Endogenous Bradykinin and the Renin and Pressor Responses to Furosemide in Humans

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ABSTRACT

In humans, bradykinin contributes to the acute renin response after ACE inhibition. To further explore the role of endogenous bradykinin in human renin regulation, we determined the effect of HOE 140, a specific bradykinin B2 receptor antagonist, on the renin response to 0.5 mg/kg i.v. furosemide in a randomized, single blind, crossover design study of 10 healthy, salt-replete volunteers. HOE 140 did not affect basal plasma renin activity, aldosterone, mean arterial pressure, or heart rate. Furosemide administration increased plasma renin activity from 1.0 ± 0.2 to 4.5 ± 1.2 ng of angiotensin l/ml/h and there was no effect of HOE 140 (from 1.1 ± 0.2 to 3.9 ± 0.8 ng of angiotensin l/ml/h). Similarly, there was no effect of HOE 140 on the diuretic response to furosemide. Mean arterial pressure increased in response to furosemide after HOE 140 (82 ± 2 to 94 ± 2 mm Hg), but not after vehicle (81 ± 3 to 85 ± 2 mm Hg), whereas heart rate was unchanged. In conclusion, activation of the B2 receptor by endogenous bradykinin does not contribute to the renin response to acute furosemide treatment in humans. However, bradykinin may contribute to blood pressure regulation under conditions in which the renin-angiotensin system is stimulated.

The renin-angiotensin system (RAS), which consists of systemic and tissue components, regulates blood pressure and sodium- and volume homeostasis. Activation of the systemic, or circulating, RAS is initiated by release of the protease renin from renal juxtaglomerular cells, specialized vascular smooth muscle cells of the afferent arteriole. Renin cleaves angiotensinogen to angiotensin (Ang) I, which is rapidly metabolized to the effector peptide Ang II by endothelial-bound angiotensin-converting enzyme (ACE) (Sealey and Laragh, 1990).

Physiologic regulation of systemic renin release involves several integrated mechanisms. The macula densa portion of the thick ascending limb of the loop of Henle is intimately apposed to juxtaglomerular cells, forming the juxtaglomerular apparatus. Decreased transport of sodium ions and chloride ions at the macula densa results in increased renin release (Hackenthal et al., 1990). Several lines of evidence indicate that prostaglandins, particularly prostacyclin, mediate the effect of decreased renal perfusion and sodium chloride delivery at the macula densa on renin release (Frolich et al., 1976; Jackson et al., 1982). Activation of the renal baroreceptor, a vascular receptor in the afferent arteriole, stimulates renin release in response to decreased renal perfusion and the sympathetic nervous system stimulates renin release via β1-adrnergic receptors located on juxtaglomerular cells (Hackenthal et al., 1990). A short feedback loop of inhibition of renin release exists due to a direct action of Ang II on the juxtaglomerular apparatus (Hackenthal et al., 1990). Intracellular mediators of renin release include increases of cAMP and decreases in Ca2+ concentrations (Churchill, 1990). Nitric oxide indirectly stimulates renin release by inhibiting phosphodiesterase-3 and, thereby preventing the breakdown of cAMP (Kurtz et al., 1998).

We have recently determined that coadministration of the bradykinin B2 receptor antagonist HOE 140 blocks the renin response to acute administration of the ACE inhibitor captopril in humans (Gainer et al., 1998). More recently, a new class of medication, called vasopeptidase inhibitors (combined ACE and neutral endopeptidase inhibitor), has entered clinical trials. Because bradykinin is degraded by both of these peptidases, the vasopeptidase inhibitors will presumably have a greater effect on bradykinin and, in humans, have been shown to cause a marked increase in plasma renin activity (PRA) compared with ACE inhibitor alone (Liao et al., 1998). Bradykinin stimulates the production of two mediators of renin release: prostacyclin (Barrow et al., 1986) and nitric oxide (Cherry et al., 1982). In the study of Gainer et al. (1998), urinary concentrations of the prostacyclin med-

ABBREVIATIONS: RAS, renin-angiotensin system; Ang, angiotensin; ACE, angiotensin-converting enzyme; PRA, plasma renin activity; PGF, prostaglandin F; Hct, hematocrit; MAP, mean arterial pressure.
tabolite 2,3-dinor-6-keto-PGF$_{1\alpha}$ were not increased after acute captopril administration, raising the possibility of a prostacyclin-independent effect of bradykinin on renin. Beierwaltes (1987) has reported a prostaglandin-independent effect of bradykinin on renin release in isolated rat glomeruli. Similarly, Wirth et al. (1997) have reported that bradykinin antagonism decreases renin in cirrhotic rats. Taken together, these data suggest the hypothesis that bradykinin regulates renin release in humans.

