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Effects of Bradykinin on Venous Capacitance in Health and Treated Chronic Heart Failure.

Prasad Gunaruwan, Abdul Maher, Lynne Williams, James Sharman, Matthias Schmitt, Ross Campbell, Michael Frenneaux

Department of Cardiovascular Medicine,

Medical School,

University of Birmingham

Edgbaston

Birmingham

United Kingdom

Short Title: Gunaruwan, Effects of bradykinin on venous capacitance Key words: bradykinin, venous capacitance, heart failure, bradykinin receptors, ACE inhibitors, angiotensin receptor blockers *Correspondence:* Dr Prasad Gunaruwan Tel: +44 121 4146926 Fax: +44 121 4141045 Email: gunaruwan@doctors.org.uk Acknowledgement: This work was supported by the British Heart Foundation

Abstract

We investigated the effects of basal and intra-arterial infusion of bradykinin on unstressed forearm vascular volume (a measure of venous tone) and blood flow in health (G1, n=20) and in chronic heart failure patients treated with angiotensin converting enzyme (ACE) inhibitors (G2, n=16) and angiotensin receptor blockers (ARB) (G3, n=14). We used radionuclide plethysmography to examine the effects of bradykinin and of the bradykinin antagonists B9340 (B1/B2 antagonist) and HOE140 (B2 antagonist). Bradykinin infusion increased unstressed forearm vascular volume in a similar dose dependent manner in G1 and G3 (G1 maximum 12.3±2.1%; p<0.001 vs baseline, G3 maximum 9.3±3.3%; p<0.05 vs baseline, p=NS for difference) but the increase in unstressed volume in G2 was higher (maximum 28.8±7.8%; p<0.001 vs baseline; p<0.05 for the difference between groups). In contrast, while the increase in blood flow in G1 (maximum $362\pm9\%$; p<0.001) and in G2 (maximum $376\pm12\%$; p<0.001) was similar (p=NS for the difference between groups), the increase in G3 was less (maximum 335±7%; p<0.001; p <0.05 for the difference between groups). Infusion of each receptor antagonist alone similarly reduced basal unstressed volume and blood flow in G2 but not in G1 or G3. In conclusion, bradykinin does not contribute to basal venous tone in health, but in ACE inhibitor-treated chronic heart failure it does. In ARB-treated heart failure venous responses to bradykinin are preserved but arterial responses reduced compared with healthy controls. Bradykinin mediated vascular responses in both health and heart failure are mediated by the B2 rather than the B1 receptor.

Introduction

Bradykinin is a potent endothelium-dependent dilator of the resistance vessels in health [1;2] and in chronic heart failure (CHF) [3]. In patients treated with angiotensin converting enzyme (ACE) inhibitors, bradykinin contributes to the regulation of basal vascular tone in the resistance vessels [4] and in the pulmonary circulation [5]. In health bradykinin is also a potent dilator of conduit veins [6]. However the physiology of small veins and venules that make up most of the venous volume differs markedly from that of conduit veins [7]. Since a large proportion of total blood volume is contained within these vessels even small changes in the tone of these 'capacitance' veins will influence cardiac pre-load. This is particularly relevant in CHF. Understanding the receptors involved in mediating these effects is also important since they are potential therapeutic targets. To the best of our knowledge the direct local effects of bradykinin on capacitance veins have not been assessed in health or in chronic heart failure in man.

In health, bradykinin mediates its effects primarily via its constitutively expressed type 2 receptor (B2) [8]. In arteries there is evidence to suggest that there is a proportionately higher expression of the bradykinin receptor type 1 (B1) in CHF [3,4], either due to up regulation of the B1 receptor or to down regulation of the B2 receptor [9]. The contribution of B1 and B2 receptors to bradykinin-induced changes in venous capacitance has not been evaluated in health or in CHF. Furthermore, endothelial dysfunction affects arterial vessels in CHF and an impaired dilator response to bradykinin might be expected. In contrast, we have previously shown preservation of endothelium dependent responses to carbachol [10] and also preservation of the nitric oxide-dependent response to atrial natriuretic peptide [11], in the forearm capacitance bed of patients with CHF despite attenuation of responses in arteries. Preservation of responses to bradykinin in capacitance vessels might thus be anticipated in CHF.

