

# 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 and Michael FRENNEAUX

Department of Cardiovascular Medicine, Medical School, University of Birmingham, Edgbaston, Birmingham, U.K.

## A B S T R A C T

In the present study, 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 healthy volunteers ( $n=20$ ) and in chronic heart failure patients treated with ACEIs [ACE (angiotensin-converting enzyme) inhibitors] ( $n=16$ ) and ARBs (angiotensin receptor blockers) ( $n=14$ ). We used radionuclide plethysmography to examine the effects of bradykinin and of the bradykinin antagonists B9340 [B1 (type 1)/B2 (type 2) receptor antagonist] and HOE140 (B2 antagonist). Bradykinin infusion increased unstressed forearm vascular volume in a similar dose-dependent manner in healthy volunteers and ARB-treated CHF patients (healthy volunteers maximum  $12.3 \pm 2.1\%$ ,  $P < 0.001$  compared with baseline; ARB-treated CHF patients maximum  $9.3 \pm 3.3\%$ ,  $P < 0.05$  compared with baseline;  $P =$  not significant for difference between groups), but the increase in unstressed volume in ACEI-treated CHF patients was higher (maximum  $28.8 \pm 7.8\%$ ,  $P < 0.001$  compared with baseline;  $P < 0.05$  for the difference between groups). In contrast, while the increase in blood flow in healthy volunteers (maximum  $362 \pm 9\%$ ,  $P < 0.001$ ) and in ACEI-treated CHF patients (maximum  $376 \pm 12\%$ ,  $P < 0.001$ ) was similar ( $P =$  not significant for the difference between groups), the increase in ARB-treated CHF patients was less (maximum  $335 \pm 7\%$ ,  $P < 0.001$ ;  $P < 0.05$  for the difference between groups). Infusion of each receptor antagonist alone similarly reduced basal unstressed volume and blood flow in ACEI-treated CHF patients, but not in healthy volunteers or ARB-treated CHF patients. In conclusion, bradykinin does not contribute to basal venous tone in health, but in ACEI-treated chronic heart failure it does. In ARB-treated heart failure, venous responses to bradykinin are preserved but arterial responses are 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 CHF [chronic HF (heart failure)] [3]. In patients treated with ACEIs [ACE (angiotensin-converting enzyme) 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

**Key words:** angiotensin-converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), bradykinin, bradykinin receptor, heart failure, venous capacitance.

**Abbreviations:** ACEI, ACE (angiotensin-converting enzyme) inhibitor; ANCOVA, analysis of covariance; ANP, atrial natriuretic peptide; ARB, angiotensin receptor blocker; B1 receptor, bradykinin type 1 receptor; B2 receptor, bradykinin type 2 receptor; BP, blood pressure; CHF, chronic heart failure; FBF, forearm blood flow; FVV, forearm venous volume; HF, heart failure; HR, heart rate; NYHA, New York Heart Association; PG, prostaglandin; RBC, red blood cell.

**Correspondence:** Dr Prasad Gunaruwan (email gunaruwan@doctors.org.uk).

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 CHF in humans.

In health, bradykinin mediates its effects primarily via its constitutively expressed B2 receptor (type 2 receptor) [8]. In arteries there is evidence to suggest that there is a proportionately higher expression of the B1 bradykinin receptor (type 1 receptor) 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 NO-dependent response to ANP (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 ACEIs, but not in healthy volunteers or in CHF patients treated with ARBs (angiotensin receptor blockers). Secondly, we hypothesized that in CHF patients the response to bradykinin is impaired in resistance vessels, but preserved in capacitance vessels. Thirdly, we hypothesized that B1-mediated effects are greater in both resistance and capacitance vessels in CHF patients when compared with healthy controls.

## MATERIALS AND METHODS

### Subjects

We studied 20 healthy volunteers and 30 patients with stable treated CHF associated with LV (left ventricular) systolic dysfunction (ejection fraction <40%) who had NYHA (New York Heart Association) class II–III symptoms. Of the 30 patients with stable treated CHF, 16 patients were on maximal tolerated doses of ACEIs and 14 patients were on maximally tolerated doses of ARBs. Medications and doses are listed in Table 1. All subjects were on an *ad libitum* diet, but refrained from caffeine for at least 12 h 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 h prior to the study. Patient and subject characteristics are summarized in Table 1. Written informed consent was provided by all subjects, and the study was approved by the local research ethics committee.

