Bradykinin Contribution to Renal Blood Flow Effect of Angiotensin Converting Enzyme Inhibitor in the Conscious Sodium-Restricted Dog

Ben G. Zimmerman, Pauline C. Raich, R.J. Vavrek, and J.M. Stewart

We examined the relative contribution of renin-angiotensin system blockade and bradykinin potentiation to the renal hemodynamic effect of the angiotensin converting enzyme inhibitor enalaprilat in sodium-deprived dogs. Six conscious dogs instrumented for monitoring of blood pressure (BP) and renal blood flow (RBF) were employed in five groups of experiments. In group 1, enalaprilat alone was administered, and it decreased BP by \(-24\pm3\) mm Hg and increased RBF by \(135\pm15\) ml/min. During a constant intravenous infusion of saralasin (group 2), enalaprilat decreased BP by \(-7\pm3\) mm Hg and increased RBF by \(84\pm7\) ml/min (\(\Delta BP\) and \(\Delta RBF\), \(p<0.01\) vs. group 1 by analysis of variance). During a constant intrarenal arterial infusion of saralasin (group 3), the respective changes in BP and RBF after enalaprilat were \(-10\pm3\) mm Hg and \(69\pm12\) ml/min, and these results did not differ from those of group 2. The infusion of saralasin intravenously or intrarenal arterially decreased BP slightly and increased RBF. In the presence of an intravenous infusion of a specific bradykinin antagonist, d-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-d-Phe-Thi-Arg-TFA (B5630) (group 4), enalaprilat decreased BP by \(-28\pm4\) mm Hg and increased RBF by \(82\pm24\) ml/min (\(\Delta RBF\), \(p<0.01\) vs. group 1). The largest attenuation of the RBF increase to enalaprilat was seen in group 5, in which intravenous saralasin and intrarenal arterial B5630 were administered concurrently; the BP decrease was \(-10\pm4\) mm Hg, and RBF increase was \(38\pm7\) ml/min (\(\Delta RBF\), \(p<0.01\) vs. group 1, \(p<0.05\) vs. groups 2 and 4). The results clearly indicate that while blockade of the renin-angiotensin system accounted for a significant part of the increase in RBF caused by an angiotensin converting enzyme inhibitor, a substantial component was contributed by endogenous bradykinin. (Circulation Research 1990;66:242–248)

Although it is well known that angiotensin converting enzyme (ACE) inhibitors exert a potentiating effect on the vasodilator response to exogenous bradykinin by inhibiting kininase II, there has been mainly suggestive evidence for participation of bradykinin in the hemodynamic actions of these agents. Difficulty in the measurement of plasma bradykinin and, until recently, the absence of effective bradykinin antagonists have presented obstacles to the progress of this research. With the introduction of specific bradykinin antagonists with little or no agonist properties, clarification of the role of bradykinin in the cardiovascular actions of these drugs has become possible. Recently, it has been shown using such an antagonist that bradykinin contributes to the hypotensive response of enalapril in the conscious normal rat and to the autoregulation of glomerular filtration rate in the sodium-restricted dog, and also participates in papillary blood flow regulation in the anesthetized rat. Previous results from our laboratory had suggested a role for a hormonal vasodilator, possibly bradykinin, in the renal hemodynamic effect of ACE inhibitors in the conscious sodium-restricted dog. We found that the angiotensin antagonist saralasin blocked only partially the renal blood flow (RBF) increase due to the administration of ACE inhibitors in this high renin model. Thus, contribution of active vasodilation to the increase in RBF caused by ACE inhibition seemed likely and, interestingly, only when the plasma renin level was high. Saralasin abolished the RBF effect of the ACE inhibitors in the sodium replete dog.
The present investigation was undertaken to further examine the renal vasodilator effect of an ACE inhibitor that remains after saralasin blockade in the sodium-restricted dog. First, we wished to compare the ability of saralasin administered intrarenally arterially with that given intravenously, to test for greater blockade of the effect of intrarenally generated angiotensin II. Secondly, a specific bradykinin antagonist, having little or no agonist action, was used to examine the role of bradykinin. Because of the influence of anesthetics and surgical trauma on vascular reactivity and on the release of hormonal substances, the use of conscious instrumented dogs was considered important in this investigation.

