Response of Atrial Natriuretic Factor to Acute and Chronic Increases of Atrial Pressures in Experimental Heart Failure in Dogs

Role of Changes in Heart Rate, Atrial Dimension, and Cardiac Tissue Concentration

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Background. This study evaluated the role of changes in heart rate, atrial pressure, volume, and cardiac tissue atrial natriuretic factor (ANF) concentration in the modulation of plasma ANF concentration in a model of pacing-induced heart failure.

Methods and Results. The effects of acute right ventricular pacing (250 beats/min), acute volume expansion (35 ml/min), and volume expansion after 1 week of right ventricular pacing on plasma ANF concentration were compared in eight dogs (group 1). As shown during right ventricular pacing previously, volume expansion produced significant increases in cardiac filling pressures and left atrial volume. Right ventricular pacing and volume expansion produced similar increments in plasma ANF concentration: from 32±12 to 168±153 pg/ml (p<0.05) and from 32±9 to 137±113 pg/ml (p<0.05), respectively. When pacing was initiated after volume expansion, there were no significant further increases in left atrial volume and plasma ANF concentrations (from 332±121 to 407±113 pg/ml despite significant increases in filling pressures. Atrial and ventricular tissue samples were also obtained from 21 dogs paced to severe heart failure (group 2) and from 14 normal dogs (controls). In all groups, atrial ANF was higher than ventricular ANF concentration. At 1 week (group 1), left atrial appendage ANF concentration (6.2±2.5 versus 16.1±10.3 ng/mg) was reduced, whereas left ventricular free wall ANF concentration (0.62±0.31 versus 0.24±0.16 pg/mg) was increased compared with that of controls (both p<0.001). At severe heart failure (group 2), atrial ANF remained low, whereas ventricular ANF concentration was similar to that of the controls.

Conclusions. These data indicate that in pacing-induced heart failure, changes in heart rate, atrial pressure, and volume all contribute to the increased plasma ANF concentration. However, by 1 week (early heart failure), ANF release is attenuated, perhaps because of the inability of the atria to be stretched further and because of reduced atrial ANF concentration. In addition, the ventricle may be an additional source of ANF. (Circulation 1991;83:1780–1787)

Atrial natriuretic factor (ANF) is a recently discovered hormone of cardiac origin.1 Its multiple biological effects suggest that the hormone is implicated in the regulation of blood pressure and volume homeostasis.2–5 Plasma concentrations of ANF are elevated in patients with heart failure in association with increased cardiac filling pressures,4–7 suggesting that the hormone also plays a pathophysiological role in this condition.

Chronic, rapid right ventricular pacing induces severe heart failure in dogs.8–11 In this canine model of heart failure, we previously demonstrated that...
plasma ANF concentration peaks early after 1 week of pacing and fails to increase further as severe heart failure develops, that is, despite progressive increases in atrial pressures; atrial dimensions; and plasma concentrations of norepinephrine, renin, and aldosterone. These findings suggest that there may be an attenuated release of the hormone as severe heart failure develops. The mechanism for this attenuation is unclear but could relate to 1) limitation of the atrium to further stretching after chronic increases in heart rate or atrial pressures and 2) reduced atrial storage of ANF due to a chronic increase in demand of ANF. Given our previous observation that the natriuretic activity from the right atrium is reduced in this model of heart failure as recent reports of increased ventricular ANF concentration in heart failure, it seems timely to explore the relation of cardiac tissue and plasma ANF concentrations during evolving heart failure.

Therefore, the objectives of this study were 1) to compare ANF responses during acute rapid right ventricular pacing, acute volume expansion followed by initiation of rapid pacing, and acute volume expansion after 1 week of chronic rapid ventricular pacing and 2) to compare regional cardiac tissue atrial natriuretic factor concentrations in dogs at two different stages of heart failure.

Methods

Induction of Heart Failure

The methods of insertion of the permanent pacemaker and induction of heart failure have been described previously. In brief, under general anesthesia, a unipolar pacemaker lead was advanced through the external jugular vein to the right ventricular apex, and a programmable pulse generator (SX-5985, Medtronic, Richmond, British Columbia) was inserted into a subcutaneous cervical pocket. In some dogs, during the same procedure, a chronic indwelling cannula was positioned in the aorta through the carotid artery. The animals were allowed to recover from the effects of general anesthesia for at least 1 week before the initial study measurements were obtained. Thereafter, chronic pacing (250 beats/min) was maintained either for 1 week (early heart failure, group 1) or until the development of severe heart failure (group 2), a floating biological end point as described previously.

