bjh research paper

Impaired bradykinin response to ischaemia and exercise in patients with mild congestive heart failure during angiotensin-converting enzyme treatment. Relationships with endothelial function, coagulation and inflammation

Massimo Cugno,[1,2] Piergiuseppe Agostoni,[3] Daniela Mari,[1,2] Pier Luigi Meroni,[1,4] Luisa Gregorini,[5] Maurizio Bussotti,[3] Gian Battista Anguissola,[5] Francesco Donatelli[5] and Jürg Nussberger[6]

[1]Department of Internal Medicine, University of Milan, [2]IRCCS Maggiore Hospital, [3]Institute of Cardiology, Centro Cardiologico Monzino IRCCS, University of Milan, [4]Istituto Auxologico Italiano IRCCS, [5]Cardiovascular Diseases Institute-MultiMedica, University of Milan, Milan, Italy, and [6]Department of Internal Medicine, University Hospital, Lausanne, Switzerland

Received 17 February 2005; accepted for publication 28 April 2005

Correspondence: Massimo Cugno, MD, Department of Internal Medicine, University of Milan, Via Pace, 15, 20122 Milan, Italy.
E-mail: massimo.cugno@unimi.it

Summary

Inflammation and endothelial dysfunction play important roles in the pathophysiology of congestive heart failure (CHF), and the peptide bradykinin, generated during inflammation, may act as a defence mechanism by inducing vasodilation. Plasma bradykinin levels are increased in experimental heart failure but low in patients with advanced chronic CHF despite treatment with angiotensin-converting enzyme (ACE) inhibitors. It is not currently known how bradykinin behaves in less severe phases of CHF controlled by long-term ACE inhibitor treatment. We studied 10 male patients with clinically stable chronic CHF [New York Heart Association (NYHA) class II] on long-term ACE inhibitor treatment and 10 normal sex- and age-matched control subjects. High performance liquid chromatography/radioimmunoassay methods were used to evaluate plasma levels of bradykinin in relation to an array of parameters of endothelial function, coagulation and inflammation before and after stimuli of forearm arterial occlusion and physical exercise. CHF patients had higher levels of bradykinin ($P = 0{\cdot}008$), activated factor XII ($P = 0{\cdot}049$), interleukin-6 ($P = 0{\cdot}050$) and tumour necrosis factor receptor II (sTNFRII) ($P = 0{\cdot}026$) than controls. Arterial occlusion and exercise significantly increased bradykinin and von Willebrand factor levels in controls but not in CHF patients. The increase in brachial artery diameter after arterial occlusion was less in CHF patients ($P = 0{\cdot}036$) and inversely related to baseline plasma levels of bradykinin ($r = -0{\cdot}855$, $P = 0{\cdot}002$) and sTNFRII ($r = -0{\cdot}780$, $P = 0{\cdot}008$). NYHA class II CHF patients during long-term treatment with ACE inhibitors have increased bradykinin levels and signs of inflammation. They are unable to respond adequately to stimuli of ischaemia and physical exercise which both require vasodilation.

Keywords: bradykinin, heart failure, exercise, ischaemia, endothelium.

There is a growing body of evidence indicating that inflammation and endothelial dysfunction play an important role in the pathophysiology of congestive heart failure (CHF) (Levine *et al*, 1990; Testa *et al*, 1996; Chong *et al*, 2004), in addition to the well known neuroendocrine activation including norepinephrine and the renin–angiotensin system (Francis *et al*, 1990). Inflammation may be detrimental in CHF because proinflammatory cytokines and chemokines participate in cardiac depression and progression of heart failure (Torre-Amione *et al*, 1996; Kelly & Smith, 1997), and cytokine levels may be high even before the clinical onset of CHF (Vasan *et al*, 2003). However, activation of the kallikrein–kinin system, which is involved in the inflammatory process and leads to the production of the vasoactive peptide bradykinin, may be beneficial in heart failure because bradykinin reduces arterial resistance, has positive inotropic and lusitropic effects

  doi:10.1111/j.1365-2141.2005.05569.x

and decreases myocardial oxygen consumption and ischaemia (Cheng *et al*, 1998; Cleland *et al*, 2000). Bradykinin exerts its effect through endothelial B2 receptors constitutively expressed on arteries, and through B1 receptors induced by tissue injury or proinflammatory cytokines (Prado *et al*, 2002). Bradykinin is efficiently degraded by kininases, the most important of which, in humans, is the angiotensin converting enzyme whose presence in plasma and on the endothelium represents a link between the renin–angiotensin and the kallikrein–kinin systems (Schmaier, 2003).

In dogs, endogenous immunoreactive kinins quadruple after the acute induction of heart failure by pacing (Cheng *et al*, 1998), an increase that preserves cardiovascular function and may partially offset the detrimental effects of activation of the renin–angiotensin system (Su *et al*, 1998).

In contrast with the animal model, we found that humans with advanced CHF have normal plasma bradykinin levels (Cugno *et al*, 2000) even in presence of parallel coagulation activation, endothelial dysfunction and increased proinflammatory cytokines levels (Cugno *et al*, 2000, 2004). No differences in plasma bradykinin levels were found between the patients untreated or treated with angiotensin-converting enzyme (ACE) inhibitors. However, in less severe phases of CHF controlled by long-term ACE inhibitor therapy, it is not known how bradykinin behaves in relation to coagulation or inflammation, and after stimuli, such as ischaemia or physical exercise (both of which require vasodilation).

