during angiotensin II receptor blockade. Blood pressure and renal blood flow were monitored in eight groups of pentobarbital-anesthetized rabbits. In group 4, captopril alone was administered, and it decreased blood pressure by 14±4 mm Hg and increased renal blood flow by 21±4 ml/min. After a bolus injection and a constant intravenous infusion of the imidazole derivative angiotensin II receptor antagonist DuP 753 (group 5), captopril decreased blood pressure by 9±2 mm Hg and increased renal blood flow by 8±1 ml/min (12±1% change in renal blood flow, p<0.05 versus group 4). In the presence of a constant intravenous infusion of saralasin (group 6), captopril decreased blood pressure by 13±5 mm Hg and increased renal blood flow by 7±2 ml/min (17±5% change in renal blood flow, p<0.05 versus group 4). These results did not differ from those in group 5. During a constant intrarenal arterial infusion of a B2 bradykinin receptor antagonist, DArg°,[Hyp3-Thi8-DPhe7]-bradykinin (BkA) (group 7), captopril decreased blood pressure by 14±4 mm Hg and increased renal blood flow by 10±4 ml/min. Combined administration of DuP 753 intravenously and BkA intra-arterially (group 8) eliminated the effect of captopril. In group 8, captopril caused insignificant changes in blood pressure and renal blood flow. The results indicate that DuP 753 and saralasin antagonize the renin-angiotensin system to a comparable extent in vivo. Although blockade of the latter system accounted for a significant part of the increase in renal blood flow caused by captopril, the remaining component was contributed by endogenous bradykinin. (Hypertension 1991;17:504-509)

The administration of captopril inhibits the enzyme kininase II, resulting in the potentiation of the action of bradykinin, and this effect gives rise to actions thought to be mediated by enhanced levels of endogenous kinins.1 Recently, it was shown in normal rats that bradykinin does not contribute to the hypotensive effect of angiotensin converting enzyme (ACE) inhibitors.2 However, in two-kidney, one clip hypertensive rats one third of the hypotensive effect was mediated by bradykinin.3 Kinins in the urine have been reported to be increased after the administration of ACE inhibitors, which indicates an increase in their concentration in renal tissue.4–6 However, an increase in plasma kinins has not been consistently obtained.7 An increase in renal kinins may participate in the antihypertensive effect of ACE inhibitors by altering renovascular resistance and by increasing sodium and water excretion. Previous results from our laboratory on conscious sodium-restricted dogs indicated that combined administration of the angiotensin antagonist saralasin and the specific bradykinin antagonist DArg°,[Hyp3-Thi8-DPhe7]-bradykinin (BkA) prevented almost completely the renal hemodynamic effect of the ACE inhibitor enalaprilat.8 Based on the results of this investigation, it appeared that blockade of the renin-angiotensin system and bradykinin potentiation contributed equally to the increase in renal blood flow (RBF) caused by enalaprilat. However, the use of saralasin as a specific tool may be questioned since evidence exists for the potentiation of the depressor response to bradykinin by saralasin in conscious rats.9 Thus, the effects of saralasin in vivo may not be solely related to angiotensin II (Ang II) receptor blockade.

In the present study, we sought to compare the nonpeptide angiotensin antagonist DuP 753 with saralasin as a probe of the renal effect of the ACE inhibitor captopril in anesthetized rabbits. DuP 753 has advantages over the peptide Ang II antagonists in that it lacks partial agonist activity and does not cause bradykinin potentiation.10 We also used BkA to determine the bradykinin contribution to the renal vasodilator effect of captopril that remains after Ang
II receptor blockade. The use of these specific kinin and Ang II receptor antagonists should enable better delineation of the mechanism of ACE inhibitors than has been possible before.

