Atrial BNP endocrine function during chronic unloading of the normal canine heart

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Lisy, Ondrej, Margaret M. Redfield, John A. Schirger, and John C. Burnett Jr. Atrial BNP endocrine function during chronic unloading of the normal canine heart. Am J Physiol Regul Integr Comp Physiol 288: R158–R162, 2005; doi:10.1152/ajpregu.00444.2004.—The goal of the current investigation was to determine the effect of chronic unloading of the normal heart on atrial BNP. We performed these integrative studies in a canine model of chronic cardiac unloading produced by thoracic inferior vena cava constriction (TIVCC). We closely examined the impact of unloading on atrial secretory granules and BNP storage in granules, together with an assessment of atrial BNP mRNA and the second messenger to BNP, cGMP. We also assessed the ability of the chronically unloaded heart to release BNP in response to acute reloading. These investigations therefore advance our understanding of physical and biochemical mechanisms that modulate BNP.

METHODS

Surgical procedure. Studies were performed in male mongrel dogs (20–26 kg), conformed to the guidelines of the American Physiological Society, and were approved by the Mayo Clinic Animal Care and Use Committee. Eleven dogs were randomly divided into two groups. After receiving prophylactic antibiotic treatment, the surgery was performed, and an adjustable band was placed around the thoracic inferior vena cava (IVC). Group 1-sham had the band placed without constriction (n = 5). Group 2-TIVCC had the band constricted (n = 6) to create ≈50% reduction in the IVC diameter, as previously described (3, 11, 29). Dogs were allowed to recover for 10 days. An additional group of dogs (n = 7) was also studied in which the constriction of the thoracic IVC was released on the 11th day during an acute experiment (TIVCC-RL).

Acute experiment. Dogs were anesthetized with pentobarbital sodium (30 mg/kg) and ventilated on room air supplemented with oxygen. The right jugular vein was exposed, and a thermomodulation catheter was advanced in the pulmonary artery. The right femoral artery was cannulated for blood pressure measurement and blood sampling. In the third group of dogs (TIVCC-RL), after a 30-min equilibration period (baseline), the inflatable band around the thoracic IVC was released, and the right atrial pressure (RAP) and pulmonary capillary wedge pressure (PCWP) were recorded at 3-, 5-, 10-, 15-,

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Table 1. **Cardiovascular hemodynamics and neurohumoral function**

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<thead>
<tr>
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<th>Sham</th>
<th>TIVCC</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>109±4</td>
<td>114±8</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.8±0.4</td>
<td>2.4±0.2*</td>
</tr>
<tr>
<td>SVR, mmHg·l⁻¹·min⁻¹</td>
<td>30±3</td>
<td>49±4*</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>2.9±0.5</td>
<td>−0.8±0.6*</td>
</tr>
<tr>
<td>ET, pg/ml</td>
<td>4±1</td>
<td>13±1*</td>
</tr>
<tr>
<td>NE, pg/ml</td>
<td>176±14</td>
<td>694±146*</td>
</tr>
<tr>
<td>ANG II, pg/ml</td>
<td>10±3</td>
<td>267±29*</td>
</tr>
<tr>
<td>BNP, pg/ml</td>
<td>13±3</td>
<td>23±8</td>
</tr>
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</table>

Data are expressed as means ± SE. TIVCC, thoracic inferior vena cava constriction; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; RAP, right atrial pressure; ET, endothelin; NE, norepinephrine; BNP, brain natriuretic peptide. *P < 0.05 vs. sham.

30- and 60-min intervals after the release with a simultaneous arterial blood sampling for plasma BNP.

Dogs were euthanized with KCl. Hearts from sham and TIVCC groups were removed, and all chambers were dissected and weighed. The left ventricular (LV) weight was used for calculating LV mass index (LV mass index = LV wt/body wt). The atrial weight was used for calculation of atrial mass index (atrial mass index = atrial wt/body wt). Because dogs with TIVCC developed fluid retention and ascites, the body weight at the time of TIVCC or sham surgery was used for this calculation. Left atrial sections were taken from the full thickness of the free wall, fixed in 10% buffered formalin, and later paraffin embedded. The left atrial and ventricular tissue samples were frozen in liquid nitrogen and stored at −80°C until processing.

