Differential atrial and ventricular expression of myocardial BNP during evolution of heart failure

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To address this hypothesis, we assessed BNP gene expression in atrial and ventricular myocardium, as well as circulating BNP and cardiac tissue BNP in a...
canine model of progressive LV dysfunction that evolves from early compensated LV dysfunction to overt CHF.

METHODS

Study protocol. Fifteen male mongrel dogs were used for the study. Ten dogs underwent implantation of a programmable cardiac pacemaker (Medtronic, Minneapolis, MN). Under pentobarbital sodium anesthesia (30 mg/kg iv) and artificial ventilation (Harvard respirator, Harvard Apparatus, Millis, MA), the heart was exposed via a small left lateral thoracotomy and pericardiotomy, and a screw-in epicardial pacemaker lead was implanted into the right ventricle. The pacemaker was implanted subcutaneously into the left chest wall and connected to the pacemaker lead. Five dogs additionally underwent implantation of a chronic femoral artery catheter (model GPV Vascular-Access Port, Access Technologies, Skokie, IL), implanted via the left femoral artery and subcutaneously connected to a port above the left upper hindlimb. All dogs were allowed to recover for at least 10 days after surgery before the pacemaker was started for the induction of heart failure. All studies were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic and were conducted in accordance with the Animal Welfare Act.

Five dogs (overt CHF group) underwent pacing with a stepwise increase of stimulation frequencies over 38 days. During the first 10 days, animals were paced at 180 beats/min. As previously described (22, 26), this protocol results in early LV dysfunction as defined by significant systolic dysfunction with decreased cardiac output, cardiac enlargement, and increased filling pressures, but maintained systemic perfusion pressure and renal sodium excretion and no clinical signs of heart failure. The pacing rate was then increased weekly to 200, 210, 220, and 240 beats/min, and early LV dysfunction evolved to overt CHF (17, 25). At baseline (control), after being paced at 180 beats/min for 10 days (early LV dysfunction), and at the end of the protocol (overt CHF), conscious mean arterial pressure (MAP) was measured via the port catheter, a two-dimensional guided M-mode echocardiogram was obtained, and arterial blood was drawn. In addition, cardiac filling pressures and cardiac output by thermodilution (American Edwards Laboratories, model 9510-A) were measured in the conscious dog at baseline (control) and at the end of the pacing protocol (overt CHF). Arterial blood was collected in EDTA tubes and immediately placed on ice. Dogs were then killed (Sleepaway euthanasia solution iv, Fort Dodge Laboratories, Fort Dodge, IA) for rapid tissue harvesting. Hearts were rapidly trimmed, snap-frozen in liquid nitrogen, and stored at −80°C until further processing. Blood was centrifuged at 2,500 revolutions/min and 4°C, and the plasma was stored at −20°C until analysis as described in Analytical methods. All pacemakers were checked at the time of programming and then weekly and at the day of death for proper pacing.

A second group of five dogs was paced at 180 beats/min for 10 days only and served as tissue donor for the early LV dysfunction group, and a third group of five healthy normal dogs served as tissue donor for the control group. Again, invasive hemodynamic measurements were obtained to assess cardiac function, arterial blood was drawn, dogs were killed, and tissue was rapidly harvested and deep-frozen.

Analytical methods. BNP, cGMP, and plasma renin activity (7) were determined by standard radioimmunoassay (RIA) technique. Because of species variability in BNP, we employed a polyclonal antibody specific for canine BNP (4).

For extraction of tissue BNP, 15 left atrial and 15 LV samples were pulverized frozen, boiled for 5 min in 10 vols of 1 M acetic acid-20 mM HCl, and homogenized at high speed (PT 1200, Polytron). The homogenate was then ultracentrifuged at 27,000 g at 4°C, and the supernatant was stored at −20°C until RIA. Before centrifugation, a sample of the homogenate was taken for measurement of tissue protein content according to the Folin-phenol method by Lowry et al. (16). Immunoreactive BNP in tissue was measured as picograms per milliliter homogenate, normalized for protein content, and expressed as picograms BNP per milligram tissue protein.