Methods
Thirty-three adult New Zealand White rabbits (3–4 kg) were used. The rabbits were fed normal rabbit chow (0.35% NaCl, complete blend; Purina, St. Louis, Mo.) and were allowed free access to water. Anesthesia was induced with sodium pentobarbital (30 mg/kg). Two PE-50 catheters were placed in the jugular vein. One catheter was used for continuous infusion of sodium pentobarbital at 6 mg/kg/hr during the experiment; the other, a twoline catheter was used for intravenous delivery of drugs. Mean arterial blood pressure was monitored from a Statham P23AA pressure transducer (Gould Instruments, Oxnard, Calif.) attached to a catheter in the left carotid artery. The rabbits were suspended from a thoracic vertebral spinous process by a clamp attached to a metal stand. The left renal artery was exposed through a left retroperitoneal flank incision. A precalibrated electromagnetic blood flow probe (5 mm in circumference, Carolina Medical Electronics, Inc., King, S.C.) was affixed to the renal artery. A 30-gauge needle attached to PE-10 tubing was inserted into the renal artery, and isotonic saline was infused at 0.15 ml/min intra-arterially to maintain fluid balance and for the intra-arterial administration of drugs. The flow probe was zeroed and 10 minutes were allowed for stabilization. Measurements were made after reaching stable RBF and blood pressure levels. RBF rather than renal vascular resistance changes were analyzed because an ACE inhibitor-induced decrease in blood pressure can reduce renal vascular resistance via renal autoregulation. We have assumed that an increase in RBF will reflect the renal action of the ACE inhibitor instead of an indirect effect of autoregulation. Rather than increasing RBF, renal autoregulation maintains flow unchanged over a wide range of blood pressure. The contribution of autoregulation to a reduction in renal vascular resistance produced by the ACE inhibitors cannot be assessed in these experiments.

In total, eight groups of animals were studied. In group 1 (n=4), two bolus doses of Ang II (CIBAGEIGY, Summit, N.J.), 0.15 and 0.3 µg/kg i.v., were given after control measurements of blood pressure and RBF were made. Then, DuP 753 (E. I. du Pont de Nemours & Co., Wilmington, Del.) (4 mg/kg bolus injection and 2 mg/kg/hr i.v. continuous infusion) was administered. This dosage regimen of DuP 753 established a maximal blockade of the response to Ang II. Subsequently, after obtaining a stable RBF level (15–20 minutes), the bolus doses of Ang II were administered again to evaluate the degree of Ang II receptor blockade.

Group 2 (n=4) was treated identically to group 1 with the exception that saralasin (1 µg/kg/min; Sigma Chemical Co., St. Louis, Mo.) was infused intravenously instead of DuP 753. This dose of saralasin also established a maximal blockade of the response to Ang II.

In group 3 (n=4), after control measurements of blood pressure and RBF were obtained, two doses of bradykinin (triacetate salt, 0.02 and 0.05 µg/min i.a.; US Biochemicals, Cleveland, Ohio) were infused for five minutes each. BkA (kindly supplied by R.J. Vavrek and J.M. Stewart, Denver, Colo.) was then infused continuously at 0.5 µg/min i.a., and after a transient increase in RBF, which occurred over a 15–20-minute interval, RBF returned to the control level. Then, to assess the extent of bradykinin receptor blockade, both doses of bradykinin were again infused.

In group 4 (n=4), after establishing the control blood pressure and RBF levels, isotonic saline was continuously infused (0.11 ml/min i.v.) for the remainder of the experiment as vehicle for Ang II antagonist. Saline vehicle control for BkA was started 10 minutes later, given as an intra-arterial infusion, and after 10 minutes, captopril (kindly supplied by the Squibb Medical Institute, Princeton, N.J.), was administered as a 2 mg/kg bolus and 1 mg/kg/hr intravenous infusion.

In group 5 (n=4), after the control period, DuP 753 (4 mg/kg bolus injection and 2 mg/kg/hr i.v.) was given continuously as the saline vehicle above. On the establishment of a stable RBF level (15–18 minutes), a 10-minute infusion of saline vehicle for BkA intra-arterially was given, after which captopril was administered as described above.

Group 6 (n=4) experiments were identical to those in group 5 with the exception that saralasin (1 µg/kg/min i.v.) was given instead of DuP 753.

In group 7 (n=5), after the control period, isotonic saline was continuously infused intravenously as vehicle for Ang II antagonist. Ten minutes afterward, the infusion of BkA (0.5 µg/min i.a.) was begun and was continued for the remainder of the experiment. After allowing the first 15–20 minutes for the RBF to stabilize, captopril was then administered.

In group 8 (n=4), after the control period, intravenously administered DuP 753 was given continuously. When a stable RBF level was obtained (15–18 minutes), the infusion of BkA was begun and was given continuously intra-arterially for 15–20 minutes, and then captopril was administered.

