

Bradykinin B₂ Receptor Does Not Contribute to Blood Pressure Lowering during AT₁ Receptor Blockade

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ABSTRACT

This study tested the hypothesis that endogenous bradykinin contributes to the effects of angiotensin AT₁ receptor blockade in humans. The effect of the bradykinin B₂ receptor antagonist D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg (HOE-140) (18 μg/kg/h i.v. for 6 h) on hemodynamic and endocrine responses to acute and chronic (1-month) treatment with valsartan (160 mg/day) was determined in 13 normotensive and 12 hypertensive salt-deplete subjects. Acute valsartan increased plasma renin activity (PRA) from 5.3 ± 9.9 to 15.6 ± 19.8 ng of angiotensin (Ang) l/ml/h ($P < 0.001$) and decreased aldosterone from 18.3 ± 10.5 to 12.0 ± 9.6 ng/dl ($P < 0.001$). Chronic valsartan significantly increased baseline PRA (10.5 ± 15.5 ng of Ang l/ml/h; $P = 0.004$) but did not affect baseline angiotensin-converting enzyme activity or aldosterone. HOE-140 tended to increase the PRA response to valsartan, and it attenuated the

decrease in aldosterone following chronic valsartan ($P = 0.03$). Acute valsartan decreased mean arterial pressure 12.7 ± 6.9% (from 100.2 ± 8.4 to 87.5 ± 9.8 mm Hg in hypertensives and from 82.4 ± 8.6 to 70.3 ± 8.4 mm Hg in normotensives). HOE-140 did not affect the blood pressure response to either acute (effect of valsartan, $P < 0.001$; effect of HOE-140, $P = 0.98$) or chronic (valsartan, $P = 0.01$; HOE-140, $P = 0.84$) valsartan. Plasma cGMP was increased significantly during chronic valsartan ($P = 0.048$) through a bradykinin receptor-independent mechanism (effect of HOE-140, $P = 0.13$). Both acute ($P < 0.001$) and chronic ($P < 0.001$) valsartan increased heart rate. HOE-140 augmented the heart rate response to chronic valsartan ($P = 0.04$). These data suggest that endogenous bradykinin does not contribute significantly to the blood pressure-lowering effect of valsartan through its B₂ receptor.

The availability of the specific bradykinin B₂ receptor antagonist D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg (HOE-140) as well as the combined B₁ and B₂ antagonist D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Igl-Oic-Arg (B9340) has allowed investigators to determine the contribution of endogenous bradykinin to the effects of angiotensin-converting enzyme (ACE) inhibitors in animals and humans. For example, Gainer et al. (1998) demonstrated that coadministration of HOE-140 attenuated the hemodynamic and endocrine effects of acute ACE inhibition in normotensive subjects and hypertensive subjects of both African-American and Caucasian race. Likewise, HOE-140 attenuated the acute hypotensive response to perindoprilat in normotensive subjects (Squire et al., 2000).

In congestive heart failure patients treated chronically with ACE inhibitor, combined B₁ and B₂ inhibition, but not B₂ inhibition alone, caused vasoconstriction (Davie et al., 1999; Witherow et al., 2001).

Whether bradykinin contributes to the blood pressure-lowering effect of AT₁ receptor blockade remains to be determined. Unlike ACE inhibition, acute AT₁ receptor blockade does not potentiate the vasodilator effects of exogenous bradykinin in the human forearm vasculature (Cockcroft et al., 1993). However, this does not exclude the possibility that endogenous bradykinin contributes to the effects of either acute or chronic administration of an AT₁ receptor antagonist. Indeed, studies in vitro and in animals suggest a mechanism whereby bradykinin may contribute to the effects of AT₁ receptor blockade. During AT₁ receptor blockade, excess angiotensin II (Ang II) increases bradykinin and subsequently NO through an AT₂ receptor-dependent mechanism in the aorta (Gohlke et al., 1998; Tsutsumi et al., 1999) as well as the kidney (Siragy and Carey, 1997). Moreover, the

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ABBREVIATIONS: HOE-140, D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg; B9340, D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Igl-Oic-Arg; ACE, angiotensin-converting enzyme; Ang, angiotensin; PAH, *para*-aminohippurate; PRA, plasma renin activity; BK1–5, Arg-Pro-Pro-Gly-Phe; RVR, renal vascular resistance; MAP, mean arterial pressure; bpm, beats per minute.

absence of AT₂ receptors in genetically modified mice has been associated with an increase in ACE activity and decreased sensitivity to exogenous bradykinin infusion (Hunley et al., 2000).