To assess the role of bradykinin and to identify the receptors involved in mediation of its effects on capacitance veins in both health and in treated CHF, we tested three hypotheses. First, that bradykinin modulates basal forearm venous capacitance in CHF patients treated with ACE inhibitors but not in health or in CHF patients treated with angiotensin receptor blockers (ARB). Secondly, we hypothesised that in CHF patients the response to bradykinin is impaired in resistance vessels but preserved in capacitance vessels. Thirdly, that B1-mediated effects are greater in both resistance and capacitance vessels in CHF patients when compared to healthy controls.

Subjects and Methods

We studied 20 healthy volunteers and 30 patients with stable treated CHF associated with left ventricular systolic dysfunction (ejection fraction <40%) who had NYHA class II-III symptoms. 16 patients were on maximal tolerated doses of ACE inhibitors and 14 patients were on maximally tolerated doses of ARB. Medications and doses are listed in Table1. All subjects were on an ad-libitum diet, but refrained from caffeine for at least 12 hours prior to the study. Smoking was an exclusion criterion for the volunteers, and there were no current smokers in the patient group. All medications were withheld for at least 24 hours. Patient and subject characteristics are summarised in Table 1. Written informed consent was provided by all subjects, and the study was approved by the local research ethics committee.

Subjects rested supine in a temperature controlled laboratory (21-22°C). An 18-gauge cannula was inserted into a vein in the ante cubital region of the dominant arm and radio-labelling of red blood cells was carried out as described previously [7]. A 27-gauge arterial needle (Coopers Engineering, Birmingham) mounted onto a 16-gauge epidural catheter was then inserted into the non-dominant brachial artery, and kept patent by continuous infusion of 0.9% saline. Following 20 minutes rest and a 15-minute intra-brachial 0.9% saline infusion, baseline forearm blood flow (FBF) and forearm venous volume (FVV) was measured.

FBF was measured using standard mercury in silastic strain gauge plethysmography (Hokanson, Bellevue, WA, USA), as described elsewhere [12;13]. The changes in the infused arm were corrected for changes in the control arm.

FVV was measured by combining venous occlusion plethysmography, and equilibrium bloodpool scintigraphy, as described previously [7;11]. In brief: following modified ex-vivo radiolabelling of red cells with Technetium (Tc^{m99}), blood pool volume/pressure relations were constructed for both forearms, by inflating upper arm cuffs to 10, 20 and 30mmHg for one minute at each venous occlusion pressure. Dynamic images were acquired continuously, firstly during infusion of normal saline and then during each of the infusions as described in the experiments below. After correction for physical decay, scintigraphic vascular volume was plotted against occluding cuff pressure. Linear regression was performed and a linear model was adopted if the R^2 value was >0.9. Parallel shifts of the relation indicate a change in venous tone. The intercept of the regression curve with the Y-axis reflects 'unstressed' venous volume (i.e. the volume that would exist at a theoretical zero transmural pressure gradient). The unstressed volume during normal saline infusion was arbitrarily denoted as 100%, and subsequent readings



were expressed as a percentage of this value. Changes in the infused arm were then corrected for those occurring in the control arm.

Measurements of FBF and FVV were repeated after each infusion of bradykinin and receptor antagonists in the experiments described below.

Vascular response to exogenous bradykinin: In all subjects, bradykinin (Clinalfa, Switzerland) was then infused incrementally at 31.8 ng/min (30 pmol/min) and 318 ng/min (300 pmol/min) for 6 minutes at each dose.

Assessment of antagonism of exogenous bradykinin: We studied the effects of the specific B2 receptor antagonist HOE140 (Clinalfa, Switzerland) in 12 healthy volunteers and 15 patients (n=10 on ACE inhibitors and n=5 on ARB) and the non-specific B1/B2 receptor antagonist B9340 (Clinalfa, Switzerland) in 8 healthy volunteers and 15 patients (n=6 on ACE inhibitors and n=9 on ARB). Following assessment of vascular response to exogenous bradykinin, HOE140 or B9340 was co-infused at 13.5 nmol/min (17.6 µg/min and 17.8 µg/min respectively) with bradykinin 318ng/min (300 pmol/min) for 6 minutes. A 40:1 antagonist to bradykinin ratio was maintained to ensure adequate local antagonist concentration.