In the present investigation, we studied plasma levels of bradykinin and a series of markers of activation of the kinin system, coagulation, fibrinolysis and cytokines, as well as endothelial function, in 10 accurately selected patients with CHF in stable, functional New York Heart Association (NYHA) class II during long-term treatment with ACE inhibitors before and after forearm arterial occlusion as ischaemic stimulus and physical exercise with a cycle ergometer.

Patients and methods

We studied 10 male patients aged 54–70 years (mean ± SD, 63 ± 6 years) with chronic CHF (NYHA class II) who had been in a clinical stable condition for at least 3 months and were receiving optimised treatment, which included diuretics, angiotensin-converting enzyme (ACE)-inhibitors and beta-blockers. To avoid any interference with treatment, no patient was on statins, aspirin or other antiplatelet therapy. All patients were in regular sinus rhythm. The patient's clinical characteristics and therapies are shown in Table I. Ten healthy male subjects age-matched with the patients served as normal controls. The study was approved by the local Ethics Committee, and conducted in accordance with our institutional guidelines and the principles described in the Declaration of Helsinki. All of the subjects gave their written informed consent before taking part in the study.

Table I. Clinical parameters in 10 patients with mild chronic heart failure.

Patients (*n*)	10
Age (years, mean ± SD)	63 ± 6
Males (*n*)	10
NYHA class II (*n*)	10
Aetiology	
Ischaemic (*n*)	4
Idiopathic (*n*)	6
Blood pressure (mmHg, mean ± SD)	
Systolic	115 ± 10
Diastolic	74 ± 5
Left ventricular ejection fraction (% mean ± SD)	28·6 ± 2·7
Treatment	
Enalapril	10–20 mg
Furosemide	12·5–50 mg
Spironolactone	25 mg
Carvedilol	12·5–25 mg

Arterial occlusion test

The test was performed in a supine position after a rest of 10 min. An antecubital vein was canulated with a Venflon catheter 18GA (Becton Dickinson, Helsingborg, Sweden) and a baseline blood sample was obtained. The brachial artery diameter was measured on two-dimensional ultrasound images, recorded using a 7 MHz linear array transducer and a standard 125XP/10 system (Acuson, Mountain View, CA, USA) (Celermajer *et al*, 1992; Corretti *et al*, 2002), after which a sphygmomanometer cuff was inflated to a pressure of 300 mmHg for 3 min. Ninety seconds after cuff deflation, a second blood sample was obtained and the artery diameter was re-measured using the artery bifurcation as an anatomic marker.

Exercise test

Two research exercise tests were performed in a sitting position on a cycle ergometer: the first test was at a constant workload exercise of 25-W, and the second test using an individualised ramp protocol aimed at reaching maximal load within 10 min (Guazzi *et al*, 1997). In order to determine the individual ramp rates for the second test, we used a baseline test to identify each subject's maximal oxygen uptake: the patients were encouraged to exercise until they felt unable to continue because of dyspnoea or fatigue (symptom-limited maximal exercise). During the test, the electrocardiogram was continuously monitored and arterial blood pressure measured by means of cuff sphygmomanometry. Breath-by-breath analyses of ventilation and expiratory gases (CO_2 production and O_2 consumption) were made at rest and throughout the exercise period (Sensor Medics 2900 Metabolic Measurement Cart; Sensor Medics, Yorba Linda, CA, USA). Blood samples were obtained from an antecubital vein canulated with a Venflon catheter 18GA under baseline conditions, during constant

exercise load (6th minute), and at the maximal load. We performed two research exercise tests to analyse bradykinin, coagulation and inflammation response to light constant exercise (below lactic acidosis) and to a maximal exercise (above lactic acidosis).

Blood sampling

Before and after the two stimulation tests (arterial occlusion and exercise), blood samples were collected in the morning into polypropylene tubes containing: (1) sodium citrate (3·8%), to measure the levels of tissue plasminogen activator (tPA), plasmin–antiplasmin (PAP) complexes, D-dimer fragment, prothrombin fragment F1 + 2, thrombin–antithrombin (TAT) complexes, fibrinogen, von Willebrand factor (VWF) antigen, factor VII (FVII), activated factor XII (FXIIa) and thrombomodulin; (2) ethylenediaminetetraacetic acid sodium salt (EDTA) to determine the levels of interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), soluble TNF receptor II (sTNFRII), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble endothelial selectin (sE-selectin); (3) prechilled sodium citrate at pH 4·3 (Stabilyte; Biopool, Umea, Sweden) for PAI–1 assay; (4) a cocktail of protease inhibitors (100 mmol/l trisodium citrate, 67 mmol/l citric acid and 2% dextrose, pH 4·5, 100 mmol/l benzamidine, 400 μg/ml hexadimethrine bromide, 2 mg/ml soybean trypsin inhibitor, 263 μmol/l leupeptin and 20 mmol/l aminoethylbenzenesulphonylfluoride) to evaluate cleaved high-molecular-weight kininogen (HK). The samples were immediately centrifuged at 2000 ***g***, at 4°C, aliquoted, frozen and stored at −80°C until testing.

For the bradykinin measurements, blood samples were collected into prechilled syringes containing a mixture of protease and peptidase inhibitors to obtain final concentrations of 21 μmol/l aprotinin, 73 μg/ml chickenegg–albumin trypsin inhibitor, 305 μg/ml hexadimethrine bromide, 4·5 mmol/l 1,10-phenanthroline, and 4·5 mmol/l edetic acid. The blood samples were rapidly transferred into prechilled polypropylene tubes and centrifuged at 2°C. Plasma was immediately precipitated in ethanol and supernatants stored at 80°C until analysis.

