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Secretion of Prohormone of B-Type Natriuretic Peptide, proBNP$_{1\text{-}108}$, Is Increased in Heart Failure

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Objectives Using a novel, specific assay for proBNP$_{1\text{-}108}$, this study tested the hypotheses that proBNP$_{1\text{-}108}$ is secreted by both nonfailing and failing human hearts and that proBNP$_{1\text{-}108}$ secretion is increased in failing hearts.

Background The prohormone of B-type natriuretic peptide (proBNP$_{1\text{-}108}$) is a 108-amino acid peptide produced primarily by the heart and cleaved into biologically active BNP$_{1\text{-}32}$ and the biologically inactive NT-proBNP$_{1\text{-}76}$. It is unknown to what extent increased cardiac proBNP$_{1\text{-}108}$ secretion compared to reduced peripheral processing is responsible for elevated proBNP$_{1\text{-}108}$ levels in patients with heart failure (HF) compared to subjects without HF.

Methods The transcardiac gradient of proBNP$_{1\text{-}108}$ was determined by collecting arterial blood and blood from the coronary sinus (CS). Samples from subjects without overt heart disease (n = 9) were collected during cardiac catheterization after coronary artery disease had been excluded. Samples from HF patients (n = 21) were collected during implantation of a biventricular pacemaker. ProBNP$_{1\text{-}108}$ was measured with a new assay. Values are medians (25th/75th percentiles).

Results The gradient of proBNP$_{1\text{-}108}$ across the nonfailing hearts was 8 (2/20) ng/l (aorta: 15 [1/25] ng/l; CS: 24 [8/41] ng/l; p = 0.018). The transcardiac gradient of proBNP$_{1\text{-}108}$ in the failing hearts was 326 (96/482) ng/l (arterial: 381 [201/586] ng/l; CS: 709 [408/1,087] ng/l; p<0.001). The transcardiac gradient was greater in failing than nonfailing hearts (p = 0.001).

Conclusions ProBNP$_{1\text{-}108}$ is secreted by nonfailing and failing human hearts, but more so in the latter. It remains to be established where peripheral processing of proBNP$_{1\text{-}108}$ occurs and how this is affected by disease.
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Mature B-type natriuretic peptide (BNP) has vasodilating, natriuretic, antihypertrophic, antifibrotic, and metabolic properties. Human BNP is initially synthesized as the 134-amino-acid precursor, pre-proBNP. A 26-amino-acid signal peptide is cleaved and the 108-amino-acid prohormone, proBNP$_{1\text{-}108}$, is formed. The proBNP$_{1\text{-}108}$, which is less biologically active than mature BNP, is then cleaved enzymatically, presumably by corin and furin, to the biologically inactive amino terminal fragment NT-proBNP$_{1\text{-}76}$ and the biologically active, mature peptide BNP$_{1\text{-}32}$ (1–3). BNP is secreted primarily by the heart in response to increased wall stress; thus, assays for BNP or NT-proBNP were developed as cardiovascular disease biomarkers (4–13).

It was initially assumed that the "mature," most bioactive BNP form (BNP$_{1\text{-}32}$) was the only form secreted into the circulation (14). Now it is known that some of the circulating BNP immunoreactivity in normal humans is proBNP$_{1\text{-}108}$ and various proBNP$_{1\text{-}108}$ derivatives, including BNP$_{1\text{-}32}$ (15–22). Also, proBNP$_{1\text{-}108}$ is cleaved in human plasma to produce BNP$_{1\text{-}32}$ (3). Conventional assays for BNP are not specific because antibodies directed against BNP$_{1\text{-}32}$ and NT-proBNP$_{1\text{-}76}$ cross-react with proBNP$_{1\text{-}108}$ (23). In some disease states, BNP$_{1\text{-}32}$ levels measured by mass spectrometry are dramatically lower than the BNP immunoreactivity measured by conventional BNP assays (24,25). Indeed, proBNP$_{1\text{-}108}$, and not BNP$_{1\text{-}32}$, is the major BNP molecular form in heart failure (HF) (19–21). This may explain the

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Abbreviations and Acronyms

ANP = atrial natriuretic peptide
BNP = B-type natriuretic peptide
CS = coronary sinus
EF = ejection fraction
HF = heart failure
LOD = lower limit of detection
LV = left ventricular
mAB = monoclonal antibody
NT-proBNP = amino terminal-proBNP
NYHA = New York Heart Association

paradox seen in HF of high circulating BNP levels, but reduced BNP activity.

A specific assay for uncleaved, full-length proBNP$_{1-108}$ was recently developed. It is directed against the proBNP$_{1-108}$ hinge region, which is only present in the uncleaved prohormone, proBNP$_{1-108}$, and not in BNP$_{1-32}$ or NT-proBNP$_{1-76}$ (16) (Fig. 1). Using this assay, we and others reported that proBNP$_{1-108}$ circulates in healthy subjects' plasma and is elevated in cardiovascular disease, including HF (10,16,26).