**Immunoelectron microscopy.** Left atrial free wall samples were excised from paraffin blocks, deparaffinized in xylene, rinsed in absolute ethanol, and reembedded in Quetol resin. Thin sections were mounted on nickel grids and dried overnight. Sections were incubated for 15 min at room temperature in PBS + 0.05% Tween 20 (PBST) with 0.1 M glycine and 2% normal goat serum added. After nonspecific sites were blocked, the sections were incubated in 1:500 rabbit polyclonal antibodies to canine BNP in PBST for 3 h at room temperature, rinsed in PBST, and incubated for 60 min in goat anti-rabbit antibodies conjugated to 10 nm colloidal gold. Sections were rinsed in PBST and water, dried, and stained in lead citrate and uranyl acetate. Subsequently, the sections were examined by transmission electron microscopy (Phillips CM-10). Representative secretory granules (n = 80) in each sample of left atrial myocardium were examined at a magnification of 66,000 and photographed. The number of gold particles over each secretory granule was counted, and the area of secretory granules was calculated. In control sections incubated without the primary antibodies, no gold particles were found.

Radioimmunoassays. Arterial blood for hormone analysis was collected in sodium EDTA tubes, placed on ice, and centrifuged at 2,500 rpm at 4°C. Plasma endothelin (ET) was determined using an ET-1 assay (Amersham International, Buckinghamshire, UK), as previously described (31). Plasma norepinephrine (NE) was measured by HPLC. ANG II and cGMP were measured by RIA, as previously described (11, 12). Plasma BNP was determined by a sensitive and specific RIA, as previously described (12). Tissue homogenates were ultra-centrifuged for 30 min at 15,000 rpm and 4°C, and the supernatant was stored at −20°C until RIA, with a sample taken for protein measurement.

**Northern blot analysis.** For analysis of myocardial BNP gene expression, mRNA was extracted from left atrial and ventricular samples (Fast-track Kit; Invitrogen). mRNA (4 μg/extract) was loaded on 1.2% agarose-formaldehyde gel and electrophoresed for 2–3 h at 75 volts. For amplification of canine BNP cDNA fragment, the following primers were selected: sense, 5′-GAAGAGGGAGGAGTTTCAGACGCGTG-3′; antisense, 5′-AAAGACCCCTGACTTTG-GCATC-3′. BNP probe was random primed with [32P]dCTP (random-primed DNA labeling kit; Boehringer-Mannheim Biochemical) and column purified. To control for loading conditions and mRNA transfer to 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.

**Statistical analysis.** Results of quantitative studies are expressed as means ± SE. Statistical comparisons between groups were performed by unpaired Student’s t-test. Statistical comparisons within groups were performed by use of repeated-measures ANOVA, followed by a post hoc Dunnett’s test. Statistical significance was accepted at P < 0.05.

**RESULTS**

**Characteristics of the model.** Our model of myocardial unloading produced by TIVCC was characterized by a significant decrease in cardiac output and right atrial pressure compared with a sham group (Table 1). No difference in blood pressure was observed, whereas systemic vascular resistance increased in the unloaded heart group. There was no significant difference in plasma BNP between the groups, whereas plasma ANG II, ET-1, and NE markedly increased. Atrial and LV mass index obtained at autopsy was significantly lower in the unloaded heart group compared with sham (0.7 ± 0.1 vs. 1.0 ± 0.1 g/kg and 3.4 ± 0.1 vs. 4.6 ± 0.2 g/kg, respectively). Right ventricular mass index was also lower in the unloaded group (1.2 ± 0.1 vs. 1.8 ± 0.1 g/kg). Thus these findings were consistent with atrophy of atrial and ventricular myocardium.

**Cardiac BNP in unloaded and sham hearts.** Immunoelectron microscopy demonstrated that the atrial secretory granule area of the unloaded atrium was unchanged compared with sham operated (Table 2). In contrast, immunogold staining of BNP revealed a marked increase in BNP in atrial secretory granules in the unloaded hearts compared with sham (Table 2 and Fig. 1). Left and right atrial tissue concentration of the second messenger of BNP, cGMP, was elevated in the unloaded hearts compared with sham (38.2 ± 8.7 vs. 52.1 ± 3.8 fmol cGMP/mg protein and 30.0 ± 4.5 vs. 7.6 ± 3.8 fmol cGMP/mg protein, respectively). Northern blot analysis of BNP gene expression in atrial and ventricular myocardium revealed decreases in BNP mRNA in left atrial myocardium of the unloaded hearts (Fig. 2). No change in already lower ventricular BNP mRNA was observed.

