Increase in plasma concentrations of cardiodilatin (amino terminal pro-atrial natriuretic peptide) in cardiac failure and during recumbency

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SUMMARY  Plasma concentrations of cardiodilatin, the peptide sequence at the amino terminal of the pro-atrial natriuretic peptide, in 17 normal subjects ranged from 59 to 202 (mean 118 (SEM) (9)) pmol/l. Recumbency increased the mean (SEM) concentration to 160 (13) pmol/l. The plasma concentration of cardiodilatin in 24 patients with congestive cardiac failure was much higher (964 (175) pmol/l) than in the normal subjects. It was highest in those with heart failure in New York Heart Association functional classes III and IV and the concentration correlated both with atrial natriuretic peptide concentrations and left ventricular ejection fraction. Concentrations rose during induced tachycardia in three patients tested. Chromatography showed a single clean peak of plasma cardiodilatin immunoreactivity. It seems that cardiodilatin is a second circulating cardiac peptide that is jointly released with atrial natriuretic peptide by common stimuli.

Other workers have reported that, like atrial natriuretic peptide, three partial cardiodilatin sequences can stimulate renal particulate guanylate cyclase and increase cyclic guanosine monophosphate. The simultaneous release of cardiodilatin in higher circulating concentrations than atrial natriuretic peptide may be relevant to the finding that appropriate concentrations of exogenous atrial natriuretic peptide alone do not produce the full renal effects associated with endogenous peptide release.

Cardiodilatin is present in the atrium1-3 as part of the 126 amino acid residue prohormone and in the plasma45 as α-atrial natriuretic peptide, the last 28 amino acids of the carboxy terminal end of the prohormone (fig 1). Its release into the circulation is stimulated by atrial distension caused by plasma volume expansion,67 and concentrations are raised in congestive cardiac failure8-10 and chronic renal failure.1112 Diuresis and natriuresis associated with endogenous release of atrial natriuretic peptide13-15 are greater than when similar plasma concentrations are produced by exogenously administered peptide.91617 This discrepancy could be explained by the additional renal stimulation induced by the altered cardiac and renal nervous activity resulting from atrial distension.18-20 It is also possible that a second cardiac peptide is released with atrial natriuretic peptide and augments its actions. The term cardiodilatin (fig 1) was initially used to describe a porcine cardiac peptide21; it is now used to refer to the amino terminal sequence of the pro-atrial natriuretic peptide.22-24 The suggestion that cardiodilatin is the

Fig 1  Schematic presentation of the atrial natriuretic peptide prohormone. α-Atrial natriuretic peptide is the 28 amino acid residue at the carboxy terminal of the prohormone (Ser99-Tyr126). Cardiodilatin is the peptide derived from the amino terminal sequence of the prohormone. The Asn1-Lys16 sequence (cardiodilatin 1-16) is the fragment used to develop the radioimmunoassay used in this study. The Asn1-Arg87 sequence is cardiodilatin 1-67.

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second cardiac peptide is supported by a recent study that showed that three different amino terminal fragments of the prohormone stimulated renal particulate guanylate cyclase activity and increased cyclic guanosine monophosphate in vitro.25

We developed a specific radioimmunoassay for circulating concentrations of cardiodilatin. We used it to study normal subjects during postural change and patients with congestive cardiac failure and during tachycardia.

**Patients and methods**

**Cardiodilatin radioimmunoassay and chromatography**

We used pure synthetic human Asn1-Lys18 amino terminal pro-atrial natriuretic peptide (cardiodilatin 1–16 (fig 1) Peninsula Laboratories, Merseyside) to develop the radioimmunoassay.26 Antisera were raised in rabbits and the one used in the assay (final dilution 1:14000) showed 44% cross reactivity with Asn1-Arg67 pro-atrial natriuretic peptide (cardiodilatin 1–67 (fig 1) but no cross reactivity with α-atrial natriuretic peptide. Its cross reactivity with the prohormone or with the complete cardiodilatin 1–98 molecule is not known (synthetic standards were not available). Therefore, the cardiodilatin concentrations measured by the assay, and reported here, are expressed as cardiodilatin 1-16 immunoreactivity equivalents. The radioactive tracer was produced by chloramine T iodination of cardiodilatin 1-16 (sodium iodide123, Amersham IMS 30) and purified by reverse phase high pressure liquid chromatography. Standard curves were constructed with cardiodilatin 1–16.

