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C type natriuretic peptide production by the human kidney is blunted in chronic heart failure

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Abstract

C-type natriuretic peptide (CNP) is a vasodilatory peptide produced by vascular endothelium and human heart with a short half-life. CNP has been identified within the human kidney. Few data are available on whether the human kidney is a systemic source of CNP. The aim of the current study was to establish whether CNP is secreted by human kidney and whether synthesis is blunted in chronic heart failure (CHF). 20 male subjects (age 57 ± 2 years, mean \pm SEM) undergoing CHF assessment ($n=13$) or investigation of paroxysmal supraventricular arrhythmia ($n=7$, normal left ventricular function, in sinus rhythm during procedure) were recruited. Renal CNP production was determined from concomitant plasma concentration in aorta and renal vein. When considering all subjects, a significant step-up in plasma CNP was found from aorta to renal vein (3.0 ± 0.3 vs 8.3 ± 2.4 pg/mL, respectively, $p=0.0045$). The mean increase in CNP was 5.3 ± 2.4 pg/mL (range -0.9 to 45.3 pg/mL). In patients with CHF, aortic concentration was 3.3 ± 0.4 pg/mL as compared with renal vein concentration 4.3 ± 0.6 pg/mL ($p=0.11$). In those with normal left ventricular function the respective values were 2.5 ± 0.5 pg/mL and 15.7 ± 6.0 pg/mL ($p=0.01$). CNP is synthesised and secreted into the circulation by the normal human kidney, where it may have paracrine actions. Net renal secretion of CNP appears blunted in patients with CHF.

Introduction

The natriuretic peptide family comprises structurally related vasoactive peptides with similar physiological properties. Clinical and research interests have focused on atrial (ANP) and B-type natriuretic peptide (BNP) and their role in circulatory homeostasis [1]. Much less is known about the pathophysiological role of C-type natriuretic peptide (CNP) in humans [2]. C-type natriuretic peptide exerts its biological effects via an interaction with a specific transmembrane receptor (natriuretic peptide receptor-B). In comparison to ANP and BNP, CNP has a much shorter circulatory half-life [3]. CNP has been detected at low concentration in the plasma of healthy human subjects, but, unlike ANP and BNP, initial studies found that peripheral venous plasma levels of CNP do not appear to be significantly elevated in patients with chronic heart failure (CHF) [4,5].

C-type natriuretic peptide has been identified in a number of human tissues. CNP immunoreactivity has been demonstrated in cultured human endothelial cells, whilst its receptor is located in high concentrations in adjacent vascular smooth muscle cells [6]. Studies with isolated arteries and veins have confirmed vasodilator effects of CNP [7]. These findings, together with its short circulatory half-life, have led to the suggestion that CNP exerts effects locally within the vascular wall.

We have previously shown that in patients with CHF, the heart synthesises CNP [8]. The presence of CNP in human atrial and ventricular myocardial tissue has been confirmed by immunohistochemistry and radioimmunoassay; atrial and ventricular myocardial levels of CNP were significantly elevated (100 – 200%) in patients with CHF in comparison to controls ($p < 0.05$) [4]. mRNA transcripts for the CNP receptor have also been detected in the human heart [9]. Thus CNP may have important local paracrine effects adjacent to its site of production.

In the human kidney, CNP is located in cells of all tubular segments and glomeruli [10]. Studies in humans with CHF have provided conflicting results on whether urine CNP is altered or not [11-13]. To clarify the issue of renal CNP production, we measured CNP simultaneously in both the aorta and the renal vein in subjects with normal ventricular function and in patients with CHF. We hypothesised that renal production would be greater in patients with CHF.

Further studies were undertaken to assess urinary CNP excretion in both healthy individuals and those with CHF.

Methods

Assessment of renal CNP production

Renal CNP production was assessed by determining the difference in plasma concentration between the aorta and renal vein. The objectives of the study were to establish whether or not the kidney produces CNP and whether this is different in patients with CHF as compared with healthy subjects. Patients undergoing diagnostic right and left heart catheterisation as part of heart failure assessment were recruited. The diagnosis of CHF was based on symptoms, examination and relevant investigations confirming left ventricular dilatation and impairment of systolic

function (chest radiograph, echocardiogram and cardiac magnetic resonance imaging) in accordance with published guidelines [14].