ANF Response to Volume Expansion and Pacing

To assess the impact of acute and chronic increases of heart rate and atrial pressures (volume loading) on ANF release, the eight dogs of the early heart failure group (group 1) underwent three sequential study protocols during the 1-week period.

Protocol A (response to acute temporary pacing). On day 1 (after 1 week of recovery from general anesthesia), the pacer was programmed to an asynchronous mode at a rate of 250 beats/min for 30 minutes. Arterial plasma samples for ANF concentrations were obtained at baseline (prepacing, 0 minutes) and at 15 and 30 minutes after initiation of pacing. Thereafter, the pacer was reprogrammed to allow resumption of sinus rhythm. Although changes in cardiac filling pressures and left atrial dimension associated with acute right ventricular pacing were not performed in protocol A of the present study, they have been characterized previously.

Protocol B (baseline acute volume expansion followed by acute pacing). On day 2, animals underwent right heart catheterization with a Swan-Ganz catheter introduced by way of the right femoral vein under lidocaine local anesthesia. Arterial pressure was obtained from the chronic indwelling aortic cannula. After obtaining baseline hemodynamic and echocardiographic measurements, and arterial plasma samples for ANF and norepinephrine measurements, 6% dextran in isotonic saline was infused at a rate of 35 ml/min. Hemodynamic and echocardiographic measurements and plasma samples were obtained every 10 minutes; the aim was to increase pulmonary capillary wedge pressure by 10–15 mm Hg. When the target filling pressure was reached, the infusion was stopped, and the pacer was activated at 250 beats/min. All measurements were then repeated at 15 and 30 minutes after the initiation of pacing. Pacing was then maintained for 1 week.

Protocol C (repeated volume expansion after 1 week of pacing). On day 8 (after 1 week of pacing), the volume expansion study was repeated during ventricular pacing. The dextrose and saline infusion was delivered at the same rate as in the baseline volume expansion study. Measurements were obtained every 5 minutes because it was anticipated the target filling pressure would be reached earlier than that in the baseline study. After the volume expansion study, the dogs were allowed to recover for 24 hours so that the effects of volume expansion would dissipate. Pacing was maintained during this time. The animals were then killed so that cardiac tissue ANF concentrations could be measured.

Cardiac Tissue ANF Concentrations

Cardiac tissue samples for measuring ANF concentration were obtained from three groups of dogs. Group 1 dogs (n=8) were paced for 1 week before being killed, and this was the same group that underwent the above sequential physiological studies. Group 2 dogs (n=21) were paced until severe heart failure occurred (4.5±2.1 weeks). Severe heart failure was defined as 25% or more increase in radiographic planimetered heart size, accompanied by pulmonary edema and/or 10% or more increase in body weight. Fourteen normal dogs served as the control group. All dogs underwent hemodynamic and echocardiographic study 24 hours before death. Details of hemodynamic and echocardiographic assessments have been described. Left atrial volume was derived from the formula: 3/4×LA area×LA length, where LA area is the planimetered cross-sectional left atrial area obtained from the
Assays

Plasma and tissue ANF concentrations were determined by radioimmunoassay with a commercially available antibody kit (Peninsula Laboratories, Belmont, Calif.). Details of the method have been reported in detail.17,18 Plasma renin activity was determined by radioimmunoassays19 whereas plasma norepinephrine concentration was determined by high-performance liquid chromatography.19

Statistical Analysis

Changes over time were measured by repeated measures analysis of variance followed by Dunnett’s t test. Comparisons between groups were made by one-way analysis of variance followed by Dunnett’s t test. Relations between study variables were assessed by linear regression analysis. Data are presented as mean±SD. A p value of less than 0.05 was considered significant.

