Circulating β–Atrial Natriuretic Factor in Congestive Heart Failure in Humans

Chi-Ming Wei, MD, PhD; Pai C. Kao, PhD; Jiann-Trzuo Lin, PhD; Denise M. Heublein, Hartzell V. Schaff, MD; John C. Burnett, Jr, MD

Background. β-Atrial natriuretic factor (β-ANF) is an antiparallel dimer of α-ANF (α-ANF) with diminished cyclic GMP generation in vitro. To date, the presence of β-ANF in the circulation of humans with severe congestive heart failure (CHF) remains controversial. The current study was designed to determine the presence and magnitude of circulating β-ANF in severe CHF, to correlate plasma β-ANF with the degree of ventricular dysfunction, and to investigate the role of human plasma and atrial tissue in the degradation of β-ANF.

Methods and Results. Venous plasma samples were obtained from patients (n=12) with severe CHF and normal volunteers (n=8). Total plasma ANF was measured by radioimmunoassay. α-ANF and β-ANF in nonextracted plasma were separated by gel filtration chromatography using a P-6 column. Right atrial tissue samples (n=5) were collected from a different group of patients at the time of open-heart surgery. 125I β-ANF and 131I ANF were incubated with atrial tissue or plasma. The corresponding peak areas of β-ANF were determined by Tamaya Digital Planimeter. β-ANF represented 61% of total plasma ANF in CHF patients and was not detected in normal human plasma. The elevation of β-ANF correlated with the severity of ventricular dysfunction. Thirty percent of β-ANF and 100% α-ANF were converted to smaller peptide fragments in atrial tissue with no conversion in plasma.

Conclusions. β-ANF is the principal form of circulating ANF in patients with severe CHF and correlates with the degree of left ventricular dysfunction. β-ANF is not generated from α-ANF and may be degraded rapidly in atrial tissue to smaller peptide fragments that do not occur in plasma. As β-ANF is reported to have reduced biological action, the current studies may support the conclusion that the ANF system in CHF has reduced functional activity despite increases in circulation concentrations. (Circulation. 1993;88:1016-1020.)

KEY WORDS • natriuretic factors • heart failure • atrium

Atrial natriuretic factor (ANF) is an endogenous peptide hormone of cardiac origin that is stored in atrial granules as a 126-amino-acid pro-ANF molecule.1-5 In response to atrial stretch, pro-ANF is cleaved by a membrane-bound protease and released into the circulation as a 28-amino-acid peptide.6,7 Studies support a biological role for ANF in the regulation of cardio renal homeostasis in which physiological concentrations of ANF may increase sodium excretion, inhibit the renin-angiotensin-aldosterone system (RAAS), and decrease venous return.8

Repeated studies have demonstrated that ANF synthesis and secretion by the heart are markedly enhanced in congestive heart failure, resulting in elevation of circulating concentrations.3,5-11 While elevated plasma ANF may serve to promote sodium excretion and antagonize the RAAS,12 severe symptomatic congestive heart failure is a clinical syndrome that demonstrates avid sodium retention and marked activation of the RAAS. Thus, severe symptomatic congestive heart failure is characterized by renal and endocrine hyporesponsiveness to endogenous ANF as well as to exogenous ANF.13,14 While multiple mechanisms have been advanced to explain the biological resistance to endogenous ANF in congestive heart failure, such as receptor down-regulation, the opposing actions of angiotensin II, and increased degradation of ANF,15-17 the release of an altered biologically active form of ANF has been proposed.18,19

Studies by Sugawara and coworkers20 have reported the presence of an antiparallel dimer form of ANF termed β-ANF in atrial tissue of humans with severe congestive heart failure with minimal concentrations in patients with mild congestive heart failure. Recent in vitro studies have demonstrated that β-ANF has decreased biological activity compared with α-ANF, suggesting a potential pathophysiological role for this altered form of ANF in severe congestive heart failure.21 Reports of low plasma concentrations of β-ANF in plasma of patients with valvular heart disease but without congestive heart failure demonstrate that β-ANF exists as a circulating hormone in addition to its known presence in cardiac tissue of the failing heart.22-26 To date, the presence and magnitude of circulating β-ANF and its relationship to ventricular function have not been defined in humans with severe congestive heart failure.

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TABLE 1. Parameters of New York Heart Association Functional Class IV Congestive Heart Failure Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73</td>
<td>M</td>
<td>Idiopathic dilated cardiomyopathy</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>M</td>
<td>Idiopathic dilated cardiomyopathy</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>M</td>
<td>Idiopathic dilated cardiomyopathy</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>M</td>
<td>Ischemic cardiomyopathy</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>M</td>
<td>Ischemic cardiomyopathy</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>M</td>
<td>Ischemic cardiomyopathy</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>M</td>
<td>Ischemic cardiomyopathy</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
<td>M</td>
<td>Ischemic cardiomyopathy</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>F</td>
<td>Post–cardiac transplant, cardiomyopathy</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>M</td>
<td>Carcinoid cardiomyopathy</td>
</tr>
<tr>
<td>11</td>
<td>84</td>
<td>F</td>
<td>Hypertensive cardiomyopathy</td>
</tr>
<tr>
<td>12</td>
<td>72</td>
<td>M</td>
<td>Hypertensive cardiomyopathy</td>
</tr>
</tbody>
</table>

The current study was therefore designed to determine the presence and relative concentration of β-ANF in the plasma of patients with severe chronic congestive heart failure and normal volunteers. Second, the magnitude of elevation of this altered form of ANF was correlated with the degree of left ventricular dysfunction. Third, we investigated the role of human plasma and atrial tissue in the degradation of β-ANF.