It is notoriously difficult to recruit a homogeneous population of healthy control subjects for a catheter laboratory based study. Exposure to ionising radiation and invasive catheterization raises major ethical concerns if there is no clinical mandate to undertake evaluation of any sort. We wished to make a comparison of patients with CHF with a group with essentially normal cardiac structure, and in particular normal left ventricular function. Otherwise healthy subjects with paroxysmal supraventricular arrhythmia (in sinus rhythm at the time of study procedure and without symptoms > 24hours) undergoing diagnostic or therapeutic electrophysiology study were recruited as the 'control' population. Sampling was performed *prior to* induction of arrhythmia.

The study conforms to the principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Royal Brompton Hospital local ethics committee. Written informed consent was obtained from all participants.

Confirmation of renal vein sampling was made by selective renal angiography. Blood from the renal vein and aorta was collected into chilled EDTA tubes containing aprotinin (trasylo). Samples were centrifuged for 15 minutes at 3000 rpm and plasma phase stored at -80°C. CNP measurements were performed by a blinded operator using standard competitive radioimmunoassay kits (Peninsula Laboratories, Bachem Ltd UK) after solid phase extraction from plasma proteins as described previously [5]. The intra-assay coefficient of variation was 11.4%. There was no demonstrable cross-reactivity of the anti-CNP antibody used with human ANP or BNP. The lower limit of detection for the CNP assays is 0.1 pg/mL.

Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation [15].

Patients with chronic heart failure

Thirteen patients with CHF were recruited for the study. Baseline characteristics are shown in Table 1 (all male, age 60±3 years). No patient had significant renal failure (mean creatinine 100±7 µmol/L), evidence of active infection, inflammation or diabetes. All were receiving an angiotensin converting enzyme inhibitor (n=12) or an angiotensin receptor blocker (n=1), 8 were receiving beta-blockers and 9 diuretic therapy (see Table 1 for doses of furosemide).

Subjects with paroxysmal supraventricular arrhythmia and normal left ventricular function (control group)

Nine subjects were recruited (Table 2). Of these two were in arrhythmia at the time of sampling. As such they were excluded from the main analysis, although individual data are presented for interest. The remaining 7 subjects were all male (age 53±4 years). Five had history of paroxysmal atrial fibrillation and two atrioventricular nodal tachycardia. All had normal resting left ventricular systolic function assessed by echocardiograph, and were asymptomatic when not in arrhythmia. One individual (patient 3) had undergone prior angioplasty to his right coronary artery and was subsequently well without angina and had a normal exercise test prior to study. Another (patient 7) had history of atrioventricular node ablation and dual chamber pacemaker implant. A further individual (patient 4) was found by transthoracic echo

to have a small patent foramen ovale/atrial septal defect but had normal right heart size and pressures (see Table 2 for details of the respective individuals).

Three of the 7 seven subjects in sinus rhythm at the time of sampling were receiving anti-arrhythmic medication. Two (subjects 1 and 3, Table 2) were on Amiodarone, whilst one individual (subject 5) was on a combination of flecainide and sotalol.

Assessment of urinary CNP excretion

Subsequent to the analysis of the data from the cross kidney sampling study we felt it important to undertake a further study to clarify whether urinary CNP excretion is different in patients with CHF and healthy individuals. Prior to recruiting subjects to this additional study we determined whether urinary CNP is influenced by the methodology relating to collection conditions. A series of studies demonstrated good agreement of CNP measurements irrespective of whether urine was collected into containers containing aprotinin or surrounded by ice, according to methodology recommended by standard Bland and Altman plots (data not shown). For ease of convenience we performed standard 24 hour urine collections thereby enabling the study to be performed on an outpatient basis.

A different cohort of patients with stable CHF (n=16) on standard medication and healthy control subjects (n=7, asymptomatic, no history of prior cardiovascular disease, normal cardiovascular assessment) were recruited. Patients and healthy control subjects were similar in terms of age (68 ± 2 versus 67 ± 2 years, respectively, $p=0.8$) and renal function (estimated glomerular filtration rate, 68 ± 6 versus 79 ± 5 ml/min/1.73m², respectively, $p=0.23$).