Plasma samples (50 or 100 μl) were assayed directly for cardiodilatin immunoreactivity. Assays were incubated at 4°C for four days and separated by dextran coated charcoal (12 mg charcoal:1-2 mg dextran). The detection limit of the assay was 2 fmol/tube and the intra and inter assay coefficients of variation were 7% and 11% respectively. Plasma atrial natriuretic peptide was measured after plasma extraction as described previously.12

Plasma cardiodilatin immunoreactivity was characterised by gel permeation chromatography on a column containing G-100 Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden) and by fast protein liquid chromatography with a peptide reversed phase chromatography high resolution 5/5 (PepRPC HR5/5) C-18 column (Pharmacia). Samples of neat plasma were loaded onto the gel column. This was eluted with assay buffer (containing 0-2 mol/l sodium chloride) at 1-6 ml per 20 minutes per fraction. With each sample run, dextran blue, cytochrome c, and sodium iodide123 were included as markers of the void volume, molecular size, and total volume. Samples of each fraction were assayed for cardiodilatin immunoreactivity and the elution position was determined.27 Plasma samples were treated with ethanol to precipitate excess protein before fast protein liquid chromatography. After centrifugation the supernatant was dried in a Savant vacuum centrifuge and the resulting pellet was reconstituted in water (with 0-05% trifluoroacetic acid), passed through a 0-2 μm filter (Minisart, Sartorius-Instruments, Surrey) and the filtrate was injected onto the column. This was equilibrated with 20% (v/v) acetonitrile in water (each with 0-05% trifluoroacetic acid). After injection of a sample the column was eluted with a gradient of acetonitrile from 20% to 40% (v/v) in water over one hour at 1 ml per minute per fraction. Samples of each fraction were assayed for cardiodilatin immunoreactivity. Both columns were calibrated with cardiodilatin 1–67 before and after a series of sample runs.

**Posture study**

We studied 17 normal subjects (14 men, three women, aged 21–42, mean (SD) (28 (5) yr) between 0900 and 1000 after an overnight fast. We collected three samples of peripheral venous blood at the start of the study with the subjects sitting and after one hour in the supine position with a 30° passive elevation of the legs. All samples were collected into edetic acid tubes and centrifuged immediately. The plasma was separated and stored at −20°C until assay.

**Patients**

We studied 24 patients (21 men, three women) aged 23–77 (54 (9)) with stable chronic congestive cardiac failure. The aetiology of the cardiac failure was ischaemic (n = 15) or dilated (n = 9) cardiomyopathy. Three patients were in New York Heart Association functional class I, eight in class II, four in class III, and three in class IV. Left ventricular ejection fraction was measured in 21 patients by radionuclide ventriculography 25 within 48 hours of blood sampling. The patients were on maintenance drug treatment as follows: diuretics (24), angiotensin converting enzyme inhibitors (10), nitrates (4), digoxin (4), aspirin/antiaggregants (10), β-blockers (1), and amiodarone (2). Samples of peripheral venous blood were taken between 0800 and 0900 after an overnight fast with the patient in the supine position and processed as described above. Samples were also taken from three patients before and during tachycardia, induced by an extra systole and maintained for 10 minutes, in the course of electrocardiological physiological studies for the investigation of tachycardias.
Plasma cardiodilatin in cardiac failure

Statistical Analysis
Concentrations of plasma cardiodilatin (expressed as cardiodilatin 1-16 immunoreactivity equivalents) and atrial natriuretic peptide are given as mean (SEM). We used a two-tailed Student’s t test for paired or unpaired variables and linear regression analysis of log transformed data (least squares method).

Results

Effect of Posture Change
The basal plasma concentrations of cardiodilatin in normal subjects ranged from 59 to 202 pmol/l. Mean (SEM) plasma cardiodilatin increased significantly from 118 (9) pmol/l in the sitting position to 160 (13) pmol/l (range 99–264) when the subject was supine (p < 0.001).

