Role of Endothelium-Derived Hyperpolarizing Factor in 
ACE Inhibitor-Induced Renal Vasodilation in Vivo

Hiroto Matsuda, Koichi Hayashi, Shu Wakino, Eiji Kubota, Masanori Honda, Hirobumi Tokuyama, Ichiro Takamatsu, Satoru Tatematsu, Takao Saruta

Abstract—Although the angiotensin-converting enzyme (ACE) inhibitor-induced bradykinin enhances nitric oxide (NO) release, bradykinin may also stimulate the production of an additional vasodilator, endothelium-derived hyperpolarizing factor (EDHF). This study examined the role of EDHF in mediating the NO-independent action of ACE inhibitors in canine renal microcirculation in vivo. We used intravital CCD camera videomicroscopy that allowed direct visualization of renal microcirculation in superficial and juxtamedullary nephrons in an in vivo, in situ, and relatively intact setting. In the presence of E4177 (an angiotensin receptor blocker), cilazaprilat (30 μg/kg) had no effect on diameter of superficial afferent arterioles (Aff), but it increased renal contents of bradykinin and nitrate plus nitrite, and it elicited dilation of juxtamedullary Aff (from 24.0±0.2 to 28.2±0.8 μm), juxtamedullary efferent arterioles (Eff) (from 24.2±0.2 to 28.0±0.8 μm), and superficial Eff (from 18.2±0.2 to 19.7±0.2 μm). These changes in diameters were prevented by Nω-adamantaneacetyl-D-Arg-[Hyp3,Thi5,8,D-Phe7]bradykinin, a bradykinin receptor antagonist. The pretreatment with nitro-L-arginine methylester (L-NAME) plus E4177 eliminated the dilator response of juxtamedullary/superficial Eff and the increase in renal nitrate plus nitrite levels induced by cilazaprilat. In contrast, in the presence of E4177+L-NAME, cilazaprilat still caused 8%±3% dilation of juxtamedullary Aff, which was completely eliminated by proadifen, a cytochrome-P450 and KCa channel blocker. Collectively, the ACE inhibitor exerts multiple vasodilator mechanisms, including the inhibition of angiotensin II formation; blockade of angiotensin II activity appears to be a dominant mechanism in superficial Aff, whereas the bradykinin-induced NO acts on superficial Eff and juxtamedullary Aff/Eff. Furthermore, a putative EDHF is an additional mechanism for the ACE inhibitor-induced vasodilation of juxtamedullary Aff in vivo. (Hypertension. 2004;43:603-609.)

Key Words: angiotensin-converting enzyme ■ bradykinin ■ endothelium-derived factors ■ arterioles ■ nitric oxide

It has been established that the renin–angiotensin system constitutes a vital determinant of the progression of renal disease, and the inhibition of this system by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) offers beneficial action on renal injury.1-4 These two pharmacological tools, however, exert different activity on the renal microcirculation. Thus, the ACE inhibitor accumulates renal bradykinin by inhibiting kininase II (a kinin-degrading enzyme) with greater contents in the medulla than in the cortex,5,6 and the elevated bradykinin would be anticipated to elicit renal arteriolar dilation.6,7 In contrast, we have recently demonstrated that ARB fails to increase the renal bradykinin content in canine kidneys in an in vivo setting, and these differences contribute to the distinct renal microvascular responsiveness to the ACE inhibitor and ARB.6 Although it is obvious that bradykinin participates in the distinct actions of ACE inhibitors and ARB, the precise mechanism entailing the divergent arteriolar responsiveness to the ACE inhibitor and ARB in the renal microcirculation remains fully undetermined.