The contribution of endogenous bradykinin to the effects of AT₁ receptor blockade in humans is not well defined. Two groups have reported no effect of HOE-140 or B9340 on vasodilation in congestive heart failure patients treated chronically with the AT₁ receptor antagonist losartan (Davie et al., 1999; Cruden et al., 2004). In contrast, Hornig et al. (2003) observed that HOE-140 attenuated the effect of candesartan on flow-mediated vasodilation in patients with coronary artery disease. Campbell et al. (2005) reported that losartan, but not eprosartan, increased circulating bradykinin concentrations in hypertensive subjects. In the current study, we tested the hypothesis that bradykinin contributes to the blood pressure-lowering effect of either acute or chronic (1-month) administration of the AT₁ receptor antagonist valsartan in salt-deplete normotensive and hypertensive subjects.

Materials and Methods

Overall Study Protocol. The single-blind, crossover design protocol was approved by the Vanderbilt Institutional Review Board, and all subjects gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki. Following screening history and physical examination, subjects were given placebo for 2 to 4 weeks. Any antihypertensive medications were discontinued or withdrawn during this period such that all hypertensive subjects were off β -blockers, ACE inhibitors, or AT₁ receptor blockers for at least 3 weeks, and they were off all other medications for at least 1 week before the first infusion study day. During the last week of placebo run-in, subjects were provided a low (10 mmol/day) sodium diet for 8 days. On the 5th and 7th days of diet, subjects collected their urine for 24 h for measurement of sodium excretion. On the 6th and 8th days of diet, subjects presented to the Vanderbilt General Clinical Research Center (Nashville, TN) for oral administration of valsartan and intravenous administration of either HOE-140 or vehicle. Following the second infusion day, subjects were provided valsartan at 160 mg/day for 4 weeks. During the last week of valsartan administration, the low sodium diet, 24-h urine collections, and HOE-140 and vehicle infusions were repeated.

To control for the effect of time and HOE-140 alone on the study variables, seven subjects (five male, two female; five white, two black American; four hypertensive, three normotensive) completed a second protocol, identical to the first except that subjects were given placebo rather than valsartan during HOE-140 and vehicle infusion days, and the chronic phase was omitted.

HOE-140/Vehicle Infusion Protocol. Subjects reported to the Vanderbilt General Clinical Research Center in the fasting state at 7:00 AM. An intravenous catheter was placed in the antecubital vein of each arm, one catheter for drawing blood and the other catheter for administering drugs. Thirty minutes later subjects were given a loading dose of *p*-aminohippurate (PAH; 8 mg/kg) followed by a constant infusion of 12 mg/min for measurement of renal blood flow. One hour after PAH was started, subjects were given valsartan 160 mg (time 0), and HOE-140 or vehicle was infused. HOE-140 was given as a loading dose of 22 μ g/kg, followed by a continuous infusion of 18 μ g/kg/h for 6 h, a dose that has been shown previously to attenuate the vasodilator effect of bradykinin (Cockcroft et al., 1994). In a pilot study, this dose of HOE-140 was confirmed to block the potentiating effect of enalaprilat on bradykinin-induced vasodilation in the forearm. The duration of HOE-140 infusion was chosen to continue until the peak hypotensive effect of valsartan had been achieved. Blood pressure and heart rate were monitored every 5 min

throughout the study using an automated blood pressure cuff, and rates were averaged over 1-h intervals.

Blood for measurement of plasma renin activity (PRA), ACE activity, Ang II, aldosterone, cGMP, NO metabolites, bradykinin and its metabolite BK1-5, and PAH was obtained at 0, 2, 4, 6, and 8 h following administration of valsartan. All samples from at least the first five subjects were assayed to establish the time course for each variable. Thereafter, only the samples from time 0 (designated baseline in the tables) and from the time associated with the maximum effect following valsartan (post) were assayed. For Ang II, the peak effect occurred at 2 h following valsartan. For all other variables, this was at 6 h following losartan. Urine collected 2 h before and from 6 to 8 h following valsartan was assayed for NO metabolites and BK1-5.