Subjects rested in a supine position 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 radiolabelling of RBCs (red blood cells) was carried out as described previously [7]. A 27-gauge arterial needle (Coopers Engineering) mounted on to 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 min rest and a 15 minute intra-brachial 0.9% saline infusion, baseline FBF (forearm blood flow) and FVV (forearm venous volume) were measured.

FBF was measured using standard mercury in silastic strain gauge plethysmography (Hokanson), as described previously [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 blood-pool scintigraphy, as described previously [7,11]. In brief, following modified *ex vivo* radiolabelling of RBCs with technetium ( $Tc^{m99}$ ), blood pool volume/pressure relationships were constructed for both forearms, by inflating upper-arm cuffs to 10, 20 and 30 mmHg for 1 min at each venous occlusion pressure. Dynamic images were acquired continuously, first during infusion of normal saline and then during each of the infusions as described below. After correction for physical decay, the scintigraphic vascular volume was plotted against the 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 relationship indicates 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) was infused incrementally at 31.8 ng/min (30 pmol/min) and 318 ng/min (300 pmol/min) for 6 min at each dose.

**Table 1 Subject characteristic**

Values are means  $\pm$  S.E.M. or mean (range). The total daily doses of the drugs are listed. DCM, dilated cardiomyopathy; IHD, ischaemic heart disease; MUGA EF, multiple-gated acquisition ejection fraction.

Parameter	Healthy volunteers ( $n = 20$ )	ACEI-treated CHF patients ( $n = 16$ )	ARB-treated CHF patients ( $n = 14$ )
Age (years)	58 (52–68)	64 (50–80)	66 (50–80)
Gender (male/female)	11:9	14:2	8:6
Aetiology (IHD/DCM)		11:5	12:2
NYHA class (II/III)		14:2	11:3
Treated hypertension ( $n$ )		8	6
Treated diabetes ( $n$ )		4	3
Medication ( $n$ )			
$\beta$ -Blockers		10	11
Diuretics		12	12
Aspirin		11	3
Nitrate		5	6
Lipid-lowering drugs		11	10
Digoxin		3	3
Warfarin		6	5
Ramipril (dose 10 mg)		5	
Ramipril (dose 5 mg)		4	
Perindopril (dose 4 mg)		3	
Lisinopril (dose 20 mg)		2	
Lisinopril (dose 2.5 mg)		1	
Fosinopril (dose 40 mg)		1	
Losartan (dose 50 mg)			4
Candesartan (dose 8 mg)			4
Losartan (dose 100 mg)			2
Losartan (dose 25 mg)			2
Valsartan (dose 160 mg)			2
MUGA EF (%)	54 $\pm$ 1	38 $\pm$ 2	35 $\pm$ 3
Baseline FBF ( $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml of forearm volume}^{-1}$ )	1.8 $\pm$ 0.2	1.7 $\pm$ 0.2	1.7 $\pm$ 0.3
Baseline FVV (radioactive counts in the infused forearm)	956 $\pm$ 50	899 $\pm$ 148	947 $\pm$ 41

### Assessment of antagonism of exogenous bradykinin

We studied the effects of the specific B<sub>2</sub> receptor antagonist HOE140 (Clinalfa) in 12 healthy volunteers and 15 patients ( $n = 10$  on ACEIs and  $n = 5$  on ARBs) and the non-specific B<sub>1</sub>/B<sub>2</sub> receptor antagonist B9340 (Clinalfa) in eight healthy volunteers and 15 patients ( $n = 6$  on ACEIs and  $n = 9$  on ARBs). Following assessment of the vascular response to exogenous bradykinin, HOE140 or B9340 was co-infused at 13.5 nmol/min (17.6  $\mu\text{g}/\text{min}$  and 17.8  $\mu\text{g}/\text{min}$  respectively) with bradykinin at 318 ng/min (300 pmol/min) for 6 min. A 40:1 antagonist/bradykinin ratio was maintained to ensure an 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 equalize between

the infused and control arms. HOE140 or B9340 was then infused at 13.5 nmol/min (17.6  $\mu\text{g}/\text{min}$  and 17.8  $\mu\text{g}/\text{min}$  respectively) for a further 6 min.