For analysis of myocardial BNP gene expression, mRNA was extracted from 15 left atrial and 15 LV samples (Fasttrack Kit, Invitrogen). Briefly, tissue was homogenized (Polytron PT 1200) in a detergent-based buffer containing ribonuclease-protein degrader and incubated in a slow-shaking waterbath. DNA was precipitated and sheared, and oligo(dT) cellulose was added for adsorption of polyadenylated mRNA. DNA, proteins, cell debris, and nonpolyadenylated RNA were washed off, and mRNA eluted off the oligo(dT) cellulose. The yield of mRNA was determined in a spectrophotometer by absorption of 260-nm ultraviolet light. Ten micrograms of mRNA per extract were loaded on a 1.2% agarose-formaldehyde gel and electrophoresed for 2−3 h at 75 V. The gel was blotted downward overnight (Turbo-Blotter, Schleicher & Schuell) onto a nylon membrane (maximum strength Nytran membrane, Schleicher & Schuell). A 3-kilobase (kb) Hind III DNA restriction fragment containing the 1,804-base pair (bp) gene for canine BNP (24) was random-primed with [32P]dCTP (random-primer DNA labeling kit, Boehringer-Mannheim Biochemical) and column-purified. Membranes were prehybridized (QuickHyb Hybridization Solution, Stratagene) for 10 min at 68°C and then hybridized with the labeled probe for 80 min at 68°C. Membranes were then washed stringent [2× standard saline citrate (SSC)-0.1% sodium dodecyl sulfate (SDS) at 22°C for 5 min, then 0.2× SSC-0.1% SDS at 22°C for 5 min, then 0.2× SSC-0.1% SDS at 55°C for 20 min] and exposed to an X-ray film. To control for loading conditions and mRNA transfer onto the membranes, we rehybridized blots with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe. The respective autoradiographic bands for BNP and GAPDH were quantified with a scanning spectrophotometer, and BNP mRNA was expressed in arbitrary units as the ratio of autoradiographic density of the BNP band to that of the GAPDH band.

Echocardiography. A short-axis echocardiogram (Toshiba, Ottawara, Japan) was performed by an expert echocardiographer from the right parasternal window. LV end-diastolic (LVEDD) and end-systolic diameters (LVESD) and diastolic LV posterior wall thickness were determined from three repeated two-dimensional guided M-mode tracings. From those measurements, ejection fraction (EF) was calculated as

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EF = \frac{[(LVEDD)^2 - (LVESD)^2]}{(LVEDD)^2} \times 100
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Statistical analysis. Results of the quantitative studies were expressed as means ± SE. Comparisons between the control, early LV dysfunction, and overt CHF groups were performed by analysis of variance followed by Fisher’s least significant difference test. Statistical significance was defined as \( P < 0.05 \).

RESULTS

LV function and geometry. Early LV dysfunction was characterized by significant eccentric LV dilatation and LV dysfunction with no change in systemic mean arterial pressure. In overt CHF, ventricles were further dilated and LV ejection fraction was further decreased.
Cardiac filling pressures progressively increased (right atrial pressure 14.5 ± 1.2 mmHg in overt CHF vs. 5.6 ± 1.2 mmHg in control, \( P < 0.03 \); pulmonary capillary wedge pressure 23.5 ± 1.8 mmHg in overt CHF vs. 10.1 ± 1.0 mmHg in control, \( P < 0.01 \)), whereas cardiac output (2.4 ± 0.4 l/min in overt CHF vs. 5.2 ± 0.5 l/min in control, \( P = 0.057 \), not significant (NS)) and systemic arterial pressure were decreased in overt CHF (Table 1).