Materials and Methods

The experiments were conducted on six dogs of either sex weighing 21–25 kg. Dogs had been quarantined for 2 weeks and inoculated before experimentation. Instrumentation procedures were as reported in detail previously and will be briefly described. Two surgical procedures were conducted on the dogs while they were anesthetized with 30 mg/kg i.v. of sodium pentobarbital. Polyethylene catheters (PE-50) were placed in the femoral artery and vein for recording arterial blood pressure (BP) and withdrawal of arterial blood samples, and to administer drugs intravenously. One to two weeks later, after training the dogs, a second operation was carried out to install a blood flow probe (6 mm in diameter, Zepeda Instruments, Seattle, Washington) and a renal arterial catheter (PE-10) to permit intrarenal arterial infusion of drugs. A retroperitoneal flank incision was made to expose the renal artery. The renal nerves were carefully avoided during dissection and manipulation of the renal artery. After three dogs were killed, the possibility of renal denervation by the probe implantation procedure was checked by determining the renal cortical norepinephrine concentration in the monitored and untouched kidneys, using a fluorometric method. In no case was the norepinephrine concentration decreased in the monitored compared with the untouched kidney, thus indicating that the renal innervation remained intact.

The three implanted catheters were filled with heparin sodium, 1,000 U/ml, plugged, and the ends of the catheters and probe leads passed subcutaneously to exit high on the dog's back. Transpose tape (3M, St. Paul, Minnesota) and a nylon (Alice King Chatham, Los Angeles, California) or a canvas jacket were employed to protect the exposed leads and catheter ends at all times except during experiments. The care and handling of the animals was as prescribed in the Guide for Care and Use of Laboratory Animals (DHEW Publication No. NIH 73-23).

Protocol

The minimum amount of time between the second operative procedure and the first experiment reported in the “Results” was 7 days, which was sufficient for full recovery from surgery. Two of the six dogs had been instrumented for a much longer time, several months, before the experiments were conducted. Recording of BP and RBF was accomplished usually with the dog lying in a relaxed position on a padded table or while in a standing position in a sling. The animals were well trained and accustomed to people entering and leaving the laboratory, and no effort was made to shield the dogs from light or noise. We felt that it was important to allow the dog to be exposed to normal everyday stimuli. Connections were made between the arterial catheter and a P23AA Statham pressure transducer for pressure, and between the probe leads and a SWF-4 Zepeda flowmeter for flow recording. These parameters were registered continuously on a polygraph (Grass Instruments, Quincy, Massachusetts). Heart rate was measured by increasing the paper speed and counting BP or flow pulses. The probe factor was set depending on the hematocrit value and based on in vitro calibration of the probe before implantation. At the start of the experiment, the probe was balanced electronically and then checked intermittently during an experiment. At the conclusion of an experiment, angiotensin II (CIBA-GEIGY, Summit, New Jersey), 2–2.5 μg, was injected into the renal arterial catheter to produce the true zero RBF reading to confirm the validity of the balance setting. In these experiments, the electronic zero agreed within 7.3±10.3% (SD). A similar pharmacological method of obtaining the reference zero flow compared favorably with the occlusive zeroing method. We prefer not to implant an occluder on the renal artery because of length limitation and the greater chance for constriction of the artery. After the BP and RBF had stabilized in the control period, which occurred in approximately 10 minutes, a plasma sample was taken for determination of plasma renin activity (PRA). Five different experiments (groups 1–5) were conducted on each of the six instrumented dogs, and the order in which the experiments were run was varied for each animal. Data obtained in two identical protocols on the same dog were averaged. The dogs were placed on a low sodium diet for 5–7 days before and while the experiments were being conducted. The diet consisted of H/D prescription canned meat and H/D dry chow (Hills, Topeka, Kansas), which provided approximately 6 meq NaCl/day. Furosemide was administered in the dose of 1 mg/kg subcutaneously on the first day and 0.5 mg/kg on the fifth to seventh day of the diet. If the PRA was found to decrease, additional daily doses of furosemide (0.1–0.2 mg/kg) were administered to elevate the PRA. Generally, the five experiments on each dog were completed within a 1–2-week period, and at least 24 hours was allowed between experiments for drug effects to wear off completely. We have reported before that 24 hours after a dose of enalaprilat had been given there was no residual blocking effect on the BP and RBF response to angiotensin I, and in the present study...
Table 1. Control Blood Pressure, Renal Blood Flow, Renal Vascular Resistance, and Plasma Renin Activity in Five Groups of Sodium-Restricted Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>RVR (mm Hg·ml⁻¹·min⁻¹)</th>
<th>PRA (ng Al·ml⁻¹·hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>104±6</td>
<td>239±15</td>
<td>0.44±0.01</td>
<td>6.7±1</td>
</tr>
<tr>
<td>2</td>
<td>104±5</td>
<td>261±21</td>
<td>0.41±0.02</td>
<td>5.1±0.8</td>
</tr>
<tr>
<td>3</td>
<td>100±5</td>
<td>242±22</td>
<td>0.42±0.03</td>
<td>7.1±1.9</td>
</tr>
<tr>
<td>4</td>
<td>103±5</td>
<td>258±28</td>
<td>0.42±0.05</td>
<td>8.4±1.9</td>
</tr>
<tr>
<td>5</td>
<td>107±5</td>
<td>229±20</td>
<td>0.49±0.05</td>
<td>9.1±1.6</td>
</tr>
</tbody>
</table>