Results

ANF Response to Volume Expansion and Pacing

Changes in hemodynamics, echocardiographically derived left atrial volume, and arterial plasma ANF and norepinephrine concentrations are summarized in Table 1. On day 1 (protocol A), acute (30 minutes) ventricular pacing was associated with a significant increase in plasma ANF concentrations within 15 minutes (32±12 to 156±155 pg/ml, p<0.05), which remained increased at 30 minutes (to 168±153 pg/ml, p<0.05). Like ANF, plasma norepinephrine concentration also increased significantly at 30 minutes.

On day 2 (protocol B), before volume expansion, plasma ANF concentration (32±9 pg/ml) was identical to the control (prepacing) value (32±12 pg/ml) of protocol A. As shown in Table 1 and Figure 1, volume expansion was associated with a gradual increase in heart rate, cardiac output, pulmonary capillary wedge and right atrial pressures, left atrial volume, and plasma ANF concentration. At the conclusion of the infusion (peak infusion, 29±14 minutes), plasma ANF concentration (137±113 pg/ml) was similar to that achieved by 30 minutes of pacing in protocol A on day 1. When pacing was initiated after the volume expansion, there was only a

short-axis view at the level of the aortic valve13 and LA length is the length of the left atrium obtained from the apical four-chamber view. In group 2 and group 3 dogs, venous instead of arterial plasma samples were obtained for measuring ANF concentrations because these dogs had no chronic indwelling aortic cannula. The animals were killed by an overdose of sodium thiopental. The hearts were removed, trimmed, and weighed. Large portions of the body and appendage of the left and right atria, the interatrial septum, the free wall, and septum of the left and right ventricles were separated and immersed immediately in liquid nitrogen. The samples were then stored in a refrigerator at −80°C. The tissue was subsequently homogenized for 1 minute with a Virtischer (Virtis Inc., Chicago) in 0.1 M acetic acid and was centrifuged again at 30,000 rpm for 20 minutes. The pellet was discarded, and the supernatant was stored at −20°C until assay.

Table 1. Effects of Pacing and Volume Expansion on Hemodynamics, Left Atrial Dimension, and Plasma Atrial Natriuretic Factor and Norepinephrine Concentrations

<table>
<thead>
<tr>
<th>Time</th>
<th>HR (beats/min)</th>
<th>RAP (mm Hg)</th>
<th>PCWP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>CO (l/min)</th>
<th>LAV (cm³)</th>
<th>[ANF] (pg/ml)</th>
<th>[NE] (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol A (acute pacing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>120±23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32±12</td>
<td>277±49</td>
</tr>
<tr>
<td>15 onset</td>
<td>250±0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>156±155*</td>
<td>-</td>
</tr>
<tr>
<td>30 onset</td>
<td>250±0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>168±153*</td>
<td>511±144†</td>
</tr>
<tr>
<td>Protocol B (baseline acute volume expansion followed by pacing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>106±27</td>
<td>7±1</td>
<td>9±1</td>
<td>136±19</td>
<td>4.2±0.9</td>
<td>21±6</td>
<td>32±9</td>
<td>343±53</td>
</tr>
<tr>
<td>10</td>
<td>151±32*</td>
<td>13±2†</td>
<td>16±2*</td>
<td>147±17</td>
<td>7.4±0.5</td>
<td>28±7</td>
<td>36±14</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>184±37†</td>
<td>19±4‡</td>
<td>24±6‡</td>
<td>155±16</td>
<td>8.1±3.2‡</td>
<td>38±8*‡</td>
<td>86±57</td>
<td>-</td>
</tr>
<tr>
<td>Peak</td>
<td>187±37†</td>
<td>21±5†</td>
<td>27±6†</td>
<td>157±19*</td>
<td>8.4±2.3†</td>
<td>40±11†</td>
<td>137±113*</td>
<td>204±68†</td>
</tr>
<tr>
<td>15 onset</td>
<td>250±0†</td>
<td>20±3‡</td>
<td>32±5†</td>
<td>144±16</td>
<td>5.2±0.9</td>
<td>45±12†</td>
<td>462±295†</td>
<td>-</td>
</tr>
<tr>
<td>30 onset</td>
<td>250±0†</td>
<td>17±3‡</td>
<td>28±4‡</td>
<td>145±15</td>
<td>6.1±2.2*</td>
<td>47±17†</td>
<td>272±156†</td>
<td>300±51</td>
</tr>
<tr>
<td>Protocol C (repeated acute volume expansion after 1 week of pacing)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>250±0</td>
<td>10±2</td>
<td>18±3</td>
<td>95±10</td>
<td>2.3±0.5</td>
<td>37±9</td>
<td>332±121</td>
<td>687±172</td>
</tr>
<tr>
<td>5</td>
<td>250±0</td>
<td>15±5*</td>
<td>26±5†</td>
<td>109±15</td>
<td>-</td>
<td>40±10</td>
<td>301±183</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>250±0</td>
<td>20±5‡</td>
<td>31±6†</td>
<td>119±13*</td>
<td>2.8±1.0</td>
<td>41±11</td>
<td>315±138</td>
<td>-</td>
</tr>
<tr>
<td>Peak</td>
<td>250±0</td>
<td>25±5‡</td>
<td>37±5†</td>
<td>125±12*</td>
<td>3.3±0.7</td>
<td>45±15</td>
<td>407±113</td>
<td>721±171</td>
</tr>
</tbody>
</table>