Measurements

Bradykinin was measured by radioimmunoassay after liquid phase extraction and high-performance liquid chromatography (Nussberger *et al*, 1998). The method had a detection limit of 0·125 pmol/l and an overall recovery of 99·7% (SD 8·4%). Intra- and inter-assay coefficients of variation were 18% at the low endogenous concentrations.

Activated factor XII (FXIIa) was measured with a sandwich enzyme-linked immunosorbent assay (ELISA; Asserachrom FXIIa, Diagnostica Stago, Asnieres, France). The intraassay- and interassay-coefficients of variation (CV) were less than 7%.

Cleaved high-molecular-weight kininogen (HK) was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting analysis. Using this method, native HK appeared as a band of Mr 130 000 and cleaved HK as two bands of Mr 107 000 and 98 000. The density of the bands was evaluated by computerised image analysis (IMAGE MASTER; Pharmacia, Uppsala, Sweden). The amount of cleaved HK (bands with molecular weights 107 000 and 98 000) was expressed as a percentage of the total HK (sum of the three bands) (Cugno *et al*, 1994).

Thrombin–antithrombin (TAT) complexes were measured using a sandwich ELISA (Enzygnost TAT Micro; Behring Diagnostics GmbH, Marburg, Germany). Intraassay- and interassay-CVs were 2·5% and 5%, respectively.

Prothrombin fragment 1 + 2 (F1 + 2) was assessed with a sandwich ELISA (Enzygnost F1 + 2; Behring Diagnostics GmbH). Intraassay- and interassay-CVs were 5% and 8%, respectively.

Factor VII (FVII) antigen was measured by a commercial ELISA (Asserachrom VII:Ag; Stago) according to the manufacturer's instructions. Intraassay- and interassay-CV were 5·2% and 6·9%.

Tissue plasminogen activator (tPA) antigen was measured by a commercial enzyme-linked immunoassay method (Imunolyse tPA; Biopool) according to the manufacturer's instructions. Intraassay- and interassay-CVs were 6·5% and 8%, respectively.

Plasminogen activator inhibitor type 1 (PAI-1) antigen was measured by a commercial ELISA (Innotest PAI-1, Byk Gulden, Konstanz, Germany). Intraassay- and interassay-CVs were 8% and 13%, respectively.

Plasmin–antiplasmin (PAP) complexes were measured using a sandwich ELISA (Enzygnost PAP Micro; Behring Diagnostics GmbH). Intraassay- and interassay-CV were 3·5% and 6·5%, respectively.

D-dimer was measured by a commercial ELISA (Enzygnost D-dimer; Behring Diagnostics GmbH) according to the manufacturer's instructions. Intraassay- and interassay-CVs were 10% and 15%, respectively.

Von Willebrand factor antigen was measured in citrated plasma by a sandwich ELISA that used two monoclonal antibodies directed against different VWF epitopes (11B6·18 and 7G10·8) (Mannucci & Coppola, 1999). Intraassay- and interassay-CVs were both lower than 8%.

Thrombomodulin was measured in plasma by a commercial sandwich ELISA (Asserachrom Thrombomodulin; Diagnostica Stago). Intraassay- and interassay-CVs were both lower than 10%.

Tumour necrosis factor-α (TNF-α) levels were measured in plasma by a direct solid-phase immunoassay (Enzyme Amplified Sensitivity Immunoassay, EASIA; Biosource, Flerus, Belgium). Intraassay- and interassay-CVs were 8% and 10%, respectively.

Soluble TNF receptor II (sTNFRII) was measured by a sandwich ELISA (Quantikine Human sTNFRII Immunoassay; R&D Systems Inc., Minneapolis, MN, USA). Intraassay- and interassay-CV were 2·5% and 5·1%, respectively.

Interleukin-6 (IL-6) was measured in plasma by a sandwich ELISA (Quantikine Human IL-6 Immunoassay; R&D Systems Inc.). Intraassay- and interassay-CVs were 4·2% and 6·4%, respectively.

Soluble intercellular adhesion molecule-1 (sICAM-1) was measured in plasma by a sandwich ELISA (Parameter Human sICAM-1 Immunoassay; R&D Systems Inc.). Intraassay- and interassay-CV were 4·8% and 10·1%.

Soluble vascular cell adhesion molecule-1 (sVCAM-1) was measured in plasma by a sandwich ELISA (Parameter Human sVCAM-1 Immunoassay; R&D Systems Inc.). Intraassay- and interassay-CVs were 5·9% and 10·2%, respectively.

Soluble endothelial selectin (sE-selectin) was measured in plasma by a sandwich ELISA (Parameter Human sE-selectin Immunoassay; R&D Systems Inc.). Intraassay- and interassay-CVs were 5·0% and 8·8%, respectively.

Statistical analysis

The descriptive statistics are given as mean values ± standard error of the mean (SEM). Differences between groups were evaluated by the Student *t*-test both for unpaired and paired data. Correlations were assessed by the Pearson test. The differences or correlations with a *P*-value of less than 0·05 were considered significant.

Results

Baseline

Baseline parameters of the contact system, coagulation, fibrinolysis and inflammation of patients with CHF are shown in Table II. Plasma levels of FXIIa and bradykinin were significantly higher in CHF patients than in controls ($P = 0{\cdot}049$ and $P = 0{\cdot}008$). The markers of inflammation were also higher in CHF patients than in controls (IL-6, $P = 0{\cdot}05$; TNFRII, $P = 0{\cdot}026$; the differences in TNF-α and C-reactive protein were not statistically significant) (Table II). There was no significant difference between the patients and controls in any of the other parameters.

