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Cardiac peptides differ in their response to exercise

Implications for patients with heart failure in clinical practice

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Aims Cardiac peptides have diagnostic and prognostic value in heart failure. Their plasma concentrations, however, are sensitive to rapid changes in haemodynamics. As blood sampling under standard conditions is not feasible in clinical practice, it is important to know which peptides are most resistant to change. Therefore, the present study investigated the differences in response to exercise between atrial natriuretic peptide, N-terminal proatrial natriuretic peptide, brain natriuretic peptide and the recently identified N-terminal probrain natriuretic peptide.

Methods and Results Fifty-two patients with chronic heart failure performed a symptom-limited graded bicycle exercise. Blood samples for determination of plasma concentrations of cardiac peptides were drawn at rest and at peak exercise. There was a significant difference in percentage increase in response to exercise between the four peptides ($P<0{\cdot}0001$). N-terminal proatrial natriuretic peptide increased less than atrial natriuretic peptide ($5 \pm 18\%$ vs $59 \pm 58\%$; $P<0{\cdot}0001$). The difference in increase between N-terminal probrain natriuretic peptide and brain natriuretic peptide was less distinct but still significant ($24 \pm 24\%$ vs $38 \pm 52\%$, $P<0{\cdot}05$).

Conclusions Both N-terminal proatrial natriuretic peptide and N-terminal probrain natriuretic peptide increased less in response to exercise than their C-terminal counterparts. This implies that the circumstances under which blood sampling for measurements of N-terminal proatrial natriuretic peptide and N-terminal probrain natriuretic peptide should be performed are more favourable than the blood sampling conditions for atrial natriuretic peptide and brain natriuretic peptide.
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Key Words: Atrial natriuretic peptide, brain natriuretic peptide, peptide fragments, exercise, heart failure.

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Introduction

Atrial natriuretic peptide is stored in atrial myocytes in the form of a prohormone (proANP) that is secreted in response to increased atrial stretch[1]. On secretion, proANP is cleaved into a C-terminal peptide, ANP(99–126), and an N-terminal peptide, N-terminal proANP(1–98)[2]. Because N-terminal proANP has a longer half-life than atrial natriuretic peptide, its plasma concentrations are higher and less sensitive to rapid changes in haemodynamics[3,4].

Brain natriuretic peptide is not only secreted from the atria, but also from the ventricles, especially in patients with chronic heart failure[5, 6]. Although brain natriuretic peptide shares many structural and functional similarities with atrial natriuretic peptide, there are still uncertainties about its circulating forms. Available evidence indicates that three forms of brain natriuretic peptide circulate in human plasma: the C-terminal peptide, BNP(77–108), a large molecular weight peptide, presumably proBNP(1–108), and its N-terminal fragment, N-terminal proBNP(1–76)[7,8]. Whether N-terminal proBNP, like N-terminal proANP, has more stable plasma concentrations than brain natriuretic peptide is not known.

Several studies have shown that elevated plasma concentrations of atrial natriuretic peptide, N-terminal proANP peptide and brain natriuretic peptide have diagnostic and prognostic value in heart failure[9–15]. Likewise, N-terminal proBNP has been shown to be a marker of left ventricular dysfunction and an

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independent predictor of prognosis after myocardial infarction[16,17]. In previous research, blood sampling for cardiac peptide measurements has been performed under standard conditions. These requirements, however, are hard to meet in daily practice. Thus, the variability in plasma concentrations due to non-cardiac influences, such as body position or exercise, should be considered when deciding which peptide is most suitable for use in clinical practice. For that reason, the present study evaluated the differences in response to exercise between atrial natriuretic peptide, N-terminal proANP, brain natriuretic peptide and N-terminal proBNP.

Methods

Patients

Within the framework of an outpatient heart failure research project, 52 patients with chronic heart failure and left ventricular systolic dysfunction (left ventricular ejection fraction <45%) underwent echocardiography, cardiopulmonary exercise testing and blood sampling for measurement of plasma concentrations of cardiac peptides. Patients were eligible when they were able to perform exercise without being limited by angina pectoris, pulmonary disorders or peripheral vascular disease. In order to standardize for diurnal variations of measurement parameters all tests were performed in the late morning, 2–3 h after administration of heart failure medication.