Values are mean±SD.

5, 10, and 20 minutes, time of volume expansion; peak, peak volume expansion (29±14 minutes for protocol B and 17±6 minutes for protocol C); 15 minutes onset and 30 minutes onset, time after initiation of pacing; HR, heart rate; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; MAP, mean arterial pressure; CO, cardiac output; LAV, left atrial volume; [ANF], plasma atrial natriuretic factor concentration; [NE], plasma norepinephrine concentration.

*p<0.05, †p<0.01 versus baseline (0 minutes) of each protocol.
trend for pulmonary capillary wedge pressure to increase further at 15 minutes (32±5 mm Hg, p=0.08 compared with peak infusion), and there was no further increase in left atrial volume and right atrial pressure. On the other hand, there was a significantly further increase in plasma ANF concentration at 15 minutes after initiation of pacing (462±295 pg/ml, p<0.01 versus peak infusion). At 30 minutes after initiation of pacing, plasma ANF concentration declined slightly from the peak value at 15 minutes after onset of pacing, but the difference was not significant (p=0.13). Unlike ANF, plasma norepinephrine concentration declined significantly (p<0.01) from baseline at peak volume expansion but then increased at 30 minutes after initiation of pacing (p<0.05 versus peak volume expansion). This increase with pacing, however, was smaller than that on day 1 (p<0.05).

On day 8 (protocol C), after 1 week of pacing, baseline (before volume expansion) ANF concentration was significantly higher (p<0.05) than the corresponding control values on days 1 and 2. However, unlike values obtained from protocol B on day 2 and despite substantial further increases in cardiac filling pressures, there was no further increase in left atrial volume or plasma ANF concentration (p=0.59, 0.49, respectively, analysis of variance) with volume expansion. Furthermore, plasma ANF concentration at peak infusion (17±6 minutes) was comparable to the peak value obtained at 15 minutes of rapid pacing after acute volume expansion on day 2. As expected, baseline (before volume expansion) plasma norepinephrine concentration after 1 week of pacing was significantly higher than the corresponding baseline values on days 1 and 2 (both p<0.01). However, unlike the value on day 2, there was no significant decline in norepinephrine concentration with volume expansion.

**Cardiac Filling Pressures and Left Atrial Volume**

Cardiac filling pressures and left atrial volume of the three study groups obtained in the conscious state are shown in Table 2. At 1 week (group 1), pulmonary capillary wedge pressure, right atrial pressure, and left atrial volume were increased significantly compared with those of the controls (p<0.01, p<0.05, and p<0.05). At the time of severe heart failure (group 2), there were further increases in all
of these parameters \((p<0.01, p<0.05, \text{ and } p<0.05\) versus group 1, respectively).

**Heart Weight and Plasma and Cardiac Tissue ANF Concentrations**

Data for the gross heart weight, heart weight normalized to body weight, and plasma and cardiac tissue ANF concentrations obtained from the four cardiac chambers of the three study groups are shown in Table 3. Arterial plasma ANF concentration at 1 week was significantly higher than the venous concentration in the controls \((p<0.01)\). At severe heart failure, plasma ANF was lower than that at 1 week \((p<0.01)\) although significantly higher than that of controls \((p<0.05)\).