BP (blood pressure) was monitored continuously with finger photo-plethysmography (TNO-TPD Biomedical Instrumentation). The HR (heart rate) was recorded throughout the study via a three-lead ECG.

### Statistics

The sample size was based on sample-size calculations, with 80% power to detect a 10% change in unstressed FVV, with a significance at the 0.05 level with a  $\sigma$  of 10%. Statistical analysis was performed using SPSS version 11.5.1. All values are expressed as means  $\pm$  S.E.M. and a  $P$  value of  $<0.05$  was considered significant. Within each subject group (controls, ARB-treated CHF patients and ACEI-treated CHF patients), one-way ANOVA 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 ANOVA

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

Values are means  $\pm$  S.E.M. indicating the percentage change from baseline. \* $P < 0.05$ , ‡ $P < 0.01$  and † $P < 0.001$  compared with baseline (measured by using one-way ANOVA)

Infusion	FBF			FVV		
	Healthy volunteers	ACEI-treated CHF patients	ARB-treated CHF patients	Healthy volunteers	ACEI-treated CHF patients	ARB-treated CHF patients
Bradykinin (at 31.8 ng/min)	231 $\pm$ 9‡	262 $\pm$ 11‡	220 $\pm$ 10‡	2.7 $\pm$ 1.6	11.2 $\pm$ 3.1*	1.1 $\pm$ 2.1
Bradykinin (at 318 ng/min)	362 $\pm$ 9†	376 $\pm$ 12†	335 $\pm$ 17†	12.3 $\pm$ 2.1†	28.8 $\pm$ 7.8†	9.3 $\pm$ 3.3*

was performed to assess between-group differences, and Bonferroni correction was applied for multiple comparisons. One-way ANOVA was carried out for the percentage changes of unstressed FVV between the infused arm and the control arm for the analysis of unstressed FVV response to bradykinin, and two-way ANOVA was performed to assess between group differences. Two-way ANCOVA (analysis of covariance) 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 the covariate. A paired sample Student's *t* test was used for the analysis of basal bradykinin effects within each group.

## RESULTS

Subject characteristics are shown in Table 1.

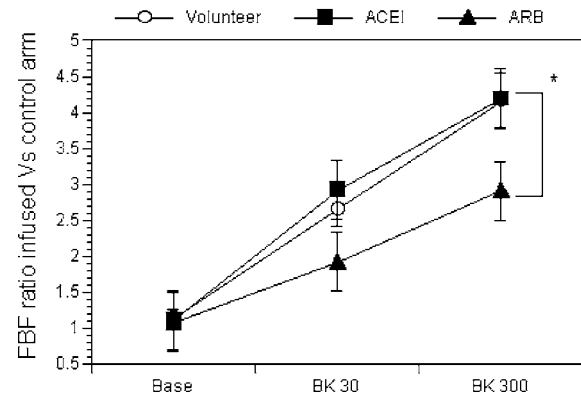
BP and HR did not change significantly from baseline during or at the end of the infusions (baseline BP 120/65  $\pm$  4/4, 110/60  $\pm$  8/6 and 115/64  $\pm$  12/8 mmHg for healthy volunteers, ACEI-treated CHF patients and ARB-treated CHF patients respectively compared with BP during final infusion 118/65  $\pm$  6/4, 118/58  $\pm$  14/10 and 112/60  $\pm$  16/12 mmHg respectively for the groups as above).

### Effects of bradykinin infusion on resistance vessels

FBF increased significantly in the infused compared with non-infused arms in healthy volunteers and in both CHF patient groups (ACEI-treated and ARB-treated) (see Table 2). The increase in FBF in healthy volunteers and ACEI-treated CHF patients was similar, but both were significantly higher ( $P < 0.05$ , measured by using two-way ANOVA) than in ARB-treated CHF patients (Figure 1).

