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

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The hypothesis of the current investigation was that atrial myocardium is the principal site of BNP mRNA in the absence of ventricular dysfunction and that a selective increase in BNP mRNA occurs in early LV dysfunction. In overt CHF, we hypothesize that a further increase in atrial BNP gene expression and the recruitment of ventricular BNP gene expression occurs in association with a further increase in circulating BNP.

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.

For 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 decreased 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 proper pacing, 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 in the conscious dog at baseline (control) and at the day of death for those measurements, ejection fraction (EF) was calculated as

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EF = \left(\frac{(LVESD^2 - LVESD^2)}{LVEDD^2}\right) \times 100
\]

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.

EF = \left(\frac{(LVESD^2 - LVESD^2)}{LVEDD^2}\right) \times 100
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)
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.

The authors acknowledge outstanding assistance by Lawrence L. Aarhus, Ross A. Aleff, Denise M. Heublein, Sharon M. Sandberg, and the Department of Veterinary Medicine.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-36634 and HL-07111, the Hearst Foundation, the Miami Heart Research Institute, the Bruce and Ruth Rappaport Program in Vascular Biology, and the Mayo Foundation. A. Luchner is a recipient of Deutsche Forschungsgemeinschaft Grant Lu 562/1–1.

This material has been presented at the 46th Annual Scientific Session of the American College of Cardiology and published in abstract form (J. Am. Coll. Cardiol. 29, Suppl. A:A25, 1997).

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Received 11 August 1997; accepted in final form 13 January 1998.

REFERENCES


Differential atrial and ventricular BNP in CHF evolution