A growing body of evidence has accrued that nitric oxide (NO) contributes to the vascular action of bradykinin as a second mediator coupling between bradykinin B2 receptors and the vascular tone regulation.5,9 Although NO is undoubtedly involved in the vasodilator mechanism of the ACE inhibitor,9 bradykinin is also reported to cause membrane hyperpolarization independently of the action of NO and is assumed to produce an endothelium-derived hyperpolarizing factor (EDHF),10,11 which would inhibit voltage-dependent calcium channels predominantly distributed at the afferent arteriole.12 These observations therefore favor the premise that the ACE inhibitor elicits vasodilation by multiple mechanisms including NO-induced and EDHF-induced smooth muscle relaxation, in addition to the angiotensin II blockade. Nevertheless, there is no investigation demonstrating a possible role of EDHF in mediating the ACE inhibitor-induced renal vasodilation in an in vivo and in situ setting. Furthermore, the kidney possesses unique characteristics manifesting zonal differences in the autocrine/paracrine distribution within the kidney,5,13 and exhibits marked heterogeneity in...
Experimental Protocol
After the insertion of a CCD probe into the kidney, the animals were allowed to equilibrate for 60 minutes before initiating experimental protocols.

Effect of Cilazaprilat in the Presence of ARB
We previously reported that 30 \( \mu \text{g/kg} \) E4177 (Eisai, Tokyo), an ARB, caused a maximal action on renal arterioles. We therefore confirmed the effect of cilazaprilat (30 \( \mu \text{g/kg} \); Nippon Roche, Tokyo) on the renal microcirculation in the presence of E4177. After 20 minutes of the E4177 (30 \( \mu \text{g/kg} \), intravenous) administration, the effect of cilazaprilat on the renal microcirculation and renal contents of bradykinin and NOx was evaluated and was compared with the effect in the absence of E4177.

Next, the effect of a bradykinin receptor antagonist on cilazaprilat-induced renal arteriolar vasodilation was examined. After the combined treatment with N\textsubscript{ω}-adamantanemethyl-L-Arg (L-NAME; 1 mg/kg, IV), cilazaprilat at a dose of 30 \( \mu \text{g/kg} \) was injected. Whether the ACE inhibitor-induced vasodilation was mediated by NO was examined. Similarly, the effect of cilazaprilat on renal contents of bradykinin and NOx was also evaluated.

Role of NO in Cilazaprilat-Induced Vasodilation
Twenty minutes after the treatment with E4177 (30 \( \mu \text{g/kg} \), intravenous) and nitro-L-arginine methylester (L-NAME; 1 mg/kg, IV), cilazaprilat at a dose of 30 \( \mu \text{g/kg} \) was injected. Whether the ACE inhibitor-induced vasodilation was mediated by NO was examined. Similarly, the effect of cilazaprilat on renal contents of bradykinin and NOx was also evaluated.

Role of EDHF in Cilazaprilat-Induced Vasodilation
In additional series of experiments, the effect of proadifen (an inhibitor of cytochrome P450 and a blocker of \( \text{K}_c \) channels) on the cilazaprilat-induced dilation of renal microvessels was evaluated. Initially, the dogs were pretreated with the combination of E4177 (30 \( \mu \text{g/kg} \), L-NAME (1 mg/kg), and proadifen (5 mg/kg; Sigma, St Louis, Mo). Thereafter, whether cilazaprilat dilated renal arterioles was assessed, and these effects were compared with those in the presence of E4177 plus L-NAME.

Statistics
Data are expressed as the mean±SEM. Data were analyzed by 2-way ANOVA with repeated measures, followed by Bonferroni post hoc test. \( P<0.05 \) was considered statistically significant.

Results
Effect of Cilazaprilat in the Presence of ARB
Cilazaprilat caused a decrease in MAP (Table 1, control) and elicited marked increases in diameters of superficial (afferent, from 16.5±0.4 to 19.3±0.2 \( \mu \text{m} \), \( P<0.01 \), \( n=6 \); efferent, from 15.1±0.4 to 19.2±0.3 \( \mu \text{m} \), \( P<0.01 \), \( n=6 \)) and juxtamedullary arterioles (afferent, from 20.0±0.4 to 26.0±0.9 \( \mu \text{m} \), \( P<0.01 \), \( n=6 \); efferent, from 18.4±0.3 to 25.4±0.8 \( \mu \text{m} \), \( P<0.01 \), \( n=6 \)) and an elevation in RBF (Table 1).