Exercise protocol

Symptom-limited exercise was performed on an upright bicycle ergometer (Lode, Groningen, the Netherlands) at a constant pedalling speed of 60 r . m^{-1} with workload increments of 10 Watts . min^{-1}. Heart rate, blood pressure and a 12-lead ECG were monitored during the test. Gas volume and gas concentrations were measured continuously using a breath-by-breath system and a face-mask with a digital volume sensor (Oxycon Champion, Mijnhardt Oxycon Systems, Bunnik, The Netherlands). Oxygen consumption and carbon dioxide production were recorded every 30 s. Peak oxygen consumption (defined as the maximal value measured at the end of the test) was expressed as absolute value divided by actual weight (ml . kg^{-1} . min^{-1}).

Cardiac peptide measurements

Venous blood samples were drawn from a catheter inserted in the left cubital vein after 30 min of supine rest and at peak exercise. The samples were collected in pre-chilled polystyrene tubes containing EDTA and aprotinin, placed immediately on ice and centrifuged within 10 min from sampling in a refrigerated centrifuge at 3000 ***g***. Plasma was separated and stored at −80 °C. Atrial natriuretic peptide measurements were performed in the Cardiovascular Research Laboratory, University Hospital Rotterdam, The Netherlands. Commercially available kits were used for measurement of plasma concentrations of atrial natriuretic peptide (Nichols Institute, Wijchen, The Netherlands) and N-terminal proANP (Biotop, Oulu, Finland), as described previously[18]. Brain natriuretic peptide measurements were performed in the Institute for Surgical Research, University of Oslo, Norway. For this purpose, the plasma neurohormone samples had been shipped on dry-ice to Norway. Plasma brain natriuretic peptide concentrations were measured using an immunoradiometric assay for human brain natriuretic peptide (Shionoria BNP kit, Shionogi & Co. Ltd. Japan)[6]. Plasma N-terminal proBNP concentrations were measured by radioimmunoassay directly in plasma utilizing polyclonal antiserum raised in a rabbit immunized with proBNP(1–21) (Cat no. 9076, Peninsula Laboratories Inc., CA, U.S.A.). The standard curve was set up by serial dilution of proBNP(1–21) in assay buffer. For tracer proBNP (Tyr-1-21) (Medprobe, Oslo, Norway) was iodinated by the Chloramine-T method. There was no detectable cross-reactivity with atrial natriuretic peptide, brain natriuretic peptide or N-terminal proANP. The assay has a limit of detection of 9·7 pmol . l^{-1}, an intra-assay coefficient of variation of 7·3% (sample of 430·7 pmol . l^{-1}) and a recovery of 81·5% of added peptide.

Echocardiography

Two-dimensional echocardiographic examinations were performed in all patients. Left ventricular end-diastolic and end-systolic volumes and ejection fractions were calculated from the apical four-chamber and long-axis views using a biplane disk method. Mean values were obtained by averaging three consecutive beats.

Statistical analysis

Cardiac peptide concentrations are expressed as median (range). Other values are presented as mean ± SD. Cardiac peptide concentrations at rest and at peak exercise were compared using a non-parametric test for paired samples (Wilcoxon). The differences in percentage change between cardiac peptides was tested using a non-parametric test for several related samples (Friedman). All analyses were performed using SPSS for Windows (version 6.1). Statistical significance was defined as $P<0{\cdot}05$.

Results

Study population

The study population consisted of 52 patients, 44 men and eight women, aged 61 ± 10 years. At the time of the

Table 1 Cardiac peptides at rest and at peak exercise

	Rest	Exercise	*P*
ANP (pmol . l^{-1})	83 (14–288)	117 (23–539)	<0·0001
N-terminal proANP (pmol . l^{-1})	592 (99–2417)	628 (116–2507)	0·11
BNP (pmol . l^{-1})	30 (2–383)	38 (2–480)	<0·0001
N-terminal proBNP (pmol . l^{-1})	67 (10–550)	83 (13–699)	<0·0001

Data are presented as median (range). ANP=atrial natriuretic peptide; BNP=brain natriuretic peptide.

study, 10 patients were in New York Heart Association class I, 35 in class II and seven in class III. The mean left ventricular ejection fraction was 32 ± 6%, the mean left ventricular end-diastolic volume was 234 ± 78 ml and the mean left ventricular end-systolic volume was 158 ± 65 ml. The cause of left ventricular systolic dysfunction was ischaemic heart disease in 37 patients and idiopathic dilated cardiomyopathy in 15 patients. All patients were in sinus rhythm and none of the patients had renal failure (serum creatinine ranged from 59 to 148 μmol . l^{-1}). The majority of the patients (n=48) used angiotensin converting enzyme inhibitors, 37 diuretics and 25 digoxin.