In the controls, there was significant intra-atrial regional differences \((p<0.05, \text{ analysis of variance})\) in tissue ANF concentrations (Table 3). The concentration tended to be higher in the appendages than in the bodies of the atria and higher in the left than in the right atria. The mean concentration in the ventricles was approximately \(\sqrt{100,000}\) that of the atria, and there were no significant regional variations.

Similarly, at 1 week, there was still significant regional variation in atrial tissue ANF concentration. However, the concentrations in the appendages and bodies of both atria were lower than those in the controls \((all \ p<0.05)\). The concentrations in the left and right ventricular free walls were higher than the corresponding concentrations in the controls \(both\ p<0.05\), and the concentration was higher in the left than in the right ventricle \((p<0.05)\).

In contrast to the values obtained in the controls and group 1, in group 2, there were no regional variations in atrial tissue ANF concentrations. ANF concentration from all regions of the two atria were significantly lower than the corresponding values of the controls but similar to those of group 1. ANF concentration was higher in the right ventricle than in the left ventricle or the interventricular septum. In all ventricular regions, ANF concentrations were significantly lower than those from group 1 and were similar to those in the controls.

**Relations of Cardiac Filling Pressures, Left Atrial Volume, and Tissue ANF Concentrations**

Tissue ANF concentrations from all three study groups were correlated with their corresponding chamber pressures and left atrial ANF concentrations with left atrial volume obtained before death. ANF concentrations from the left atrial appendage correlated inversely with pulmonary capillary wedge pressure \((r=-0.43, p<0.05)\) and left atrial volume \((r=-0.49, p<0.05)\), whereas ANF concentrations from the right atrial appendage correlated inversely with the right atrial pressure \((r=-0.50, p<0.05)\). ANF concentrations from the body of the left atrium correlated only modestly with pulmonary capillary wedge pressure \((r=-0.38, p<0.05)\) and not with left atrial volume \((r=-0.28, \text{ NS})\). ANF concentration from the body of the right atrium also correlated only modestly with right atrial pressure \((r=-0.34, p<0.05)\).

**Discussion**

Our study provides new information regarding 1) the relative contributions of acute versus chronic increases in heart rate, atrial pressures, and left atrial volume to the release of ANF and 2) the regional cardiac tissue ANF concentrations in the atria and the ventricles in normal dogs and dogs with early (1 week) and advanced heart failure.

**Relative Contribution of Acute and Chronic Increases of Heart Rate, Atrial Pressures, and Left Atrial Volume to ANF Release**

We previously documented that acute right ventricular pacing increases plasma ANF concentration, accompanied by increased pulmonary capillary wedge pressure and left atrial dimension.\(^{13}\) Our present study demonstrates that acute right ventricular pacing (protocol A) increases plasma ANF concentrations to similar levels achieved by acute volume expansion (protocol B). Moreover, acute pacing after volume expansion (protocol B) increases plasma ANF concentration even more, in the absence of significant further increases in atrial pressures and left atrial volume. After 1 week of pacing, atrial pressures, left atrial volume, and ANF concentration were all increased. Although acute volume expansion after 1 week of pacing (protocol C) produces further increases in atrial pressures, this is no longer associated with increases of left atrial volume or plasma ANF concentration. Of note, the peak ANF concentration achieved during volume expansion at 1 week was comparable to the maximal ANF value obtained after volume expansion and onset of pacing at day 2 (protocol B). These findings indicate that 1) rapid right ventricular pacing contributes to the release of ANF and 2) the release of ANF is attenuated after 1 week of pacing. Based on the left
TABLE 3. Heart Weight and Plasma and Cardiac Tissue Atrial Natriuretic Factor Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g)</td>
<td>191±37 (14)</td>
<td>180±26 (8)</td>
<td>203±33 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>Normalized heart weight (g/kg)</td>
<td>8.3±1.1 (14)</td>
<td>9.0±1.0 (8)</td>
<td>8.2±1.2 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma ANF (pg/ml)</td>
<td>98±25 (9)</td>
<td>332±120 (8)</td>
<td>182±62 (21)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Atrial tissue ANF (ng/mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA appendage</td>
<td>16.1±10.3 (14)</td>
<td>6.2±2.5 (8)</td>
<td>6.2±2.7 (21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA body</td>
<td>10.4±8.3 (14)</td>
<td>5.3±2.1 (8)</td>
<td>5.2±1.7 (21)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Septum</td>
<td>9.0±4.3 (9)</td>
<td>4.7±1.3 (8)</td>
<td>4.5±1.6 (12)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RA appendage</td>
<td>10.0±4.1 (15)</td>
<td>4.0±1.7 (8)</td>
<td>5.4±2.1 (21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RA body</td>
<td>8.7±6.8 (15)</td>
<td>4.2±1.4 (8)</td>
<td>4.4±1.6 (21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-ventricular tissue ANF (pg/mg)</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LV free wall</td>
<td>0.24±0.16 (10)</td>
<td>0.62±0.31 (8)</td>
<td>0.19±0.17 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Septum</td>
<td>0.14±0.11 (5)</td>
<td>0.81±0.52 (8)</td>
<td>0.05±0.03 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV free wall</td>
<td>0.20±0.18 (10)</td>
<td>0.37±0.18 (8)</td>
<td>0.23±0.20 (16)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD; sample sizes are in parentheses. Group 1, paced for 1 week; group 2, paced to severe heart failure.
Plasma samples from the controls and group 2 were obtained from the peripheral vein, whereas samples from group 1 were obtained from the aorta.
ANF, atrial natriuretic factor; LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle.
Probabilities were obtained from analysis of all groups or sites.

