Zonal Heterogeneity in Action of Angiotensin-Converting Enzyme Inhibitor on Renal Microcirculation: Role of Intrarenal Bradykinin

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Abstract. The present study examined the role of intrarenal bradykinin in angiotensin-converting enzyme inhibitor (ACEI)-induced dilation of renal afferent (AFF) and efferent arterioles (EFF) in vivo, and further evaluated whether ACEI-stimulated bradykinin activity differed in superficial (SP) and juxtamedullary nephrons (JM). Arterioles of canine kidneys were visualized with an intravital charge-coupled device camera microscope. E4177 (an angiotensin receptor antagonist, 30 μg/kg) dilated AFF and EFF in SP (15 ± 3% and 19 ± 5%) and JM (15 ± 3% and 18 ± 4%). Subsequently, cilazaprilat (30 μg/kg) caused further dilation of both AFF (29 ± 4%) and EFF (36 ± 4%) in JM, whereas in SP it dilated only EFF (29 ± 3%). Similarly, in the presence of E4177, cilazaprilat caused further increases in sodium excretion. This cilazaprilat-induced vasodilation and natriuresis was abolished by a bradykinin antagonist (Nα-adamantaneacetyl-d-Arg-[Hyp3, Thi5,8, D-Phe7]-bradykinin). In parallel with these results, cilazaprilat increased renal bradykinin content, more greatly in the medulla than in the cortex (5.7 ± 0.4 versus 4.6 ± 0.1 ng/g). Similarly, cilazaprilat elicited greater bradykinin-dependent increases of nitrite/nitrate in the medulla. In conclusion, zonal heterogeneity in renal bradykinin/nitric oxide levels and segmental differences in reactivity to bradykinin contribute to the diverse responsiveness of renal AFF and EFF to ACEI. ACEI-enhanced kinin action would participate in the amelioration of glomerular hemodynamics and renal sodium excretion by ACEI.

Although the renin-angiotensin system is indispensable in maintaining renal homeostatic responses including glomerular filtration and BP regulation, this system is also recognized as a detrimental factor in the progression of renal diseases (1–3). Indeed, recent clinical studies have demonstrated that angiotensin-converting enzyme inhibitors (ACEI) exert salutary action in retarding the progression of renal diseases (4–7). Since the development of orally active angiotensin receptor antagonists (AII-A), it has been extensively disputed whether ACEI, which raises kinin activity, protects the kidney more favorably than AII-A (8). In addition, the increased bradykinin could augment renal nitric oxide (NO) production. Despite these biochemical formulas, the effect of ACEI-induced bradykinin on renal hemodynamics is reported to be divergent. Several lines of investigation favor an important role of bradykinin in ACEI-induced renal hemodynamic changes (9–12). In contrast, it was also demonstrated that bradykinin receptor (B2) antagonist (BKA) reduced the ACEI-induced increases in renal blood flow (RBF) only slightly (13,14). Thus, the role of bradykinin in ACEI-induced renal vasodilation in vivo remains undetermined. Furthermore, the effect of ACEI on renal production of NO has not been delineated.

Despite a well-established fact that bradykinin causes renal vasodilation, the precise site of this action on the renal microvasculature is the subject of controversy. Thomas et al. (15) demonstrated that intra-arterial infusion of bradykinin primarily reduced renal preglomerular resistance in superficial nephrons and caused a slight decrease in postglomerular resistance. In contrast, in isolated renal microvessels, bradykinin is reported to induce selective efferent arteriolar dilation (16). Furthermore, Kon et al. (9) demonstrated that in superficial nephrons, enalapril caused preferential efferent arteriolar dilation, and this effect was offset by BKA. In addition to these discrepant findings observed in afferent and efferent arterioles of superficial nephrons, laser Doppler studies demonstrated that BKA markedly blunted the ACEI-induced increase in papillary blood flow originating from the juxtamedullary nephrons, but had only a modest effect on cortical blood flow (11). Collectively, the renal microvascular action of bradykinin...
may vary, depending not only on the type of vascular segments (i.e., afferent versus efferent arterioles), but also on the zonal localization of nephrons (i.e., superficial versus juxtamedullary). This presumption, however, is drawn from the results obtained in a number of different experimental models (9–16), and therefore requires a more direct demonstration observed under the same experimental conditions, to establish the heterogeneous action of ACEI-induced bradykinin within the kidney in vivo.