Laboratory Analysis. All blood samples were centrifuged for 20 min immediately following blood drawing, and the plasma or serum was stored at -80°C until the time of analysis. Blood for PRA and aldosterone was drawn in chilled tubes containing EDTA. PRA was measured by radioimmunoassay for Ang I formation at pH 7.4 and 37°C (Workman et al., 1979). Aldosterone was measured using a commercially available radioimmunoassay (Diagnostic Products, Los Angeles, CA). Blood for Ang II determination was collected in chilled tubes containing a cocktail of protease inhibitors, and Ang II measurements were made by radioimmunoassay, as described previously (Kohara et al., 1991; Senananyake et al., 1994). Serum ACE activity was determined by a three-step colorimetric assay in which ACE hydrolyzes the substrate *p*-hydroxybenzoyl-glycyl-L-histidyl-L-leucine, and subsequent reactions lead to the formation of quinone-imine dye, which was measured spectrophotometrically (Fujirebio America Inc., Fairfield, NJ). Bradykinin was measured by enzyme immunoassay (Peninsula Laboratories, Belmont, CA and Bachem California, Torrance, CA), and BK1-5 was measured using liquid chromatography-mass spectroscopy, as described previously (Murphy et al., 2001). PAH was measured in 18 subjects as described previously (Shoback et al., 1983). Renal vascular resistance (RVR) was calculated as mean arterial pressure (MAP)/renal blood flow. Venous plasma and urine concentrations of NO metabolites were measured using a modified Griess reaction (Actif Motif, Carlsbad, CA). cGMP was measured by enzyme-linked immunosorbent assay, using a commercially available kit (Amersham Biosciences, Piscataway, NJ).

Statistics. Data are presented as means \pm S.D. in the text and tables and as means \pm S.E.M. in the figures. The effect of treatment on blood pressure and heart rate was assessed using repeated measures analysis of variance in which the within-subject variables were acute or chronic valsartan therapy, the presence or absence of HOE-140, and time. Missing data (e.g., 27 of 900 MAP time points) were replaced by series means. For neurohumoral variables, changes pre- and postvalsartan during acute and chronic valsartan or in the presence or absence of HOE-140 were compared using a paired Student's *t* test or Wilcoxon signed rank test, as appropriate. A two-sided *P* value < 0.05 was considered significant.

Results

Thirteen normotensive and 12 hypertensive subjects completed the full study. Their baseline characteristics are shown in Table 1. Hypertensive subjects were heavier and had a higher MAP compared with normotensive subjects. An additional subject (a 39-year-old white female, not included in the data analysis) was excluded after her first study day (valsartan + HOE-140), after she developed pulmonary emboli, and it was discovered that she had begun taking an oral contraceptive and she was heterozygous for the Factor V Leiden mutation. The data and safety monitoring committee of the study concluded that the adverse event was probably not related to study drug.

TABLE 1
Subject characteristics

Characteristic	Normotensive (n = 13)	Hypertensive (n = 12)	P Value
Gender (male/female)	6:7	7:5	N.S.
Race (white/black)	9:4	5:7	N.S.
Age (years)	42.9 ± 9.8	46.3 ± 7.5	N.S.
Body mass index (kg/M ²)	25.1 ± 2.3	27.8 ± 1.8	0.003
MAP (mm Hg)	83.3 ± 8.3	99.2 ± 6.9	<0.001
Heart rate (bpm)	63.6 ± 9.6	67.9 ± 7.6	N.S.

N.S., not significant.

Table 2 provides baseline hemodynamic and renal data in the normotensive and hypertensive subjects on each of the four study days. Baseline parameters were similar on the vehicle and HOE-140 study days during both acute and chronic valsartan. MAP was significantly higher in the hypertensive subjects than in the normotensive subjects on all 4 study days. In both normotensive and hypertensive subjects, chronic valsartan decreased baseline MAP. Baseline RVR was significantly higher in hypertensive subjects than in normotensive subjects before treatment with acute valsartan but not during chronic valsartan. Chronic valsartan therapy significantly decreased baseline RVR in hypertensive subjects but not in normotensive subjects. There was no difference in baseline heart rate or urine sodium excretion between normotensive and hypertensive subjects or between acute and chronic valsartan study days.