Assessment of endogenous bradykinin activity: Following assessment of the effects of the receptor antagonists, 0.9% saline was infused, and measurements were allowed to return to baseline and equalise between the infused and control arms. HOE140 or B9340 was then infused at 13.5 nmol/min (17.6 µg/min and 17.8 µg/min respectively) for another 6 minutes.

Blood pressure was monitored continuously with finger photo-plethysmography (TNO-TPD Biomedical Instrumentation, Amsterdam, Netherlands). Heart rate was recorded throughout the study via a 3 lead ECG.

Statistics

Sample size was based on sample size calculations with 80% power to detect a 10% change in unstressed FVV with significance at 0.05 level with a sigma of 10%. Statistical analysis was performed using SPSS version 11.5.1. All data are expressed as mean \pm SEM and a probability (p) value of <0.05 was considered significant. Within each subject group (controls, ARB-treated CHF, ACE inhibitor-treated CHF), one-way analysis of variance was carried out for the absolute FBF ratios between the infused and the control arms for the analysis of FBF response to bradykinin. Two-way analysis of variance was performed to assess between group differences and Bonferroni correction was applied for multiple comparisons. One-way analysis of variance was carried out for the % changes of unstressed FVV between the infused arm and the control arm for the analysis of unstressed FVV response to bradykinin, and two-way analysis of variance (ANCOVA) was carried out for the analysis of the two antagonists B9340 and HOE140, between each pair of the three subject groups, using the FBF and unstressed FVV differences at maximum bradykinin induced dilatation as covariate. A paired sample *t*-test was used for the analysis of basal bradykinin effects within each group.



Results

Subject characteristics are shown in Table 1.

Blood pressure and heart rate did not change significantly from baseline during or at the end of the infusions (Baseline blood pressure \pm SEM 120/65 \pm 4/4, 110/60 \pm 8/6 and 115/64 \pm 12/8mmHg for volunteers, ACE inhibitor-treated CHF and ARB-treated CHF respectively vs blood pressure during final infusion \pm SEM 118/65 \pm 6/4, 118/58 \pm 14/10 and 112/60 \pm 16/12 mmHg respectively for the groups above).

Effects of bradykinin infusion on resistance vessels: FBF increased significantly in the infused vs non-infused arms in healthy subjects and in both CHF groups (ACE inhibitor-treated and ARB-treated) (see Table 2). The increase in FBF in healthy volunteers and ACE inhibitor-treated CHF patients was similar, but both were significantly higher (p<0.05, two-way ANOVA) than in ARB-treated CHF patients (Figure 1).

Effects of bradykinin infusion on capacitance vessels: Unstressed FVV increased significantly in all 3 groups. The percentage increases from baseline are summarised in Table 2. The unstressed FVV increase in ACE inhibitor-treated CHF patients was significantly higher (p<0.05, two-way ANOVA) than in both healthy volunteers and ARB-treated CHF patients (Figure 2).

Co-infusion of bradykinin with receptor antagonists: B9340 and HOE140 both attenuated the FBF and unstressed FVV responses to 318ng/min (300 pmol/min) infusion of bradykinin, to a similar extent in all 3 groups (P<0.05, ANCOVA; Figures 3 and 4). There was no significant difference between B9340 and HOE140 (P>0.05 ANCOVA).

Baseline bradykinin activity: Infusion of B9340 or HOE140 did not reduce the FBF or unstressed FVV in healthy volunteers or in ARB-treated patients (p>0.05, Paired *t*-test; Figures 5, 6, 7 and 8). For HOE140 the percentage changes in FBF±SEM were $-4.4\pm11.2\%$ and $4.6\pm12.8\%$ and percentage changes in unstressed FVV±SEM were $-0.4\pm1.8\%$ and $-0.7\pm1.9\%$ respectively (p>0.05, Paired *t*-test) for normal volunteers and for ARB-treated patients. However, both B9340 and HOE140 reduced FBF and unstressed FVV in ACE inhibitor-treated patients (p<0.05, paired *t*-test; Figures 5, 6, 7 and 8). For HOE140 the percentage change in FBF±SEM was $-27.8\pm10.8\%$ (p>0.05, Paired *t*-test) and percentage change in unstressed FVV±SEM was $-4.0\pm1.8\%$ (p>0.05, Paired *t*-test) ACE inhibitor-treated patients.