In CHF patients, plasma levels of bradykinin were significantly correlated with levels of FXIIa ($r = 0{\cdot}818$; $P = 0{\cdot}025$) and tPA ($r = 0{\cdot}827$; $P = 0{\cdot}006$).

Arterial occlusion test

The brachial artery response to the occlusion test is shown in Fig 1. Before the stimulation test, brachial artery diameter was larger in CHF patients (0·546 ± 0·021 cm) than in controls (0·488 ± 0·012 cm) ($P = 0{\cdot}028$) (the weight and height of the patients were not significantly different from those of the

Table II. Parameters of contact system, coagulation, fibrinolysis and inflammation in 10 patients with CHF in basal conditions and 10 normal controls.

	CHF patients ($n = 10$)	Controls ($n = 10$)	Statistical significance
FXIIa (ng/ml)	2·76 ± 0·44	1·67 ± 0·22	$P = 0{\cdot}049$
Cleaved HK (% of total HK)	14·9 ± 1·6	12·6 ± 2·9	N.S.
Bradykinin (pmol/l)	13·90 ± 2·02	6·22 ± 1·50	$P = 0{\cdot}008$
FVII antigen (ng/ml)	82 ± 7	96 ± 8	N.S.
F1 + 2 (ng/ml)	0·94 ± 0·29	0·90 ± 0·18	N.S.
TAT complexes (ng/ml)	3·3 ± 0·4	3·1 ± 0·3	N.S.
VWF (% of normal)	148 ± 27	118 ± 15	N.S.
tPA antigen (ng/ml)	12·4 ± 1·6	9·7 ± 1·4	N.S.
PAI-1 antigen (ng/ml)	60 ± 6	66 ± 7	N.S.
PAP complexes (ng/ml)	291 ± 57	206 ± 27	N.S.
D-dimer (ng/ml)	22 ± 5	14 ± 1	N.S.
IL-6 (pg/ml)	7·9 ± 2·6	2·6 ± 1·6	$P = 0{\cdot}05$
TNF (pg/ml)	18·2 ± 1·1	16·2 ± 1·1	N.S.
sTNFRII (pg/ml)	3184 ± 525	1892 ± 75	$P = 0{\cdot}026$
sICAM (ng/ml)	344 ± 47	277 ± 13	N.S.
sVCAM (ng/ml)	645 ± 69	678 ± 58	N.S.
sE-selectin (ng/ml)	60·9 ± 9·5	42·0 ± 5·7	N.S.
Thrombomodulin (ng/ml)	40·2 ± 10·3	37·7 ± 6·2	N.S.
CRP (μg/l)	2·28 ± 0·78	1·04 ± 0·23	N.S.

Mean values ± SEM.

CHF, chronic heart failure; FXIIa, activated factor XII; HK, high molecular weight kininogen; FVII, factor VII; F1 + 2, prothrombin fragment F1 + 2; TAT, thrombin–antithrombin; VWF, von Willebrand factor; tPA, tissue type plasminogen activator; PAI, plasminogen activator inhibitor; PAP, plasmin–antiplasmin; IL-6, interleukin-6; TNF, tumour necrosis factor; sTNFRII, soluble tumour necrosis factor receptor II; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; E-selectin, endothelial-selectin; CRP, C reactive protein; N.S., not significant.

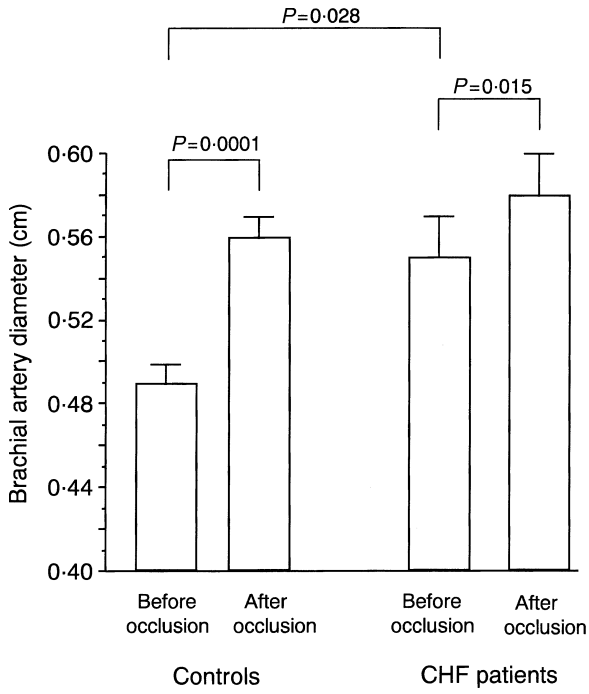

Fig 1. Brachial artery diameter expressed as centimetre in 10 normal controls and in 10 patients with chronic congestive heart failure (CHF) before and after the arterial occlusion test.