In the present study, we directly examined the role of bradykinin in mediating the ACEI-induced vasodilation of the renal microcirculation in vivo, and determined whether ACEI-stimulated bradykinin action differs in superficial and juxtamedullary nephrons. To clarify these important issues, we developed a novel approach to visualize directly the renal microcirculation, an intravital needle-type charge-coupled device (CCD) camera microscope technique (17,18) that allowed direct visualization of renal microvessels not only in an in vivo and in situ experimental setting, but also under the same experimental conditions. Furthermore, alterations in renal interstitial bradykinin and nitrite/nitrate (NOx) contents were evaluated, which may be directly related to the renal microvascular action of ACEI.

Materials and Methods

Renal Hemodynamic Studies

Measurement of Systemic and Renal Hemodynamics. All experimental procedures in this study were conducted according to the guideline of the Animal Care Committee of Keio University. Fifty adult male mongrel dogs (8 to 13 kg) were fed a standard diet (Oriental Yeast Co., Tokyo, Japan) and were anesthetized with sodium pentobarbital (30 mg/kg). After intratracheal intubation, each animal was ventilated with an artificial respirator and placed on a heating blanket to maintain body temperature at 37°C. A 7-Fr catheter was inserted through the right femoral artery and placed on a heating blanket to measure mean arterial pressure (MAP) and heart rate (HR), and the left radial vein was catheterized for infusion of drug. A 7-Fr catheter (Create Medic, Tokyo, Japan) was placed in the bladder for clearance study.

Through a retroperitoneal incision, the kidney was exposed, and an electromagnetic flow probe was placed around the renal artery for measurement of RBF. Data on systemic and renal hemodynamics (MAP, HR, and RBF) were analyzed with a Macintosh Laboratory system (Mac Lab, Analog-Digital Instruments, Castle Hill, Australia) (19,20).

Visualization of Renal Microcirculation. Renal microcirculatory responses to vasoactive agents were observed with an intravital needle-type CCD camera; the prototype of this CCD camera had been developed for the assessment of cardiac microcirculation (17,18). This probe (4.5 mm in diameter) contained 18 optical fibers. The CCD had a spatial resolution of 0.87 μm. The spatial resolution of this CCD system was measured as described previously (17). In brief, sequential images of renal microvessels were captured by a Macintosh computer with a freeze-frame modality, and the density in the gray scale mode was digitized along the scanning line across the vessel. The difference between the peak and noise level was divided into quarters, and the position with the density of a quarter higher value above the noise level was identified as an inner diameter (17,18). A vessel segment approximately 15 μm in length was scanned, and mean vessel diameter was determined by averaging at least five measurements during the plateau of the response. The spatial resolution of this CCD system is 0.87 μm.

Experimental Protocol. After the insertion of a CCD probe into the kidney, the animals were allowed to equilibrate for 60 min before initiating experimental protocols. Each experimental period required 40 min to obtain stable responses in renal clearance.

Protocol 1: Effects of ACEI and All-A on Renal Microcirculation. After the 30-min observation of basal hemodynamics, cilazaprilat (Nippon Roche Ltd., Tokyo, Japan) or E4177 (type I All-A; Eisai Co., Ltd., Tokyo, Japan) (21) was added intravenously at doses of 3, 10, and 30 μg/kg (cilazaprilat) and 3, 10, 30, and 100 μg/kg (E4177). The effects of these agents on whole kidney hemodynamics and renal vascular tone were assessed.

Protocol 2: Role of Bradykinin in ACEI-Induced Vasodilation. To elucidate the role of bradykinin in ACEI-induced dilation, Nα-adamantaneacetyl-d-Arg-[Hyp5, Thi8, D-Phe7]-bradykinin (NAAB; 0.5 nmol/kg per min, Sigma, St. Louis, MO), a BKA, was initially administered, and the ability of cilazaprilat (30 μg/kg) to dilate renal microvessels was assessed. This dose of NAAB had been confirmed to completely block the renal action of the intrarenal infusion of bradykinin (100 ng/kg per min, data not shown).