atrial volume data, this attenuation may be related to an inability of the atria to be stretched further or to a finite limit of ANF release.

Previous studies suggested that pacing alone releases ANF. In rat atrial strips, pacing from 2 to 4 Hz was associated with a 46% increase in ANF concentration.20 In conscious dogs with surgically induced atrioventricular block, atrial pacing produced a significant rise in coronary sinus ANF concentrations even in the absence of an increase in ventricular rate and cardiac filling pressures.21 The impact of volume expansion has been examined in two groups of pentobarbital-anesthetized dogs: one group after acute (25 minutes) and the other after chronic (14–16 days) right ventricular pacing.22 Volume expansion failed to increase plasma ANF concentration in both groups of dogs. Of note, volume expansion in these studies was administered after instead of before acute pacing as in our study. Therefore, the limit for release of ANF may have already been reached after acute pacing. Interpretation of these data is further confounded by the potential effect of general anesthesia and the lack of concomitant atrial dimension measurements.

The increased heart rate and mean arterial pressure after volume loading on day 2 is consistent with activation of the Bainbridge reflex.23,24 The increase in cardiac output is likely modulated both by the increased heart rate and by the Frank-Starling mechanism augmenting stroke volume through increased cardiac filling pressures.

Plasma norepinephrine increased significantly 30 minutes after acute right ventricular pacing on day 1 (protocol A). This increase in plasma norepinephrine concentration may reflect a reflex-mediated increase in sympathetic nerve activity in response to an abrupt decrease in stroke volume induced by rapid pacing.25 On the other hand, the decrease in plasma norepinephrine concentration after volume expansion on day 2 (protocol B) and the smaller increase after onset of pacing compared with that on day 1 likely reflects the stimulation of the cardiopulmonary reflex and, thereby, an inhibition of sympathetic outflow.26,27 The lack of a change in plasma norepinephrine concentration after volume expansion after 1 week of pacing (protocol C) signifies an attenuation of the cardiopulmonary reflex. Indeed, preliminary data in this model from our laboratory have documented an attenuation of cardiopulmonary baroreflex by 1 week of pacing.28

Cardiac Tissue ANF Concentration

Our study is the first that compares cardiac regional tissue ANF concentrations in normal dogs and in dogs with early and advanced heart failure. In normal dogs, the atrial appendages have the highest ANF concentration, suggesting that they may act as a storage site for the hormone. Immunoreactive ANF, although detectable in the ventricles, is less than 1/100,000 of that in the atria, suggesting that the ventricles are not the primary site of production of the hormone under normal conditions.

Alterations in atrial tissue concentration of ANF may in part explain the attenuated response to volume expansion after 1 week of pacing. In these dogs paced for 1 week (early heart failure), plasma ANF is increased, whereas immunoreactive ANF in the atria is markedly reduced compared with controls, possibly because of increased demand. However, there remain significant interatrial variations,
with the left atrial appendage having the highest ANF concentration. In marked contrast to the atria, immunoreactive ANF concentrations in the ventricles are significantly higher than the corresponding concentrations in controls. The latter observation suggests that in dogs with early heart failure, the ventricle is stimulated to increase the production of ANF, presumably in an attempt to sustain an elevated plasma ANF concentration.