Figure 1 shows the effect of treatment on MAP. Although MAP was higher in the hypertensive subjects studied, the change in MAP normalized as a percentage of baseline MAP was statistically similar in the normotensive and hypertensive subjects studied; therefore, the data for the two groups are presented together. Neither placebo nor HOE-140 alone significantly affected MAP. Acute administration of valsartan lowered MAP 12.7 ± 6.9% ($P < 0.001$, from 82.4 ± 8.6 to 70.3 ± 8.4 mm Hg in normotensive subjects and from 100.2 ± 8.4 to 87.5 ± 9.8 mm Hg in hypertensive subjects), with a peak effect at 6 h. Following chronic valsartan, the relative change in MAP was less than following acute dosing ($P = 0.01$ for effect of valsartan, $P < 0.001$ versus acute valsartan), but the baseline MAP was lower. Bradykinin receptor blockade did not affect the blood pressure response to valsartan.

TABLE 2
Baseline hemodynamic and renal data by study day

Parameter	Acute		Chronic	
	Valsartan	Valsartan + HOE-140	Valsartan	Valsartan + HOE-140
MAP (mm Hg)				
Normotensive	82.4 ± 8.6	84.1 ± 9.4	75.5 ± 7.4*	75.1 ± 8.5*
Hypertensive	100.2 ± 8.4†	98.2 ± 7.6†	91.1 ± 8.8*†	92.0 ± 7.4*†
Heart rate (bpm)				
Normotensive	63.0 ± 10.5	64.1 ± 8.9	62.6 ± 8.0	63.8 ± 9.6
Hypertensive	68.1 ± 6.9	67.6 ± 8.8	67.5 ± 10.0	68.9 ± 12.6
RVR (mm Hg/[ml/min/1.73 M ²])				
Normotensive	0.129 ± 0.026	0.122 ± 0.020	0.115 ± 0.021	0.109 ± 0.022
Hypertensive	0.162 ± 0.039‡	0.166 ± 0.036§	0.132 ± 0.032	0.135 ± 0.036*
24-h Urine sodium (mmol)				
Normotensive	18.4 ± 9.8	17.6 ± 14.4	21.3 ± 15.2	25.6 ± 22.2
Hypertensive	39.8 ± 55.6	28.2 ± 11.9	33.8 ± 16.2	30.7 ± 18.8

* $P < 0.05$ versus acute.

† $P < 0.001$ versus normotensive.

‡ $P < 0.05$ versus normotensive.

§ $P < 0.005$ versus normotensive.

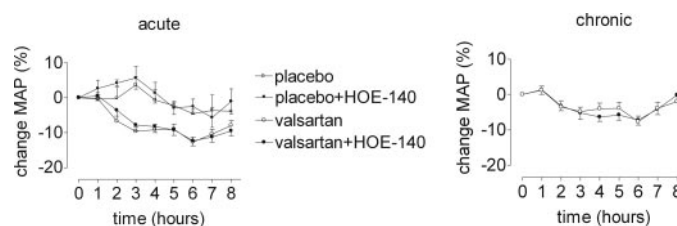


Fig. 1. Left, change in MAP in response to an acute dose of 160 mg of valsartan (circles) or placebo (squares) in the presence (filled) and absence (open) of HOE-140. $P < 0.001$ for effect of valsartan. Right, change in MAP in response to 160 mg of valsartan in the presence or absence of HOE-140 after chronic (1-month) dosing. $P = 0.01$ for effect of valsartan; $P < 0.001$ versus acute valsartan.

Heart rate increased significantly following both acute ($P < 0.001$) and chronic ($P < 0.001$) administration of valsartan but not following placebo or placebo + HOE-140 (Fig. 2). After adjustment for age, bradykinin receptor blockade significantly enhanced the heart rate response to chronic valsartan ($P = 0.04$) but not to acute valsartan ($P = 0.38$). In subgroup analysis, the effect of HOE-140 on the heart rate response to chronic valsartan was seen in hypertensive subjects ($P = 0.04$) but not in normotensive subjects ($P = 0.16$).

