Plasma bradykinin levels in human chronic congestive heart failure

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ABSTRACT

Induction of congestive heart failure by high-frequency pacing has been reported to increase plasma levels of immunoreactive kinins in dogs. In the present study, we evaluated plasma bradykinin levels in human heart failure. Utilizing a recently developed method, we specifically measured plasma levels of bradykinin-(1–9) nonapeptide in 21 patients with chronic congestive heart failure (New York Heart Association (NYHA) stages III and IV). At the same time, we measured plasma atrial natriuretic peptide levels and plasma renin activity, and, as a marker of inflammation, plasma levels of tumour necrosis factor. In addition, 18 healthy subjects matched for gender and age served as normal controls. Plasma bradykinin concentrations were not higher in patients with chronic congestive heart failure (median 2.1 fmol/ml) than in healthy subjects (2.6 fmol/ml). In contrast, plasma atrial natriuretic peptide levels were clearly higher (patients, 63 fmol/ml; controls, 24 fmol/ml; \( P < 0.0001 \)), despite diuretic treatment and in the presence of high plasma renin activity (patients, 13.0 ng \( \cdot \) h\(^{-1} \cdot \) ml\(^{-1} \); controls, 0.3 ng \( \cdot \) h\(^{-1} \cdot \) ml\(^{-1} \); \( P < 0.0001 \)). Tumour necrosis factor was elevated in heart failure patients in NYHA class IV only (27 pg/ml, compared with 21 pg/ml in controls; \( P = 0.013 \)). Bradykinin, atrial natriuretic peptide and plasma renin activity levels were not correlated with the severity of the disease, as assessed by NYHA classification. These results indicate that a rather selective cytokine activation, without concomitant stimulation of the kallikrein–kinin system, occurs in human chronic congestive heart failure.

INTRODUCTION

In severe heart failure, neurohumoral vasoconstrictor systems (such as the sympathetic system and the renin–angiotensin system) and vasodilator systems (such as the kallikrein–kinin system) may play important roles. Activation of the former contributes to sodium retention, cardiac remodelling and depression of left ventricular function [1], thus increasing the severity of heart failure. Activation of the kallikrein–kinin system, resulting in the production of bradykinin (BK), might be beneficial in heart failure, because BK reduces both systemic and coronary resistance, has positive inotropic and lusitropic effects, may decrease myocardial oxygen consumption and ischaemia, and may increase gas exchange in the lungs of patients with heart failure [2,3]. In healthy individuals, the responses to BK are thought to be mainly mediated by nitric oxide. In heart failure, however, endothelium-derived vasodilatation is blunted [4]. In such disease states, BK may act through endothelium-independent mechanisms. In fact, it has been demonstrated in dogs with pacing-induced heart failure that prostaglandins mediate sympathetic activation in response to epicardial BK application [5]. Recently it has

Key words: ACE inhibitors, bradykinin, cytokines, heart failure, renin–angiotensin system.

Abbreviations: ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; BK, bradykinin; CHF, congestive heart failure; NYHA, New York Heart Association; PRA, plasma renin activity; TNF, tumour necrosis factor.

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been demonstrated in the same dog model that exogenous and endogenous BK maintain the vasodilator effects [6]. It has also been shown that plasma levels of immunoactive kinins are elevated 4-fold in conscious animals with pacing-induced heart failure [2]. Thus endogenous BK may play a role in preserving cardiovascular function in heart failure, and increased levels of BK may partially offset the detrimental effects of activation of other neurohormonal systems [2]. However, we have no data on BK levels in humans with congestive heart failure. Indeed, measurement of BK is difficult, due to its extremely short half-life in plasma and to its easy generation and degradation in vitro. Furthermore, several issues limit the translation of the experimental data to the clinical field, including the lack of complete reliability of the acute heart failure model, which does not correspond to chronic congestive heart failure (CHF) in humans.

In the present study, we used a new method to measure plasma BK levels [7] in 21 patients with CHF and in 18 matched healthy controls. At the same time, we measured plasma atrial natriuretic peptide (ANP), plasma renin activity (PRA) and plasma tumour necrosis factor (TNF). TNF is one of the pro-inflammatory cytokines recently suggested to be a mediator in the progression of heart failure [8].

METHODS

Subjects

We studied 21 patients (14 men and seven women; age 58–84 years) with chronic CHF who were in a stable clinical condition and with defined drug regimens (Table 1). The diagnosis of CHF and its aetiology were based on clinical, echocardiographic and angiographic criteria. The aetiology was ischaemic in seven patients, valvular in three patients, hypertensive in one patient and idiopathic in 10 patients (normal coronary angiography). Of the CHF patients, eight were in New York Heart Association (NYHA) class III and 13 were in class IV. The left ventricular ejection fraction was < 35% in all patients except patient 4 (50%). This patient suffered from mitral stenosis. Echocardiograms and cardiac catheterization had been performed in the 6 months preceding the study, while the clinical evaluation was carried out on the day of blood sampling. In addition, 18 healthy subjects (12 men and six women; age 53–86 years) served as normal controls. The following plasma measurements were performed for each patient: BK, PRA, ANP and TNF.