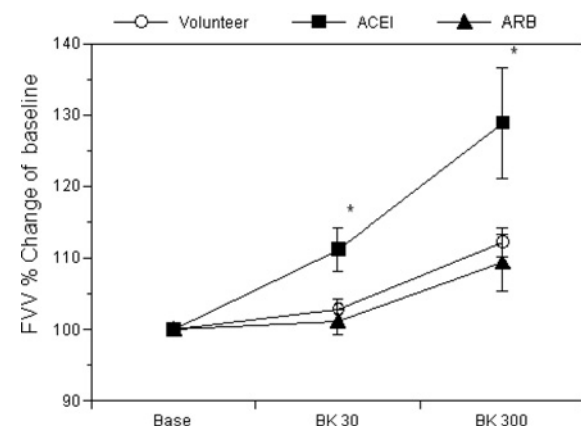
### Effects of bradykinin infusion on capacitance vessels

Unstressed FVV increased significantly in all three groups. The percentage increases from baseline are summarized in Table 2. The unstressed FVV increase



**Figure 1** Absolute changes in the FBF ratio between the infused and control arms in response to bradykinin

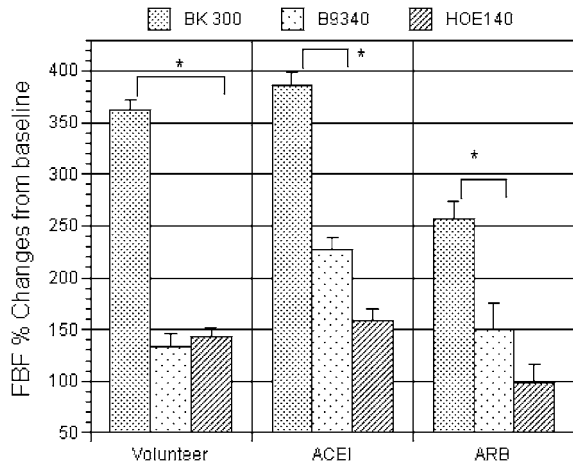
BK 30, FBF during the infusion of bradykinin at 30 pmol/min; BK 300, FBF during the infusion of bradykinin at 300 pmol/min. \* $P < 0.05$  (measured by using two-way ANOVA).



**Figure 2** Changes in FVV as a percentage of the baseline in response to bradykinin

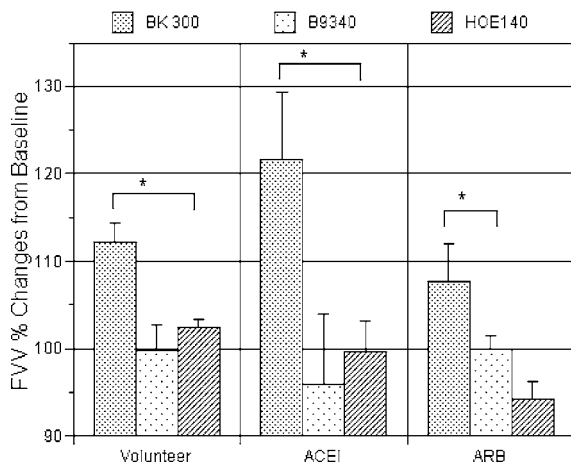
BK 30, FVV during the infusion of bradykinin at 30 pmol/min; BK 300, FVV during the infusion of bradykinin at 300 pmol/min. \* $P < 0.05$  (measured by using two-way ANOVA).

in ACEI-treated CHF patients was significantly higher ( $P < 0.05$ , measured by using two-way ANOVA) than in both healthy volunteers and ARB-treated CHF patients (Figure 2).



**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. \* $P < 0.05$  (measured by using two-way ANCOVA).



**Figure 4** Changes in FVV as a percentage of the baseline during infusion of bradykinin alone or co-infusion with B9340 or HOE140

BK 300, FVV during the infusion of bradykinin at 300 pmol/min. \* $P < 0.05$  (measured by using two-way ANCOVA).