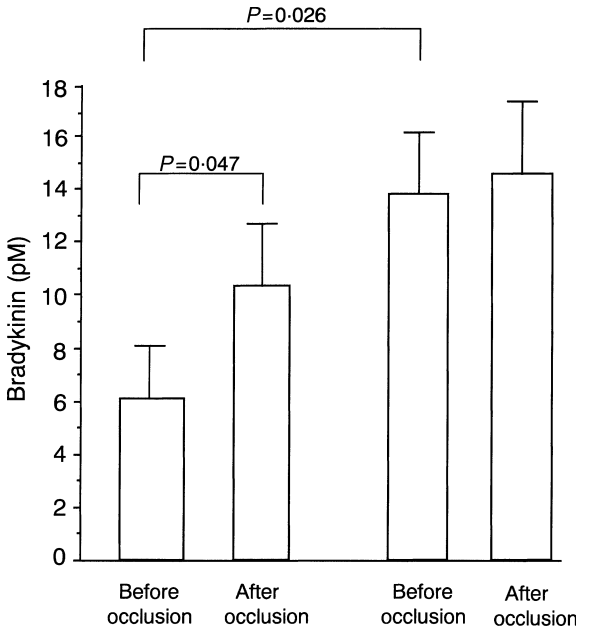

Fig 2. Bradykinin plasma levels expressed as pmol/l in 10 normal controls and in 10 patients with chronic congestive heart failure (CHF) before and after the arterial occlusion test.

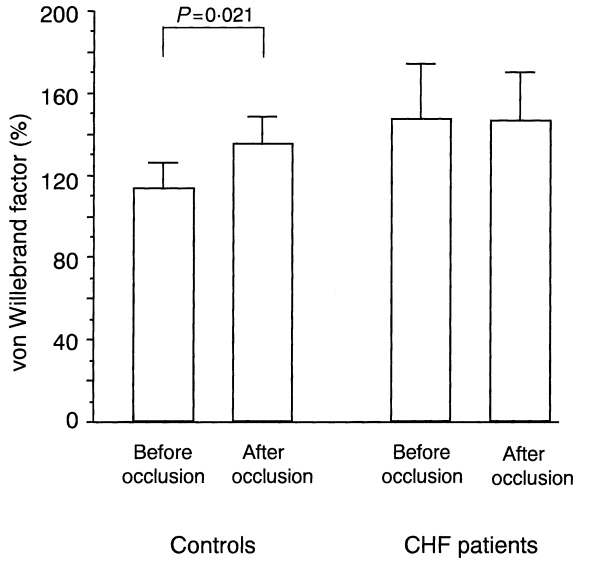

Fig 3. Plasma levels of von Willebrand factor expressed as percentage of normal in 10 normal controls and in 10 patients with chronic congestive heart failure (CHF) before and after the arterial occlusion test.

controls). After occlusion, the percentage increase was less in CHF patients (7·5 ± 2·5%) than in controls (14·7 ± 1·7%) ($P = 0·036$).

After stimulation, bradykinin plasma levels significantly increased in normal subjects ($P = 0·047$) and remained unchanged high in CHF patients (Fig 2). At the same time, VWF also increased in normal controls ($P = 0·021$) and remained unchanged high in CHF patients (Fig 3). All of the other parameters remained unchanged after arterial occlusion both in CHF patients and controls.

In our CHF patients, the percentage increase in brachial artery diameter after the occlusion test was inversely correlated with the baseline plasma levels of both bradykinin ($r = -0·855$, $P = 0·002$) (Fig 4) and TNFRII ($r = -0·780$, $P = 0·008$).

Exercise test

Exercise capacity was significantly lower in our CHF patients. Peak exercise oxygen consumption (peak VO_2) was 2048 ± 422 ml/min in controls and 1352 ± 338 ml/min in patients ($P = 0·001$); the corresponding weight-corrected values were 27·0 ± 5·5 ml/kg/min in controls and 18·3 ± 4·2 ml/kg/min in patients ($P = 0·001$), which indicated that our patients were not too seriously compromised.

In normal controls, the exercise test induced an increase in plasma bradykinin levels after both constant ($P = 0·05$) and maximal load ($P = 0·07$), but a decrease in the CHF patients ($P = 0·04$ and $P = 0·015$ respectively, Fig 5). Two markers of endothelial stimulation (VWF and tPA) increased in normal controls during the exercise test: VWF was significantly increased after both constant and maximal load (Fig 6), whereas tPA was increased only after maximal load (from 11·9 ± 1·3 to 16·2 ± 2·1 ng/ml, $P = 0·01$). In contrast, the levels of both VWF and tPA remained unchanged in the CHF patients.

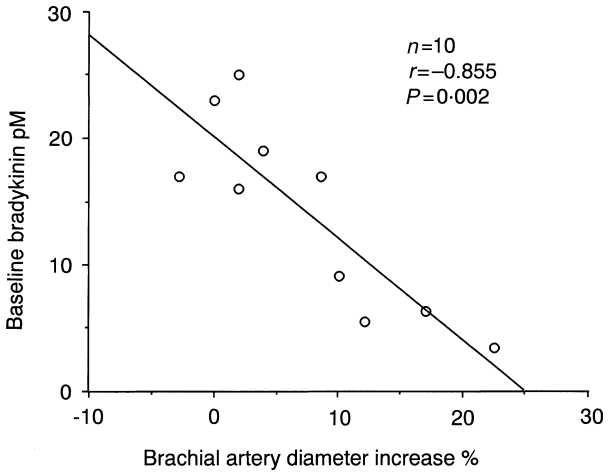

Fig 4. Correlation between baseline plasma levels of bradykinin (expressed as pmol/l) and brachial artery diameter increase after the occlusion test (expressed as percentage increase) in 10 patients with chronic congestive heart failure. The Pearson's correlation coefficient '*r*' is reported.