Acute valsartan decreased RVR from 0.147 ± 0.037 to 0.115 ± 0.044 mm Hg/[ml/min/1.73 M²] ($P < 0.001$) at a nadir of 6 h in the normotensive and hypertensive subjects combined, from 0.129 ± 0.026 to 0.094 ± 0.037 mm Hg/[ml/min/1.73 M²] ($P = 0.01$) in the normotensive subjects, and from 0.162 ± 0.039 to 0.130 ± 0.042 mm Hg/[ml/min/1.73 M²] ($P = 0.01$) in hypertensive subjects. During chronic valsartan, RVR decreased from 0.124 ± 0.028 to 0.104 ± 0.035 mm Hg/[ml/min/1.73 M²] ($P = 0.001$) 6 h following drug dosing in the combined groups, from 0.115 ± 0.021 to 0.094 ± 0.031 mm Hg/[ml/min/1.73 M²] ($P = 0.03$) in normotensive subjects, and from 0.132 ± 0.032 to 0.113 ± 0.037 mm Hg/[ml/min/1.73 M²] ($P = 0.06$) in hypertensive subjects. There was no effect of bradykinin receptor blockade on the change in RVR following acute ($P = 0.23$) or chronic ($P = 0.85$) valsartan either in all subjects combined or within hypertensive or normotensive subjects.

Table 3 shows the effect of acute and chronic valsartan on the renin-angiotensin-aldosterone and kallikrein-kinin systems in the presence and absence of the bradykinin receptor

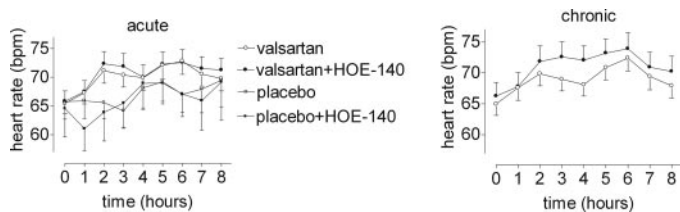


Fig. 2. Left, effect of an acute dose of 160 mg of valsartan (circles) or placebo (squares) on heart rate in the presence (filled) or absence (open) of HOE-140. $P < 0.001$ for effect of valsartan. Right, effect of 160 mg of valsartan in the presence or absence of HOE-140 after chronic (1-month) dosing. $P < 0.001$ for effect of valsartan; $P = 0.04$ for effect of HOE-140.

antagonist HOE-140. Table 4 provides control data for the effect of placebo in the presence or absence of HOE-140. All baseline parameters were similar on vehicle and HOE-140 study days, during either acute or chronic valsartan administration. ACE activity decreased slightly after administration of acute and chronic valsartan as well as following placebo; the magnitude of this change was similar in the presence and absence of HOE-140. There was no effect of chronic valsartan therapy on baseline ACE activity. Acute valsartan administration significantly increased PRA compared with either baseline or placebo. Chronic valsartan therapy significantly increased baseline morning PRA, and there was a further increase following valsartan dosing. HOE-140 tended to accentuate the change in PRA following valsartan ($P = 0.05$). Ang II concentrations increased following either acute or chronic valsartan administration but not following placebo. Mean baseline Ang II concentrations were significantly higher during chronic valsartan compared with before acute valsartan ($P = 0.02$). There was no effect of HOE-140 on Ang II concentrations. Serum aldosterone concentrations decreased after dosing of acute or chronic valsartan as well as after placebo. HOE-140 did not affect the decrease in aldosterone following acute valsartan. Chronic valsartan therapy tended to decrease baseline morning aldosterone concentration ($P = 0.07$). Bradykinin receptor antagonism blunted the decrease in aldosterone following chronic valsartan ($P = 0.03$).

There was no effect of acute or chronic valsartan or of bradykinin receptor blockade on plasma bradykinin, BK1-5 concentrations, or the ratio of BK1-5 to bradykinin, or on urine BK1-5 concentrations (data not shown). There was no effect of placebo or HOE-140 alone on plasma cGMP. Plasma cGMP decreased following both acute and chronic valsartan administration ($P = 0.04$ for effect of time). Overall, however, plasma cGMP concentrations were significantly higher ($P = 0.048$) during chronic valsartan administration than during acute valsartan administration. There was no difference in cGMP concentrations between normotensive and hypertensive subjects ($P = 0.85$). There was no effect of bradykinin receptor blockade on plasma cGMP during valsartan ($P = 0.13$). There was no effect of placebo, valsartan or bradykinin receptor antagonism on plasma NO metabolites (data not shown) or on urinary excretion of NO metabolites.