Patients with Congestive Cardiac Failure
Plasma concentrations of cardiodilatin were much higher (143–3280 (964 (175)) pmol/l (p < 0.001) in patients with congestive cardiac failure. Plasma atrial natriuretic peptide was 34 (9) (4–190) pmol/l. The correlation (fig 2a) between the two peptides was highly significant (r = 0.74, p < 0.001). Both cardiodilatin (fig 2b) and atrial natriuretic peptide showed strong negative correlations with left ventricular ejection fraction (r = −0.66 and −0.67 respectively; p < 0.001). Both peptides were significantly higher in patients in New York Heart Association classes III and IV than in classes I and II (cardiodilatin 1340 (280) and 519 (81) pmol/l (p < 0.02); atrial natriuretic peptide 54 (15) and 13 (4) pmol/l (p < 0.02).

Tachycardia
A mean (SD) rise in heart rate from a basal level of 71 (5) to 180 beats/min was accompanied by a peak increase in plasma cardiodilatin of 601 (33%), 47 (26%), and 291 (74%) pmol/l from basal values in the three patients studied.

Chromatography
Gel permeation chromatography (fig 3a and b) of plasma samples from normal volunteers (n = 8) and patients with cardiac failure (n = 4) showed a single discrete peak of cardiodilatin immunoreactivity with identical elution coefficients of 0.36, eluting earlier than synthetic cardiodilatin 1–67 (elution coefficient of 0.44). The total cardiodilatin immunoreactivity recovered in this peak was 85–105% of the concentrations measured in the original direct plasma assay.

Fast protein liquid chromatography (fig 3c and d) confirmed the presence of a single peak of cardiodilatin immunoreactivity that was identical in normal plasma and in plasma from patients with cardiac failure and eluted at a greater acetonitrile concentration than synthetic cardiodilatin 1–67.

Discussion
Atrial natriuretic peptide and cardiodilatin are the two peptides present at the carboxy and amino terminals of the atrial natriuretic peptide prohormone respectively. Although atrial cells in culture secrete the prohormone, studies on the isolated perfused mammalian heart and intact animals indicate that atrial natriuretic peptide alone and not the prohormone is secreted by the atrium. This was confirmed by chromatography and amino acid sequence analysis of the circulating peptide in man and in the rat.

The present data demonstrate that cardiodilatin also circulates in man. In the human atrium cardiodilatin immunoreactivity is present not only as part of the prohormone but also as a separate
molecular form that lacks atrial natriuretic peptide immunoreactivity and represents the cleaved amino terminal portion of the prohormone.\(^2^6\) Atrial and plasma cardioldilatin immunoreactivity are chromatographically identical and correspond to a smaller and less hydrophobic molecule than pro-atrial natriuretic peptide but larger and more hydrophobic than cardioldilatin 1–67. Therefore the Arg\(^9^7\) residue of the prohormone (fig 1) is presumably not a site for proteolysis as was previously suggested.\(^2^3\) Instead, the Arg\(^9^8\)–Ser\(^9^9\) bond seems the most likely cleavage site, simultaneously generating atrial natriuretic peptide\(^3^1\) \(^3^8\) and cardioldilatin, with the latter containing the entire amino-terminal sequence of the prohormone. Preliminary evidence of coeluting cardioldilatin immunoreactive peaks, on chromatography, detected by the present antisera and by an antiserum raised to the sequence lys\(^9^7\)–arg\(^9^8\) of the prohormone supports this claim (L Meleagros et al, unpublished observations).

A second, circulating, cardiac peptide derived from the atrial natriuretic peptide prohormone would be expected to show a similar pattern of release as atrial natriuretic peptide itself. Atrial distension in vitro\(^3^2\) \(^3^3\) and in vivo\(^3^6\) \(^3^7\) \(^3^3\) is a recognised stimulus for such release. We found that in normal subjects a supine posture with passive leg elevation increased plasma cardioldilatin. This stimulus is also known to increase atrial natriuretic peptide\(^4^1\) \(^3^7\) \(^3^8\) and was attributed to increased intrathoracic blood volume causing atrial distension.