The study was performed according to institutional guidelines, was approved by the local ethical committee and conformed with the principles outlined in the Declaration of Helsinki (1989) of the World Medical Association. All subjects gave their informed consent prior to participation.

**Determination of plasma BK levels**

After an overnight fast, blood was drawn through an indwelling venous catheter after a supine rest of 30 min. As described previously in detail [7], samples for BK measurement were collected into prechilled syringes containing a mixture of protease and peptidase inhibitors. The blood samples were transferred rapidly into prechilled polypropylene tubes and centrifuged at 2 °C. Plasma was immediately precipitated in ethanol, and supernatants were stored at −80 °C until analysed [7]. Plasma BK-(1–9) nonapeptide was measured specifically by RIA after liquid-phase extraction and subsequent HPLC. The limit of detection in plasma is 0.2 fmol/ml. Both intra-assay and interassay coefficients of variation were 18% at the low endogenous concentrations [7]. The antiserum used was raised in a New Zealand White rabbit by one of us (J. N.), and was found to be specific for the C-terminal end of the BK-(1–9) nonapeptide [7].

**Measurement of ANP, PRA and TNF**

For ANP, PRA and TNF determinations, blood was collected into chilled EDTA tubes. The samples were centrifuged rapidly and the plasma was stored at −80 °C until analysed.

ANP was measured by RIA using a very sensitive rabbit antiserum produced in our laboratory. Immunoactive ANP was extracted from plasma by reversible adsorption on to phenylsilyl-silica prior to the RIA [9]. Recovery losses were below 20%, and samples were corrected individually by comparison with internal standards. The limit of detection in plasma is 1.1 fmol/ml. Intra-assay and interassay coefficients of variation were 8% and 12% respectively.

PRA was measured by antibody trapping and by subsequent RIA of angiotensin I that was generated from the endogenous substrate angiotensinogen [10,11]. The limit of detection is 0.05 ng·h⁻¹·ml⁻¹. Both intra-assay and interassay coefficients of variation were below 12% [11].

TNF levels were measured by a direct solid-phase immunoassay in unextracted plasma [Enzyme Amplified Sensitivity Immunoassay (EASIA); Biosource, Flerus, Belgium]. The method uses a mixture of monoclonal antibodies directed against different epitopes of TNF-α in order to avoid hyperspecificity and to provide high sensitivity [12]. The limit of detection is 3 pg/ml. Intra-assay and interassay coefficients of variation were 8% and 10% respectively.

**Statistical analysis**

Descriptive statistics are reported as median and minimum and maximum values, because of the skewed distribution of the investigated variables. Differences between groups were evaluated by the non-parametric
Table 1  Clinical parameters for 21 patients with CHF

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>NYHA class</th>
<th>EF (%)</th>
<th>Aetiology</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>f</td>
<td>IV</td>
<td>26</td>
<td>Valvular</td>
<td>Furosemide, nitrates</td>
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<td>2</td>
<td>63</td>
<td>m</td>
<td>III</td>
<td>20</td>
<td>Idiopathic</td>
<td>Furosemide, spironolactone, cardiac glycosides, enalapril, losartan</td>
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<td>3</td>
<td>58</td>
<td>m</td>
<td>IV</td>
<td>17</td>
<td>Hypertensive</td>
<td>Furosemide, spironolactone, cardiac glycosides</td>
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<td>4</td>
<td>74</td>
<td>f</td>
<td>III</td>
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<td>5</td>
<td>84</td>
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<td>6</td>
<td>70</td>
<td>m</td>
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<td>7</td>
<td>78</td>
<td>m</td>
<td>IV</td>
<td>33</td>
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<td>Furosemide, cardiac glycosides, enalapril</td>
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<tr>
<td>8</td>
<td>83</td>
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<td>IV</td>
<td>31</td>
<td>Ischaemic</td>
<td>Furosemide, chlortalidone, cardiac glycosides, losartan</td>
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<td>9</td>
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<td>m</td>
<td>IV</td>
<td>25</td>
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<td>10</td>
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<td>Furosemide, cardiac glycosides, enalapril</td>
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<td>III</td>
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<td>Furosemide</td>
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<td>Furosemide</td>
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Wilcoxon–Mann–Whitney test. A P value of < 0.05 was considered significant.

RESULTS

Plasma levels of BK, TNF, ANP and PRA for 21 patients with severe chronic CHF (NYHA classes III and IV) and for 18 control subjects are depicted in Figure 1. Levels of TNF, ANP and PRA were significantly increased in patients with CHF, but BK levels were not altered. ANP and PRA levels were the same in CHF patients of NYHA classes III and IV, whereas TNF was higher in class IV patients only (Table 2).