To delineate more directly the role of bradykinin in ACEI-induced vasodilation, the renal vascular effect of ACEI was assessed in the presence of All-A. After the administration of E4177 (30 μg/kg), cilazaprilat (30 μg/kg) was added, and whether it exerted additive renal vasodilator action was examined. After 40 min, NAAB (0.5 nmol/kg per min) was infused, and the effect of NAAB on the cilazaprilat-induced dilation was assessed.

Renal Medullary and Cortical Kinins and NOx

Renal bradykinin levels were assessed by measuring bradykinin contents in tissues obtained with renal biopsies; a biopsy needle (Bard Biopryt-Cut Needle with Spacer, 16-gauge; C. R. Bard, Inc., Coving-
ton, GA) was inserted into the cortex and medulla, and the specimen was obtained at each dose of cilazaprilat (3, 10, and 30 µg/kg) or E4177 (3, 10, 30, and 100 µg/kg). The tissue was immediately fixed with a mixture of 100% ethanol, TCA (100 mol/L; Sigma), ethylenediaminetetra-acetic acid (3.5 mol/L; Sigma), 1,10-phenanthroline (0.5 mmol/L; Sigma), p-hydroxymercuribenzoate sodium salt (1 mmol/L; Sigma), and pepstatin (0.12 mmol/L; Sigma), and was frozen at −80°C until measurement by RIA (22). The localization of the renal tissue was confirmed by histologic examination.

Renal interstitial NOx levels were determined by a microdialysis technique (23–25). A microdialysis tube (0.5 mm in diameter and a 10-kD transmembrane diffusion cutoff; Eicom, Kyoto, Japan) was inserted into the cortex and medulla at a depth of 2 and 10 mm, respectively, from the renal surface. The microdialysis tube was perfused with lactated Ringer’s solution (147 mEq/L Na, 4 mEq/L K, 5 mEq/L Ca, 156 mEq/L Cl) at 2 µl/min. At this rate, in vitro recovery was 78 ± 3% for nitrite and 70 ± 4% for nitrate. A 180-min stabilization period was allowed before the experiments. The effluent was collected at −20°C for nitrite/nitrate measurements. Nitrite and nitrate concentrations were evaluated with the Griess reaction (26,27), and the sum of these constituents was considered as a marker of renal NOx levels.

Statistical Analyses

Data are expressed as mean ± SEM. Data were analyzed by two-way ANOVA with repeated measures, followed by the Bonferroni post hoc test. P < 0.05 was considered statistically significant.

Results

Observation of Renal Microcirculation in Vivo

The experimental model used in the present study allowed direct observation of the renal microvasculature in vivo (Figures 1 and 2). Both afferent and efferent arteriolar reactivity was visible in situ within the kidney. Furthermore, the microvascular response was accessible not only in the superficial but also in the juxtamedullary cortex.

Protocol 1: Effects of ACEI and AII-A. The effects of cilazaprilat and E4177 on systemic and renal hemodynamics are shown in Table 1. The administration of 3 µg/kg cilazaprilat failed to alter MAP, but increased RBF. A higher dose of 10 µg/kg cilazaprilat reduced MAP, and at 30 µg/kg cilazaprilat had no additional effect on MAP or RBF. GFR did not change at any dose of cilazaprilat.

E4177 at 3 µg/kg reduced MAP and increased RBF. At doses above 10 µg/kg, E4177 caused no further changes in MAP or RBF. GFR remained unchanged at any dose of E4177.

Figure 3 summarizes the effects of cilazaprilat on renal microvascular diameter. In superficial nephrons, 3 µg/kg cilazaprilat caused significant afferent (from 16.5 ± 0.3 to 18.2 ± 0.5 µm, P < 0.05, n = 6) and efferent arteriolar dilation (from 15.1 ± 0.5 to 17.6 ± 0.6 µm, P < 0.05, n = 6). At 30 µg/kg, cilazaprilat-induced dilation was greater in the
Figure 2. Cilazaprilat-induced vasodilation of renal microvessels in canine kidneys. Cilazaprilat infusion (30 μg/kg) dilated both afferent and efferent arterioles.