Presented in the results of groups 4, 5, 6, 7, and 8 are the blood pressure and RBF changes at the 20-minute interval of captopril administration.

Statistics
Data were analyzed by either one-way analysis of variance (ANOVA) with repeated measures, factorial ANOVA, or paired t test where appropriate. Differences between means after ANOVA were determined by Scheffe’s test. The Arcsin transformation for proportions was used to compare between percentages. A value of p<0.05 was considered sta-
TABLE 1. Effect of DuP 753 or Saralasin on the Renal Blood Flow and Blood Pressure Responses Evoked by Two Doses of Angiotensin II

<table>
<thead>
<tr>
<th>Absolute change</th>
<th>DuP 753 Before</th>
<th>After DuP 753</th>
<th>Saralasin Before</th>
<th>After saralasin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARBF (ml/min)</td>
<td>LD</td>
<td>HD</td>
<td>LD</td>
<td>HD</td>
</tr>
<tr>
<td>ΔBP (mm Hg)</td>
<td>14±5</td>
<td>22±4</td>
<td>1±1*</td>
<td>1±2*</td>
</tr>
</tbody>
</table>

Ang II, angiotensin II; LD, low dose Ang II (0.15 μg/kg i.v.); HD, high dose Ang II (0.3 μg/kg i.v.); ΔRBF, change in renal blood flow; ΔBP, change in blood pressure.

* p<0.05 vs. before DuP 753.
† p<0.05 vs. before saralasin.

Statistically significant. Values in graphs, tables, and text are presented as mean±SEM.

Results

Angiotensin II Receptor Blockade by DuP 753 and Saralasin and Their Attenuation of Captopril Response

The doses of DuP 753 and saralasin administered in groups 1 and 2 were effective in totally blocking the blood pressure and RBF changes produced by both doses of Ang II (Table 1).

In group 4, the mean increase in RBF and decrease in blood pressure after captopril were 21±4 ml/min (p<0.05) and 14±4 mm Hg (p<0.05), respectively (Figures 1 and 2). In the experiments conducted in group 5, DuP 753 increased RBF by 16±6 ml/min (p<0.05) and decreased blood pressure by 11±5 mm Hg (p<0.05) (Figures 1 and 2). After DuP 753, there was no additional increase in RBF during the intra-arterial infusion of vehicle. The administration of captopril after DuP 753 caused an additional 8±1 ml/min increase in RBF (p<0.05) and 9±2 mm Hg decrease in blood pressure (p<0.05). Saralasin given intravenously in group 6 decreased RBF transiently by 11 ml/min. At 8–12 minutes after the beginning of saralasin infusion, RBF was not significantly different from the control level (43±6 ml/min versus 40±4 ml/min). Subsequent to saralasin, no additional increase in RBF was detected during the intra-arterial infusion of vehicle. The mean increase in RBF and decrease in blood pressure produced by captopril in group 6 were 7±2 ml/min (p<0.05) and 13±5 mm Hg, respectively (p<0.05) (Figures 1 and 2). Thus, although both saralasin and DuP 753 attenuated the effect of captopril, there still remained a significant effect on RBF and blood pressure.

Bradykinin Receptor Blockade by Bradykinin Antagonist and Its Attenuation of Captopril Response

In group 3, BkA completely blocked the RBF responses to both doses of bradykinin (Table 2).

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\text{FIGURE 1. Bar graph showing effect of angiotensin II (AII) receptor blockade or bradykinin antagonist (BkA), or both, on the renal blood flow (RBF) effect of captopril. Results are mean ± SEM.} \]

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\text{FIGURE 2. Bar graph showing effect of angiotensin II (AII) receptor blockade or bradykinin antagonist (BkA), or both, on the decrease in blood pressure (BP) evoked by captopril. Results are mean ± SEM.} \]

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\text{GROUP 4} \quad \text{(n=4)}
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\text{GROUP 5} \quad \text{(n=4)}
\]

\[
\text{GROUP 6} \quad \text{(n=4)}
\]

\[
\text{GROUP 7} \quad \text{(n=5)}
\]

\[
\text{GROUP 8} \quad \text{(n=4)}
\]
TABLE 2. Effect of Bradykinin Antagonist on the Renal Blood Flow and Blood Pressure Changes Evoked by Two Doses of Bradykinin