Circulating hormones. Early LV dysfunction was characterized by an activation of circulating BNP and cGMP without activation of renin. Overt CHF was associated with further increased BNP and cGMP in association with an activation of plasma renin activity (Table 1 and Fig. 2).

Cardiac BNP gene expression. A positive signal for BNP mRNA was detected in all left atrial samples and in LV samples from overt CHF dogs. Representative autoradiographies are depicted (Fig. 1). By quantitative analysis, early LV dysfunction was characterized by a selective increase in left atrial BNP mRNA (0.65 ± 0.11 arbitrary units (AU) vs. 0.23 ± 0.05 AU, \( P < 0.01 \)), whereas LV BNP mRNA was unchanged (0.07 ± 0.05 vs. 0.01 ± 0.01 AU). Overt CHF was characterized by a further increase in left atrial BNP mRNA (0.99 ± 0.09 AU, \( P < 0.02 \) vs. early LV dysfunction) and a strong recruitment of LV BNP mRNA (1.23 ± 0.29 AU, \( P < 0.01 \) vs. control and early LV dysfunction; Fig. 2).

Cardiac BNP tissue concentrations. BNP immunoreactivity was present in atrial and ventricular myocardium, and left atrial BNP (13.0 ± 7.6 pg/mg protein)
BNP (1.93 ± 0.76 pg/mg protein, P < 0.01 vs. control) but unchanged LV BNP (0.45 ± 0.10 vs. 0.39 ± 0.03 pg/mg protein, NS). In contrast, overt CHF was characterized by increased LV BNP (1.93 ± 0.21 pg/mg protein, P < 0.01 vs. control and early LV dysfunction), whereas left atrial BNP did not increase further (88.7 ± 13.8 pg/mg protein, P < 0.01 vs. control; NS vs. early LV dysfunction).

DISCUSSION

The current study was designed to define the cardiac endocrine role with respect to BNP gene expression and tissue concentrations during progressive experimental heart failure. Left atrial and ventricular BNP gene expression was assessed by Northern analysis with a canine-specific probe (24), and immunoreactive BNP in plasma and tissue was measured utilizing a canine-specific polyclonal antibody (4). Before LV dysfunction, BNP gene expression was selective for atrial myocardium. In early LV dysfunction, characterized by eccentric LV dilatation, impaired systolic LV function, and maintained mean arterial pressure, atrial BNP gene expression was increased in association with increased BNP concentrations in plasma and atrial tissue. Ventricular BNP gene expression and tissue BNP remained unchanged in early LV dysfunction. In overt CHF, characterized by further impaired systolic LV function, further LV dilatation, decreased mean arterial pressure, and activation of circulating renin, BNP gene expression was further increased in atrial myocardium with additional recruitment from ventricular myocardium. The onset of ventricular BNP gene expression was associated with increased ventricular tissue BNP and further increased plasma BNP. The current studies demonstrate that in the absence of ventricular dysfunction and during early experimental LV dysfunction atrial myocardium is the predominant site of BNP gene expression and production and that circulating BNP closely parallels atrial BNP gene expression. Furthermore, the present studies demonstrate that overt CHF is characterized by a further increase in atrial BNP gene expression as well as an additional recruitment of BNP gene expression and production from ventricular myocardium.

Although there is agreement as to the presence of significant BNP gene expression and immunoreactivity in atrial tissue under normal conditions (1, 11, 18, 30), controversy persists regarding the BNP gene expression in normal ventricular tissue. Indeed, conflicting studies both in the human and in animals either describe strong ventricular BNP gene expression (18, 21) or report no or only faint BNP gene expression (2, 29) in normal ventricular myocardium. Our current findings support the latter studies and a predominant role for atrial myocardium in the gene expression and production of BNP in the absence of ventricular dysfunction, as we found significant transcription and tissue concentrations of BNP only in atrial myocardium, whereas significant ventricular BNP transcription was not detectable and ventricular tissue concentrations were low.