The pretreatment with E4177 decreased MAP and increased RBF (Table 1) and renal arteriolar diameters (Table 2). Furthermore, E4177 abolished the cilazaprilat-induced increases in diameters of superficial afferent arterioles (from 18.4±0.4 to 19.1±0.3 \( \mu \text{m} \), \( n=6 \); triangles, Figure 1) and the decrease in MAP (Table 1). Similarly, in the presence of E4177, the cilazaprilat-induced vasodilation of superficial efferent arterioles (from 18.2±0.2 to 19.7±0.2 \( \mu \text{m} \), \( P<0.05 \), \( n=8 \)), juxtamedullary afferent arterioles (from 24.0±0.2 to 28.0±0.8 \( \mu \text{m} \), \( P<0.05 \), \( n=8 \)), and juxtamedullary efferent arterioles (from 24.2±0.6 to 27.7±0.7 \( \mu \text{m} \), \( P<0.05 \), \( n=8 \)), as well as the increase in RBF, were diminished when compared with...
with those in the absence of E4177 (8%±3% versus 27±5%, 17%±2% versus 30%±5%, 14%±5% versus 38%±6%, and 7%±2% versus 14%±3%, respectively; P<0.05). Thus, these results precisely coincided with our previous observations.6

The baseline MAP, RBF, and renal arteriolar diameters in the presence of E4177 and NAAB were nearly the same as those in the presence of E4177 alone (P>0.5; Tables 1 and 2). Nevertheless, the combined pretreatment with E4177 plus NAAB completely prevented the cilazaprilat-induced changes in vessel diameter (n=5 for each arteriole; open circles, Figure 1) and RBF (n=7, Table 1).

Table 1. Effect of Cilazaprilat on Systemic and Renal Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>MAP (mm Hg)</th>
<th>Renal Blood Flow (mL/min)</th>
<th>RVR (mm Hg · mL⁻¹ · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Cilazaprilat</td>
<td>Baseline</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>86±1</td>
<td>75±3*</td>
<td>148±3</td>
</tr>
<tr>
<td>E4177 (n=8)</td>
<td>72±2†</td>
<td>71±3</td>
<td>162±3†</td>
</tr>
<tr>
<td>E4177+NAAB (n=7)</td>
<td>73±3†</td>
<td>73±2</td>
<td>159±3†</td>
</tr>
<tr>
<td>E4177+L-NAME (n=6)</td>
<td>87±3‡</td>
<td>84±2‡</td>
<td>135±6‡</td>
</tr>
<tr>
<td>E4177+L-NAME + proadifen (n=5)</td>
<td>86±3‡</td>
<td>85±1†‡</td>
<td>134±6‡</td>
</tr>
</tbody>
</table>

Results are the mean±SEM.

MAP indicates mean arterial pressure; RVR, renal vascular resistance.

Role of NO in Cilazaprilat-Induced Vasodilation

When compared with the responses in the presence of E4177 alone, the combined pretreatment with E4177 plus L-NAME elevated MAP and elicited marked reductions in RBF (Table 1) and diameters of all arterioles (Table 2). Furthermore, this pretreatment prevented the cilazaprilat-induced changes in RBF (Table 1) and diameters of superficial efferent arterioles (from 12.2±0.7 to 13.1±0.6 µm, n=5, P<0.5; filled triangles, Figure 2) and juxtamedullary efferent arterioles (from 16.6±0.3 to 16.9±0.3 µm, n=5, P<0.5). Similar to the effect in the presence E4177 alone, cilazaprilat did not alter the superficial afferent arteriolar diameter during the combined pretreatment with E4177 and L-NAME.