BP, blood pressure; RBF, renal blood flow; RVR, renal vascular resistance; PRA, plasma renin activity; Al, angiotensin I.

enalaprilat exerted no lasting effect on RBF 24 hours later.

Group 1 (n=6). After establishing the control BP and RBF levels, a time interval (X=14 minutes) was allowed, which was approximately equivalent to the duration of saralasin (Bachem, Torrance, California, or Sigma Chemical, St. Louis, Missouri) infusion in group 2. Sterile water vehicle was then infused intrarenally for 5 minutes, to control for the intrarenal arterial infusion in groups 4 and 5, followed by enalaprilat (0.2 mg/kg i.v.) injected over a 1-minute period. Recording of BP and RBF was continued for at least 10 minutes after enalaprilat administration, which was the period of time required for stabilization of these parameters after ACE inhibition. This dose of enalaprilat was shown previously to totally block the BP and RBF response to angiotensin I, 0.02–0.05 μg/kg i.v.10

Group 2 (n=6). After obtaining the control BP and RBF readings, saralasin infusion, 1 μg/kg/min i.v., was begun and continued for the remainder of the experiment. A time interval of X=12 minutes was allowed for the BP and RBF to stabilize after the start of the saralasin infusion. An intrarenal arterial vehicle infusion was begun, and 5 minutes later enalaprilat was administered.

Group 3 (n=6). In these experiments, an identical protocol as that in group 2 was followed with exception that the saralasin infusion was given intrarenally arterially instead of intravenously.

Group 4 (n=6). After obtaining stable control levels of BP and RBF, d-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-d-Phe-Thi-Arg·TFA (B5630, where Thi is β-(2-thienyl)-l-alanine, Hyp is l-4-hydroxyproline, and TFA is trifluoroacetic acid salt) was administered intrarenally arterially at 27 μg/min for X=7 minutes. Enalaprilat was then injected intravenously with the infusion of B5630 continuing during and after the administration of enalaprilat.

Group 5 (n=6). These experiments were conducted as those in group 4 with the exception that in addition, saralasin infusion, 1 μg/kg/min, was begun after BP and RBF had stabilized in the control period, and the infusion continued for the remainder of the experiment.