Cardiopulmonary exercise testing

All patients performed symptom-limited exercise; the reason for discontinuing exercise was either shortness of breath or fatigue. In all cases, the ratio between carbon dioxide production and oxygen consumption exceeded 1·0, indicating that each patient had made a valid effort. Mean exercise duration was 10 ± 3 min. Heart rate increased from 74 ± 10 beats . min^{-1} at rest to 143 ± 18 beats . min^{-1} at peak exercise. Systolic blood pressure increased from 128 ± 18 mmHg to 176 ± 28 mmHg. Mean oxygen consumption at peak exercise was 17 ± 4 ml . kg^{-1} . min^{-1}.

Cardiac peptides

Plasma concentrations of atrial natriuretic peptide, N-terminal proANP, brain natriuretic peptide and N-terminal proBNP at rest and at peak exercise are presented in Table 1. Exercise induced an increase in atrial natriuretic peptide, brain natriuretic peptide and N-terminal proBNP concentrations. The rise in N-terminal proANP, however, was not significant. There was a difference in percentage increase in response to exercise between the four peptides (P<0·0001, Fig. 1). N-terminal proANP increased less than atrial natriuretic peptide (5 ± 18% vs 59 ± 58%; P<0·0001). The difference in increase between N-terminal proBNP and brain natriuretic peptide was less distinct but still significant (24 ± 24% vs 38 ± 52%, P<0·05). Finally, atrial natriuretic peptide increased more than brain natriuretic peptide (59 ± 58% vs 38 ± 52%, P<0·05) and N-terminal proANP increased less than N-terminal proBNP (5 ± 18% vs 24 ± 24%, P<0·0001).

Discussion

The present study demonstrated that both N-terminal proANP and N-terminal proBNP increased less in response to exercise than their C-terminal counterparts. The rise in plasma concentrations of cardiac peptides in response to exercise can be attributed to augmented secretion due to an increase in filling pressures and heart rate on the one hand, and reduced clearance due to

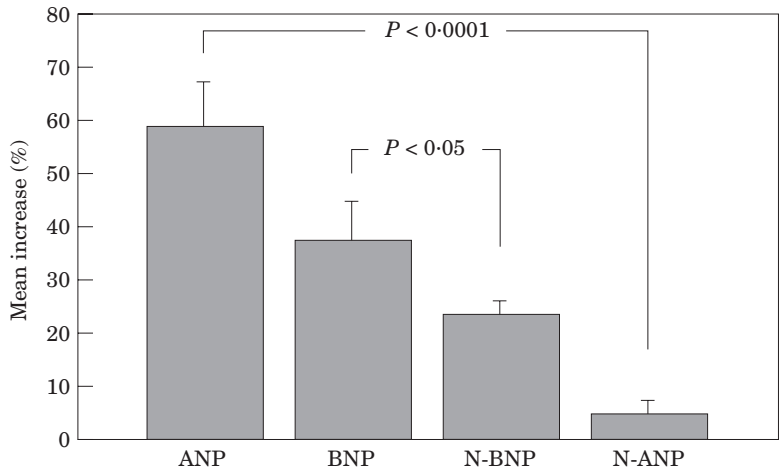

Figure 1 **Percentage increase in atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), N-terminal proBNP (N-BNP) and N-terminal proANP (N-ANP) in response to exercise. Mean values+1 standard error.**

redistribution of blood flow on the other[19]. Previous research showed that both atrial natriuretic peptide and N-terminal proANP increase with exercise in patients with heart failure[20]. As in the present study, the rise in atrial natriuretic peptide was larger than the rise in N-terminal proANP. Similar discrepancies between the responses of N-terminal proANP and atrial natriuretic peptide were found in healthy subjects[2]. Thus, despite the fact that N-terminal proANP and atrial natriuretic peptide are secreted on an equimolar basis, plasma concentrations of N-terminal proANP and atrial natriuretic peptide do not seem to show an equal response to exercise. Because of its short half-life of 2·5 min, atrial natriuretic peptide is rapidly cleared from plasma and its concentrations are low[3]. N-terminal proANP, with its longer half-life, has higher and more stable plasma concentrations[4]. Consequently, the percentage increase in plasma concentrations of N-terminal proANP will be smaller compared to atrial natriuretic peptide. In addition, when the performance of the applied assays is taken into account as well, small changes in N-terminal proANP concentrations will be more difficult to measure than comparable changes in atrial natriuretic peptide concentrations. Half-life differences are therefore likely to be responsible for the differences in absolute and relative changes in response to exercise between the two peptides.