Discussion

The primary focus of bradykinin related research in the past has been on the peripheral resistance vasculature [1-4], the coronary arteries [14], and the pulmonary circulation [5]. A number of studies have examined the effects of bradykinin on the dorsal hand vein [6;15]. However it is increasingly clear that such conduit veins may have different physiological characteristics to the small veins and venules that contribute most to the capacitance vasculature [7]. Although Mason et al [16] examined the effects of systemic infusions of bradykinin on venous capacitance, two important caveats should be considered. First, systemic infusions of bradykinin lead to stimulation of baro-reflexes and other peripheral and systemic compensatory responses. Indeed, there is evidence that bradykinin may alter baro-reflex sensitivity [17]. Second, venous capacitance was measured using strain gauge venous occlusion plethysmography. Bradykinin is known to affect capillary permeability, thus interpretation of limb volume changes as being due to changes in vascular volume may be very misleading [7]. To our knowledge, this present study is the first to directly measure changes in venous tone and regional vascular volume in response to local infusions of bradykinin in healthy subjects. In addition to demonstrating modulation of FVV by exogenous bradykinin, our results demonstrate that endogenous bradykinin does not contribute to the regulation of basal resistance or capacitance vascular tone in the human forearm in health. We also confirm that in health, bradykinin exerts its effects on both resistance and capacitance vessels through the B2 receptor. In CHF patients treated with ACE inhibitors forearm resistance vessel responses to bradykinin were similar to those of healthy controls, but in patients treated with ARB they were significantly reduced. The most likely explanation for these observations is that endothelial dysfunction in CHF patients reduces responsiveness of resistance vessels to bradykinin, and that ACE inhibitor treatment increases local concentrations to a greater extent than ARB (by inhibition of bradykinin breakdown), resulting in 'preservation' of the response to infused bradykinin. ACE inhibitors and ARB may ameliorate endothelial dysfunction in some disease states [18;19], but they do not normalise it. Another possible explanation is that B2 receptors are 're-sensitized' in the presence of ACE inhibitors via the influences of angiotensin₁₋₉ and angiotensin₁₋₅ [20]. Our finding of preservation of resistance vessel response to exogenous bradykinin in ACE inhibitor treated patients is consistent with those of Maguire et al [21], but our finding that responses in ARB-treated patients are impaired is, to the best of our knowledge novel. Davie et al [3] observed reduced responsiveness in CHF patients treated with ARB vs

those treated with ACE inhibitors but there was no healthy control group comparator.

Importantly, in contrast with our findings in resistance vessels, we found that venous responses to bradykinin in CHF patients treated with ARB were similar to those of healthy controls, and that responses in the ACE inhibitor treated patients were higher than those of healthy controls. Although the mechanism is unproven we propose that this observation is most likely due to preservation of endothelial function in capacitance vessels in CHF patients. We previously demonstrated that in ACE inhibitor-treated CHF patients, there was marked arterial endothelial dysfunction, but preservation of endothelium dependent responses to carbachol in the capacitance vessels [10]. Furthermore in another study we showed that much of the responses of forearm resistance and capacitance vessel response to atrial natriuretic peptide is mediated via endothelium dependent nitric oxide (NO) release and that in CHF there was blunting of this NO dependent component in the resistance, but not the capacitance vessels [13].

There is histological evidence that the expression of B1 and B2 receptors may shift towards preferential B1 expression in CHF, with a reduction in B2 receptor expression in end stage CHF [9]. The studies of Davie et al [3] and Witherow et al [4] together indicate that bradykinin exerts basal effects on forearm resistance vessels in CHF patients treated with ACE inhibitors and that these effects are mediated via B1 rather than B2 receptors. In contrast, in a study using B1 receptor agonists, Cruden et al [22] refuted any significant resistance vessel role for the B1 receptor in CHF. Our findings are consistent with the latter and demonstrate that both receptor antagonists (B9340 and HOE140) negated the effects of exogenous bradykinin equally, in resistance and capacitance vessels of the forearm to a similar extent. Most of our patients were only moderately symptomatic (NYHAII). Most of Cruden et al [22] patients also had relatively mild (NYHA II) symptoms. In contrast, patients in Witherow at al [4] study were mainly severely symptomatic (NYHA III or IV). Thus our patient group may not manifest the shift of receptor expression. We draw the conclusion that in treated CHF patients with moderate symptomatic limitation the effects of exogenous bradykinin are primarily mediated via the B2 receptor. It is possible that in more severe heart failure B1 mediated effects may be more important.

