Roles of ANG II and bradykinin in the renal regional blood flow responses to ACE inhibition in sodium-depleted dogs

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Methods

Experiments were conducted on 7 mongrel dogs, weighing 18–20 kg. The dogs were fed a low-salt diet (0.01%) for 5 days before the experiment, as previously described (12). On the day of the experiments, the dogs were anesthetized with pentobarbital sodium with an initial dose of 30 mg/kg body wt, intravenous, and supplementation was given as needed during the course of the experiment. Positive pressure ventilation was provided via a cuffed endotracheal tube with an

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artificial respirator at a rate of 18 strokes per min, and a stroke volume of 15 ml/kg body wt. Body temperature was maintained within the range of 99° to 101° F using an electric heating pad placed under the dog. Mean arterial pressure was measured from a catheter placed in the abdominal aorta inserted via the right femoral artery and recorded on a polygraph (model 7D; Grass Instruments, Quincy, MA). The left femoral artery was cannulated for the collection of blood samples. The femoral and jugular veins were cannulated for the administration of drugs, infusion of saline and inulin solutions, and additional doses of anesthesia as needed.

The left kidney was exposed through a flank incision, and the renal artery separated from the surrounding tissue. Renal denervation was performed by cutting all visible nerves projecting to the kidney from the aortico-renal ganglion. The rationale for renal denervation was to minimize the effects of alterations in renal sympathetic activity due to possible changes in arterial pressure following administration of candesartan and/or enalaprilat. The ureter was cannulated for the collection of urine samples. RBF was measured with an electromagnetic flow probe placed around the renal artery and connected to a square wave flowmeter (Carolina Medical Electronics, Kings, NC). The zero flow was obtained at the beginning and the end of the experiment by momentarily occluding the renal artery. A curved 23-gauge needle was inserted in the renal artery and connected with a pressure transducer to measure renal arterial pressure (RAP). In the present study, RAP was not reduced with an occluding clamp; therefore, RAP and mean arterial pressure are essentially the same, and thus only RAP is reported. A catheter connected to this needle cannula was used for the direct renal infusion of drug solutions and heparinized saline at the rate of 0.4 ml/min.

A dual channel laser-Doppler flowmeter (LDF; Periflux 4001, Perimed, Stockholm, Sweden) with two needle probes (500 μm diameter) was used to measure relative changes in blood flows in the renal cortex and medulla (4, 5). The cortical probe was inserted to a depth of 5 mm into the kidney to position the tip in the mid-cortical region; the medullary probe was inserted to a depth of ~15 mm to position the tip at the junction of the inner and outer medulla. At the end of each experiment, the positions of the tips of the needle probes were confirmed by dissecting the kidney and locating the needle tracts. The flow probes were calibrated with a motility standard of a colloidal suspension of latex particles (10-μm microspheres). Brownian motion of the latex particles provides a standard value of 250 perfusion units (PU), with one PU corresponding to an analog output of 10 mV. The data are reported as percent of the basal levels recorded during the control periods, although the absolute PU values were also monitored and recorded. The zero flow recordings of the LDF probes were determined by occluding the renal artery momentarily at the beginning and the end of each experiment. To avoid respiratory movement artifacts in the recording of LDF signals, the kidney was maintained in a fixed position by placing it on a plastic holder similar to that used for micropuncture studies. Care was taken not to cause any changes in the basal RBF due to such fixation. After the completion of surgery, a 2.5% solution of inulin in normal saline was administered via the jugular vein for at least 45 min before the beginning of the experimental protocol. An initial dose of 1.6 ml/kg body wt was followed by a continuous infusion of 0.03 ml · min⁻¹ · kg body wt⁻¹.

The experimental period was started with urine collections for two consecutive 10-min control periods, with an arterial blood sample (2 ml) taken at the mid-point of each collection period. This was followed by a 100-μg intra-arterial bolus of candesartan. This dose was effective in blocking blood flow responses to 100 ng of ANG II administered intra-arterially into the kidney of 8 dogs. The RBF changes in response to ANG II prior to candesartan administration averaged $-63 \pm 6\%$, compared to $-0.03 \pm 0.02\%$ observed after candesartan administration, indicating complete blockade of AT₁ receptors. After a 10-min stabilization period following candesartan administration, two 10-min collections of urine were made. An intra-arterial infusion of enalaprilat was then started at a dose of 33 μg · kg⁻¹ · min⁻¹ and continued for the duration of the experiment. After 10 min of stabilization, another two 10-min urine samples were collected. The BK B₂ receptor blocker icatibant was then given as a 300-μg bolus systemic dose followed by a 10-min stabilization and two 10-min clearance periods.