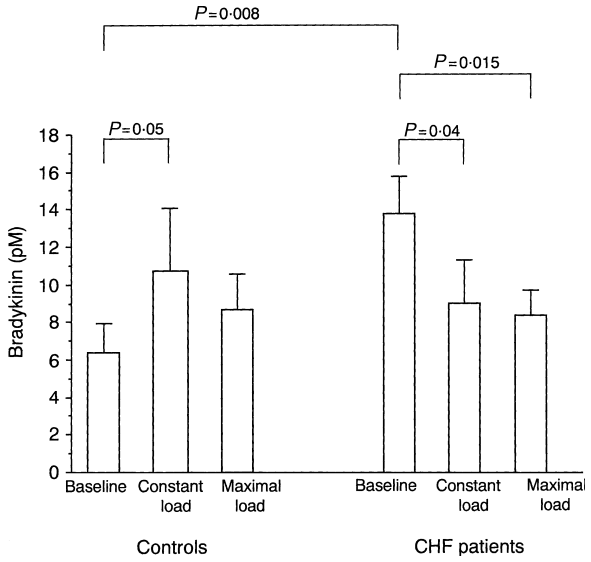

Fig 5. Plasma levels of bradykinin expressed as pmol/l in 10 normal controls and in 10 patients with chronic congestive heart failure (CHF) under baseline conditions and during the exercise test after constant load and maximal load.

Discussion

The results of our study show that CHF patients (NYHA class II) undergoing long-term ACE inhibitor treatment have high plasma bradykinin levels. After ischaemic and physical exercise stimuli, plasma levels of bradykinin and endothelial markers increased in normal subjects but not in CHF patients, whose endothelial-dependent vasodilation was reduced and inversely correlated with the levels of bradykinin and the sensitive inflammation marker TNFRII.

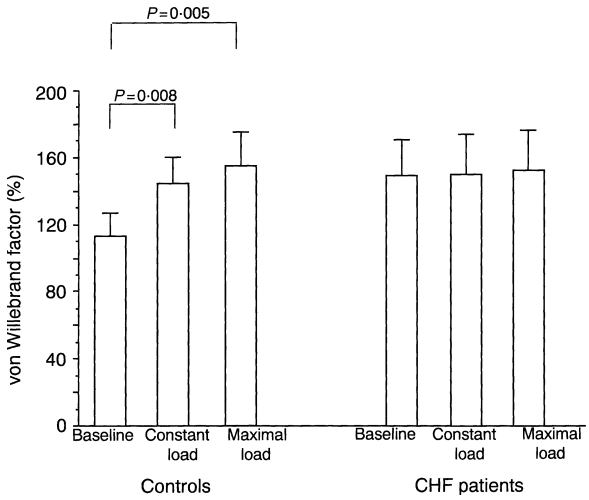

Fig 6. Plasma levels of von Willebrand factor expressed as percentage of normal in 10 normal controls and in 10 patients with chronic congestive heart failure (CHF) under baseline conditions and during the exercise test after constant load and maximal load.

The small number of patients in our study was chosen because a large series of tests was to be carried out to simultaneously compare the involvement of the contact system, coagulation, fibrinolysis, inflammation and endothelial function. Our patients were accurately selected to have a clinically stable condition and a well standardised long-term treatment with ACE inhibitors, diuretics and low-dose beta-blockers.

Before the stimulation tests, the high bradykinin levels of our CHF patients significantly correlated with the plasma levels of FXIIa, a sensitive marker of contact system activation. Bradykinin is physiologically generated during contact system activation as a result of the action on HK of kallikrein, an enzyme derived from the zymogen prekallikrein as a result of the action of FXIIa, and which in turn activates FXII to FXIIa (Kaplan *et al*, 2002). Thus our data indicate that the increase in bradykinin may be because of activation of the contact system. Our CHF patients also had slightly increased cleaved HK levels, however the sensitivity of the method we used for measuring HK (Western blot analysis) was low and even a very small cleavage of HK could generate the femtomols of bradykinin that were clearly detected by the bradykinin assay. The observed direct correlation between bradykinin and tPA may be because of the fact that bradykinin induces tPA release from human endothelium via a B2 receptor-dependent, NO synthase-independent and cyclooxygenase-independent pathway, as previously demonstrated by Brown *et al* (2000).

High bradykinin levels have been previously described after measuring immunoreactive kinins in animal models of pacing-induced heart failure (Cheng *et al*, 1998) and, in these models, bradykinin exerted vasodilator effects (Su *et al*, 1998). Bradykinin was also involved in the beneficial effects of ACE inhibition on ejection fraction, heart volumes and remodelling in rats with CHF induced by myocardial infarction (Liu *et al*,

2000). A number of studies of patients with heart failure, using bradykinin receptor antagonists, have shown that bradykinin contributes to the haemodynamic effects of ACE inhibition (Witherow *et al*, 2001; Fujii *et al*, 2002; Cruden *et al*, 2004). So far, our group (Cugno *et al*, 2000) and Duncan *et al* (2000) have directly measured the bradykinin 1–9 peptide in CHF patients. We found that chronic CHF patients in NYHA class III and IV have normally low bradykinin levels (about 2 pmol/l) even when they are taking ACE inhibitors (Cugno *et al*, 2000), and Duncan *et al* (2000) also found a bradykinin concentration of 2 pmol/l in the arterial blood of severe heart failure patients taking ACE inhibitors.