Discussion

This study tested the hypothesis that endogenous bradykinin contributes to the blood pressure-lowering effect of either acute or chronic AT₁ receptor blockade in salt-deplete normotensive and hypertensive subjects. Angiotensin AT₁

TABLE 3
Effects of treatment on parameters of the renin-angiotensin-aldosterone and kallikrein-kinin systems

Parameter	Acute				Chronic			
	Valsartan		Valsartan + HOE-140		Valsartan		Valsartan + HOE-140	
	Baseline	Post-	Baseline	Post-	Baseline	Post-	Baseline	Post-
ACE (IU/ml)	23.9 ± 10.5	22.7 ± 9.0	25.8 ± 10.4	24.1 ± 10.2*	24.0 ± 10.2	22.4 ± 8.4 [†]	24.0 ± 10.2	23.1 ± 9.6
PRA (ng Ang I/ml/h)	5.3 ± 9.9	15.6 ± 19.8 [‡]	4.6 ± 7.5	15.1 ± 22.5*	10.5 ± 15.5 [§]	14.8 ± 17.2*	11.4 ± 16.5 [§]	23.0 ± 30.7 [‡]
Ang II (pg/ml)	83.1 ± 53.9	186.5 ± 193.5 [†]	78.7 ± 57.0	159.6 ± 179.9*	117.1 ± 96.7 [§]	175.5 ± 146.3 [†]	125.8 ± 93.0 [§]	212.9 ± 197.4 [‡]
Aldosterone (ng/dl)	18.3 ± 10.5	12.0 ± 9.6 [†]	16.8 ± 8.3	11.8 ± 13.2 [†]	15.6 ± 11.1	9.9 ± 5.7*	13.3 ± 8.7	11.7 ± 6.9 [‡]
Bradykinin (fmol/ml)	5.5 ± 3.6	6.6 ± 5.8	6.9 ± 6.7	5.6 ± 4.2	6.0 ± 4.1	8.6 ± 14.3	7.8 ± 8.0	5.3 ± 4.3
Bradykinin 1-5 (fmol/ml)	12.7 ± 15.3	14.4 ± 17.4	12.6 ± 14.1	11.3 ± 11.5	10.1 ± 10.9	10.9 ± 10.5	11.3 ± 9.9	9.7 ± 8.4
Plasma cGMP (nM)	14.8 ± 5.2	13.7 ± 3.3	14.3 ± 3.8	12.9 ± 2.3*	15.9 ± 4.6	15.0 ± 4.2	15.0 ± 4.7	14.4 ± 3.5 [‡]
Urine nitrate + nitrite (μmol/g Cr)	290.3 ± 249.1	294.9 ± 270.7	266.4 ± 200.8	323.6 ± 282.2	256.1 ± 205.7	327.8 ± 268.0	259.5 ± 235.0	311.7 ± 350.8

* $P < 0.05$, [†] $P < 0.01$, and [‡] $P < 0.001$ versus baseline.

[§] $P < 0.05$ for baseline during chronic versus before acute valsartan.

^{||} $P < 0.05$ for the change following chronic valsartan + HOE-140 versus following chronic valsartan.

[¶] $P < 0.05$ versus during acute valsartan + HOE-140.

TABLE 4

Effects of placebo and HOE-140 on parameters of the renin-angiotensin-aldosterone and kallikrein-kinin systems