In dogs with severe heart failure, ANF concentration is reduced throughout all regions of the atria, including the left atrial appendage, and no interatrial differences are evident. The concentrations in the ventricles are similar to those in the controls, indicating reduced content of the hormone compared with early heart failure. These observations in this model of heart failure may explain the failure of plasma ANF concentration to increase further after 1 week, despite progressive increases in atrial pressures and dimensions.12,13

Previous morphological studies suggested that the atrial appendages contain the highest number of granules,20 supporting the concept that the atrial appendages are the storage depot for the hormone. Indeed, in conscious monkeys, atrial appendectomy practically abolishes the increase in plasma ANF concentration during atrial distension.21 Our observations of the regional differences in tissue immunoreactive ANF are consistent with those from the morphological study20 and a previous study on normal dogs.31 In the latter, immunoreactive ANF concentrations were also highest in the atrial appendages and lowest in the ventricle.

Reduced ANF content in the atria has been reported in other experimental models of heart failure.15,32–37 Aside from reduced immunoreactivity,15,35–37 reduced natriuretic activity on bioassay2,10,30,31 and reduced number of granules on immunohistochemical staining14,34 suggest that there is reduced atrial storage of the hormone in heart failure. The reduced storage is likely due to excessive release secondary to increased demand of the hormone, as suggested by the observations of hypertrophied golgi apparatus in the atria of dogs with pacing-induced heart failure.38

Under normal conditions, ANF content in the ventricle is extremely low relative to the atria.14,15,37 However, in hamsters and rats with heart failure, the number of granules,14 tissue immunoreactive ANF concentration,14,15,36 and Langendorff effluent of immunoreactive ANF39 from the ventricles have all been found to be markedly increased compared with controls. In the myopathic hamsters15 and rats with volume overload,40 ANF messenger RNA was significantly increased, indicating that the increase in ANF occurs at the level of gene expression. However, these previous studies differ from ours in that the tissues were obtained from animals with severe heart failure and no attempts were made to correlate tissue ANF concentrations with severity of heart failure.

Potential Limitations

Certain points in the design and observations of our study deserve further comments. First, blood samples for plasma ANF determination were obtained from the aortic cannula in group 1, whereas samples were obtained from the peripheral vein in group 2 and the controls. Therefore, plasma ANF concentration in group 2 may, conceivably, have been lower if venous samples had been used. However, given the ratio of arterial to venous plasma ANF concentration (1.2 to 1) that we previously documented in patients with heart failure, plasma ANF concentration would still have been significantly higher in group 2 than in group 1 and the controls. Second, because weights of individual cardiac chambers were not measured in our study, one may argue that total tissue ANF content might not have been altered in the heart failure dogs if hypertrophy had occurred. This seems unlikely because 1) total heart weight did not differ among the study groups, 2) left ventricular hypertrophy does not occur in this model,11,41 and 3) studies using other models have demonstrated both reduced ANF content and concentration,35,36 as well as granularity14 in the atrium in severe heart failure.

In summary, in the model of pacing-induced heart failure, increases in heart rate, cardiac filling pressure, and atrial dimension all appear to contribute to the increase in plasma ANF concentration. In addition, the response of ANF to an acute increase in atrial pressures is attenuated after 1 week of rapid pacing. This attenuation may be due to the inability of the atria to be stretched further or to reduced atrial ANF storage. The plasma and tissue ANF data suggest the following paradigm for the contribution of cardiac tissue ANF to the changes in plasma ANF concentration during evolving heart failure. In normal dogs, plasma ANF concentration is low, and the atria almost entirely contribute to the circulating ANF. After 1 week of pacing, cardiac filling pressures and left atrial volume are increased, and plasma ANF concentration is markedly elevated. Because atrial tissue ANF concentration is then reduced, the increased ventricular ANF concentration may reflect an additional source of ANF production, helping to sustain a high plasma ANF concentration. At severe heart failure, both the atrial and ventricular storage of ANF are reduced, thus accounting for the failure of plasma ANF concentration to increase further.

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