**Effect of reloading of the unloaded heart on BNP secretion.** In the third group of dogs (TIVCC-RL), acute reloading of chronically unloaded myocardium resulted in increases in RAP and PCWP (Fig. 3). This acute reloading was associated with a significant increase in plasma BNP within 10 min.

**DISCUSSION**

The goal of the current study was to define the effect of chronic unloading of the normal heart on atrial endocrine Table 2. **Concentration of BNP in secretory granules and area of secretory granules in LA myocardium of sham and TIVCC by immunoelectron microscopy**

<table>
<thead>
<tr>
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<th>Sham-LA</th>
<th>TIVCC-LA</th>
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<tr>
<td>BNP gold particle/μm² secretory granule</td>
<td>31±9</td>
<td>154±22*</td>
</tr>
<tr>
<td>Area of secretory granules, μm²</td>
<td>0.0140±0.0005</td>
<td>0.0143±0.0006</td>
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</table>

Data are means ± SE. LA, left atrial myocardium; BNP, brain natriuretic peptide. *P < 0.05 vs. sham.
function, with a focus on BNP. Specifically, we determined BNP during cardiac unloading but in association with marked activation of vasoconstrictors and growth-promoting pathways. First, this investigation revealed that chronic mechanical myocardial unloading of the normal heart is characterized by atrial and ventricular atrophic remodeling, despite neurohumoral stimulation by ET-1, ANG II, and NE. Examination of atrial myocardium by immunochemistry in response to increased vasoconstrictor and growth-promoting pathways, such as the ubiquitin pathway, revealed no decrease in atrial secretory granule area but did reveal an increase in storage of BNP in granules through imaging of the immunogold-labeled BNP complex. This increased storage occurred with an increase in atrial cGMP, the second messenger for BNP, and with a decrease in atrial BNP mRNA, consistent with enhanced local BNP activation of natriuretic peptide-A receptor in atrial myocardial cells.

The current study extends previous investigation of ventricular atrophic remodeling with mechanical unloading and reports for the first time that unloading of the normal heart results in atrophic atrial remodeling and preservation of atrial secretory granules with increased storage of BNP. Unlike activation of fetal gene programs in ventricular myocardium during unloading, such as the ubiquitin pathway with ventricular atrophy (4, 26), atrial BNP mRNA was significantly decreased despite increases in ANG II and ET-1, which are known to activate BNP. Although in the current study we have not observed a significant increase in plasma BNP in the unloaded hearts when compared with sham, there was a trend suggesting that, with a larger sample size, plasma BNP may be higher in the unloaded hearts, as discussed below. Nonetheless, this increase would not achieve the markedly increased levels associated with cardiac volume overload, such as in congestive heart failure.

Our finding is consistent with the predominance of load vs. biochemical stimulation in control of atrial BNP. Moreover, acute reloading of the atrophic atrium also was characterized by a prompt release of BNP, again showing the importance of atrial load. In spite of this, we cannot completely exclude that the neurohumoral factors and cardiac load itself may play a role in the control of atrial BNP, especially regarding the regulation of its release. The current study focused on BNP in the unloaded heart, whereas the previous study (11) examined the impact of cardiac load and neurohumoral stimulation as it relates to tissue and plasma ANP. In the previous study, we have reported that myocardial unloading tended to decrease ANP gene expression with an increase in ANP atrial stores. In the current study, BNP mRNA in the unloaded atrial myocardium was lower and tissue concentration was higher when compared with sham-operated animals. Thus our investigation underscores the similarities in the regulation of ANP and BNP as it relates to myocardial unloading. Furthermore, because the unloading and decreased atrial stretch should decrease BNP secretion from the heart, one could speculate that neurohumoral mechanisms could be equally important for BNP secretion and is keeping the plasma level from falling below normal concentrations.

BNP is synthesized and secreted in both atrial and ventricular myocardium (7, 12, 19). BNP gene is constitutively expressed in adult atrial and ventricular myocytes, and higher levels of peptide and mRNA are generally observed in the atria (6, 19). BNP is co-stored with ANP in secretory granules, secreted, and released in the circulation (24). Under basal conditions, the amount of BNP in tissue and plasma is relatively low compared with ANP. However, some pathological conditions, such as congestive heart failure (CHF), are associated with elevation of tissue and plasma BNP. Samples obtained during catheterization of the human heart have shown that BNP originates from both atria and ventricles and that,
during CHF, the ventricles may contribute more than the atria to the rise in plasma levels (19). BNP levels in heart failure and after myocardial infarction may exceed those of ANP (20, 22). Relative contribution of the atria vs. the ventricles to plasma BNP in the unloaded hearts is unknown. Thus further studies using selective cannulations of the cardiac veins may address this issue, which is beyond the scope of the current study. Although the assessment of BNP mRNA in all four chambers of the heart should be addressed in future studies focusing on unloading, current findings of changes in BNP synthesis and storage in left atrium and ventricles are so consistent that no major difference in the right side of the heart is expected.