To further elucidate the role of bradykinin in the regulation of systemic renin release in humans we measured the effect of acute i.v. administration of furosemide on PRA in the presence and absence of the bradykinin B$_2$ receptor antagonist HOE 140. We chose to study furosemide as it causes a well characterized rapid increase in PRA in humans (Rosenthal et al., 1968; Patak et al., 1975). Additionally, furosemide-stimulated renin release is mediated in part through prostacyclin (Jackson et al., 1982), suggesting a possible role for bradykinin in this response. Our data indicate that bradykinin activation of the B$_2$ receptor does not play a role in the renin response to furosemide.

### Materials and Methods

**Subjects.** Ten (nine male, one female) healthy subjects age 20 to 48 years, body mass index 25.3 ± 1.1 kg/m$^2$, were studied. Each subject underwent a medical history, physical examination, and laboratory screening. Subjects were excluded for any medical condition or for pregnancy. Subjects took no prescription or over-the-counter medications for 2 weeks before the study. Written informed consent was obtained from each subject. The protocol was approved by the Vanderbilt University Medical Center Institutional Review Board and was in accordance with the Declaration of Helsinki. All subjects tolerated the protocol without serious side effects.

**Protocol.** Each subject participated in a randomized, single blind, crossover study and was supplied a controlled diet (150 mmol/day sodium, 75 mmol/day potassium, 1000 mg/day calcium, methykanthine free) for a total of 10 days. On day 5 of the diet, subjects reported to the Vanderbilt General Clinical Research Center having fasted since the night before and having collected a 24-h urine specimen for determination of urinary volume, sodium, potassium, and creatinine. An i.v. catheter was placed in each antecubital vein for blood sampling and for drug infusion, at which time blood for baseline potassium concentration was obtained. After 1 h supine, blood was sampled for PRA, aldosterone, and hematocrit (Hct). HOE 140 (100 μg/kg, a gift of Hoechst, Frankfurt, Germany) or vehicle (5% dextrose in water) was then infused in a total volume of 50 ml over 1 h. We (Brown et al., 2000) and others (Cockcroft et al., 1994) have reported that bradykinin antagonism decreases renin in cirrhotic rats. Taken together, these data suggest the hypothesis that bradykinin regulates renin release in humans.

**Diuretic Response.** Twenty-four-hour urine excretion of creatinine, sodium, and potassium did not differ before either study arm (Table 1). Furosemide caused a marked diuresis in the 1st h. For collections during both the 1st and 2nd h after furosemide, urinary volume, creatinine, sodium, potassium, and 2,3-dinor-6-keto-PGF$_{1\alpha}$ excretion did not differ between the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test). Consistent with the HOE 140 and vehicle study days (Table 1). Basal Hct did not differ between study days ($P = .69$, $t$ test).
with volume contraction, the Hct increased after furosemide (F_{2,7} = 38.9, P < .001, Table 2); however, there was no effect of HOE 140 on the increase in Hct (F_{1,8} = .88, P = .38). Basal serum potassium did not differ between study days (P = .23, t test, Table 2). Serum potassium was decreased after furosemide administration (F_{2,7} = 17.6, P = .002) but this change was not affected by treatment with HOE 140 (F_{1,8} = .25, P = .63).

**Hemodynamic Response.** Basal MAP and heart rate were similar on each study day (P = .17 and P = .24, respectively, by t test, Fig. 1) and there was no effect of HOE 140 on basal MAP or heart rate (F_{1,8} = .63, P = .45 and F_{1,8} = .97, P = .36, respectively). There was a significant interactive effect of furosemide-HOE 140 on MAP (F_{2,4} = 20.8, P = .008, Fig. 1A). Thus, MAP increased in response to furosemide after bradykinin antagonism (F_{2,5} = 6.77, P = .038), but not after vehicle administration (F_{2,7} = 3.37, P = .094). Contrary to the MAP response, heart rate was not affected by either furosemide administration (F_{2,4} = 3.34, P = .14) or by HOE 140 treatment (F_{1,5} = .08, P = .79, Fig. 1B) and there was no interaction between bradykinin antagonism and furosemide administration (F_{2,4} = .41, P = .69).