Table 1. Effects of cilazaprilat and E4177 on systemic and renal hemodynamics

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>RBF (ml/min)</th>
<th>GFR (ml/min)</th>
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<tr>
<td>Control (n = 7)</td>
<td>77 ± 4</td>
<td>119 ± 13</td>
<td>139 ± 10</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>NAAB</td>
<td>79 ± 4</td>
<td>120 ± 13</td>
<td>137 ± 10</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>85 ± 1</td>
<td>123 ± 4</td>
<td>140 ± 3</td>
<td>29 ± 1</td>
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<td>CLZ</td>
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<tr>
<td>3 μg/kg</td>
<td>78 ± 3</td>
<td>122 ± 4</td>
<td>156 ± 5</td>
<td>29 ± 2</td>
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<tr>
<td>10 μg/kg</td>
<td>69 ± 4c</td>
<td>119 ± 4</td>
<td>170 ± 6c</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>30 μg/kg</td>
<td>67 ± 4c</td>
<td>118 ± 4</td>
<td>171 ± 2c</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>84 ± 1</td>
<td>119 ± 10</td>
<td>136 ± 3</td>
<td>30 ± 1</td>
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<tr>
<td>E4177</td>
<td></td>
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<tr>
<td>3 μg/kg</td>
<td>76 ± 2b</td>
<td>112 ± 9</td>
<td>156 ± 3b</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>10 μg/kg</td>
<td>72 ± 2c</td>
<td>110 ± 9</td>
<td>159 ± 6c</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>30 μg/kg</td>
<td>71 ± 2c</td>
<td>110 ± 11</td>
<td>163 ± 6c</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>100 μg/kg</td>
<td>71 ± 2c</td>
<td>110 ± 10</td>
<td>163 ± 5c</td>
<td>33 ± 2</td>
</tr>
</tbody>
</table>

*MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; NAAB, N-alpha-adamantaneacetyl-d-Arg-[Hyp³, Thi⁵, 8, D-Phe⁷]-bradykinin; CLZ, cilazaprilat.

b P < 0.05 versus control.

c P < 0.01 versus control.

As illustrated in Figure 4, in superficial nephrons, 10 μg/kg E4177 elicited significant dilation of afferent (from 16.3 ± 0.4 to 18.3 ± 0.4 μm, P < 0.005, n = 7) and efferent arterioles (from 15.4 ± 0.5 to 17.3 ± 0.4 μm, P < 0.005, n = 7). At 30 μg/kg, E4177-induced dilation was similar in afferent (15.0 ± 4.0% increment) and efferent arterioles (19.3 ± 3.1% increments). In juxtamedullary nephrons, significant vasodilation was observed at 3 μg/kg in afferent (from 21.8 ± 0.3 to 23.9 ± 0.7 μm, P < 0.05, n = 7), and at 30 μg/kg in efferent arterioles (from 20.7 ± 0.5 to 24.4 ± 0.8 μm, P < 0.05, n = 7). For both superficial and juxtamedullary nephrons, increased doses of E4177 from 30 to 100 μg/kg had no additive vasodilator action.

Protocol 2: Role of Bradykinin in ACEI-Induced Renal Vasodilation. NAAB alone had no effect on whole kidney hemodynamics (Table 1) or arteriolar diameter in superficial (afferent, from 16.7 ± 0.5 to 15.9 ± 0.5 μm, P > 0.1, n = 7; efferent, from 15.8 ± 0.3 to 15.0 ± 0.2 μm, P > 0.1, n = 7) or juxtamedullary nephrons (afferent, from 19.2 ± 0.9 to 18.9 ± 0.9 μm, P > 0.5, n = 7; efferent, from 18.1 ± 1.0 to 16.2 ± 1.0 μm, P > 0.05, n = 7) (Figure 5).

In the presence of NAAB, cilazaprilat elicited arteriolar vasodilation in both superficial (11.6 ± 2.5 and 13.0 ± 3.2% increments from NAAB for afferent and efferent, respectively) and juxtamedullary nephrons (11.6 ± 4.0 and 6.9 ± 2.8% increments from NAAB for afferent and efferent, respectively) (Figure 5). When compared with responses in the absence of NAAB, however, the cilazaprilat-induced dilation was diminished at juxtamedullary afferent and efferent arterioles and superficial efferent arterioles. Thus, although in the absence of NAAB, the cilazaprilat-induced dilation of these vascular segments was greater than that induced by E4177, this difference was lost in the presence of NAAB. Of note, no difference in superficial afferent arteriolar responses was observed between E4177, cilazaprilat, and cilazaprilat + NAAB (P > 0.2).