At the end of each experiment, the electromagnetic flow probe was calibrated in situ by timed collections of blood into a graduated cylinder from a catheter placed in the renal artery. The kidney was then removed, stripped of all surrounding tissue, blotted dry and weighed so that the calculated parameters could be expressed per gram of kidney weight. Flame photometry (Instrumentation Laboratory, Watertown, MA) was used to determine the sodium concentrations in plasma and urine. Inulin concentrations in the samples were determined by the anthrone colorimetric technique (Gilford Instruments, Oberlin, Ohio).

Values are reported as means ± SE. Statistical comparisons of differences in the responses were conducted with the use of ANOVA, followed by the Newman-Keuls test. Differences in the mean values were deemed significant at $P \leq 0.05$.

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**Fig. 1.** Responses of total renal blood flow (RBF) and renal vascular resistance (RVR) to candesartan (CAND), enalaprilat (ENAL) and icatibant (ICAT). Values are means ± SE; $n = 8$ each. *$P \leq 0.05$ vs. control (Cont). **# $P \leq 0.05$ vs. candesartan.
RESULTS

Figures 1 and 2 summarize the RBF, renal vascular resistance (RVR), CBF and MBF responses to candesartan, enalaprilat, and icatibant. As shown in Fig. 1A, AT1 receptor blockade with candesartan resulted in a significant increase in RBF by 21% (3.8 ± 0.4 to 4.6 ± 0.4 ml • min⁻¹ • g⁻¹, P < 0.01). ACE inhibition after AT1 receptor blockade further increased RBF to 5.7 ± 0.5 ml • min⁻¹ • g⁻¹ (24%, P < 0.001). B2 receptor blockade with icatibant returned RBF back to 5.0 ± 0.5 ml • min⁻¹ • g⁻¹. Figure 1B shows the effects of AT1 receptor blockade, ACE inhibition, and B2 receptor blockade on RVR. Candesartan significantly decreased RVR by 22% (34.6 ± 3.8 to 26.9 ± 3.3 mmHg • ml⁻¹ • min⁻¹ • g⁻¹, P < 0.001), and enalaprilat resulted in a further decrease of 34% to 17.8 ± 2.4 mmHg • ml⁻¹ • min⁻¹ • g⁻¹. RVR partially returned with icatibant significantly below the RVR during enalaprilat to a value that was not significantly different from that observed with candesartan alone (21.9 ± 3.6 mmHg • ml⁻¹ • min⁻¹ • g⁻¹, P < 0.001) although this value was still significantly different from the control value.

Figure 2 shows the responses in CBF and MBF. As shown in Fig. 2A, AT1 receptor blockade increased CBF (+20 ± 2%, P < 0.05), and administration of enalaprilat resulted in a further increase of 21% (to +41 ± 8%). Blockade of B2 receptors returned CBF to +24 ± 8%, an average not significantly different from those observed with candesartan administration alone. Figure 2B summarizes MBF responses to candesartan, enalaprilat, and icatibant. Candesartan increased MBF by 22 ± 7% (P < 0.05). Enalaprilat infusion following candesartan further increased MBF by 40% (to +61 ± 10%, P < 0.01). Blockade of B2 receptors returned MBF to levels not different from those seen prior to ACE inhibition (+14 ± 8%). The relative responses of MBF to enalaprilat after candesartan administration were noted to be significantly greater than those of CBF (+21% vs. +41%, respectively, P < 0.01).

Table 1 summarizes the responses of RAP, glomerular filtration rate (GFR), urine flow (UV), and sodium excretion (UNaV). AT1 receptor blockade decreased RAP with a further decrease due to subsequent administration of enalaprilat. B2 receptor blockade using icatibant returned the responses to levels not different from those observed with candesartan administration alone. GFR, UV, UNaV, and fractional excretion of sodium (FENa%) were not significantly altered by any of the treatments (Table 1).