Peripheral venous plasma samples were also taken for N-terminal proBNP in this cohort of subjects. These were analysed in the biochemistry laboratory at the Royal Brompton Hospital, London using the Roche Diagnostics NT proBNP kit on a Roche 1010 immunoassay analyser. Cross reactivity with ANP, BNP and CNP is all $<0.001\%$. The within assay co-efficient of variation is approximately 2.2%.

The 24-hour urine collection started with the first urine specimen in the morning of day 1 of the study and included all samples up to but not including the first morning specimen on day 2. At the end of the collection the total urine volume was noted and samples aliquoted from each container frozen and stored at -80°C until analysis.

Statistics

Data were analyzed with StatView 4.5 (Abacus Concepts Inc) and are expressed as mean \pm SEM. Because of the skewness of CNP concentrations, logarithmic transformation was used. Differences between sample sites were assessed by paired *t* test. Unpaired *t* test was used to assess differences between groups. In view of the fact comparisons were made between a number of *t* tests, analysis with repeated measures ANOVA was also performed. A probability value of <0.05 was considered statistically significant.

Results

When considering all individuals (n=20, all male) a significant step up from aorta to renal vein was seen (3.0 ± 0.3 vs 8.3 ± 2.4 pg/mL, respectively, $p=0.0045$). The mean increase in CNP was 5.3 ± 2.4 pg/mL (range -0.9 to 45.3 pg/mL).

Patients with CHF were similar to the control group in terms of age ($p>0.15$) and renal function (Creatinine 100 ± 7 vs 94 ± 6 $\mu\text{mol/L}$, eGFR 75 ± 5 vs 79 ± 5 mL/min/1.73m², respectively, both $p=0.5$). In patients with CHF there was no significant difference in CNP concentration between aorta (3.3 ± 0.4 pg/mL) and renal vein (4.3 ± 0.6 pg/mL, $p=0.11$), (Table 1, Figure 1). In contrast individuals with normal left ventricular function in sinus rhythm demonstrated a significant step up from aorta (2.5 ± 0.5 pg/mL) to renal vein (15.7 ± 6.0 pg/mL, $p=0.01$), (Table 2, Figure 2).

Concentration of CNP in the aorta was similar in patients with CHF and individuals with normal left ventricular function ($p=0.28$). In contrast, CNP concentration was significantly higher in the renal vein in those with normal left ventricular function when compared to patients with CHF ($p=0.025$).

Additional analysis with repeated measures ANOVA was performed. The difference between renal vein and aortic CNP concentration ' ΔCNP : renal vein-aorta, pg/mL' was the dependent variable, the absence/presence of heart failure as a factor and eGFR as a covariate. This confirmed a significantly higher step up in plasma CNP from aorta to renal vein in the subjects without heart failure (i.e. controls) as compared with the patients with CHF (mean difference 12.3 pg/mL between groups, $p=0.017$).

Two patients in the 'control' group went into tachyarrhythmia (heart rate of 180 for patient 8 and 150 for patient 9) at the time of the study, prior to sampling. Whilst they were excluded from the main analysis, relevant data are presented in Table 2 and Figure 2. A step up in plasma CNP concentration from aorta to renal vein was seen in both individuals.

No relation was seen between the step up in CNP from aorta to renal vein and renal function (as estimated by serum Creatinine or eGFR, $p>0.9$). There was no relation between resting heart rate and concentration of CNP in renal vein ($p>0.2$).

Urinary CNP

24 hour urine volumes (1756 ± 148 vs 2219 ± 259 mL, respectively, $p=0.11$) and urine CNP concentration (28.6 ± 2.0 vs 23.9 ± 1.7 pg/mL, respectively, $p=0.17$) were similar in patients with CHF and control subjects. The rate of urinary CNP excretion was similar in patients with CHF and healthy control subjects (32.4 ± 1.9 versus 35.3 ± 2.4 pg/mL/min, respectively, $p=0.3$). Plasma concentrations of CNP were not significantly different between groups, although values on the whole were low (data not shown). In contrast, patients with CHF exhibited higher concentrations of circulating N-terminal brain natriuretic peptide when compared to the control group (281.1 ± 123.8 versus 39.1 ± 24.5 pmol/L, $p<0.05$). There was no relation between the rate of urinary CNP excretion and estimated glomerular filtration rate ($R^2=0.005$, $p=0.7$).

