Elevated plasma C-type natriuretic peptide concentrations in patients with chronic renal failure

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INTRODUCTION

The natriuretic peptide family consists of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) [1]. These peptides have potent hypotensive, diuretic and natriuretic activities. The hypotensive, diuretic and natriuretic effects of CNP are not so potent as those of ANP in rats [1]. However, subsequent studies using dogs [2, 3] showed that CNP has potent hypotensive effects, but does not have diuretic and natriuretic effects when infused continuously or injected as a bolus.

ANP and BNP are cardiac hormones. High concentrations of immunoreactive (IR-) ANP and IR-BNP are present in the cardiac tissue [4, 5], and the plasma concentrations of these peptides are elevated in patients with diseases accompanied by volume overload, such as heart failure and chronic renal failure (CRF) [5-7].

On the other hand, CNP is thought to be either a neuropeptide or a vascular peptide. CNP was originally isolated from porcine brain [1]. High concentrations of IR-CNP are found in the central nervous system [8, 9], but CNP concentrations in cardiac tissue are very low or undetectable [8]. Recent studies have shown that CNP is also produced by the vascular endothelial cell [10, 11].

Thus, the pathophysiological roles of CNP seem to be different from those of ANP and BNP. The presence of IR-CNP in human plasma has recently been demonstrated [11], and plasma IR-CNP concentrations are not elevated in patients with congestive heart failure (CHF) [12]. However, plasma IR-CNP concentrations in patients with various diseases have not been studied and the IR-CNP in plasma has not been characterized by h.p.l.c. We have developed a sensitive and specific r.i.a. for CNP [9] which is able to detect IR-CNP in human plasma. In the present study, plasma IR-CNP concentrations were studied in patients with CHF and patients with CRF, in whom plasma IR-ANP and IR-BNP concentrations were known to be elevated.

Key words: atrial natriuretic peptide, brain natriuretic peptide, C-type natriuretic peptide, chromatography, haemodialysis, heart failure, plasma, radioimmunoassay, renal failure.

Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHF, congestive heart failure; CNP, C-type natriuretic peptide; CRF, chronic renal failure; IR, immunoreactive.

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The peptides were extracted from plasma with a Sep-Pak C<sub>18</sub> cartridge (Waters, Milford, MA, U.S.A.) as described previously [11]. The recoveries were 78 ± 4% to ANP-28, 86 ± 4% to human BNP-32 and 85 ± 2% to CNP-22 (n = 6, means ± SEM). The plasma extract was reconstituted in assay buffer [0.1 mol/l phosphate buffer, pH 7.7, containing 0.1% (v/v) human serum albumin, 0.2% (v/v) Triton X-100 and 0.1% (w/v) sodium azide] and assayed.

Plasma concentrations of ANP, human BNP and CNP were measured with the respective r.i.a. as reported previously [6, 9, 14]. The ANP assay showed less than 0.01% cross-reaction with human BNP-32, CNP-22 and CNP-53. The human BNP assay showed less than 0.01% cross-reaction with human ANP-28, CNP-22 and CNP-53. CNP-22 was used as standard in the CNP assay, which showed 100% cross-reaction with CNP-53 and less than 0.01% cross-reaction with human ANP-28 and human BNP-32. Intra- and inter-assay coefficients of variation were less than 10% and 12%, respectively, in all r.i.a.s.

**Methods**

**Subjects**

Blood samples were obtained from 59 subjects (34 males and 25 females, aged 17–86 years, mean 49.7 years), including 26 normal subjects (17 males and nine females, 31.0 ± 15.2 years old, mean ± SD), 11 patients with CHF (New York Heart Association class II–IV; five males and six females, 74.0 ± 7.9 years old), nine non-dialysed patients with CRF (five males and five females, 58.4 ± 12.7 years old) and 13 haemodialysis patients with CRF (eight males and five females, 61.8 ± 7.2 years old). Serum creatinine levels in non-dialysed patients with CRF ranged from 204 to 1282 μmol/l (548 ± 362 μmol/l), whereas those in normal subjects and patients with CHF were within the normal range (<110 μmol/l). Pulmonary congestion was noted on the chest roentgenogram in six out of 11 patients with CHF, but not in non-dialysed patients with CRF and dialysed patients with CRF. The clinical characteristics of patients with CRF are summarized in Table 1. Informed consent was obtained from each subject.

The blood was collected in a chilled tube containing EDTA (1 mg/ml of blood) and Trasylol (500 units/ml of blood), and was centrifuged at 3000 rev./min for 10 min at 4°C. The plasma was stored at −30°C until extraction.

**Extraction and r.i.a.**

The peptides were extracted from plasma with a Sep-Pak C<sub>18</sub> cartridge (Waters, Milford, MA, U.S.A.) as described previously [11]. The recoveries were 78 ± 4% to ANP-28, 86 ± 4% to human BNP-32 and 85 ± 2% to CNP-22 (n = 6, means ± SEM). The plasma extract was reconstituted in assay buffer [0.1 mol/l phosphate buffer, pH 7.7, containing 0.1% (v/v) human serum albumin, 0.2% (v/v) Triton X-100 and 0.1% (w/v) sodium azide] and assayed.

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**Chromatography**

Chromatographical characterization of the IR-ANP, IR-human BNP and IR-CNP in plasma was performed by reverse-phase h.p.l.c. using a column of μ-Bondapak C<sub>18</sub> (3.9 mm × 300 mm; Waters). Pooled plasma from normal subjects (20–33 ml) and haemodialysis patients (3–22 ml) was extracted by Sep-Pak C<sub>18</sub> cartridges and loaded on to the column. The column was eluted with a linear gradient from 10% to 60% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min per fraction over 50 min. Fractions (1 ml) were collected, dried in air, reconstituted and assayed. CNP-22 and CNP-53 eluted in an identical position in this acetonitrile gradient system [9]. Therefore, IR-CNP in plasma extract was also examined with a linear gradient from 30% to 70% methanol in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min per fraction over 50 min.