Abnormal atrial distension in congestive cardiac failure was also associated with raised concentrations of atrial natriuretic peptide.\(^6^\) \(^9^\) \(^1^0^\) The present study shows that plasma cardioldilatin was also greatly increased and correlated with atrial natriuretic peptide in patients with congestive cardiac failure. The relations between atrial natriuretic peptide and left ventricular ejection fraction\(^8^9\) \(^3^9\) and New York Heart Association functional class\(^2^0\) \(^4^0\) were confirmed in our study. We also found that cardioldilatin was significantly related to both these variables. Because cardioldilatin, unlike atrial natriuretic peptide, does not require an initial laborious extraction step,\(^5^\) \(^1^5\) \(^3^1\) \(^3^2\) direct assay of plasma cardioldilatin may be a useful index of disease severity and possibly of the chronic response to treatment in congestive cardiac failure. Supraventricular tachycardia increases atrial pressure and circulating atrial natriuretic peptide.\(^2^2\)

Similarly, in the present study, tachycardia was associated with a rise in plasma cardioldilatin.

Thus the evidence suggests that two cardiac peptides, atrial natriuretic peptide and cardioldilatin, behave similarly both during physiological atrial distension and pathological atrial distension. Cardioldilatin circulates as a single molecular form, perhaps containing the entire amino terminal sequence of the pro-atrial natriuretic peptide. Any amino terminal fragment of the prohormone in the rat had a longer plasma half life\(^4^2\) and this may be the reason why we found higher concentrations of cardioldilatin than atrial natriuretic peptide.

Atrial natriuretic peptide was first recognised as a physiological regulator of sodium and water balance when the correlation between changes in its plasma concentrations and alterations in plasma volume was identified.\(^6^\) \(^4^3\) The present data show the same relation between cardioldilatin and sodium and water balance. There is still uncertainty about why a modest rise in endogenous\(^1^3\) \(^1^4\) \(^1^5\) plasma atrial natriuretic peptide has a much greater effect on the kidney than similar concentrations achieved by exogenous infusion.\(^4^4\) Plasma concentrations need to be at least twice as high\(^1^7\) to mimic the renal effects associated with endogenously released atrial natriuretic peptide.

Other factors that are independent of atrial natriuretic peptide could influence renal function in physiological circumstances but not during exogenous peptide infusions. Certainly, cardiac denervation abolishes the diuresis and natriuresis induced by stretching the atrium.\(^1^8\) \(^1^9\) This observation, however, does not diminish the role that atrial natriuretic peptide may have in the regulation of renal function.
This is because atrial stretching in these studies\textsuperscript{18,19} probably did not continue long enough\textsuperscript{16} for the actions of atrial natriuretic peptide to become manifest. Cardiac denervation abolishes the inhibitory effect of vagal afferent stimulation upon renal sympathetic tone,\textsuperscript{20} and this may have resulted in the persistence of renal vasoconstriction that could not be overridden by the actions of atrial natriuretic peptide. In one study no renal effects were observed after atrial stretch that caused a 400\textperthousand increase in plasma atrial natriuretic peptide, but an increase in plasma concentration of almost 1500\textperthousand after exogenous peptide infusion was also ineffective.\textsuperscript{18} Thus either the experimental period was too short, or the dog model, which was used exclusively in these experiments,\textsuperscript{18,19} may be unsuitable. The finding that atrial natriuretic peptide blockade by monoclonal antibodies abolishes the renal effects of atrial distension,\textsuperscript{40} further emphasises the role of this peptide.

It seems likely that the renal changes that follow atrial stretch are the result of cardiac nerve stimulation and the actions of atrial natriuretic peptide. In addition to these, a second cardiac peptide may also influence renal function. It is therefore of interest that three different amino terminal fragments of proatrial natriuretic peptide, which together make up almost the entire cardiodilatin sequence, were recently reported to be nearly as potent as the atrial natriuretic peptide itself in stimulating particulate guanylate cyclase activity and increasing cyclic guanosine monophosphate in renal tissues in vitro.\textsuperscript{25} This finding together with the evidence presented in this paper, of concomitant release of atrial natriuretic peptide and cardiodilatin, suggest a possible role for this second cardiac peptide in potentiating the effects of endogenous atrial natriuretic peptide and may help to explain why exogenous peptide does not have the same renal effects as endogenous peptide.

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