Discussion

The current study has shown that CNP is synthesised and secreted by the human kidney. In the normal kidney, the excess CNP produced by the kidney exits the kidney through the renal vein more than through the urine. In CHF CNP production by the kidneys and release into the venous system appears blunted when compared to individuals with normal left ventricular function.

As expected there was intersubject heterogeneity within both groups of subjects. The pattern was much more consistent in the healthy controls. Although we found in patients with CHF a step up in CNP concentration from aorta to renal vein of similar magnitude to that from aorta to coronary sinus seen in our previous study [8], this did not reach statistical significance. This may relate in part to the small number of patients included in this study. However, the fairly consistent finding of greater magnitude of step up in plasma CNP concentration from aorta to renal vein in the subjects with normal left ventricular function suggests that renal production does genuinely appear blunted in patients with CHF.

Our findings share similarities with those seen in a sheep model of heart failure. Charles et al. [16] demonstrated a significant step up in plasma CNP across the heart, kidney and liver in healthy sheep (suggesting net production). This was in contrast to ANP and BNP where the heart was the key source of circulating peptide and net extraction of these peptides was seen across the other organ beds. After induction of heart failure (rapid pacing) a significant step up in CNP remained across the heart. Whilst arterial levels were still higher than respective venous levels across the kidney and liver these no longer reached statistical significance.

Extensive research has helped elucidate the central role for ANP and BNP in the pathophysiology of CHF [17]. In contrast, the role of CNP in health and diseased states remains less certain. Initial studies of patients with CHF demonstrated plasma CNP concentration to be similar to that of control subjects [4,5]. More recently Del Ry and colleagues found that CNP levels were increased in patients with CHF as a function of clinical severity [18].

In terms of urinary excretion of CNP, there are conflicting data with some studies finding elevated urine CNP in CHF and others finding little difference [11-13]. Mattingly and colleagues assessed urinary CNP in a timed collection (4 hours) with urine collected on ice [11]; Bentzen et al used a standard 24-hour collection [12]; Ng et al. evaluated urinary CNP on a spot sample [13]. We performed a series of studies and demonstrated good agreement of CNP measurements irrespective of whether urine was collected into containers containing aprotinin or surrounded by ice. Our data showed similar urinary CNP excretion rate in patients with CHF and control subjects.

Acute intravascular overload in healthy dogs resulted in elevation in pulmonary capillary wedge pressure and plasma ANP, without change in plasma BNP and CNP concentration [19]. In contrast, a significant increase in urinary CNP but not ANP or BNP was seen after acute intravascular loading. This raises the possibility that the discrepant results of urinary CNP in CHF may be because the CHF patients in each study were in a different state of fluid retention at the time of the study. In our

studies, the CHF patients appeared normovolaemic clinically. It is therefore unlikely that current volume expansion impacted significantly upon the urinary CNP results.

The appearance of CNP in urine will depend upon the relative contributions of renal filtration, secretion across tubular cells from post glomerular blood and local production within the kidney with direct secretion into the tubules. Counteracting this are the two pathways involved in metabolism of CNP: removal by the clearance receptor (natriuretic peptide receptor-C) [20] and hydrolysis by neutral endopeptidase [21]. Neutral endopeptidase has a wide tissue distribution and in particular is located in the vascular endothelium and at high levels in the kidney brush border [20]. In vitro, CNP appears to be more rapidly hydrolysed by neutral endopeptidase than the other natriuretic peptides [22]. The lack of increase in renal vein CNP seen in CHF in our study could in theory be due to kidneys in patients with CHF having higher levels of neutral endopeptidase than normal kidneys. This has previously been shown in rat models of heart failure [23].

Renal blood flow is reduced in CHF [24]. As such the lower renal arterio-venous difference found in patients with CHF cannot be explained by an increased renal blood flow and unchanged renal production. Lower flow could predispose to enhanced renal clearance thereby contributing to reduced CNP concentration within the venous effluent in patients with CHF. We found no relation between renal function and step up in plasma CNP from aorta to renal vein.

It is plausible that medication such as diuretics and inhibitors of the renin-angiotensin-aldosterone system might impact on renal CNP production and release in CHF. In the current study all patients with CHF were receiving either angiotensin converting enzyme inhibitors or angiotensin receptor blockers which do alter intra-renal haemodynamics. However, it seems intuitively unlikely, but not impossible, that the large differences we saw in renal vein CNP between CHF and controls were due to ACE inhibitors. There was no relation between diuretic dose and difference in CNP between aorta and renal vein in patients with CHF.