### Co-infusion of bradykinin with receptor antagonists

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

### Baseline bradykinin activity

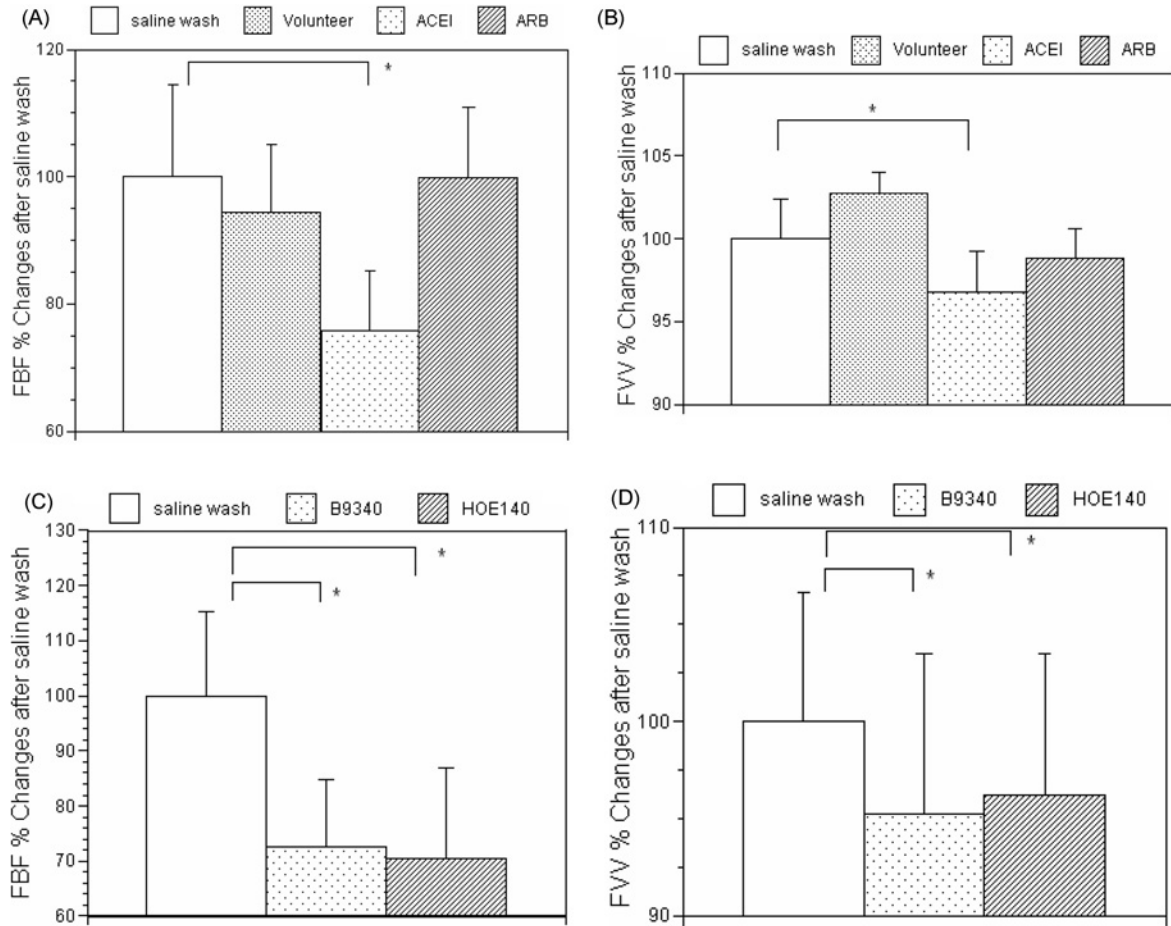
Infusion of B9340 or HOE140 did not reduce the FBF or unstressed FVV in healthy volunteers or in ARB-treated

CHF patients ( $P > 0.05$ , measured by using a paired  $t$  test; Figures 5A–5D). For HOE140 the percentage changes in FBF were  $-4.4 \pm 11.2$  and  $4.6 \pm 12.8$ %, and the percentage changes in unstressed FVV were  $-0.4 \pm 1.8$ % and  $-0.7 \pm 1.9$ % respectively ( $P > 0.05$ , measured by using a paired  $t$  test) for normal healthy volunteers and for ARB-treated CHF patients; however, both B9340 and HOE140 reduced FBF and unstressed FVV in ACEI-treated CHF patients ( $P < 0.05$ , measured by using a paired  $t$  test; Figures 5A–5D). For HOE140 the percentage change in FBF was  $-27.8 \pm 10.8$ % ( $P > 0.05$ , measured by using a paired  $t$  test) and the percentage change in unstressed FVV was  $-4.0 \pm 1.8$ % ( $P > 0.05$ , measured by using a paired  $t$  test) in ACEI-treated CHF 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 and Melmon [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 baroreflexes and other peripheral and systemic compensatory responses. Indeed, there is evidence that bradykinin may alter baroreflex sensitivity [17]. Secondly, 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, the 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, the results of the present study 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 ACEIs, the forearm resistance vessel responses to bradykinin were similar to those of healthy controls, but in patients treated with ARBs 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 ACEI treatment increases local concentrations to a greater



**Figure 5** Changes in FBF and FVV in healthy volunteers compared with ACEI-treated CHF patients and ARB-treated CHF patients.