In the presence of E4177 and L-NAME, juxtamedullary afferent arterioles exhibited a markedly diminished vasodilator response to cilazaprilat (from 18.0±0.4 to 19.6±0.3 µm, n=5, P<0.05; filled triangles, Figure 2) when compared with that in the presence of E4177 alone (open triangles; 8%±3% versus 17.2%, P<0.05). Of note, however, the vasodilator response to cilazaprilat was still retained even under the dual blockade of angiotensin II and NO.

Role of EDHF in Cilazaprilat-Induced Vasodilation

Because cilazapril elicited the vasodilator response of juxtamedullary afferent arterioles in the presence of E4177 and L-NAME, a potential role of EDHF in mediating this remaining vasodilator action was assessed with the use of proadifen. Thus, in the presence of E4177, L-NAME, and proadifen, hemodynamic parameters (MAP and RBF; Table 1) and the baseline diameters of juxtamedullary afferent arterioles (Table 2) were nearly the same as those in the presence of E4177 and L-NAME. In contrast, the triple combination pretreatment completely abrogated the vasodilator action of cilazaprilat on juxtamedullary afferent arterioles (from 17.8±0.8 to 17.9±0.6 µm, n=5, P<0.05; Figure 2). Of note, proadifen had no effect on basal (ie, with no vasodilators or vasoconstrictors) vascular tone of any arteriolar segments (data not shown).

Cilazaprilat-Induced Changes in Renal Bradykinin and NOx Contents

Figure 3 illustrates the effect of cilazaprilat on renal cortical and medullary contents of bradykinin and NOx during the...
treatment with E4177 or E4177 plus L-NAME. In the absence of E4177 and L-NAME, cilazaprilat elevated renal bradykinin and NOx contents, with greater increments observed in the medulla (n=5) than in the cortex (n=5, filled circles). The pretreatment with E4177 had no effect on the baseline level or the cilazaprilat-induced changes in these substances (n=5, open triangles). Similarly, the combined treatment with E4177 plus L-NAME did not alter the baseline levels or the cilazaprilat-induced changes in renal bradykinin contents (n=5, filled triangles). In contrast, this pretreatment markedly reduced baseline NOx levels and completely prevented the cilazaprilat-induced increase in renal NOx contents.

In summary, cilazaprilat-induced blockade of angiotensin II activity appears to be a dominant mechanism in superficial afferent arterioles, whereas bradykinin-induced NO dilates superficial efferent arterioles and juxtamedullary afferent and efferent arterioles. Finally, in juxtamedullary afferent arterioles, bradykinin can produce an additional vasodilator factor, most likely an EDHF.

**Discussion**

It has been well established that bradykinin and NO contribute substantially to the regulation of the renal microcirculation, and the renal action of the ACE inhibitor is attributed at least in part to these intrarenal vasoactive substances. Kon et al demonstrated a predominant efferent arteriolar dilation by enalapril in superficial nephrons, and this action was offset by a bradykinin antagonist. Furthermore, a bradykinin antagonist was shown to have only a

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**TABLE 2. Baseline Arteriolar Diameters in the Absence or Presence of Various Inhibitors**

<table>
<thead>
<tr>
<th></th>
<th>Superficial</th>
<th>Juxtamedullary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Afferent</td>
<td>Efferent</td>
</tr>
<tr>
<td>Control (n)</td>
<td>16.5±0.4 (6)</td>
<td>15.1±0.49 (6)</td>
</tr>
<tr>
<td>E4177</td>
<td>18.4±0.4* (6)</td>
<td>18.2±0.2* (6)</td>
</tr>
<tr>
<td>E4177+NAAB (n)</td>
<td>18.0±0.4* (5)</td>
<td>17.3±0.6* (5)</td>
</tr>
<tr>
<td>E4177+L-NAME (n)</td>
<td>14.1±0.6*† (5)</td>
<td>12.2±0.7*† (5)</td>
</tr>
<tr>
<td>E4177+L-NAME +proadifen (n)</td>
<td>14.2±0.5† (5)</td>
<td>12.3±0.5† (5)</td>
</tr>
</tbody>
</table>