In an additional series of experiments, the effect of ACEI was assessed under the angiotensin II blockade by E4177 (Table 2, Figure 6). In the presence of E4177 (30 μg/kg), efferent arteriole (30.3 ± 3.3% increment, i.e., 19.7 ± 0.6 μm, P < 0.001) than in the afferent arteriole (20.4 ± 2.2% increment, i.e., 19.8 ± 0.3 μm, P < 0.001). In juxtamedullary nephrons, basal arteriolar diameters were greater than those of superficial nephrons (afferent, 19.8 ± 0.4 μm, n = 6, P < 0.001; efferent, 18.2 ± 0.4 μm, n = 6, P < 0.001). In response to 3 μg/kg cilazaprilat, afferent and efferent arterioles exhibited 17.3 ± 1.7% (23.2 ± 0.3 μm, P < 0.05) and 16.7 ± 2.3% (21.3 ± 0.3 μm, P < 0.005) dilation, respectively. At 30 μg/kg, cilazapril elicited nearly the same magnitude of afferent (30.8 ± 3.9% increment, i.e., 25.8 ± 1.3 μm, P < 0.001) and efferent arteriolar dilation (35.4 ± 3.1% increment, i.e., 24.7 ± 0.9 μm, P < 0.001).
cilazaprilat (30 μg/kg) had no effect on MAP, but increased RBF (Table 2). This increase was completely blocked by the subsequent addition of NAAB. Figure 6 illustrates the role of bradykinin in ACEI-induced dilation of renal arterioles. Under the angiotensin II blockade by E4177, cilazaprilat elicited further dilation of afferent (from 25.1 ± 0.6 to 28.2 ± 0.8 μm, n = 8, P < 0.05) and efferent arterioles (from 24.4 ± 0.7 to 28.0 ± 0.7 μm, n = 8, P < 0.001) in juxtamedullary nephrons. In superficial nephrons, by contrast, cilazaprilat induced additive dilation only at the efferent arteriole (from 18.3 ± 0.3 to 19.8 ± 0.2 μm, n = 8, P < 0.05), but had no significant effect on the afferent arteriole (from 18.6 ± 0.3 to 19.6 ± 0.5 μm, n = 8). Furthermore, the cilazaprilat-induced additive vasodilation was abolished by the subsequent addition of NAAB (superficial efferent, 17.0 ± 0.6 μm, P < 0.001; juxtamedullary afferent, 24.6 ± 1.0 μm, P < 0.005; juxtamedullary efferent, 21.8 ± 0.5 μm, P < 0.001).

Figure 7 illustrates the effect of E4177 and cilazaprilat on urinary sodium excretion. E4177 increased the urinary sodium excretion from 7.8 ± 0.3 to 11.6 ± 0.8 μEq/min (P < 0.05, n = 7). The subsequent addition of cilazaprilat further enhanced the natriuresis to 16.4 ± 1.5 μEq/min (P < 0.01), and this increment was partially inhibited by NAAB (to 14.0 ± 1.5 μEq/min, P < 0.05).

Role of Local Vasodilator Substances

The ACEI-induced alterations in tissue bradykinin levels are illustrated in Figure 8 (left panel). Basal bradykinin concentrations were similar in the medulla (4.3 ± 0.4 ng/g, n = 13) and the cortex (3.6 ± 0.3 ng/g, n = 11, P > 0.1). In the medulla, 10 μg/kg cilazaprilat caused 32 ± 6% increments in bradykinin levels (5.7 ± 0.2 ng/g, P < 0.05). In contrast, in the cortex 10 μg/kg cilazaprilat failed to increase bradykinin, and a significant increase was observed at 30 μg/kg (4.6 ± 0.1 ng/g, P < 0.01), a dose higher than that required to increase the medullary bradykinin (i.e., 10 μg/kg). Furthermore, cilazapril-
lat produced greater bradykinin levels in the medulla than in the cortex (3 μg/kg, P < 0.06; 10 μg/kg, P < 0.01; 30 μg/kg, P < 0.05).

