Myocardial Production of C-Type Natriuretic Peptide in Chronic Heart Failure

Paul R. Kalra, MA, MRCP; Jonathon R. Clague, MD, MRCP; Aidan P. Bolger, BSc, MRCP; Stefan D. Anker, MD, PhD; Phillip A. Poole-Wilson, MD, FRCP; Allan D. Struthers, MD, FRCP; Andrew J. Coats, DM

Background—C-type natriuretic peptide (CNP) is a vasodilator produced by the vascular endothelium. It shares structural and physiological properties with the cardiac hormones atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), but little is known about its pathophysiological role in chronic heart failure (CHF). We assessed the hypothesis that CNP is produced by the heart in patients with CHF.

Methods and Results—Myocardial CNP production was determined (difference in plasma levels between the aortic root and coronary sinus [CS]) in 9 patients undergoing right and left heart catheterization as part of their CHF assessment (all male, age 59±9 years; New York Heart Association class 2.2±0.1; left ventricular ejection fraction 29±5%; creatinine 105±8 μmol/L [all values mean±SEM]). BNP, established as originating from myocardium, was assessed from the same samples as a positive control. Analyses were performed by a blinded operator using a standard competitive radioimmunoassay kit (Peninsula Laboratories, Bachem Ltd UK). A step-up (29%) in plasma CNP concentration was found from the aorta to the CS (3.55±1.53 versus 4.59±1.54 pg/mL, respectively; P=0.035). The mean increase in CNP was 0.90±0.35 pg/mL (range 0.05 to 2.80 pg/mL). BNP levels increased by 57% from aorta to CS (86.0±20.5 versus 135.0±42.2 pg/mL; P=0.01). CS CNP levels correlated with mean pulmonary capillary wedge pressure (r=0.82, P=0.007).

Conclusions—We have shown that CNP is produced by the heart in patients with CHF. Although further evaluation is required to define its full pathophysiological role in this condition, CNP may represent an important new local mediator in the heart. (Circulation. 2003;107:571-573.)

Key Words: heart failure ■ hormones ■ myocardium ■ natriuretic peptides

Ten patients undergoing right and left heart catheterization as part of their CHF assessment were recruited. The diagnosis of heart failure was based on symptoms, examination, and appropriate investigations (chest radiograph, echocardiogram, and cardiac MRI). All patients had evidence of left ventricular (LV) dilatation (LV end-diastolic dimension ≥60 mm). Myocardial CNP production was determined from the difference in plasma levels between the aortic root and coronary sinus (CS). BNP, established as originating from myocardium, was assessed from the same samples to serve as a positive control. It was technically not possible to cannulate the CS in 1 patient; data are presented for the remaining 9 patients (all male, age 59±9 years; New York Heart Association class 2.2±0.1; Table). No patient had significant renal failure (creatinine 105±8 μmol/L) or active infection/inflammation. Patients were receiving conventional medication for heart failure: ACE inhibitor or angiotensin II receptor blocker (n=9), diuretics (n=7), and β-blockers (n=4). Ethical approval was granted by the local ethics committee. Written informed consent was obtained from all participants.

After confirmation of the catheter position by fluoroscopy (advanced past the CS ostium), blood was collected into chilled EDTA tubes containing aprotinin. Samples were immediately centrifuged.
for 15 minutes at 3000 rpm, and the plasma phase was stored at −80°C. CNP and BNP 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. The intra-assay coefficient of variation was 11.4% for CNP and 12.2% for BNP. The anti-CNP antibody used had no cross-reactivity with human ANP or BNP, and the specific rabbit antiserum for human BNP-32 had no cross-reactivity with human ANP or CNP. The lower limit of detection for both peptide assays is 0.1 pg/mL.

Statistics
Data were analyzed with StatView 4.5 (Abacus Concepts Inc) and are expressed as mean±SEM. Because of the skewness of CNP levels, a logarithmic transformation was used. Differences between sample sites were assessed by paired t test. Simple regression analysis was performed. A probability value of <0.05 was considered statistically significant.

Results
Patients had evidence of LV dysfunction (LV end-diastolic dimension 67±3 mm, ejection fraction 29±5%, and pulmonary capillary wedge pressure [PCWP] 19±2 mm Hg). A significant step-up (29%) in plasma CNP concentration was found from the aorta to the CS (3.55±1.53 versus 4.59±1.54 pg/mL, respectively; P=0.035; Figure 1 and the Table). The mean increase in CNP was 0.90±0.35 pg/mL (range 0.05 to 2.80 pg/mL). A mean increase in plasma BNP of 49.1±28.9 pg/mL (57%) was seen when levels in the aorta were compared with those in the CS (86.0±20.5 versus 135.0±42.2 pg/mL, respectively, P<0.05).