Results are the mean±SEM. *P<0.05 vs control; †P<0.05 vs E4177.
modest effect on cortical blood flow but caused a marked reduction in papillary blood flow that originates from juxtamedullary nephrons. Similarly, intrarenal NO serves to counter the angiotensin II-induced afferent arteriolar constriction of superficial nephrons, but this effect is absent in the efferent arteriole. In addition to the stimulation of NO synthesis, bradykinin is also demonstrated to produce EDHF, which subsequently inhibits voltage-dependent calcium channels and arteriolar constriction. However, no investigations have been conducted examining the zonal (superficial versus juxtamedullary) and segmental (afferent versus efferent) heterogeneity in the action of the ACE inhibitor and its vasodilator mechanisms in the same experimental setting.

The present study demonstrates that multiple mechanisms contribute to the ACE inhibitor-induced vasodilatation of the renal microcirculation in an in vivo and in situ setting, with the use of an intravital needle-type CCD camera videomicroscopy technique. This experimental system is unique in that direct observation of relatively intact renal microvascular effect is readily available by the insertion of a needle-type CCD camera probe without impairment in renal microvascular integrity or disruption of tubuloglomerular feedback mechanism. Using this system, we have observed that the ACE inhibitor not only blocks angiotensin II activity by reducing its synthesis but also enhances the renal bradykinin activity, with the increment greater in the medulla (Figure 3). This action is particularly important in the superficial efferent arterioles, juxtamedullary efferent arterioles and juxtamedullary effenter arterioles, with its contribution greater in juxtamedullary nephrons (Figure 1). In this regard, it was shown that although bradykinin caused a relatively selective dilator action on the efferent arteriole, higher concentrations of bradykinin could dilate afferent and effenter arterioles. Furthermore, because we have shown that the NO inhibition by L-NAME blunts or abolishes the cilazaprilat-induced renal vasodilatation and the increase in renal NOx level during the blockade of angiotensin II activity (Figure 2 and 3), it is most likely that NO conveys a vasodilatory signal for bradykinin and plays an important role in mediating the ACE inhibitor-induced renal vasodilatation. Thus, the present observations extend previous findings with regard to vasodilator mechanisms for the ACE inhibitor and further provide detailed information on the intrarenal action of the ACE inhibitor. Because E4177 completely abolished the cilazaprilat-induced changes in MAP, the alterations in afferent arteriolar diameter and RBF are mediated in large part by the direct vascular action of cilazaprilat, per se, but are not related to the autoregulatory (e.g., myogenic) response of renal arterioles.

Although the present study clearly demonstrates an important role of NO in mediating the ACE inhibitor-induced vasodilatation, there still remains a vasodilator component during the blockade of NO and angiotensin II. Thus, in the presence of E4177 and L-NAME, cilazaprilat increased renal bradykinin contents but did not alter the renal NOx level (Figure 3). In this setting, cilazaprilat failed to dilate superficial arterioles or juxtamedullary efferent arterioles (Figure 2). Nevertheless, this agent caused a significant dilation of juxtamedullary afferent arterioles, although the degree of the cilazaprilat-induced dilation was diminished compared with that in the presence of E4177 alone (8% ± 3% versus 17 ± 2%). In concert with the fact that the bradykinin blockade completely inhibited the cilazaprilat-induced dilation in the presence of E4177 (Figure 1), it follows that the remaining vasodilator activity is coupled with additional mechanisms associated with bradykinin but is independent of NO.