Basal NOx concentration was higher in the medulla (11.1 ± 0.3 nmol/L, n = 14) than in the cortex (9.0 ± 0.2 nmol/L, n = 12, P < 0.001) (Figure 8, right). Medullary NOx levels increased to 14.0 ± 0.7 nmol/L (n = 15, P < 0.05) with 3 μg/kg cilazaprilat, and further addition of cilazaprilat (i.e., 30 μg/kg) elicited 38 ± 7% increments (15.1 ± 0.7 nmol/L, n = 14, P < 0.001). In contrast, cortical NOx levels increased significantly only with 30 μg/kg (12.5 ± 0.9 nmol/L, n = 12, P < 0.05). Thus, a higher dose was required to obtain a significant increase in cortical NOx content. Furthermore, the NOx levels in the cortex were lower than in the medulla at all doses examined. Finally, these cilazaprilat-induced increments in NOx levels were abolished by the subsequent addition of NAAB (medulla, 8.6 ± 1.2 nmol/L, n = 7, P < 0.001; cortex, 7.1 ± 0.4 nmol/L, n = 7, P < 0.001).

In striking contrast, E4177 had no effect on renal bradykinin levels in the cortex (n = 10) or the medulla (n = 10) (Figure 9, left). In comparison, E4177 did not alter cortical (n = 10) or medullary NOx levels (n = 11) (Figure 9, right).

**Discussion**

Recent investigations have demonstrated that ACEI exerts renal protective action in various renal diseases (4–7). It is well established that the renal protective action of ACEI is attributed in part to the amelioration of glomerular hemodynamics by decreasing predominantly the efferent arteriolar tone (8,9). Although the ACEI-induced renal hemodynamic action is mediated in large part by the inhibition of renin-angiotensin system, ACEI also acts as a kininase II inhibitor, leading to renal bradykinin accumulation (12,28). Furthermore, ACEI-induced efferent arteriolar dilation is inhibited by BKA in superficial nephrons (9), suggesting a bradykinin-mediated dilation of this vessel. Although numerous studies have been...
conducted examining the renal action of ACEI (9–15,28–30), it has not been clarified whether ACEI-induced renal arteriolar action differs in superficial and juxtamedullary nephrons. Furthermore, the contribution of kininase II inhibition to the ACEI action has not been delineated in these nephron populations.

It has been extremely difficult to visualize renal microvascular responsiveness of superficial and juxtamedullary (deep cortical) nephrons under the same condition. To counter this technical problem and to characterize the renal microvascular action of ACEI in both superficial and juxtamedullary nephrons in vivo, we used an intravital needle-type CCD camera microscope. This system, previously introduced for evaluation of the coronary microcirculation (17,18), has unique characteristics, which under an in vivo, in situ, and intact setting allows direct observation of the coronary microvascular responsiveness to vasoactive stimuli. We have further extended this experimental system for the investigation of the renal microcirculation. With the use of this system, both superficial and juxtamedullary renal microcirculation can be accessible in the same experimental model (Figure 1). Furthermore, this system enables us to evaluate the renal microvascular response during concomitant assessment of whole kidney hemodynamics.

The present studies demonstrated that cilazaprilat caused greater dilation of renal arterioles than E4177. Furthermore, the blockade of bradykinin action markedly impaired the cilazaprilat-induced vasodilation. Finally, during the blockade of angiotensin receptors, cilazaprilat caused additional increases in RBF and dilation of superficial and juxtamedullary arterioles; these hemodynamic changes were abolished by BKA (Figure 6).


table

Figure 6. Changes in afferent and efferent arteriolar diameters induced by E4177, cilazaprilat, and a bradykinin receptor antagonist in canine kidneys. In the presence of E4177, cilazaprilat caused dilation of juxtamedullary afferent and efferent arterioles, and superficial efferent arterioles, whereas it had no effect on superficial afferent arterioles. The subsequent addition of a bradykinin antagonist, NAAB, inhibited the cilazaprilat-induced dilation. CLZ, cilazaprilat. †P < 0.05 versus control; ††P < 0.01 versus control; *P < 0.05; **P < 0.01.