In separate experiments on all six dogs, the effect of B5630 was studied on the RBF responses to intrarenal arterial infusions of 5 and 10 ng/kg/min bradykinin (United States Biochemicals, Cleveland, Ohio). Because of the short half-life of B5630, the antagonist and bradykinin were combined in a single solution and infused into the renal arterial catheter. Control responses to bradykinin were first elicited and then B5630, 27 μg/min, was infused for approximately 5 minutes to induce blockade (Figure 2). This was followed by intrarenal arterial infusion of bradykinin, 5 and 10 ng/kg/min, plus B5630, 27 μg/min.

In four of the dogs, the specificity of bradykinin blockade by B5630 was tested. Acetylcholine bromide (Sigma Chemical) was infused intrarenally arterially before and during infusion of B5630, 27 μg/min. Two doses of acetylcholine were administered in each experiment. The low dose was either 0.5 or 1 μg/min, and the high dose was 1 or 2 μg/min, depending on the vascular responsiveness of the dog.

Statistics

Data were analyzed by one-way analysis of variance (ANOVA) with repeated measures or two-factor ANOVA with repeated measures. Differences between means following ANOVA were determined by Duncan’s new multiple range test. A value of p<0.05 was considered statistically significant. Values in graphs, in tables, and in the text are presented as mean±SEM.

Results

BP, RBF, RVR, and PRA values, taken before any intervention was imposed, for the five groups of sodium-restricted dogs are in Table 1. There were no significant differences in these control parameters among the five groups of dogs. BP and RBF remained stable in group 1, that is, did not vary significantly from control, whereas saralasin administered either intravenously (group 2) or intrarenally (group 3) increased RBF 34±10 and 46±12 ml/min, respectively, and only decreased BP slightly, −5±3 and −4±1 mm Hg, respectively. The changes in RBF and BP did not differ between the two routes of administration of saralasin. This dose of saralasin administered intravenously was previously shown to block totally the RBF and BP changes produced by angiotensin II, 0.0125–0.05 μg/kg i.v.10 No change in either BP or RBF was seen during the intrarenal arterial infusion of B5630 when given alone (group 4) or with the intravenous infusion of saralasin (group 5), which suggested the absence of any agonistic effects.
BP and RBF values obtained immediately before and 10 minutes after the administration of enalaprilat in all five groups of experiments are presented in Figure 1. The difference between the mean values before and after enalaprilat represents the effect of the ACE inhibitor on BP and RBF. A marked increase in RBF and decrease in BP was elicited by enalaprilat in group 1, and as indicated in “Materials and Methods,” these parameters had reached a stable level at the 10-minute interval after drug administration. The mean increase in RBF of 135±15 ml/min 10 minutes after enalaprilat in group 1 surpassed that obtained in the other four groups (p<0.01). As described above, intravenous infusion of saralasin increased RBF and decreased BP, but it only attenuated the effect of enalaprilat (Figure 1). The mean changes in BP and RBF in group 2 were significantly less than those in group 1 (see legend of Figure 1 for statistical analysis). Within the 12-minute interval of saralasin infusion and during the intrarenal arterial saline vehicle infusion, BP and RBF had reached a stable level before giving enalaprilat.

As with the intravenous infusion of saralasin, intrarenal arterial infusion of the antagonist also increased RBF and blocked only partially the effect of enalaprilat (group 3, Figure 1). The mean increases in RBF and decreases in BP after enalaprilat were 84±7 and 69±12 ml/min and −7±3 and −10±3 mm Hg in groups 2 and 3, respectively. There was no significant difference in the effect of enalaprilat between groups 2 and 3 indicating that intrarenal arterial saralasin had no greater efficacy than intravenous saralasin in blocking the renal hemodynamic action of enalaprilat.