Like ANP, the expression of BNP is regulated by changes in intracardiac pressure and/or stretch. Mantymaa et al. (15) reported that, in the perfused isolated heart preparation, atrial stretch induced rapid stimulation of both synthesis and secretion of BNP. In addition to mechanical stimulus, expression and/or secretion of BNP can be regulated by humoral factors, such as ET, ANG II, vasopressin, and phenylephrine (1, 12, 13, 23). Bruneau et al. (1) demonstrated that ET-1 induces a marked increase in BNP gene expression in the atrial myocardium. Furthermore, Magga et al. (13) reported that vasopressin and phenylephrine rapidly stimulates atrial and ventricular BNP mRNA synthesis in normal and hypertensive rats. Finally, Ogawa et al. (25) demonstrated that both ET-1 and phenylephrine are potent stimuli of secretion of BNP from atrial myocardium.

The understanding of chronic unloading on BNP synthesis is less known but may have clinical relevance based on unloading strategies, such as ventricular assist devices (VAD), in addition to pharmacological strategies for CHF that have gained more clinical use. VADs are currently used to unload terminally failing hearts as a bridge to heart transplantation. The functional consequences of unloading are not clearly established, and the endocrine function of the heart under this condition is unknown. Studies have reported that hemodynamic unloading by an implanted VAD may improve cardiac contractile and mitochondrial function and reduce cardiac hypertrophy (21). Several studies have also reported that myocardial unloading leads to cardiac atrophy and a decrease in myocyte volume (8, 9, 27, 32). Morawietz et al. (18) reported that myocardial unloading by VAD in humans has been associated with a suppression of a LV pro-ANP gene expression. Sodian et al. (28) have recently shown that 2 mo of myocardial unloading of the failing heart with VAD is associated with a decrease in plasma BNP. However, characterization of synthesis of this biologically active cardiac hormone during myocardial unloading of the normal heart is poorly understood. Thus our current study provides insight into atrial BNP regulation and atrial endocrine function in the unloaded heart. Specifically, we have shown that, in the unloaded normal heart, the atrial synthesis of BNP is decreased, with no change in already lower BNP mRNA in ventricles, whereas storage is increased. However, we recognize that the current sample size may not have sufficient power to exclude significant reduction in ventricular BNP mRNA in the unloaded hearts. The observed increase in atrial BNP storage may also explain the higher level of tissue cGMP. The decreased atrial synthesis of BNP occurred despite the dramatic activation of known neurohumoral stimulators of BNP. Thus our study underscores the primacy of load as a mechanism in the control of the endocrine function of the heart vs. neurohumoral stimulation. Furthermore, despite atrial atrophy, we report that the release mechanism of BNP in the unloaded heart is preserved during atrial reloading.

In summary, the current study has both physiological and clinical implications related to BNP as a cardiac hormone. First, as it relates to unloading of the heart, atrial BNP is clearly influenced primarily by mechanical load and thus is similar to the control of ANP. This strongly supports the concept that the BNP in the atrial myocardium is primarily a volume-regulated hormone. Furthermore, the rapid release of BNP with reloading suggests that this cardiac hormonal system remains sensitive to load, even in the presence of atrophic atrial remodeling. These observations continue to support the utility of plasma BNP as a biomarker for cardiac volume. Nonetheless, we have to recognize that there may be some species differences in terms of magnitude of activation of myocardial BNP. Furthermore, we cannot totally exclude an important contribution of neurohumoral stimulation to maintaining plasma levels of BNP.
despite atrial and ventricular atrophy. The present study also supports the concept that myocardial unloading of the normal heart results in a quiescence of atrial BNP despite intense neurohumoral stimulation by ET, ANG II, and catecholamines, known potent activators of BNP synthesis, and underscores the importance of mechanical load not biochemical modulators in the control of atrial BNP.

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GRANTS

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REFERENCES