In the present study, in patients with CHF in NYHA class II during treatment with ACE inhibitor, we found that the concentrations of bradykinin were clearly high (13·9 pmol/l). The difference in bradykinin concentrations between the present and previous studies may be because of the different severity of CHF, and we therefore hypothesise that the increase in bradykinin acts as a defence mechanism that is active in the less severe phases of heart failure, but disappears with the progression of the disease. It is interesting to note that, before occlusion, the diameter of the brachial artery in CHF patients was larger than in controls (Fig 1), which is in line with the vasodilating effect of the increased levels of bradykinin. After arterial occlusion, the increase in brachial artery diameter was less in the CHF patients than in normal controls and, unlike the increase observed in the controls, the bradykinin levels of the patients remained unchanged (Fig 2). This suggests that the kinin system is activated or bradykinin catabolism is reduced in CHF patients and that they can no longer respond adequately to the ischaemic stimulus. This view is further supported by the inverse correlation between baseline plasma bradykinin levels and the percentage increase in brachial artery diameter after the occlusion test (Fig 4).

The postocclusion increase in brachial artery diameter also inversely correlated with baseline plasma levels of TNFRII, one of the most sensitive markers of inflammation. Our CHF patients had higher levels of inflammation markers than controls, and this may contribute to endothelial dysfunction. In a model of human umbilical vein endothelial cells, Agnoletti *et al* (1999) demonstrated endothelial dysfunction induced by CHF patient serum that is partially antagonised by anti-TNF antibodies. Moreover, Fichtlscherer *et al* (2001) showed impaired systemic endothelial vasodilatory capacity in patients with advanced heart failure, which was improved by the anti-TNF drug, etanercept. Endothelial involvement in our CHF patients was also supported by plasma levels of the endothelial marker VWF, which were higher in CHF patients than in normal controls and did not increase after the ischaemic stimulus (Fig 3). We did not observe the activation of coagulation described in patients with advanced CHF (Cugno *et al*, 2004), but there was a slight, non-significant increase in the markers of coagulation activation.

Plasma bradykinin levels were increased by physical exercise in normal subjects and reduced in CHF patients, who thus showed a defect in an exercise-related vasodilatory mechanism (Fig 5). The high levels of bradykinin in normal controls after exercise, similar to the baseline levels in CHF patients, could be because of contact system activation and/or reduced bradykinin metabolisation. It should be noted that the lack of exercise-induced vasodilation in CHF patients was observed both below (constant workload exercise) and above the anaerobic threshold (peak exercise of a ramp protocol). The bradykinin response at peak exercise might be influenced by the different workload achieved by controls and patients. However, in CHF patients, the same impairment of the bradykinin response to exercise was observed with both maximal and constant workload exercise, and the latter was performed at the same load (25 W) in patients and controls. This weakens the hypothesis that the intensity of exercise was the major cause of the differences observed between CHF patients and controls.

The reduced endothelial response to exercise in CHF was also supported by the finding that plasma levels of VWF and tPA (two endothelial markers) remained unchanged after exercise in the patients while they significantly increased in the normal controls (Fig 6). The effects of exercise on VWF and tPA in normal subjects are well known (Paton *et al*, 2004) and a defect in exercise-induced VWF release that could be normalised by physical training has recently been observed in CHF patients (Sabelis *et al*, 2004). Concerning bradykinin, ours is the first study evaluating the response to exercise in CHF patients to demonstrate not only the absence of an increase in bradykinin levels, but also a significant decrease in contrast to the significant bradykinin increase observed in normal controls. The latter may represent a vasodilating defence mechanism during physical exercise, as hypothesised more than 20 years ago by James and Donaldson (1981).

References

Agnoletti, L., Curello, S., Bachetti, T., Malacarne, F., Gaia, G., Comini, L., Volterrani, M., Bonetti, P., Parrinello, G., Cadei, M., Grigolato, P.G. & Ferrari, R. (1999) Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor-alpha. *Circulation*, **100,** 1983–1991.

Brown, N.J., Gainer, J.V., Murphey, L.J. & Vaughan, D.E. (2000) Bradykinin stimulates tissue plasminogen activator release from human forearm vasculature through B(2) receptor-dependent, NO synthase-independent, and cyclooxygenase-independent pathway. *Circulation*, **102,** 2190–2196.

Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K. & Deanfield, J.E. (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*, **340,** 1111–1115.

Cheng, C.P., Onishi, K., Ohte, N., Suzuki, M. & Little, W.C. (1998) Functional effects of endogenous bradykinin in congestive heart failure. *Journal of the American College of Cardiology*, **31,** 1679–1686.

Chong, A.Y., Blann, A.D., Patel, J., Freestone, B., Hughes, E. & Lip, G.Y.H. (2004) Endothelial dysfunction and damage in congestive heart failure. Relation of flow-mediated dilation to circulating

endothelial cells, plasma indexes of endothelial damage, and brain natriuretic peptide. *Circulation*, **110,** 1800–1804.

Cleland, J.G.F., Witte, K. & Thackray, S. (2000) Bradykinin and ventricular function. *European Heart Journal Supplements*, **2** (Suppl. H)**,** H20–H29.

Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J. & Vogel, R. (2002) Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *Journal of the American College of Cardiology*, **39,** 257–265.

Cruden, N.L., Witherow, F.N., Webb, D.J., Fox, K.A. & Newby, D.E. (2004) Bradykinin contributes to the systemic hemodynamic effects of chronic angiotensin-converting enzyme inhibition in patients with heart failure. *Arteriosclerosis Thrombosis and Vascular Biology*, **24,** 1043–1048.

Cugno, M., Cicardi, M. & Agostoni, A. (1994) Activation of the contact system and fibrinolysis in autoimmune acquired angioedema: A rationale for prophylactic use of tranexamic acid. *Journal of Allergy and Clinical Immunology*, **93,** 870–876.