Figure 7. Effect of E4177 and cilazaprilat on urinary sodium excretion (UNaV) in canine kidneys. During angiotensin blockade by E4177, cilazaprilat increased further the urinary sodium excretion, which was partially inhibited by NAAB. CLZ, cilazaprilat. †P < 0.05 versus control; ††P < 0.01 versus control; *P < 0.05; **P < 0.01.
with no appreciable effect on cortical or whole kidney hemodynamics (11). Thus, these factors may be responsible for the divergent observations on the role of bradykinin in ACEI-induced renal responses (9–14,28,29).

Although bradykinin contributes to ACEI-induced renal vasodilation, the role of bradykinin in ACEI-induced vasodilation has not been characterized fully in renal microvessels of superficial and juxtamedullary nephrons. Using the laser Doppler technique, Fenoy et al. (11) reported that in losartan-pretreated rats, captopril had no additive effects on cortical blood flow or RBF, but increased papillary blood flow. They also found that the increase in papillary flow was abolished by BKA, and suggested that ACEI-induced bradykinin contributed importantly to the medullary, but not cortical, circulation. The present study demonstrated that the ACEI-induced bradykinin caused afferent arteriolar dilation in juxtamedullary, but not superficial, nephrons, and a similar tendency was observed at the efferent arteriole (Figure 6). These zonal differences in the renal arteriolar reactivity to ACEI could be attributed to the bradykinin levels in the superficial and juxtamedullary cortex. Thus, the present study confirmed that using sequential renal biopsy, the medullary tissue contained more abundant bradykinin than the superficial cortical tissue in response to ACEI (Figure 8). In concert, the results from both renal microvascular and biochemical studies suggest that juxtamedullary arterioles are under the greater influence of bradykinin than superficial arterioles (11).

Zonal heterogeneity in bradykinin activity within the kidney may affect the renal pre- and postglomerular microvascular responsiveness to ACEI. Edwards (16) previously reported a preferential efferent arteriolar dilation by bradykinin in isolated renal microvessels from superficial cortex. Furthermore, Kon et al. (9) demonstrated that ACEI caused preferential bradykinin-dependent dilation of efferent arterioles in superficial nephrons. In the present study, we have demonstrated that in superficial nephrons, cilazaprilat-induced bradykinin causes efferent, but not afferent, arteriolar dilation. In concert, within the superficial nephrons, bradykinin exerts preferential dilation of the efferent arteriole. In contrast, in juxtamedullary nephrons, cilazaprilat-induced bradykinin produces dilation of both afferent and efferent arterioles. Thus, the segmental difference in the renal microvascular reactivity to bradykinin may

Figure 8. Effect of cilazaprilat on renal bradykinin (left) and nitrite/nitrate (NOx) contents (right) in the cortex (□) and the medulla (■). Cilazaprilat-induced medullary bradykinin and NOx levels were greater than the cortical levels. †P < 0.05 versus control; ††P < 0.01 versus control; #P = 0.06; *P < 0.05; **P < 0.01.

Figure 9. Effect of E4177 on renal bradykinin (left) and NOx contents (right) in the cortex (□) and medulla (■). E4177 had no effect on either bradykinin or NOx levels. *P < 0.05; **P < 0.01.
be related to the zonal difference in the nephron observed. In this regard, Thomas et al. (15) demonstrated that when administered via the intra-arterial route, bradykinin predominantly decreased renal preglomerular vascular resistance, with a slight decrease in postglomerular resistance in dog micropuncture studies. In this study, however, they also suggested that vaso-dilation could have been localized to the deeper nephrons (15). Thus, markedly elevated bradykinin levels within renal preglomerular vessels would dilate afferent arterioles predominantly. Similarly, in juxtamedullary nephrons the ACEI-induced increase in intrarenal bradykinin may elicit the afferent as well as efferent arteriolar dilation. In concert, the segmental (i.e., afferent versus efferent) heterogeneity in renal microvascular responsiveness to ACEI may be attributable to the differences in bradykinin levels within superficial and juxtamedullary nephrons.