Relationship of CS CNP Levels to Clinical Variables
A significant correlation was found between mean PCWP and CS CNP levels (r=0.82, P<0.007; Figure 2) but not aortic CNP levels (P>0.15). No relationship was found with pulse rate, blood pressure, or LV ejection fraction or end-diastolic dimension (all P>0.09). The relationship between CS levels of CNP and BNP just failed to reach significance (r=0.62, P=0.07).

Discussion
The central role of neurohormones, including ANP and BNP, in the pathophysiology of heart failure is firmly established. The role of CNP, however, has not been determined. It is generally thought that CNP, a vasorelaxant, originates from vascular endothelium. The biological actions of the natriuretic peptides are mediated through specific receptors (NPR) and a resulting elevation in intracellular cGMP. Several receptor subtypes exist, and NPR-B is more specific for CNP. The finding of high levels of NPR-B in vascular smooth muscle and the observation that CNP inhibits local vascular ACE have fueled speculation that CNP plays an important role in local vascular regulation.
Plasma CNP levels are not elevated in patients with stable CHF.7,10 In view of the short plasma half-life of CNP, circulating levels may not reflect tissue concentrations near the site of production. Natriuretic peptides are catabolized by 2 distinct mechanisms. NPR-C is thought to act as a clearance receptor; after binding of a natriuretic peptide to NPR-C, the resulting receptor-ligand complex undergoes endocytosis and subsequent lysosomal hydrolysis.15 The second route of elimination involves cleavage by neutral endopeptidase. This enzyme has a wide tissue distribution, including the vascular endothelium.16 In vitro, CNP appears to be particularly sensitive to hydrolysis by neutral endopeptidase.17

Early studies failed to detect CNP mRNA within human myocardium.18 Interest was renewed when mRNA transcripts for NPR-B were found within myocardial tissue, prompting speculation that CNP may influence cardiac function.6 Wei and colleagues3 confirmed the presence of CNP within atrial and ventricular myocardium by immunohistochemistry and radioimmunoassay; myocardial CNP levels were elevated (2 to 3 times) in CHF patients compared with controls (P<0.05). A discrepancy therefore exists between circulating and local levels of CNP in CHF patients.

We have shown that in CHF patients, CNP levels are significantly increased in the CS compared with the aorta. This supports the hypothesis that CNP is produced directly in the myocardium, although it does not permit differentiation between the atrium and ventricle as the principal source. It is likely that the level within the CS (or general circulation) underestimates the CNP concentration in proximity to NPR-B receptors located on endothelial cells and myocardial tissue. It is therefore difficult to relate the degree of myocardial CNP spillover with functional effects.

In vitro studies have demonstrated marked augmentation of CNP secretion from cultured endothelial cells by mediators important in the pathogenesis of CHF, such as tumor necrosis factor, lipopolysaccharide, and BNP.19,20 Little is known about the role of hemodynamic factors in CNP release. In the present study, CS CNP levels correlated with PCWP. Although both may reflect the severity of myocardial dysfunction, it is tempting to speculate that myocardial CNP production occurs in response to elevated ventricular pressure filling pressure. Larger studies with subjects exposed to different PCWPs are required to verify this.

Locally produced CNP might affect cardiac function in several ways. Discrepant results have been seen in animal models: Exogenous CNP applied to dog right atrial preparations results in an enhanced inotropic effect,8 whereas a negative inotropic effect was demonstrated on rat papillary muscles.9 Myocardial CNP production may help to regulate local vascular tone in an attempt to optimize myocardial perfusion despite alterations in filling pressures. It may also inhibit myocardial ACE activity, as it has already been shown to do in the peripheral vasculature.

Borgeson et al21 showed that acute intravascular overload in dogs resulted in the expected increase in PCWP and plasma ANP but no change in plasma BNP and CNP levels. In contrast, a marked increase in urinary CNP but not ANP or BNP was seen. It appears that a differential release of natriuretic peptides from the myocardium and kidney occurs in response to alterations in ventricular filling pressures.

In conclusion, we have shown that in CHF, the heart produces CNP. Although further evaluation is required to define its full pathophysiological role in this condition, CNP may represent an important new local autocrine and/or endocrine mediator in the heart.

Acknowledgments
Dr Kalra, Dr Bolger, and the Department of Clinical Cardiology are supported by the British Heart Foundation. Dr Kalra was previously supported by Wessex Heartbeat and the Waring Trust. Professor Coats is supported by the Viscount Royston Trust.

References
Myocardial Production of C-Type Natriuretic Peptide in Chronic Heart Failure
Paul R. Kalra, Jonathon R. Clague, Aidan P. Bolger, Stefan D. Anker, Phillip A. Poole-Wilson, Allan D. Struthers and Andrew J. Coats

Circulation. 2003;107:571-573; originally published online January 6, 2003;
doi: 10.1161/01.CIR.0000047280.15244.EB
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/107/4/571

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/