We have previously demonstrated that in patients with CHF, the heart produces CNP, and that the CNP concentration in the coronary sinus is related to pulmonary capillary wedge pressure [8]. Davidson and colleagues [30] showed that CNP is a local endogenous inhibitor of vascular angiotensin converting enzyme. The current finding adds support to the concept that the primary effects of CNP may be paracrine.

There are limitations with the current study. The control group of individuals had experienced paroxysmal atrial arrhythmia (although in sinus rhythm during the study). It is hard to justify recruitment of an asymptomatic healthy individual for a catheter laboratory based study since all procedures carry a small risk of significant complications. Since the novel aim here was to see what renal arterio-venous differences were in CNP, we needed to recruit individuals who had a clinical reason for catheterisation. Accepting this inevitable limitation the group of subjects with normal left ventricular function were all asymptomatic when not having paroxysmal arrhythmias, and none had symptoms of heart failure.

Although we assessed CNP levels within the aorta and renal vein, we did not have concomitant data on urinary CNP concentrations in these individuals nor did we

measure blood flow so as to calculate total excretion. However, in a subsequent study described here, urine CNP was much the same in CHF as in control subjects, in agreement with Bentzen et al. [12].

Conclusion

We have demonstrated that the kidney is a site of CNP production and release in humans and that the extra CNP produced spills mainly into the renal vein in normal man. The renal production of CNP is blunted in CHF. The blunted production of intrarenal CNP in CHF may contribute to the renal sodium retention that is so characteristic of CHF.

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Disclosures

We declare that there are no conflicts of interest.

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Figure legends

Figure 1:

CNP concentrations in aorta and renal vein in patients with CHF (n=13)

Figure 2:

CNP concentrations in aorta and renal vein in control subjects (n=7). The dashed grey lines represent the two subjects in tachyarrhythmia at time of sampling and are *not* included in the data analysis shown in the figure.

Table 1: Clinical characteristics of patients with chronic heart failure

Patient	Age (yrs)	Diagnosis	LVEF (%)	NYHA class	Creat ($\mu\text{mol/L}$)	ΔCNP (RV-aorta, pg/mL)	Furosemide dose (mg)
1	67	DCM	15	III	133	2.33	120
2	47	IHD	32	I	96	0.88	0
3	65	IHD	34	II	74	0.13	0
4	56	DCM	40	I	86	5.03	20
5	61	MR	57	II	99	-0.37	0
6	72	IHD	15	II	118	0.47	80
7	46	DCM	38	II	146	2.64	20
8	55	DCM	20	III	108	-0.58	80
9	48	DCM	17	II	81	-0.50	80
10	69	IHD	28	III	89	-0.80	40
11	70	IHD	21	II	81	2.24	100
12	54	IHD	20	II	82	-0.89	40
13	59	DCM	50	I	105	2.82	0

LVEF indicates left ventricular ejection fraction; NYHA, New York Heart Association; Creat, serum creatinine; ΔCNP (RV-aorta), difference in CNP levels between renal vein and aorta; IHD, ischaemic heart disease; DCM, dilated cardiomyopathy; MR, mitral regurgitation.

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Table 2: Clinical characteristics of control subjects undergoing electrophysiological studies for paroxysmal supraventricular arrhythmia. (* Patients 8 and 9 were in tachyarrhythmia at time of study and as such excluded from main study analysis).

Patient	M/F	Age (yrs)	Diagnosis	Creat ($\mu\text{mol/L}$)	Heart rate (bpm)	ΔCNP (RV-aorta, pg/mL)
1	M	54	PAF	94	60	45.3
2	M	42	AVNRT	122	67	16.5
3	M	64	PAF	91	65	13.0
4	M	60	PAF	73	50	10.1
5	M	61	AVNRT	92	42	-0.6
6	M	33	PAF	91	80	3.2
7	M	53	PAF	96	64	4.4
8*	M	54	PAF	58	180	0.78
9*	F	44	PAF	90	150	4.4

AVNRT indicates atrioventricular node re-entry tachycardia; M/F indicates male or female, respectively; PAF, paroxysmal atrial fibrillation; Creat, serum creatinine; bpm, beats per minute; ΔCNP (RV-aorta), difference in CNP levels between renal vein and aorta

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