In this regard, several in vitro studies suggest the involvement of EDHF in the renal arteriolar action of bradykinin. In the present in vivo study, we found that proadifen completely eliminated the remaining vasodilator response of juxtamedullary afferent arterioles observed during the inhibition of angiotensin II and NO. These findings therefore are compatible with previous in vitro observations that a part of the ACE inhibitor-induced vasodilatation is associated with EDHF and further allow extrapolation to the in vivo actions of this agent. Caveat is in order, because proadifen is reported to inhibit the K channel activity and the cytochrome P450-mediated EDHF production. It is of greater importance, however, that the present observation clearly demonstrates in an in vivo and in situ setting that the ACE inhibitor is capable of exerting additional vasodilator actions distinct from NO or the inhibition of angiotensin II. Finally, although the current study does not evaluate the role of prostaglandins, the complete inhibition of cilazaprilat-induced vasodilatation by the combined treatment with E4177, L-NAME, and proadifen (Figure 2) suggests that the contribution of prostaglandins to the ACE inhibitor-induced renal arteriolar vasodilatation is modest in our experimental setting.

A growing body of evidence has accrued that renal arterioles produce EDHF or EDHF-like substances. Furthermore, bradykinin is demonstrated to elicit afferent arteriolar vasodilatation that is mediated in part by EDHF in the isolated perfused rat hydronephrotic kidney and in the rat juxtamedullary nephron preparation, which therefore is in agreement with our current finding that the vasodilator action of cilazaprilat during the blockade of angiotensin II and NO is restricted to afferent arterioles. Of note, Wang et al reported that the efferent arteriole does not exhibit an EDHF-like response to acetylcholine, because the nature of EDHF requires hyperpolarization for its activity on the vascular smooth muscle, which does not appear to affect the efferent arteriolar tone. In contrast, Ren et al demonstrated a contribution of cytochrome P450 metabolites as a vasodilator for efferent arterioles. These discrepant observations could be caused by different experimental models used.

In addition to the segmental heterogeneity in the arteriolar response to bradykinin, the present study shows zonal (superficial versus juxtamedullary) differences in the vasodilator mechanism of the ACE inhibitor. Thus, when the effect of angiotensin II is eliminated, the remaining vasodilator action is greater in the juxtamedullary than in the superficial nephrons (Figure 1), and is most likely ascribed to the action of bradykinin and NO. These findings are consistent with the formulation that the medulla contains a greater amount of bradykinin and NO than the cortex in both basal and ACE inhibitor-stimulated conditions, and are associated with additive natriuresis without changes in GFR that we observed.
in our previous study. Furthermore, to the extent that afferent arterioles are reported to dilate in response to bradykinin, and to the extent that a part of this vasodilation is mediated by the EDHF-like activity, it is conjectured that the ACE inhibitor-induced elevation in the bradykinin content does reach the level required to elicit the EDHF-mediated vasodilation in the juxtamedullary, but not superficial, cortex in this present study. Of course, this speculation warrants further investigations.

Practical implication of EDHF in normal and diseased kidneys merits comment. ACE inhibitors are characterized by the augmenting action of bradykinin and NO, which contributes to the vasodilator action of the ACE inhibitor in addition to the angiotensin blockade. In renal injury, however, renal NO production is reported to be suppressed by a variety of mechanisms, including increased levels of free radicals and asymmetrical dimethylarginine (an endogenous inhibitor of NO synthase). In this setting, several studies demonstrate that the role of EDHF is maintained or rather increased. It is intriguing, therefore, to surmise that apart from angiotensin II inhibition as a main action, the mechanism of the renal action of ACE inhibitors is shifted from NO to EDHF, and the role of EDHF may be exaggerated in chronic renal disease in which the ACE inhibitor constitutes a primary tool for its treatment.

Perspectives

We have demonstrated in an in vivo, situ, and relatively intact setting that cilazaprilat elicits vasodilation of renal microvessels through multiple distinct mechanisms, including bradykinin, NO, and EDHF-like substance, in addition to the blockade of angiotensin II activity. Furthermore, the role of these vasoactive substances differs in an in vivo setting, with greater contribution observed in the juxtamedullary nephron. The responsiveness to the ACE inhibitor would characterize the heterogeneity in the renal microvasculature in vivo.

References


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