Parameter	Placebo		Placebo + HOE-140	
	Baseline	Post-	Baseline	Post-
ACE (IU/ml)	36.8 ± 17.4	22.6 ± 23.2*	25.7 ± 17.0	26.5 ± 18.9
PRA (ng Ang I/ml/h)	2.1 ± 1.9	1.7 ± 1.3 [†]	1.4 ± 1.1	1.5 ± 1.5 [‡]
Ang II (pg/ml)	70.1 ± 46.7	70.4 ± 50.9 [†]	64.0 ± 56.9	70.5 ± 41.6 [‡]
Aldosterone (ng/dl)	18.0 ± 10.4	12.1 ± 4.8	13.5 ± 4.9	9.8 ± 5.5*
Bradykinin (fmol/ml)	6.6 ± 4.0	2.6 ± 1.2	3.3 ± 3.9	7.2 ± 8.1
Bradykinin 1-5 (fmol/ml)	17.3 ± 29.2	17.3 ± 23.8	4.1 ± 2.4	7.5 ± 9.1
Plasma cGMP (nM)	14.8 ± 4.0	14.7 ± 4.0	16.8 ± 4.6	17.2 ± 6.7
Urine nitrate + nitrite (μmol/g Cr)	253.3 ± 116.7	383.8 ± 178.2	310.2 ± 179.8	292.3 ± 130.6

* $P < 0.05$ versus baseline.[†] $P < 0.05$ versus following valsartan.[‡] $P < 0.005$ versus following valsartan.

receptor blockade with valsartan did not affect baseline serum ACE activity or the ratio of BK1-5/bradykinin, a measure of vascular ACE activity (Murphey et al., 2000). Likewise, HOE-140, given at a dose that attenuated the response to exogenous bradykinin, did not attenuate the hypotensive effect of valsartan. Together, these data indicate that AT₁ receptor blockade lowers blood pressure through a bradykinin B₂ receptor-independent mechanism in salt-deplete individuals.

Studies in animal models indicate that, during AT₁ receptor blockade, Ang II increases vascular and renal cGMP via AT₂ receptor-mediated stimulation of bradykinin and nitric oxide synthesis (Siragy and Carey, 1997; Gohlke et al., 1998; Tsutsumi et al., 1999). This effect is more pronounced following administration of valsartan, the AT₁ receptor antagonist used in this study, than following losartan (Siragy et al., 2002). We found no effect of valsartan on urinary NO metabolite excretion in salt-deplete normotensive and hypertensive subjects. However, chronic valsartan administration increased circulating cGMP, and this effect was not attenuated by bradykinin receptor antagonism. This is consistent with data from Siragy and co-workers indicating that AT₁ receptor blockade can increase NO and cGMP production through an AT₂ receptor-dependent, B₂ receptor-independent mechanism under conditions of salt depletion (Abadir et al., 2003).

Previous studies report no effect of HOE-140 on heart rate during acute ACE inhibition (Gainer et al., 1998; Squire et al., 2000). Likewise, bradykinin receptor blockade did not affect heart rate response to acute valsartan administration. In contrast, HOE-140 enhanced the heart rate response to valsartan following 1-month administration. Whereas bradykinin can increase heart rate through sympathoexcitatory reflexes (Veelken et al., 1996) and through local effects on norepinephrine release (Seyedi et al., 1997), bradykinin induces a negative chronotropic effect through intrinsic cardiac cholinergic neurons in the isolated rabbit heart (Izrailtayan and Kresh, 1997) and via a direct effect on the sinus node in ganglion-blocked dogs (Ribuot et al., 1993). Bradykinin also causes bradycardia in anesthetized cats (Prostran et al., 1991) and in hypovolemic rats (Gardiner and Bennett, 1992). Genetic disruption of the B₂ receptor has been reported to increase heart rate in some studies (Emanuelli et al., 1999) but not in others (Milia et al., 2001). As in the present study in humans, HOE-140 has been reported to increase heart rate in both a rat model (Carini et al., 2002) and in captopril-treated, sodium-depleted marmosets (Panzenbeck et al., 1995). Further studies are needed to address the mechanism

through which endogenous bradykinin modulates the heart rate response to vasodilation during chronic AT₁ receptor blockade.