**DISCUSSION**

In this study, we have evaluated the total and regional blood flow responses to ACE inhibition in the presence of AT1 receptor blockade and how these are altered by blockade of B2 receptors. The objective was to examine the relative contributions of reduced ANG II influences and enhanced BK effects in the observed vasodilatory responses to ACE inhibition. Intrarenal blockade of ANG II AT1 receptors with candesartan significantly increased RBF, CBF and MBF, with the magnitude of the changes in each parameter not significantly different from each other, indicating that at the administered dose that was sufficient to effectively block ANG II-mediated vasoconstriction, CBF and MBF were equally sensitive to ANG II receptor blockade. This observation also confirmed a significant tonic role for ANG II in regulating total and regional RBF during sodium restriction. Subsequent administration of enalaprilat in the presence of candesartan resulted in further vasodilation, increasing RBF, CBF, and MBF. This further vasodilation in the presence of ANG II receptor blockade indicates that these latter re-

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**Table 1. Renal function responses to ACE inhibition in the presence of AT1 and B2 receptor blockade**

<table>
<thead>
<tr>
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<th>RAP, mmHg</th>
<th>GFR, ml • min⁻¹ • g⁻¹</th>
<th>V, µl • min⁻¹ • g⁻¹</th>
<th>UNaV, µmol • min⁻¹ • g⁻¹</th>
<th>FENa%, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>139 ± 5</td>
<td>0.72 ± 0.07</td>
<td>6.6 ± 2.8</td>
<td>0.14 ± 0.04</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Candesartan</td>
<td>131 ± 6</td>
<td>0.87 ± 0.12</td>
<td>10.7 ± 3.4</td>
<td>0.28 ± 0.12</td>
<td>0.25 ± 0.12</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>110 ± 7†</td>
<td>0.65 ± 0.11</td>
<td>6.6 ± 2.2</td>
<td>0.39 ± 0.24</td>
<td>0.35 ± 0.19</td>
</tr>
<tr>
<td>Icatibant</td>
<td>118 ± 8†</td>
<td>0.82 ± 0.09</td>
<td>11.7 ± 4.7</td>
<td>0.39 ± 0.24</td>
<td>0.32 ± 0.16</td>
</tr>
</tbody>
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Values are means ± SE. RAP, renal arterial pressure; GFR, glomerular filtration rate; V, urine flow; UNaV, urinary excretion of sodium; FENa%, fractional excretion of sodium; ACE, angiotensin-converting enzyme. *P ≤ 0.05, compared with control, †P ≤ 0.05, compared with candesartan.
sponses were due to effects other than ACE inhibitor-induced reductions in ANG II concentrations. Although it is possible that some of these effects could be partially due to inhibition of residual effects of ANG II that were not blocked by candesartan, this seems unlikely because the responses to enalaprilat were partially or completely reversed by the B2 receptor blocker icatibant, returning blood flows to levels not significantly different from those observed with candesartan alone. These results indicate that the vasodilation induced by enalaprilat in the presence of candesartan was due primarily to activation of BK B2 receptors and provide further support to the concept that BK has a significantly greater role in mediating the RBF, CBF, and MBF responses to ACE inhibition during sodium restriction. These results extend our previous findings that although icatibant had no effect on ACE inhibition-induced renal vasodilation in dogs fed a normal salt diet, the B2 receptor blocker significantly attenuated the vasodilation caused by enalaprilat in dogs maintained on a low-salt diet (3, 6, 12, 18). The increased contribution of BK B2 receptor activation to ACE inhibition-induced vasodilation in salt-restricted dogs compared with dogs on a normal salt intake support an increased role of kinins in regulating intrarenal blood flow during sodium restriction, a condition shown to increase intrarenal BK levels (11, 15). There is evidence that elevated ANG II levels tonically stimulate BK production and subsequent nitric oxide generation via activation of AT2 receptors (13–15). The increased BK-dependent influence after ACE inhibition may reflect a greater modulating action of this interaction during sodium restriction.

Although the magnitudes of CBF and MBF responses to candesartan were similar, a comparatively greater increase in MFB than in CBF was observed when enalaprilat was administered after candesartan (+41 vs. +21%, respectively). This indicates that during sodium restriction, the medullary circulation may have an enhancement of BK forming capability, an increased BK trapping ability, or an enhanced sensitivity to BK, compared with the cortical circulation. These possibilities, however, remain to be explored further.

Neither AT1 receptor blockade nor ACE inhibition significantly affected GFR, UV, and UNaV. The AT1 receptor blocker was administered as a single intrarenal dose, which was able to block the vasoconstrictor effects of 100 ng of ANG II. It may be that although the vascular receptors were completely blocked, insufficient candesartan was available to block tubular receptors, resulting in an incomplete antagonism of ANG II-mediated tubular transport.

In conclusion, the present results provide further support to the concept that during sodium restriction there is an increased kinin-dependent influence via B2 receptors, which contributes to the vasodilatory effects of ACE inhibition. Furthermore, the renal medullary circulation is more sensitive to kinin potentiation than the cortical circulation.

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