(A) Percentage changes in the FBF ratio between the infused and control arms during infusion of B9340, after the period of normal saline washout.  $*P < 0.05$  (measured by paired Student's *t* test). (B) Changes in FVV as a percentage of the baseline during infusion of B9340 after the period of normal saline washout.  $*P < 0.05$  (measured by using a paired Student's *t* test). (C) Percentage change in FBF during infusion of B9340 or HOE140 in ACEI-treated CHF patients after the period of normal saline washout.  $*P < 0.05$  (measured by using a paired Student's *t* test). (D) Changes in FVV as a percentage of the baseline during infusion of B9340 or HOE140 in ACEI-treated CHF patients after the period of normal saline washout.  $P < 0.05$  (measured by using a paired Student's *t* test).

extent than ARBs (by inhibition of bradykinin breakdown), resulting in 'preservation' of the response to infused bradykinin. ACEIs and ARBs may ameliorate endothelial dysfunction in some disease states [18,19], but they do not normalize it. Another possible explanation is that B2 receptors are 're-sensitized' in the presence of ACEIs via the influences of angiotensin-(1-9) and angiotensin-(1-5) [20]. Our finding of the preservation of the resistance vessel response to exogenous bradykinin in ACEI-treated CHF patients is consistent with those of Maguire et al. [21], but our finding that responses in ARB-treated CHF patients are impaired is, to the best of our knowledge, novel. Davie et al. [3] observed reduced responsiveness in CHF patients treated with ARBs compared with those treated with ACEIs, but there was no healthy control group for comparison.

Importantly, in contrast with our findings in resistance vessels, we found that venous responses to bradykinin in CHF patients treated with ARBs were similar to those of healthy controls, and that responses in the ACEI-treated CHF 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 ACEI-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 ANP is mediated via endothelium-dependent 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 preferentially towards 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 ACEIs 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. The findings of the present study 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 (NYHA II). Most of the patients in the Cruden et al. [22] study also had relatively mild (NYHA II) symptoms. In contrast, patients in the Witherow et 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 HF B1-mediated effects may be more important.

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

It has been suggested that due to inhibition of PG (prostaglandin) synthesis, aspirin might attenuate the beneficial effects of ACEIs [23]. Several clinical trials have shown that aspirin treatment may increase hospital admissions due to worsening HF [24–26]. However, the balance of available evidence suggests that low-dose aspirin does not impair the arterial dilation induced by ACEIs 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 PGI<sub>2</sub>, the cyclo-oxygenase-dependent second messenger for bradykinin [30]. In addition, any effect on PGI<sub>2</sub> is short lasting (less than 6 h) [31,32] in comparison with effects on thromboxane. We did not exclude patients who had been taking low-dose aspirin (< 150 mg) because the ethics committee considered it unethical to do so, but we ensured that aspirin was

withheld for at least 48 h 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 NO-dependent component. However, addition of another infusion step with LNMMA (N<sup>G</sup>-monomethyl-L-arginine) would have extended the studies beyond 3 h in duration. We have found it impossible for subjects to remain still and relaxed beyond 3 h with an arterial needle *in situ*. No dose-comparison studies exist between ACEIs and ARBs. Thus we cannot quantitatively determine whether 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 ACEIs and ARBs. Furthermore, as the patients were already on either ACEIs or ARBs, 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 patients, but is not in ACEI-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 CHF patients and increased in ACEI-treated CHF patients compared with 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 ACEI-treated CHF patients, but not in ARB-treated CHF patients.

## FUNDING

This work was supported by the British Heart Foundation [grant number RG/04/005] and by M. G. Cezar memorial scholarships from the University of Tasmania, Australia.