We found no effect of acute or chronic administration of valsartan on circulating concentrations of bradykinin, BK1-5, or the ratio of BK1-5 to bradykinin. This finding conflicts with the data of Campbell et al. (2005) who reported that losartan increased bradykinin concentrations in hypertensive subjects (Campbell et al., 2005). In contrast, the investigators did not find a significant effect of eprosartan on bradykinin concentrations at a dose that did increase plasma concentrations of Ang I, Ang II, and Ang-(2-8), raising the possibility that the effect of losartan on kinin concentrations was not a class effect. AT₁ receptor antagonists differ in their AT₁ binding properties and in their AT₁ receptor-independent properties (de Gasparo, 2004). For example, among valsartan and the two AT₁ receptor antagonists studied by Campbell et al. (2005), eprosartan and valsartan have higher affinity for the AT₁ receptor than does losartan. Losartan and eprosartan exhibit surmountable antagonism, whereas valsartan exhibits mixed surmountable-insurmountable antagonism. Losartan inhibits platelet aggregation through an AT₁-independent mechanism, whereas valsartan does not. Despite these differences, both losartan and valsartan have been shown to decrease mortality in individuals with heart disease (Cohn et al., 2001; Dahlof et al., 2002).

The acute administration of valsartan significantly increased PRA and Ang II, and basal PRA and Ang II were higher during chronic valsartan, consistent with inhibition of the short negative feedback loop on renin and Ang II production (Kurtz and Wagner, 1999). In contrast to a previous study of the effect of bradykinin receptor antagonism on responses to ACE inhibition (Gainer et al., 1998), HOE-140 did not attenuate the renin response to valsartan. However, bradykinin receptor antagonism blunted the decrease in aldosterone following chronic valsartan. This is consistent with data indicating that administration of a kallikrein inhibitor to prevent the formation of endogenous bradykinin enhances adrenocorticotrophic hormone-stimulated aldosterone synthesis (Rebuffat et al., 2000).

A few limitations of the current study warrant mention. First, although we administered HOE-140 through the period of peak response to acute and chronic administration of valsartan, the peptide nature and expense of the antagonist did not permit longer administration. The lack of availability of an orally active bradykinin receptor antagonist for administration to humans precludes studying the effect of concur-

rent chronic bradykinin receptor antagonism on the response to chronic valsartan. We studied subjects during salt depletion because we had previously demonstrated that endogenous bradykinin contributes to the blood pressure-lowering effect of ACE inhibition under these conditions (Gainer et al., 1998); thus, we cannot exclude the possibility that endogenous bradykinin contributes to the hypotensive effect of AT₁ receptor blockade in salt-replete individuals.

In addition, in a study in patients with congestive heart failure, combined B₁ and B₂ receptor blockade, but not B₂ receptor blockade alone, attenuated the hypotensive effects of ACE inhibition (Witherow et al., 2001). Likewise, Gavras and coworkers have reported that combined B₁ and B₂ receptor antagonism increased blood pressure in rats, whereas B₂ receptor antagonism alone did not (Duka et al., 2006). However, although it is possible that endogenous kinins could contribute to the hypotensive effects of valsartan through a B₁ receptor-dependent mechanism, the B₂-specific antagonist HOE-140 attenuates exogenous bradykinin-stimulated vasodilation at the dose given. Likewise the B₁ receptor agonist Lys-des-Arg⁹-bradykinin does not cause vasodilation when infused into the brachial artery of ACE inhibitor-treated subjects (Cruden et al., 2005). Moreover, the results of the present study are in agreement with data of Cruden et al. (2004) who reported no effect of the combined B₁ and B₂ receptor antagonist B9340 on the hypotensive response to AT₁ receptor blockade with losartan in patients with congestive heart failure.

In conclusion, ACE inhibitors and AT₁ receptor blockers have become the mainstay of treatment for the prevention of morbidity and mortality in patients with congestive heart failure, at risk for coronary artery disease, and with nephropathy. Previous studies indicate that endogenous bradykinin contributes to the hemodynamic effects of ACE inhibitors (Gainer et al., 1998; Witherow et al., 2001). Whereas early studies indicated that acute AT₁ receptor blockade did not potentiate the vasodilator response to exogenous bradykinin (Cockcroft et al., 1993), a more recent study suggested that AT₁ receptor blockade may enhance endothelial function through endogenous bradykinin (Hornig et al., 2003). At the same time, studies in animals provided a plausible mechanism whereby endogenous bradykinin could contribute to the hypotensive effects of chronic AT₁ receptor blockade (Siragy and Carey, 1997; Gohlke et al., 1998). The present study does not support the hypothesis that endogenous bradykinin contributes significantly to the hypotensive effect of oral AT₁ receptor blockade in humans via its B₂ receptor.

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