With respect to early heart failure, there is a lack of information regarding atrial and ventricular BNP gene expression, which contributes to the ongoing debate as to the predominant site of BNP transcription in the absence and presence of heart failure. Our finding of a selective increase of BNP gene expression and tissue concentrations in atrial tissue during early LV dysfunction is novel and underscores a predominant endocrine role of atrial but not ventricular myocardium in early heart failure. An association of increased circulating BNP concentrations in early LV dysfunction with increased atrial BNP gene expression and the absence of changes in ventricular BNP gene expression further suggests a principal role for atrial myocardium in the increased plasma concentration of BNP during this phase. The greater sensitivity of the atrial myocyte to stimulate BNP gene expression compared with the ventricular myocyte may be related to a mechanical mechanism such as the greater atrial distensibility but may in addition also reflect a principal difference in the regulation of atrial and ventricular gene transcription.

Our findings in overt experimental CHF confirm previous studies in the human and in animals as they report significant ventricular BNP immunoreactivity and gene expression (8, 18, 29, 30). They extend previous studies as they further report an additional
increase in atrial BNP gene expression as early LV dysfunction progresses to overt CHF. These increases in atrial and ventricular BNP gene expression were associated with a tendency toward further increased circulating BNP concentrations, suggesting an additional contribution of ventricular myocardium to elevated circulating BNP in overt CHF. The finding of a selective late activation of BNP gene expression in ventricular myocardium during the evolution of CHF suggests that the presence of increased LV stretch may not be the only mechanism involved in the transcriptional activation of the ventricular BNP gene during CHF. An activation of BNP gene expression should otherwise already occur in early LV dysfunction, which is also characterized by eccentric LV dilatation in association with a maintenance of arterial blood pressure and therefore chronically increased LV stretch. We speculate that an important additional component for such a late selective activation of BNP gene expression may be the additional activation of local and circulating ANG II or endothelin 1 (ET-1), which has been demonstrated to selectively occur in overt CHF in the current model (17, 25). Indeed, ANG II (5) and ET-1 (3) have been shown to induce transcription of early genes and stimulate myocyte growth. ET-1 has further been demonstrated to directly induce BNP transcription in cultured ventricular myocytes (19). Further studies are therefore warranted to unmask the role of ANG II and ET-1 in the induction of LV BNP during overt CHF in vivo.

In the current study, ventricular BNP concentrations are widely exceeded by atrial BNP concentrations under control conditions and throughout the progression of heart failure. Under control conditions and in early LV dysfunction, this observation is most likely due to the absence of BNP gene expression in ventricular myocardium. In overt CHF however, atrial BNP concentrations still widely exceed ventricular BNP concentrations despite a similarly strong activation of BNP transcription. This underscores the primary role for the atrium as the site for BNP production and release in CHF. We speculate that this phenomenon may indicate a differential posttranscriptional processing of BNP in atrial and ventricular myocardium and in particular may suggest a greater capacity of atrial myocytes to store BNP compared with ventricular myocytes.

In summary, the current studies in experimental canine CHF provide important new insight into the cardiac BNP gene expression in the presence and absence of evolving CHF. They demonstrate that in the absence of ventricular dysfunction and during early experimental LV dysfunction, atrial myocardium is the predominant site of BNP gene expression and production. They further demonstrate that an increase in circulating BNP concentrations during early LV dysfunction is associated with increased atrial BNP gene expression and production, whereas ventricular BNP gene expression and production are unchanged. Last, they demonstrate that an additional recruitment of BNP gene expression and production occurs in ventricular tissue during overt CHF in association with a further increase in circulating BNP concentrations. The current studies are the first to demonstrate a differential pattern of BNP gene expression in atrial and ventricular myocardium during the evolution of heart failure. They underscore the important endocrine role of atrial myocardium in the generation of BNP in the absence of LV dysfunction and during early LV dysfunction and further confirm a role for ventricular myocardium in the generation of BNP during overt CHF.