Although ACEI stimulates NO releases (30,32), the role of NO in the intrarenal heterogeneity in ACEI action remains undetermined. Hajj-ali and Zimmerman (29) reported that the lisinopril-induced increase in RBF was eliminated by nitro-l-arginine, and suggested a central role of NO in ACEI-induced renal vasodilation. In the present study, we found that cilazaprilat elevated renal interstitial NOx levels, with greater production in the medulla (Figure 8), and the increased NOx production was inhibited by BKA. These findings paralleled the changes in intrarenal bradykinin content, and suggested an important role of NO as a vasodilatory mechanism for ACEI-induced vasodilation of renal microcirculation. Indeed, we also found that the pretreatment with N^G-nitro-l-arginine methyl ester and AII-A completely abolished the cilazaprilat-induced dilation of superficial and juxtamedullary efferent arterioles (33). Although the present study does not directly evaluate renal interstitial NO levels, available evidence suggests that the ACEI-induced bradykinin elevates renal NO contents, and subsequently dilates renal arterioles, with different responsiveness in superficial and juxtamedullary nephrons. Of note, recent studies (33,34) also demonstrate that bradykinin-induced renal arteriolar dilation is mediated in part by arachidonic acid metabolites.

Contrary to the effect of ACEI, the present study demonstrates that AII-A fails to increase renal interstitial bradykinin or NOx levels (Figure 9). It has been traditionally accepted that AII-A does not increase bradykinin levels. In contrast, recent studies suggest that AII-A elevates bradykinin concentrations in various tissues, including the heart (35) and the aorta (36). Siragy and Carey (37) also demonstrated that angiotensin II elicited an increase in renal interstitial cyclic GMP levels that was blocked by N^G-nitro-l-arginine methyl ester and PD123319, and suggested AT2-mediated NO production in renal interstitium. They observed, however, that AT1 receptor antagonism failed to increase renal interstitial cyclic GMP, a finding in agreement with our observations. Similarly, Arima et al. (38) found that neither AT1 nor AT2 receptors affected renal NO production. Although the reason for these divergent findings is unclear, differences in the duration of the AT1 receptor blockade and variability of the AT2 receptor expression within the organ may affect the results induced by AT1 antagonists. Furthermore, chronic exposure to bradykinin may downregulate bradykinin B2 receptors (39), resulting in no appreciable effect on the reactivity to bradykinin (40).

Finally, the zonal differences in the role of bradykinin in the ACEI-induced action merit comment. In the present study, we found that both cilazaprilat and E4177 provoked natriuresis, with the former producing a greater increase in urinary sodium excretion (Figure 7). Furthermore, this augmented response was blocked by BKA, a finding in agreement with the observation by Fenoy et al. (11). Since E4177 at the dose used is sufficient to prevent the endogenous angiotensin II actions (Figure 4), the ACEI-induced increases of bradykinin and NO within the medulla most likely contribute to the additive natriuresis (41,42) by directly inhibiting the tubular sodium reabsorption. Alternatively, the ACEI-induced decrease in vascular resistance of juxtamedullary arterioles and vasa recta (43) would increase papillary blood flow (10,11), which hemodynamically inhibits tubular Na reabsorption. In contrast, in superficial nephrons the ACEI-induced bradykinin could have a greater impact on glomerular capillary pressure by predominantly dilating the efferent arteriole (Figure 5). In concert, the present studies are consistent with the formulation that ACEI exerts renal protective action not only by reducing systemic BP derived from the systemic vasodilation as well as enhanced natriuresis, but also by ameliorating the glomerular hypertension in superficial nephrons.

In conclusion, with a novel needle-type CCD camera, we have demonstrated in an in vivo setting that ACEI causes dilation of the renal microvasculature. The current demonstration clearly indicates that the ACEI-induced bradykinin activity varies, depending on the localization of nephrons and the segment of renal microvessels. Such zonal and segmental heterogeneity in bradykinin action would affect glomerular hemodynamics and medullary circulation in superficial and juxtamedullary nephrons differently, and may constitute crucial determinants in controlling the glomerular capillary pressure and renal sodium homeostasis.

Acknowledgments
This work was supported in part by a scientific research grant from the Ministry of Education, Science, and Culture (No. 09470240) (Tokyo, Japan) and by a grant from Eisai Co., Ltd.

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