<table>
<thead>
<tr>
<th>Absolute change</th>
<th>Bradykinin response (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before BkA</td>
<td>After BkA</td>
</tr>
<tr>
<td>ARBF (ml/min)</td>
<td>LD</td>
</tr>
<tr>
<td>ΔBP (mm Hg)</td>
<td>0</td>
</tr>
</tbody>
</table>

BkA, dArg6-[Hyp3-Thi5'-8-DPrie7]-bradykinin; LD, low dose bradykinin (0.02 μg/min); HD, high dose bradykinin (0.05 μg/min); ΔRBF, change in renal blood flow; ΔBP, change in blood pressure.

These low doses of bradykinin given intra-arterially exerted little or no effect on blood pressure. BkA had no action of its own on blood pressure but transiently increased RBF by 11±1 ml/min (p<0.05) at its peak effect, 6–8 minutes during infusion. This is attributable to a partial agonist action of BkA. RBF averaged 46±7 ml/min before and 47±7 ml/min for the 15–20-minute interval during BkA infusion. The corresponding mean blood pressure values were 109±8 and 110±8, respectively. In group 7, BkA increased RBF by 9 ml/min (p<0.05) at its peak effect and RBF gradually returned to the control level before administering captopril, which increased RBF by 10±4 ml/min (p<0.05) and decreased blood pressure by 14±4 mm Hg (p<0.05) (Figures 1 and 2). A more profound attenuation in the action of captopril on RBF and blood pressure was obtained when DuP 753 and BkA were given concomitantly. Captopril displayed virtually no effect on RBF and blood pressure after the combined DuP 753 and BkA regimen. The mean changes in RBF and blood pressure being a 0.25±2 ml/min increase and a 1±1 mm Hg decrease, respectively (group 8, Figures 1 and 2). The RBF increment after captopril was significant in angiotensin II-treated dogs, but because of the partial agonist properties of the antagonist, they could only speculate about the extent of participation of the renin-angiotensin system. To elucidate this last point and to substantiate results obtained with saralasin, we used DuP 753. A relatively high dose of DuP 753 was used to maximally block the influence of the renin-angiotensin system on the kidney and thus unmask any remaining vasodilator effect of the ACE inhibitor. DuP 753 and saralasin attenuated captopril's effect to a similar extent. This finding eliminated a possible role for the agonist effect of the peptide Ang II antagonist in counteracting the renal vasodilator action of captopril.

The administration of captopril after either DuP 753 or saralasin caused 12% and 17% increases in RBF, respectively. The residual effect of captopril on RBF could be attributed to several factors. Because of a high plasma renin activity, total elimination of the influence of intrarenally formed Ang II might not have been achieved. In previous experiments, the plasma renin activity was found to be on the order of 7.5±1 angiotensin 1/ml/hr after completion of the retroperitoneal flank incision; thus, the plasma renin activity and presumably the Ang II levels were high in this animal model. However, the use of maximal blocking doses of angiotensin antag-

TABLE 3. Effect of Captopril on Blood Pressure and Renal Blood Flow Changes After Various Treatments

<table>
<thead>
<tr>
<th>% change</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔRBF</td>
<td>43±14*</td>
<td>12±1†</td>
<td>17±5†</td>
<td>28±12*</td>
<td>0.25±2†</td>
</tr>
<tr>
<td>ΔBP</td>
<td>−14±4*</td>
<td>−9±3*</td>
<td>−13±3*</td>
<td>−13±3*</td>
<td>−1±1†</td>
</tr>
</tbody>
</table>

%ΔBP, mean percent decrease in blood pressure after captopril; %ΔRBF, mean percent increase in renal blood flow after captopril.

*p<0.05 vs. group 8.
†p<0.05 vs. group 4.