In group 4, B5630 had no effect of its own on BP or RBF but did decrease the RBF effect of enalaprilat (Figure 1). The increase in RBF caused by enalaprilat in this group (82±24 ml/min) was significantly decreased and the BP change was unaltered compared with group 1; however, there was variability in the effect of B5630 when given alone. A more profound and consistent attenuation was obtained when saralasin and B5630 were given concomitantly (group 5, Figure 1). Enalaprilat had a much attenuated effect on RBF, the increase amounting to only 38±7 ml/min. BP was decreased to approximately the same degree as in the experiments in which saralasin alone was administered (groups 2 and 3). The mean change in RBF obtained in group 5 was significantly less than that in groups 1, 2, and 4, whereas the mean BP reduction in group 5 was significantly less than in groups 1 and 4 (Figure 1).

A heart rate increase was obtained in five of six dogs after enalaprilat administration, and this effect was not influenced by the presence of saralasin or B5630. The increase in heart rate averaged 15±4 beats/min when all six dogs were considered, and appeared to be inversely related to the control heart rate. The heart rate before giving enalaprilat averaged 94±4 beats/min in the six dogs.

Intra-arterial administration of bradykinin and acetylcholine caused marked renal vasodilator responses (Figure 2 and Table 2). When B5630 was administered in the same dose as in the above-described experiments, the responses to bradykinin (ΔRBF, in milliliters per minute) were markedly suppressed, but the responses to acetylcholine were unaffected (Figure 2 and Table 2). In the experiment depicted in Figure 2, the responses to brady-

**Figure 1.** Mean arterial blood pressure (BP) and renal blood flow (RBF) in five groups of sodium-restricted dogs pre- and 10-minutes post-enalaprilat (0.2 mg/kg i.v.). Group 1 was given enalaprilat alone and also served as a time control; group 2 was given saralasin, 1 μg/kg/min i.v. continuously, and then enalaprilat; group 3 was given saralasin, 1 μg/kg/min i.a. continuously, and then enalaprilat; group 4 was given B5630, 27 μg/min i.a. continuously, and then enalaprilat; group 5 was given saralasin, 1 μg/kg/min i.v., and B5630, 27 μg/min i.a., and then enalaprilat. The difference between the control value and value after enalaprilat for each experiment was calculated and subjected to one-way analysis of variance with repeated measures. After analysis of variance, the mean changes were compared between the five groups of experiments by Duncan’s new multiple range test. For the blood pressure (BP) changes, p<0.01, group 1 vs. groups 2, 3, and 5; p<0.05, group 4 vs. groups 2, 3, and 5. For the renal blood flow (RBF) changes, p<0.01, group 1 vs. groups 2, 3, 4, and 5; p<0.05, group 5 vs. groups 2 and 4.
kinin were reduced by 45–63%, whereas, when all six experiments were considered the mean reduction was 73–79% (Table 2).

**Discussion**

The failure of angiotensin antagonists to block the renal hemodynamic effect of ACE inhibitors has been attributed to ACE inhibitor–induced potentiation of the renal vasodilatation caused by bradykinin.\(^{10,14}\) Alternatively, it could be postulated that saralasin incompletely antagonizes the effect of intrarenally generated angiotensin II. Evidence that bradykinin was involved rested partly on the finding of a reduction in the RBF response to the ACE inhibitor by the kallikrein inhibitor aprotinin.\(^{15}\) However, reservations have been expressed about the use of aprotinin because of its lack of enzyme inhibitory specificity.\(^{3}\) Aprotinin inhibits kallikrein as well as other enzymes.\(^{16}\) Another disadvantage of aprotinin is its failure to inhibit canine kallikrein.\(^{4}\) As an alternative means of implicating bradykinin, various investigators attempted to show that an increase in the plasma or urinary level of bradykinin correlated with the action of ACE inhibitors; however, the results reported were variable.\(^{17–22}\) The present study has provided evidence for the involvement of bradykinin in the RBF effect of ACE inhibitors under conditions in which the PRA is elevated. Previous investigators have contended that only when PRA was low did bradykinin play a role in the antihypertensive and renal effect of ACE inhibitors.\(^{23,24}\)