## REFERENCES

- 1 Cockcroft, J. R., Sciberras, D. G., Goldberg, M. R. and Ritter, J. M. (1993) Comparison of angiotensin-converting enzyme inhibition with angiotensin II receptor antagonism in the human forearm. *J. Cardiovasc. Pharmacol.* **22**, 579–584
- 2 Cockcroft, J. R., Chowienzyk, P. J., Brett, S. E., Bender, N. and Ritter, J. M. (1994) Inhibition of bradykinin-induced vasodilation in human forearm vasculature by icatibant, a potent B2-receptor antagonist. *Br. J. Clin. Pharmacol.* **38**, 317–321
- 3 Davie, A. P., Dargie, H. J. and McMurray, J. J. (1999) Role of bradykinin in the vasodilator effects of losartan and enalapril in patients with heart failure. *Circulation* **100**, 268–273

- 4 Witherow, F. N., Helmy, A., Webb, D. J., Fox, K. A. and Newby, D. E. (2001) Bradykinin contributes to the vasodilator effects of chronic angiotensin-converting enzyme inhibition in patients with heart failure. *Circulation* **104**, 2177–2181
- 5 Cruden, N. L., Witherow, F. N., Webb, D. J., Fox, K. A. and Newby, D. E. (2004) Bradykinin contributes to the systemic hemodynamic effects of chronic angiotensin-converting enzyme inhibition in patients with heart failure. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1043–1048
- 6 Dachman, W. D., Ford, G. A., Blaschke, T. F. and Hoffman, B. B. (1993) Mechanism of bradykinin-induced venodilation in humans. *J. Cardiovasc. Pharmacol.* **21**, 241–248
- 7 Schmitt, M., Blackman, D. J., Middleton, G. W., Cockcroft, J. R. and Frenneaux, M. P. (2002) Assessment of venous capacitance. Radionuclide plethysmography: methodology and research applications. *Br. J. Clin. Pharmacol.* **54**, 565–576
- 8 Farmer, S. G. and Burch, R. M. (1992) Biochemical and molecular pharmacology of kinin receptors. *Annu. Rev. Pharmacol. Toxicol.* **32**, 511–536
- 9 Kuoppala, A., Shiota, N., Kokkonen, J. O. et al. (2002) Down-regulation of cardioprotective bradykinin type-2 receptors in the left ventricle of patients with end-stage heart failure. *J. Am. Coll. Cardiol.* **40**, 119–125
- 10 Nightingale, A. K., Blackman, D. J., Ellis, G. R. et al. (2001) Preservation of venous endothelial function in the forearm venous capacitance bed of patients with chronic heart failure despite arterial endothelial dysfunction. *J. Am. Coll. Cardiol.* **37**, 1062–1068
- 11 Schmitt, M., Broadley, A. J., Nightingale, A. K. et al. (2003) Atrial natriuretic peptide regulates regional vascular volume and venous tone in humans. *Arterioscler. Thromb. Vasc. Biol.* **23**, 1833–1838
- 12 Gunaruwan, P., Schmitt, M., Taylor, J., Lee, L., Struthers, A. and Frenneaux, M. (2002) Lack of rapid aldosterone effects on forearm resistance vasculature in health. *J. Renin Angiotensin Aldosterone Syst.* **3**, 123–125
- 13 Schmitt, M., Gunaruwan, P., Payne, N. et al. (2004) Effects of exogenous and endogenous natriuretic peptides on forearm vascular function in chronic heart failure. *Arterioscler. Thromb. Vasc. Biol.* **24**, 911–917
- 14 Groves, P., Kurz, S., Just, H. and Drexler, H. (1995) Role of endogenous bradykinin in human coronary vasomotor control. *Circulation* **92**, 3424–3430
- 15 Collier, J. G., Nachev, C. and Robinson, B. F. (1972) Effect of catecholamines and other vasoactive substances on superficial hand veins in man. *Clin. Sci.* **43**, 455–467
- 16 Mason, D. T. and Melmon, K. L. (1965) Effects of bradykinin on forearm venous tone and vascular resistance in man. *Circ. Res.* **17**, 106–113
- 17 Bomtempo, C. A., Santos, G. F., Santos, R. A. and Campagnole-Santos, M. J. (1998) Interaction of bradykinin and angiotensin-(1–7) in the central modulation of the baroreflex control of the heart rate. *J. Hypertens.* **16**, 1797–1804