Cugno, M., Agostoni, P., Brunner, H.R., Gardinali, M., Agostoni, A. & Nussberger, J. (2000) Plasma bradykinin levels in human chronic congestive heart failure. *Clinical Science*, **99,** 461–466.

Cugno, M., Mari, D., Meroni, P.L., Gronda, E., Vicari, F., Frigerio, M., Coppola, R., Bottasso, B., Borghi, M.O. & Gregorini, L. (2004) Haemostatic and inflammatory biomarkers in advanced chronic heart failure: role of oral anticoagulants and successful heart transplantation. *British Journal of Haematology*, **126,** 85–92.

Duncan, A.M., Kladis, A., Jennings, G.L., Dart, A.M., Esler, M. & Campbell, D.J. (2000) Kinins in humans. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, **278,** R897–R904.

Fichtlscherer, S., Rossig, L., Breuer, S., Vasa, M., Dimmeler, S. & Zeiher, A.M. (2001) Tumor necrosis factor antagonism with etanercept improves systemic endothelial vasoreactivity in patients with advanced heart failure. *Circulation*, **104,** 3023–3025.

Francis, G.S., Benedict, C., Johnstone, D.E., Kirlin, P.C., Nicklas, J., Liang, C.S., Kubo, S.H., Rudin-Toretsky, E. & Yusuf, S. (1990) Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation*, **82,** 1724–1729.

Fujii, M., Wada, A., Tsutamoto, T., Ohnishi, M., Isono, T. & Kinoshita, M. (2002) Bradykinin improves left ventricular diastolic function under long-term angiotensin-converting enzyme inhibition in heart failure. *Hypertension*, **39,** 952–957.

Guazzi, M., Marenzi, G., Alimento, M., Contini, M. & Agostoni, P. (1997) Improvement of alveolar-capillary membrane diffusing capacity with enalapril in chronic heart failure and counteracting effect of aspirin. *Circulation*, **95,** 1930–1936.

James, F.W. & Donaldson, V.H. (1981) Decreased exercise tolerance and hypertension in severe hereditary deficiency of plasma kininogens. *Lancet*, **1,** 889.

Kaplan, A.P., Joseph, K. & Silverberg, M. (2002) Pathways for bradykinin formation and inflammatory disease. *Journal of Allergy and Clinical Immunology*, **109,** 195–209.

Kelly, R.A. & Smith, T.W. (1997) Cytokines and cardiac contractile function. *Circulation*, **95,** 778–781.

Levine, B., Kalman, J., Mayer, L., Fillit, H.M. & Packer, M. (1990) Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *New England Journal of Medicine*, **323,** 236–241.

Liu, Y.H., Yang, X.P., Mehta, D., Bulagannawar, M., Scicli, G.M. & Carretero, O.A. (2000) Role of kinins in chronic heart failure and in the therapeutic effect of ACE inhibitors in kininogen-deficient rats. *American Journal of Physiology – Heart and Circulatory Physiology*, **278,** H507–H514.

Mannucci, P.M. & Coppola, R. (1999) Von Willebrand Factor. In: *Laboratory Techniques in Thrombosis. A Manual* (ed. by J. Jespersen, R.M. Bertina & F. Haverkate), pp. 115–119. Kluwer Academic Publishers, Boston, MA.

Nussberger, J., Cugno, M., Amstutz, C., Cicardi, M., Pellacani, A. & Agostoni, A. (1998) Plasma bradykinin in angio-oedema. *Lancet*, **351,** 1693–1697.

Paton, C.M., Nagelkirk, P.R., Coughlin, A.M., Cooper, J.A., Davis, G.A., Hassouna, H., Pivarnik, J.M. & Womack, C.J. (2004) Changes in von Willebrand factor and fibrinolysis following a post-exercise cool-down. *European Journal of Applied Physiology*, **92,** 328–333.

Prado, G.N., Taylor, L., Zhou, X., Ricupero, D., Mierke, D.F. & Polgar, P. (2002) Mechanisms regulating the expression, self-maintenance, and signaling-function of the bradykinin B2 and B1 receptors. *Journal of Cellular Physiology*, **193,** 275–286.

Sabelis, L.W., Senden, P.J., Fijnheer, R., de Groot, P.G., Huisveld, I.A., Mosterd, W.L. & Zonderland, M.L. (2004) Endothelial markers in chronic heart failure: training normalizes exercise-induced vWF release. *European Journal of Clinical Investigation*, **34,** 583–589.

Schmaier, A.H. (2003) The kallikrein–kinin and the renin–angiotensin systems have a multilayered interaction. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, **285,** R1–R13.

Su, J.B., Barbe, F., Houel, R., Guyene, T.T., Crozatier, B. & Hittinger, L. (1998) Preserved vasodilator effect of bradykinin in dogs with heart failure. *Circulation*, **98,** 2911–2918.

Testa, M., Yeh, M., Lee, P., Fanelli, L., Loperfido, F. & Berman, J.W. (1996) Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension. *Journal of the American College of Cardiology*, **28,** 964–971.

Torre-Amione, G., Kapadia, S., Benedict, C., Oral, H., Young, J.B. & Mann, D.L. (1996) Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). *Journal of the American College of Cardiology*, **27,** 1201–1206.

Vasan, R.S., Sullivan, L.M., Roubenoff, R., Dinarello, C.A., Harris, T., Benjamin, E.J., Sawyer, D.B., Levy, D., Wilson, P.W. & D'Agostino, R.B. (2003) Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. *Circulation*, **107,** 1486–1491.

Witherow, F.N., Helmy, A., Webb, D.J., Fox, K.A. & 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.