In Table 3, the parameters for patients on angiotensin-converting enzyme (ACE) inhibitor treatment with
enalapril (5–20 mg per day) are compared with those of patients without such treatment. Long-term ACE inhibitor treatment (11 out of 21 patients) reduced circulating ANP levels ($P = 0.032$) and tended to further increase PRA ($P = 0.057$) in CHF patients treated chronically with diuretics. At the same time, TNF tended to be normalized by enalapril, from 27 to 22 pg/ml. BK was not modified by enalapril treatment in the CHF patients. No differences in the parameters studied were observed in patients treated with losartan, whether associated or not with enalapril.

**DISCUSSION**

Several experimental studies [2,6] suggested that plasma BK levels might be increased in human chronic CHF, and may play an important role in preserving cardiovascular function. Our results in humans do not support this hypothesis. We applied a method recently developed in our laboratory [7], combining the extreme sensitivity of our antiserum with the exclusive specificity of HPLC procedures to measure plasma levels of BK-(1–9) nonapeptide in CHF patients and in healthy controls. There were no differences in plasma BK concentrations between these groups (Figure 1), nor were BK levels related to the severity of CHF (Table 2). Chronic treatment with enalapril was not associated with an increase in plasma BK levels in patients with CHF on diuretics and digitalis glycosides (Table 3). This contrasts with reports of increased plasma BK in normal volunteers who were treated acutely with other ACE inhibitors and without diuretics [13,14]. In our CHF patients treated with enalapril, venous plasma BK concentrations were the same as those measured by Duncan et al. [15] in the arterial blood of some of their patients with severe cardiac failure. In contrast with that previous report, however, BK levels in our control samples from healthy subjects matched for age and gender and from CHF patients not receiving ACE inhibitors were the same as in the enalapril-treated CHF patients. This discrepancy may be due to differences in study design rather than in analytical procedures, although our detection limit is some 10-fold below that of Duncan et al. [15]. Despite the modest sample size of the present study, our results are meaningful in the light of the appropriate controls. The discrepancy in plasma BK levels between human CHF and experimentally induced heart failure in animals may be due to the experimental heart failure model. Indeed, our patients were in a state of chronic CHF, which is not easy to compare with the acute experimental situation in animals.

The relevance of circulating BK levels is not well established, since accurate measurement in humans became available only a few years ago. Local BK concentrations may be only partially reflected in cubital venous blood [16]. The half-life of BK in human serum in vitro was found to be 49 s, and the half-life in vivo is probably some 10–20 s [17]. Nevertheless, similar reser-
vations were held for other peptides such as angiotensin II, and it has turned out that clinically meaningful insight can be obtained from accurate peptide measurement in blood, which is more easily accessible than tissue biopsies. It requires the skill and experience of investigators to exclude sampling artefacts from the quantitative methods used [14,18]. The present report fulfills such requirements at the best possible level of the investigators involved. Nevertheless, in one patient (no. 13) the BK level was clearly outside the range observed in other subjects with CHF, and we cannot exclude an artefact. However, non-parametric testing takes care of such problems.

In the present study, the lack of an elevation in plasma BK was observed in a population with the ‘classical’ neurohumoral pattern observed in heart failure [1,19–21]. In contrast with BK, TNF was actually increased (Figure 1). Increases in TNF levels were correlated with the severity of CHF (Table 2), confirming previous reports [22–24]. ACE inhibitor treatment seems to lower plasma TNF levels (Table 3). If confirmed, the explanation for this finding requires further investigation. The difference in responses between BK and TNF is in agreement with the view expressed recently by Mann [25] of the complex and heterogeneous nature of the mechanism underlying the expression of inflammatory mediators in CHF. In this sense, as stated by Francis [26], heart failure is not generally considered to be a purely inflammatory condition, and the quantitative contribution of cytokines to the pathophysiology of heart failure is still not clear, even though some authors believe that anti-cytokine therapy may represent a new frontier for the management of heart failure [27].

Our patients had severe heart failure (NYHA classes III and IV). All were treated with diuretics, and 11 were on ACE inhibitors (enalapril) (Table 1). As suggested previously [19,21], the increase in PRA is probably related to the pharmacological treatment, since in advanced CHF PRA is normal or almost normal if patients are not taking diuretics or vasodilating agents [1,19]. Despite the fact that such treatment would tend to lower ANP levels by decreasing cardiac pre- and after-load, we still found ANP levels to be increased above normal. In particular, patients with treatment including enalapril had lower ANP levels than patients not treated with ACE inhibitors.

The kallikrein–kinin system was expected to follow the general neurohumoral and inflammatory activation described in heart failure. The present results argue against such a working hypothesis. Our results support rather selective cytokine activation without concomitant stimulation of the kallikrein–kinin system. The lack of observed changes in BK levels was not due to methodological problems, since clearly increased plasma BK levels have been measured by the same method in other clinical situations [7,28].

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REFERENCES

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