While the presence of proBNP$_{1-108}$ in the peripheral circulation is established, it remains undefined if the normal heart secretes proBNP$_{1-108}$ and if secretion increases in HF. Net cardiac secretion of BNP$_{1-32}$ immunoreactivity has previously been reported; however, the assays used were not BNP$_{1-32}$ specific and probably also detected proBNP$_{1-108}$ (27).

This study had 2 objectives. The first was to confirm that this proBNP$_{1-108}$ assay is proBNP$_{1-108}$ specific and does not detect degraded BNP forms that circulate in HF. The second objective using this novel assay was to assess invasively for the first time the actual cardiac secretion of proBNP$_{1-108}$ in humans without cardiac disease and with HF. We hypothesized that proBNP$_{1-108}$ is secreted by the normal and failing human heart and that secretion increases in HF.

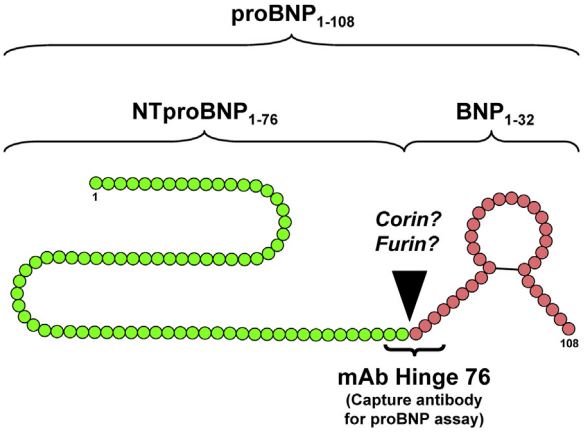

Figure 1 Schematic of proBNP$_{1-108}$ and the 2 Derivatives NTproBNP$_{1-76}$ and BNP$_{1-32}$ (proBNP$_{77-108}$)

Two putative enzymes involved in proBNP cleavage are corin and furin. The specific proBNP$_{1-108}$ assay (Bio-Rad) uses a capture antibody directed against the hinge region (i.e., the region in which proBNP$_{1-108}$ is cleaved into bioactive BNP$_{1-32}$ and inactive NTproBNP$_{1-76}$). There is a monoclonal detection antibody directed against BNP$_{1-32}$ epitopes. Thus, the proBNP$_{1-108}$ assay detects only uncleaved proBNP forms. mAb = monoclonal antibody; NT-proBNP = N-terminal -B-type natriuretic peptide.

Methods

proBNP$_{1-108}$ assay specificity. We confirmed and extended Giuliani et al. by measuring proBNP$_{1-108}$ immunoreactivity in 1 mL samples of normal human plasma with the Bio-Rad assay (16) spiked with either 1,000 pg or 10,000 pg of the following BNP molecular forms: BNP$_{1-32}$, BNP$_{8-32}$, proBNP$_{44-76}$, NT-proBNP$_{8-29}$, nesiritide (Scios, Mountain View, California), and proBNP$_{1-108}$ (Hytest, Finland). A wider range of proBNP$_{1-108}$ was used for spiking: 500 to 10,000 pg. Measurements were repeated three times.

Study population. Samples from subjects without heart disease were collected at the Mayo Clinic (Rochester, Minnesota) during cardiac catheterization after exclusion of coronary artery disease. Samples from subjects with left ventricular (LV) systolic dysfunction were collected at the Helios Clinic (Wuppertal, Germany) during implantation of a biventricular pacemaker for cardiac resynchronization therapy. All HF patients had transthoracic echocardiography performed at the Helios Clinic. All patients without heart disease had ejection fractions (EF) determined by transthoracic echocardiography or nuclear medicine scan at the Mayo Clinic. All subjects gave informed consent and had their medical records reviewed. The study was approved by the respective institutions' institutional review board or equivalent.

Sample processing and assays. The proBNP$_{1-108}$ transcardiac gradient was determined by collection of arterial and coronary sinus (CS) blood. Blood samples were placed into ethylenediaminetetraacetic acid-tubes on ice, chilled to 4°C, centrifuged at 2,500 rpm for 10 min, and the plasma stored at −80°C until used. Samples from Wuppertal were shipped to the Mayo Clinic on dry ice. ProBNP$_{1-108}$ was measured at the Mayo Clinic with the Bio-Rad assay (Bio-Rad, Hercules, California) on a commercially unavailable automated analyzer. The assay was developed by Giuliani et al. (16). The lower limit of detection (LOD) is 2 ng/l; levels below this were set as 1 ng/l (i.e., halfway between 0 and LOD). The interassay and intra-assay variabilities are 10.3% and 11.6%, respectively. All other laboratory values were measured at the respective institutions.

Statistical methods. Values are mean ± SEM for normally distributed data and as median (25th/75th percentile) for not normally distributed data. ProBNP$_{1-108}$ secretion was assessed separately in subjects with nonfailing and failing hearts by comparing arterial levels to CS levels with paired Wilcoxon signed-rank test. The transcardiac gradients i.e., difference between levels in CS versus arterial blood, were compared between the nonfailing and failing hearts by Mann-Whitney U-test. The range of transcardiac proBNP