**Statistics**

Plasma concentrations of the peptides are expressed as means ± SEM. Statistical analysis was performed by one-way analysis of variance, followed by Dunnett’s test for multiple comparisons. The paired t-test was used for paired comparisons. Linear regression analysis was used for the determination of correlations among results and clinical parameters.

**RESULTS**

IR-CNP was detected in all plasma samples examined. Dilution curves of the plasma extracts from normal subjects and haemodialysis patients were parallel with the standard curve for CNP-22 (data not shown).

The plasma IR-CNP concentration in normal subjects ranged from 1.4 to 7.5 (4.4 ± 0.4) pmol/l. Plasma IR-CNP levels were greatly elevated in patients with CRF (non-dialysed, 13.0 ± 4.2 pmol/l, P < 0.01 compared with normal subjects; haemodialysis, 16.1 ± 2.1 pmol/l, P < 0.01) but not in patients with CHF (3.0 ± 0.7 pmol/l, P > 0.05). ANP and human BNP levels were elevated in patients with CHF and haemodialysis patients (Fig. 1). ANP and human BNP levels were elevated in non-
C-type natriuretic peptide in human plasma

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Fig. 1. Plasma concentrations of IR-CNP (a), IR-ANP (b) and
IR-human BNP (c). The SEMs are represented by vertical bars, and
significant differences are indicated as *P<0.05 and **P<0.01 with respect
to the corresponding values of normal subjects and as †P<0.01 with
respect to the corresponding values 'before haemodialysis'. Abbreviation:
HD, haemodialysis.

dialysed patients with CRF, but this elevation was not statistically significant (P>0.05).

In haemodialysis patients, there was a significant correlation between plasma ANP and human BNP concentrations (r=0.84, P<0.001), but there were no significant correlations between plasma IR-CNP concentrations and plasma ANP or human BNP concentrations. Plasma levels of IR-CNP in haemodialysis patients did not change during haemodialysis sessions (from 16.1±2.1 to 14.3±1.7 pmol/l, P>0.05), whereas those of ANP (P<0.01) and human BNP (P<0.01) decreased (Fig. 1). The changes in plasma IR-CNP or IR-human BNP concentrations during haemodialysis did not correlate with the body weight losses, whereas the changes in plasma ANP concentrations correlated significantly with the body weight losses (r=0.60, P<0.05) (Fig. 2).

H.p.l.c. using the acetonitrile gradient system showed that the IR-ANP, IR-human BNP and IR-CNP in the plasma extracts from normal subjects and haemodialysis patients were mainly eluted in the positions of the respective synthetic peptides; human ANP-28, human BNP-32 and human CNP-22/human CNP-53 (Fig. 3). H.p.l.c. of the plasma extracts using the methanol gradient system revealed two major immunoreactive peaks co-migrating with synthetic human CNP-53 and CNP-22, respectively (Fig. 4).

DISCUSSION

In the present study, we have shown for the first time the greatly elevated plasma IR-CNP concentrations in patients with CRF, an approximately 4-fold increase compared with control subjects. On the other hand, plasma IR-CNP concentrations in patients with CHF were not elevated, whereas plasma IR-ANP and IR-BNP concentrations were elevated both in patients with CHF and in haemodialysis patients. The changes in plasma IR-CNP concentrations were not parallel to weight losses in haemodialysis patients, suggesting that the change in body fluid volume is not a major factor causing release of CNP. These findings may reflect the fact that CNP is not a cardiac peptide. Plasma CNP may be derived from different sources to those of ANP and human BNP, presumably from nervous tissue or vascular endothelial cells [10, 11]. H.p.l.c. has revealed that IR-CNP in plasma consists of two molecular forms identical with or very similar to CNP-22 and CNP-53. The h.p.l.c. profile is compatible with the molecular profile of IR-CNP in conditioned media of cultured bovine endothelial cells reported by Suga et al. [10].

In CRF, volume overload is caused by impaired water and electrolyte secretion from the kidney. Elevation of plasma concentrations of vasoconstrictor substances such as endothelin-1 [13] and neuropeptide Y [15] also occurs. In addition, the compensatory defence mechanism may be evoked for the overhydration and the vasoconstriction. This mechanism includes increases in circulating vasodilator peptides, ANP [6], BNP [7] and calcitonin-gene-related peptide [16]. The present study has suggested that another novel vasodilator, CNP, is also working in the mechanism of defence against overhydration and vasoconstriction in patients with CRF. CNP produced by the vascular endothelial cells may act directly on the vascular smooth muscle cells. Thus, CNP may participate in the regulation of cardiovascular and body fluid homoeostasis.
Fig. 3. Analysis of IR-CNP, IR-ANP and IR-human BNP in pooled plasma from normal subjects (A) and haemodialysis patients (B) by reverse-phase h.p.l.c. with a linear gradient of acetonitrile. The arrows indicate the elution positions of synthetic human ANP-28 (a), human BNP-32 (b) and CNP-22/CNP-53 (C1), respectively. CNP-22 and CNP-53 were eluted in an identical position in this system.

Fig. 4. Analysis of IR-CNP in pooled plasma from normal subjects (a) and haemodialysis patients (b) by reverse-phase h.p.l.c. with a linear gradient of methanol. The arrows indicate the elution positions of synthetic human ANP-28 (a), human BNP-32 (b), CNP-22 (C1) and CNP-53 (C2), respectively.

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