**Endocrine Response.** Basal PRA did not differ between study days (P = .62, t test) and there was no effect of HOE 140 on basal PRA (F_{1,9} = .03, P = .87). Furosemide significantly increased PRA after either vehicle or HOE 140 administration (F_{2,8} = 5.68, P = .03, Fig. 2); however, treatment with HOE 140 had no effect on the response to furosemide (F_{1,9} = .20, P = .66, Fig. 2). Likewise, basal plasma aldosterone concentrations did not differ between study days (P = .65, t test) and HOE 140 did not affect basal aldosterone concentration (F_{1,8} = 1.07, P = .33, Fig. 3). Aldosterone concentrations increased significantly after furosemide (F_{2,7} = 13.5, P = .004, Fig. 3) and HOE 140 did not affect the aldosterone response to furosemide (F_{1,8} = 1.68, P = .23). The relationship between change in PRA and change in aldosterone was not affected by HOE 140 treatment (data not shown). Plasma norepinephrine concentrations increased significantly in response to furosemide (F_{2,6} = 13.2, P = .006, data not shown), but were unaffected by HOE 140 treatment (F_{1,7} = .36, P = .57).

**Discussion**

Bradykinin is a potent vasoactive peptide that plays an important role in renal and cardiovascular physiology and contributes to the inflammatory response (Bhoola et al., 1992). Endogenous kinins exert their effects through kinin B₁ and B₂ G-protein-coupled receptors. The majority of cardiovascular effects of bradykinin are mediated through the constitutive B₂ receptor (Bhoola et al., 1992). In contrast, the inducible B₁ receptor has low affinity for bradykinin and quantitatively mediates a small part of physiologic responses to kinins (Marceau et al., 1998). Thus, we investigated the role of endogenous bradykinin in the regulation of the renin response to furosemide in humans using the long-acting, specific bradykinin B₂ receptor antagonist HOE 140. Our laboratory has previously reported that bradykinin B₂ antagonism attenuates the renin response to acute ACE inhibition by captopril in humans (Gainer et al., 1998). Bradykinin, acting at the B₂ receptor, is a potent stimulus for prostacyclin formation (Barrow et al., 1986) and prior studies have established that inhibition of prostacyclin and other prostaglandins by cyclooxygenase inhibitors attenuates the renin response to both ACE inhibitors (Abe et al., 1980) and to furosemide (Patak et al., 1975; Rumpf et al., 1975), suggest-

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Pretreatment</th>
<th>Post-Treatment, Furosemide</th>
<th>30 min Postfurosemide</th>
<th>60 min Postfurosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum potassium (mEq/l)</td>
<td>Vehicle</td>
<td>4.39 ± 0.08</td>
<td>N.D.</td>
<td>4.09 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HOE 140</td>
<td>4.26 ± 0.09</td>
<td>N.D.</td>
<td>3.95 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hct</td>
<td>Vehicle</td>
<td>0.401 ± 0.012</td>
<td>0.394 ± 0.012</td>
<td>0.415 ± 0.013&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HOE 140</td>
<td>0.415 ± 0.010</td>
<td>0.397 ± 0.012</td>
<td>0.420 ± 0.011&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N.D., not done.

<sup>a</sup> P < .01, <sup>b</sup> P < .001, <sup>c</sup> P < .05, vs. prefurosemide.
versus furosemide. In the same study, there was no effect of 100 µg/kg HOE 140 on basal PRA (Madeddu et al., 1993). The contribution of endogenous bradykinin to the diuretic effects of furosemide has been reported for deoxycorticosterone-treated Wistar rats. In this model, acute administration of HOE 140 attenuated the diuretic and natriuretic effects of furosemide (Madeddu et al., 1992). In contrast, we found no effect of acute bradykinin antagonism on urinary volume or sodium excretion after furosemide in normal humans. Similarly, urinary excretion of the stable prostacyclin metabolite 2,3-dinor-6-κeto-PGF<sub>1α</sub> after furosemide was not affected by HOE 140. This suggests that bradykinin-induced prostacyclin synthesis does not contribute to the renin or diuretic response to furosemide (Patak et al., 1975; Rumpf et al., 1975).