The results of the present study also emphasize the point that multiple factors contribute to the renal vasodilator effect of ACE inhibitors. A major contribution to the increase in RBF is the blockade of the renin-angiotensin system, since saralasin alone significantly attenuated this effect (Figure 1). The RBF increase was reduced from 135 to 84 ml/min by the intravenous administration of saralasin, which represented a 38% decrease in the renal response. When the bradykinin antagonist was added to the saralasin treatment a further decrease in the RBF change to 38 ml/min was obtained. Thus, the combined actions of saralasin and B5630 reduced the RBF increase by 72%. Based on the nearly equivalent decrease in the ACE inhibitor–induced RBF effect by saralasin in group 2 and by B5630 in group 4, it would appear that the renin-angiotensin system and bradykinin share equally in the response.

**Table 2. Effect of Bradykinin and Acetylcholine on Renal Blood Flow in Absence and Presence of Infusion of B5630**

<table>
<thead>
<tr>
<th>Bradykinin (n=6) (ng/kg/min)</th>
<th>Control</th>
<th>B5630</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP</td>
<td>RBF</td>
</tr>
<tr>
<td>5</td>
<td>105±7</td>
<td>212±8</td>
</tr>
<tr>
<td>10</td>
<td>104±7</td>
<td>219±11</td>
</tr>
<tr>
<td>Acetylcholine (n=4) (µg/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5–1</td>
<td>118±8</td>
<td>203±21</td>
</tr>
<tr>
<td>1–2</td>
<td>117±8</td>
<td>210±21</td>
</tr>
</tbody>
</table>

BP, blood pressure (mm Hg); RBF, renal blood flow (ml/min).

\(^*p<0.001\) vs. control by two-factor analysis of variance with repeated measures.
The possibility of an incomplete blockade of the effect of intrarenally generated angiotensin II by the intravenous administration of saralasin seems unlikely. If this were the case, we would have expected greater attenuation of the effect of enalaprilat by the same dose of saralasin administered intrarenally. In this investigation the effects of saralasin given by either route of administration were equivalent, indicating adequacy of the blockade of intrarenal angiotensin II.

What appears to be an influence of the kallikrein-kinin system on RBF after, but not before, ACE inhibition is of interest. In other words, there was no sign of renal vasodilator tone mediated by bradykinin before giving enalaprilat, since no decrease in RBF occurred during the intrarenal arterial infusion of bradykinin antagonist. This result suggests that either the basal plasma bradykinin level was below the vasodilator threshold, and this was reached only after ACE inhibition, or that the kallikrein-kinin system was activated only after the plasma PRA was increased by the ACE inhibitor. The present study does not provide an explanation for the apparent absence of bradykinin influence before ACE inhibition. Complex interactions between the renin-angiotensin system, kallikrein-kinin system, and renal prostaglandins are known to exist. Under the conditions of salt restriction, as in the present experiments, it would appear that the renin-angiotensin system plays a leading role in stimulating the activity of the kallikrein-kinin system. Further work is needed to elucidate the mechanism(s) for the stimulatory action of these potent vasoactive systems upon each other.

The present study was primarily concerned with the short-term effect of ACE inhibitor on BP and RBF and clearly indicated an involvement of bradykinin on RBF. Due to the limited supply of B5630, it was not administered intravenously, and thus we could not evaluate the contribution of bradykinin to the acute hypotensive effect of enalaprilat. Another important question is the involvement of bradykinin in the long-term effect of ACE inhibitors. This is an important consideration because of the frequent use of ACE inhibitors in patients with essential hypertension given diuretics or placed on salt restriction. The relative participation of blockade of the renin-angiotensin system and potentiation of bradykinin in the antihypertensive action of ACE inhibitors in these individuals is not known.

In summary, these results confirm the contention that bradykinin potentiation plays a role in the renal hemodynamic action of ACE inhibitors, at least under the conditions of sodium restriction. The results also indicate that blockade of the renin-angiotensin system contributes to the renal effect of these agents under these same conditions. Contribution of the kallikrein-kinin system to the acute or the long-term decrease in blood pressure caused by ACE inhibitors requires further investigation.

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References


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