We also show that endogenous bradykinin contribute to basal venous tone in ACE inhibitortreated CHF as well as confirming the previous findings [4] that endogenous bradykinin contribute to modulation of the basal forearm resistance in ACE inhibitor-treated CHF. However, in contrast to Witherow et al [4], our results show that these basal effects were also antagonised by HOE140 and B9340 to a similar extent, reinforcing our finding that B2 receptor remains to be the expressed receptor type in moderate treated CHF.



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It has been suggested that due to inhibition of prostaglandin synthesis, aspirin might attenuate the beneficial effects of ACE inhibitors [23]. Several clinical trials have shown that aspirin treatment may increase hospital admissions due to worsening heart failure [24;25;25;26]. However, the balance of available evidence suggests that low dose aspirin does not impair the arterial dilatation induced by ACE inhibitors in CHF [27], nor does it blunt the beneficial effects on mortality [28;29]. Low dose aspirin inhibits thromboxane formation and has little or no effect on prostacyclin PGI₂, the cyclo-oxygenase dependent second messenger for bradykinin [30]. In addition, any effect on PGI2 is short lasting (less then 6 hours) [31;32] in comparison to effects on thromboxane. We did not exclude patients who had been taking low dose aspirin (<150mg) because the ethics committee considered it unethical to do so, but we ensured that aspirin was withheld for at least 48 hours prior to the experiments, by which time vascular effects would have ceased.

The objective of the present study was to show that bradykinin is an important mediator of venous tone in health and in CHF. However, in dissecting the mechanisms involved in mediating the effects that we have shown, our studies are limited by the lack of direct assessment of the nitric oxide dependent component. However, addition of another infusion step with N^{G} -monomethyl-L-arginine (LNMMA) would have extended the studies beyond 3 hours in duration. We have found it impossible for subjects to remain still and relaxed beyond 3 hours with an arterial needle in-situ. No dose comparison studies exist between ACE inhibitors and ARB. Thus we cannot quantitatively determine if the doses that our patients were on inhibited the angiotensin-aldosterone axis to a comparable degree. This weakness needs to be considered when comparing the differences we identified between ACE inhibitors and ARB. Furthermore, as the patients were already on either ACE inhibitors or ARB, it is possible, at least in theory, that baseline differences in physiology may have existed prior to initiation of treatment.

In conclusion, we demonstrate that the FBF response to bradykinin is impaired in ARB-treated CHF, but is not in ACE inhibitor-treated patients. We also demonstrate that forearm venous capacitance is increased by exogenous bradykinin in both health and in CHF and that the response is preserved in ARB-treated patients and increased in ACE inhibitor-treated patients with CHF vs healthy controls. Resistance and capacitance vessel effects are mediated via the B2 receptor in both health and in CHF. Bradykinin does not play a role in modulating basal forearm venous tone in health, but contributes to the basal forearm venous tone in ACE inhibitor-treated patients with CHF.



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Table 1. Subject Characteristics

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Parameter	Volunteers	CHF+ACEI	CHF+ARB
Number	20	16	14
Age (range) years	58 (52-68)	64 (50-80)	66 (50-80)
Sex, male:female	11:9	14:2	8:6
Etiology, IHD:DCM		11:5	12:2
NYHA class II:III		14:2	11:3
Treated hypertension		8	6
Treated diabetes		4	3
Medications			
Beta blockers		10	11
Diuretics		12	12
Aspirin		11	3
Nitrate		5	6
Lipid lowering		11	10
Digoxin		3	3
Warfarin		6	5
Ramipril 10mg		5	
Ramipril 5mg		4	
Perindopril 4mg		3	
Lisinopril 20mg		2	
Lisinopril 2.5mg		1	
Fosinopril 40mg		1	
Losartan 50mg			4
Candesartan 8mg			4
Losartan 100mg			2
Losartan 25mg			2
Valsartan 160mg			2
MUGA EF %	54±1	38±2	35±3
Baseline FBF ml/min	1.8±0.2	1.7±0.2	1.7±0.3
Baseline FVV (counts)	956±50	899±148	947±41

Values are numbers (Male:Female or IHD:DCM) or range (age range) or Mean±SEM. CHF+ACEI = ACE inhibitor-treated CHF patients; CHF+ARB = ARB- treated CHF patients. Total daily doses of the drugs are listed. MUGA,EF% indicates left ventricular ejection fraction measured by multiple gated scanning. Baseline FBF is for the infused arm in mls/min/100ml of forearm volume. Baseline FVV is in radioactive counts in the infused forearm.