The same can be said for N-terminal proBNP and brain natriuretic peptide, although their plasma concentrations do not differ that much. In addition to brain natriuretic peptide and N-terminal proBNP, a high molecular weight peptide, which is presumed to be the intact proBNP, circulates in human plasma[7]. Intact proBNP contributes to the immunoreactivity of brain natriuretic peptide and thus attenuates the differences between immunoreactive brain natriuretic peptide and N-terminal proBNP concentrations. Strong correlations between N-terminal proBNP and brain natriuretic peptide concentrations suggest that N-terminal proBNP is secreted from the heart along with brain natriuretic peptide, in a similar manner to N-terminal proANP[7,8]. The clearance rate of N-terminal proBNP is not known, but higher plasma N-terminal proBNP concentrations imply that its half-life is longer than that of brain natriuretic peptide[7,8]. The present finding that plasma concentrations of N-terminal proBNP show less increase in response to exercise than brain natriuretic peptide fits well into this hypothesis.

In conformance with previous reports, brain natriuretic peptide was found to be less responsive to exercise than atrial natriuretic peptide[22,23]. Because brain natriuretic peptide has a longer half-life than atrial natriuretic peptide, its plasma concentrations may be less responsive to exercise[24]. Besides, differences in mode of secretion may play a role: atrial natriuretic peptide is pre-stored in granules and secreted rapidly in response to extracellular stimuli, whereas the secretory pattern of brain natriuretic peptide, although not fully understood, is believed to lack a quantitatively important storage mechanism[5].

Study limitations

Plasma cardiac peptide concentrations have been shown to be affected by multiple factors, including age, medication and heart rate[25,26]. For that reason, analyses of differences between patients were avoided, and analyses of differences between peptides were performed with paired tests only. Furthermore, the present study concentrated on patients with heart failure and impaired left ventricular systolic function. Previous research has indicated that changes in brain natriuretic peptide concentrations in response to exercise in these patients differ from the response to exercise in patients with normal left ventricular systolic function[22]. Whether this is also the case for N-terminal proBNP requires further investigations. Finally, in order to ensure that the change in natriuretic peptide concentrations is due to exercise and not to any diurnal pattern, time-matched non-exercising blood samples should be drawn another day.

Practical implications

Elevated plasma concentrations of cardiac peptides have been shown to be of practical use in the diagnostic and prognostic assessment of heart failure[9–17]. However, it is not clear which peptide should be used in clinical practice. In several studies it has been shown that brain natriuretic peptide is a more accurate indicator of left ventricular systolic dysfunction and heart failure than N-terminal proANP and atrial natriuretic peptide[10,11,14,15], but in other studies N-terminal proANP appeared to be a better marker for left ventricular dysfunction and early heart failure[12,13]. The clinical value of N-terminal proBNP has not yet been clarified. Nevertheless, its prognostic value after myocardial infarction and its elevated plasma concentrations and high correlations with brain natriuretic peptide concentrations in patients with heart failure suggest that N-terminal proBNP might have the same predictive qualities as brain natriuretic peptide[16,17]. Important prerequisites for the use of cardiac peptides in clinical routine are the diagnostic or prognostic accuracy of each peptide, the stability of the samples and the blood sampling conditions. Previous studies have demonstrated that, in contrast to what has been found for atrial natriuretic peptide, the in vitro stability of both N-terminal proANP and brain natriuretic peptide is sufficiently high[27–29]. In the present study the in vivo differences between atrial natriuretic peptide, N-terminal proANP, brain natriuretic peptide and N-terminal proBNP were investigated by comparing their responses to exercise. It was shown that both N-terminal peptides increased less in response to exercise than their C-terminal counterparts. This implies that the circumstances under which blood sampling for measurements of N-terminal proANP and N-terminal proBNP should be performed are more favourable than the blood sampling conditions for atrial natriuretic peptide and brain natriuretic peptide. Thus, these results confirm the

superiority of N-terminal proANP for use in clinical practice and suggest that N-terminal proBNP might be a promising new tool for the assessment of heart failure.

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