- 18 Hornig, B., Kohler, C., Schlink, D., Tatge, H. and Drexler, H. (2003) AT<sub>1</sub>-receptor antagonism improves endothelial function in coronary artery disease by a bradykinin/B<sub>2</sub>-receptor-dependent mechanism. *Hypertension* **41**, 1092–1095
- 19 Mancini, G. B., Henry, G. C., Macaya, C. et al. (1996) Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. *Circulation* **94**, 258–265
- 20 Chen, Z., Tan, F., Erdos, E. G. and Deddish, P. A. (2005) Hydrolysis of angiotensin peptides by human angiotensin I-converting enzyme and the resensitization of B<sub>2</sub> kinin receptors. *Hypertension* **46**, 1368–1373
- 21 Maguire, S. M., McAuley, D., McGurk, C., Nugent, A. G., Johnston, G. D. and Nicholls, D. P. (2001) Bradykinin infusion in chronic cardiac failure and the effects of captopril. *Eur. J. Heart Failure* **3**, 671–677
- 22 Cruden, N. L., Tse, G. H., Fox, K. A., Ludlam, C. A., Megson, I. and Newby, D. E. (2005) B<sub>1</sub> kinin receptor does not contribute to vascular tone or tissue plasminogen activator release in the peripheral circulation of patients with heart failure. *Arterioscler. Thromb. Vasc. Biol.* **25**, 772–777
- 23 Cleland, J. G., John, J. and Houghton, T. (2001) Does aspirin attenuate the effect of angiotensin-converting enzyme inhibitors in hypertension or heart failure? *Curr. Opin. Nephrol. Hypertens.* **10**, 625–631
- 24 Cleland, J. G., Findlay, I., Jafri, S. et al. (2004) The Warfarin/Aspirin Study in Heart failure (WASH): a randomized trial comparing antithrombotic strategies for patients with heart failure. *Am. Heart J.* **148**, 157–164
- 25 Al Khadra, A. S., Salem, D. N., Rand, W. M., Udelson, J. E., Smith, J. J. and Konstam, M. A. (1998) Antiplatelet agents and survival: a cohort analysis from the Studies of Left Ventricular Dysfunction (SOLVD) trial. *J. Am. Coll. Cardiol.* **31**, 419–425
- 26 Cleland, J. G., Ghosh, J., Freemantle, N. et al. (2004) Clinical trials update and cumulative meta-analyses from the American College of Cardiology: WATCH, SCD-HeFT, DINAMIT, CASINO, INSPIRE, STRATUS-US, RIO-Lipids and cardiac resynchronization therapy in heart failure. *Eur. J. Heart Failure* **6**, 501–508
- 27 Davie, A. P. and McMurray, J. J. (2002) Effect of aspirin on vasodilation to bradykinin and substance P in patients with heart failure treated with ACE inhibitor. *Br. J. Clin. Pharmacol.* **53**, 37–42
- 28 Aumegeat, V., Lamblin, N., de Groote, P. et al. (2003) Aspirin does not adversely affect survival in patients with stable congestive heart failure treated with angiotensin-converting enzyme inhibitors. *Chest* **124**, 1250–1258
- 29 Teo, K. K., Yusuf, S., Pfeffer, M. et al. (2002) Effects of long-term treatment with angiotensin-converting-enzyme inhibitors in the presence or absence of aspirin: a systematic review. *Lancet* **360**, 1037–1043
- 30 Griffoni, C., Spisni, E., Strillacci, A., Toni, M., Bachschmid, M. M. and Tomasi, V. (2007) Selective inhibition of prostacyclin synthase activity by rofecoxib. *J. Cell Mol. Med.* **11**, 327–338
- 31 Ritter, J. M., Cockcroft, J. R., Doktor, H. S., Beacham, J. and Barrow, S. E. (1989) Differential effect of aspirin on thromboxane and prostaglandin biosynthesis in man. *Br. J. Clin. Pharmacol.* **28**, 573–579
- 32 Heavey, D. J., Barrow, S. E., Hickling, N. E. and Ritter, J. M. (1985) Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature* **318**, 186–188

Received 25 March 2008/26 August 2008; accepted 18 September 2008

Published as Immediate Publication 18 September 2008, doi:10.1042/CS20080096