Studies in bovine adrenocortical cells have provided conflicting data as to the effect of endogenous bradykinin on aldosterone synthesis. Thus, Rosolowsky and Campbell (1994) reported that bradykinin stimulates aldosterone release from adrenocortical cells, whereas Chretien et al. (1998) found no stimulatory effect of bradykinin on aldosterone in similar preparations, concluding that the B<sub>2</sub> receptor density on bovine adrenocortical cells is low. In vivo, studies in Sprague-Dawley rats have failed to demonstrate an effect of chronic HOE 140 infusion or acute intra-arterial bradykinin infusion on serum aldosterone concentrations (Rudichenko et al., 1993). In the present study, there was no change in the stimulation of aldosterone by furosemide and the relationship between PRA and aldosterone after furosemide was unchanged by HOE 140. Thus, this study does not support an effect of endogenous bradykinin on Ang II-stimulated aldosterone release in humans.

We and others have previously reported that i.v. HOE 140 administered at doses that block the effects of exogenous bradykinin has no effect on resting blood pressure in humans (Cockcroft et al., 1994; Gainer et al., 1998; Brown et al., 2000). Consistent with these observations, there was no effect of HOE 140 on basal blood pressure in the current study. In contrast, we observed a significant effect of HOE 140 on the blood pressure response to furosemide, suggesting a role of bradykinin in blood pressure regulation during activation of endogenous vasopressor systems. The administration of furosemide leads to the activation of the sympathetic and the renin-angiotensin systems, and increased circulating levels of the vasoconstrictors norepinephrine and Ang II (Francis et al., 1985). In the present study, treatment with HOE 140 did not affect the plasma norepinephrine response to furosemide administration, suggesting that the effect of HOE 140 on the blood pressure responses was mediated predominantly by the renin-angiotensin system. The finding that MAP increased in response to furosemide in the presence of bradykinin suggests that endogenous bradykinin normally modulates the effect of furosemide-induced activation of the renin-angiotensin and/or sympathetic nervous systems on blood pressure. Data from studies in animals are consistent with this hypothesis. In Wistar rats, chronic B<sub>2</sub> blockade does not change basal blood pressure, but augments the slow pressor response to chronic Ang II infusion (Madeddu et al., 1994). Similarly, the kininogen-deficient Brown Norway Katholiek rat exhibits an enhanced pressor response to Ang II (Majima et al., 1994). In B<sub>2</sub> receptor knockout mice, the hypertensive response in the two-kidney/one-clip, renin-dependent model is augmented, a change that is duplicated in wild-type mice treated with HOE 140 (Madeddu et al., 1998). Taken together, these studies in animals and the present study in...
humans suggest that bradykinin plays a role in counteracting endogenous Ang II during physiologic and pharmacologic perturbations of blood pressure.

Finally, the finding that the pressor response to furosemide observed in the presence of HOE 140 did not suppress renin release suggests that endogenous bradykinin may regulate the intrarenal baroreceptor mechanism. Further studies are needed to test this hypothesis.

In conclusion, we have studied the renin response to furosemide in humans in the presence and absence of bradykinin B₂ receptor antagonism. There was no effect on the increase in PRA in response to furosemide injection after treatment with the specific bradykinin B₂ antagonist HOE 140. However, HOE 140 altered the blood pressure response to furosemide, suggesting a role of endogenous bradykinin in the regulation of blood pressure in humans.

References


Barrow SE, Dollery C, Heavey D, Hickling N, Ritter J and Vial J (1986) Effect of bradykinin B₂ receptor antagonism with the specific bradykinin B2 antagonist HOE 140. How - ever, HOE 140 altered the blood pressure response to furosemide observed in the presence of HOE 140 did not suppress renin release suggests that endogenous bradykinin may regulate the intrarenal baroreceptor mechanism. Further studies are needed to test this hypothesis.

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