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Table 2. Changes in FBF and FVV with bradykinin (corrected for control arm)

		FBF			FVV
Infusion	Volunteer	CHF+ACEI	CHF+ARB	Volunteer	CHF+ACEI CHF+ARB
Bradykinin 31.8 ng/min	231±9 [‡]	262±11 [‡]	220±10 [‡]	2.7±1.6	11.2±3.1* 1.1±2.1
Bradykinin 318 ng/min	$362\pm9^{\dagger}$	376±12 [†]	$335 \pm 17^{\dagger}$	12.3±2.1 [†]	28.8±7.8 [†] 9.3±3.3*

Values are Mean±SEM indicating percentage changes from baseline. CHF+ACEI = ACE Inhibitor-treated CHF patients; CHF+ARB = ARB-treated CHF patients. Significant changes from baseline are indicated by * p<0.05, * p<0.01, * p<0.001 (One-way ANOVA).

Figure 1



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Figure 3. 💹 BK 300 🕅 HOE140 ⊡ B9340 \star 400 FBF % Changes from baseline 350 300 250 т 200 150 100 50 Volunteer ARB ACEI Figure 4. 💹 ВК 300 🖾 HOE140 ⊡ B9340 * 130 % Changes from Baseline * 100 Ž 90 Volunteer ACEI ARB

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Figure 5







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Figure Legend

Figure 1. Absolute changes in the FBF ratio between the infused and control arms in response to bradykinin. BK 30 = FBF during the infusion of Bradykinin at 30 pmol/min and BK 300 = FBF during the infusion of Bradykinin at 300 pmol/min. ACEI = ACE inhibitor-treated CHF patients and ARB = CHF patients treated with ARB. * Denotes significant difference, p<0.05 (two-way ANOVA).

Figure 2. Changes in FVV, percentage of baseline in response to bradykinin. BK 30 = FBF during the infusion of Bradykinin at 30 pmol/min and BK 300 = FBF during the infusion of bradykinin at 300 pmol/min. ACEI = ACE inhibitor-treated CHF patients and ARB = CHF patients treated with ARB. * Denotes significant difference, p<0.05 (two-way ANOVA).

Figure 3. Percentage changes in the FBF ratio between the infused and control arms during infusion of bradykinin alone and co-infusion with B9340 or HOE140. BK 300 = FBF during the infusion of bradykinin at 300 pmol/min. ACEI = ACE inhibitor-treated CHF patients and ARB = CHF patients treated with ARB. * Denotes significant difference, p<0.05 (two-way ANCOVA).

Figure 4. Changes in FVV, percentage of baseline during infusion of bradykinin alone or coinfusion with B9340 or HOE140. BK 300 = FVV during the infusion of bradykinin at 300 pmol/min. ACEI = ACE inhibitor-treated CHF patients and ARB = CHF patients treated with ARB. * Denotes significant difference, p<0.05 (two-way ANCOVA).

Figure 5. Percentage changes in the FBF ratio between the infused and control arms during infusion of B9340, after the period of normal saline washout. ACEI = ACE inhibitor-treated CHF patients and ARB = CHF patients treated with ARB. * Denotes significant difference, p<0.05 (paired *t*-test).

Figure 6. Changes in FVV, percentage of baseline during infusion of B9340 after the period of normal saline washout. ACEI = ACE inhibitor-treated CHF patients and ARB = CHF patients treated with ARB. * Denotes significant difference, p<0.05 (paired *t*-test).

Figure 7. Percentage changes in FBF during infusion of B9340 or HOE140 in ACE inhibitortreated CHF patients after the period of normal saline washout. * Denotes significant difference, p<0.05 (paired *t*-test).

Figure 8. Changes in FVV, percentage of baseline during infusion of B9340 or HOE140 in ACE inhibitor-treated CHF patients after the period of normal saline washout. * Denotes significant difference, p<0.05 (paired *t*-test).