Discussion

Captopril, through the inhibition of kinase II, prevents the degradation of bradykinin in addition to blocking Ang II formation. The results of the present study are in agreement with numerous previous investigations showing that captopril decreases blood pressure and increases RBF in anesthetized animals. Administration of the ACE inhibitor after vehicle (group 4) resulted in a 43% increase in RBF (Table 3). The increase in RBF is attributable to vasodilatation evoked by renin-angiotensin blockade or bradykinin potentiation and not to renal autoregulation. To test whether the renal vasodilator effect of captopril is due to the elimination of the vasoconstrictor effect of Ang II, saralasin was infused intravenously to block the renal influence of Ang II. The dose of saralasin used was effective in maximally blocking the renal vasoconstrictor effect to Ang II given intravenously. Saralasin attenuated the renal vasodilatation normally seen after captopril, suggesting the contribution of Ang II receptor blockade to the increase in RBF. Clappison et al. also observed a diminished response to captopril in [Sar1, Ile8]-Ang II–treated dogs, but because of the partial agonist properties of the antagonist, they could only speculate about the extent of participation of the renin-angiotensin system. To elucidate this last point and to substantiate results obtained with saralasin, we used DuP 753. A relatively high dose of DuP 753 was used to maximally block the influence of the renin-angiotensin system on the kidney and thus unmask any remaining vasodilator effect of the ACE inhibitor. DuP 753 and saralasin attenuated captopril's effect to a similar extent. This finding eliminated a possible role for the agonist effect of the peptide Ang II antagonist in counteracting the renal vasodilator action of captopril.

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onists and the fact that the effect of the two antagonists used was similar argue against remaining Ang II–mediated vascular tone in the kidney. It is also possible that vasodilator prostaglandins, kinins, or other peptides generated through the administration of ACE inhibitors may have contributed to the further rise in RBF. Previous results from our laboratory failed to implicate a contribu-
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The failure of angiotensin antagonists to block the renal hemodynamic effect of ACE inhibitors has been attributed to ACE inhibitor–induced potentiation of renal vasodilatation by bradykinin.16,17 Moreover, using anesthetized rats, Roman and his coworkers19 found that both the kallikrein-kinin and the renin-angiotensin systems participate in the regulation of renal papillary blood flow independent of changes in outer cortical blood flow. To investigate the eventuality of a renal vasodilator role for kinins, we used the specific B2 bradykinin antagonist BkA. In contrast to results obtained on conscious dogs in our laboratory,8 results in the rabbit showed a transient increase in RBF due to the partial agonist property of BkA. There was also a BkA/bradykinin blocking dose ratio at least 10-fold less in the rabbit than in the dog. These differences suggest that the rabbit and canine renal vasculature contain bradyki-
nin B2 receptors with different affinities or different bradykinin receptor subtypes. With the passage of time, the renal vasodilator effect of BkA subsided, and captopril caused a 28% increase in RBF (group 8). Presumably, blockade of the renin-angiotensin system accounted for this increment in RBF. When intra-arterial BkA was combined with intravenous DuP 753 (group 8), the effect of captopril on RBF and blood pressure was completely eliminated. Furthermore, this was the only group in which captopril did not reduce blood pressure significantly. In groups 4 through 7, blood pressure was reduced to a similar extent (Table 3), suggesting little effect of DuP 753, saralasin, or BkA when given alone on the depressor action of captopril. Combined administration of the angiotensin and bradykinin antagonist was necessary for blockade of the hypotensive effect of captopril.

To eliminate the influence caused by different basal RBF levels between groups, comparison of the renal effect of captopril was also made by contrasting the percent changes in RBF. The percent increase in RBF due to captopril differed significantly between group 8 and groups 4, 5, 6, and 7. A significant difference was also found between the group given captopril alone (group 4) and those treated with angiotensin antagonists before captopril (groups 5 and 6). Thus, the response to captopril after vehicle did not differ from that after BkA. However, it was greater than that after either saralasin or DuP 753, which in turn, was greater than that after combined administration of DuP 753 and BkA. Therefore, it would appear that Ang II receptor blockade is more effective than bradykinin receptor blockade in attenuating the renal vasodilator effect of captopril. Complete blockade of the RBF effect of captopril in group 8 provided support for the proposition that under these experimental conditions, kinins and the renin-angiotensin system are the sole contributors to the renal effect of ACE inhibitors. The anesthetized rabbit, but to a lesser degree than the sodium-restricted dog, responds to an ACE inhibitor through a kinin-mediated action. Whether kinin-mediated renal vasodilation plays any role in the chronic renal hemodynamic effect of ACE inhibitors will be pursued in future studies.

Acknowledgments

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**KEY WORDS** • renal circulation • angiotensin antagonist • kinins • angiotensin converting enzyme inhibitors • bradykinin • captopril
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