Methods

Patients and Normal Volunteers

Eight normal subjects (mean age, 61±4 years; 6 men and 2 women) served as the control group. None had a history of cardiovascular, renal, liver, or metabolic disease, and none were taking medication. Twelve patients (mean age, 67±3 years; 10 men and 2 women) with congestive heart failure and New York Heart Association (NYHA) functional class IV were investigated. All congestive heart failure patients were on treatment, which included digitalis, diuretics, and/or vasodilators. None of the patients had intrinsic primary renal or hepatic dysfunction. The clinical profile and hemodynamic parameters of the NYHA class IV group are reported in Tables 1 and 2.

Hemodynamic Measurement

Arterial, right atrial, pulmonary wedge, left and right ventricular, and pulmonary arterial pressures were determined at the time of cardiac catheterization. Left ventricular ejection fraction was determined by left ventriculography performed at the time of cardiac catheterization. Normal left ventricular ejection fraction in the cardiac catheterization laboratory is more than 0.55. Cardiac output was determined by thermodilution. Cardiac index was determined by cardiac output divided by total body surface area.

Analysis

Blood for radioimmunoassay and cardiac hemodynamics was obtained at the time of cardiac catheterization. Venous plasma samples (3 mL) were obtained from antecubital space in normal subjects and chronic congestive heart failure patients and placed into iced EDTA tubes. Blood samples were centrifuged at 2500 rpm at 4°C for 15 minutes, and the plasma then was removed and stored at −20°C until analysis. Plasma levels of total ANF (α- plus β-ANF) were measured by an α-ANF radioimmunoassay as previously described. The radioimmunoassay had an equal molar cross-reactivity with α- and β-ANF as determined by the addition of synthetic peptides to the assay. α-ANF and β-ANF were separated from nonextracted plasma by a P-6 (Bio-Rad Laboratories, Richmond, Calif) gel-filtration column (1×13 cm). Five hundred microliters of nonextracted plasma was applied to the column and eluted with 0.5 M acetic acid buffer. Fractions of 0.5 mL were collected and dried by Speedvac. The concentration of ANF in each fraction was determined by radioimmunoassay. The P-6 column was calibrated with labeled α- and β-ANF (1125 α-ANF and 131I β-ANF) (Fig 1A). β-ANF was eluted from fractions 5 through 9 (peak fraction at 7) and α-ANF from fractions 10 through 17 (peak fraction at 12). Total ANF recovery was determined by adding the concentrations of each fraction (5 through 17) after the subtraction of background (5 pg per fraction). The amount of ANF measured in fractions 5 through 9 was calculated as β-ANF and in fractions 10 through 17 as α-ANF. The percentages of α- and β-ANF were determined from these calculated ratios. The mean column recovery of total ANF was 86%.

Right Atrial Tissue Preparation

Samples of right atrial tissue (n=5) were collected from a different group of patients from mild-to-severe congestive heart failure at the time of open-heart surgery and placed in liquid nitrogen until tissue homogenization. Atrial tissue (mean weight, 532±24 mg) was homogenized with 10 vol 20 mmol/L Na3HPO4 and 100 mmol/L NaCl (active tissue) or with 10 vol 1 mol/L acetic acid and 20 mmol/L HCl and boiled for 5 minutes (inactive tissue for control study). The tissue protein concentrations were measured with standardized protein in the supernatant. 1125 β-ANF or 131I α-ANF was incubated with supernatants of active and inactive tissue and plasma for 0 and 60 minutes at 22°C. β-ANF and α-ANF were identified with a P-6 gel filtration column. The peak areas of β-ANF and α-ANF were determined with Tamaya Planimeter. The peak area of 131I

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β-ANF or 125I-α-ANF was not changed by incubation for 60 minutes in normal saline at 22°C.

Statistical Analysis

Data for congestive heart failure and normal subjects are expressed as mean±SEM values. Comparisons between congestive heart failure and normal groups were analyzed by unpaired Student’s t-test. Statistical significance was defined as P<.05.

Results

Table 3 gives the total ANF (α- plus β-ANF) concentrations in the plasma of congestive heart failure patients and normal subjects. Total ANF was greater in the congestive heart failure group than in the normal group. Analysis by gel filtration of the molecular forms of ANF in the plasma demonstrated that β-ANF represented 61±2% of total plasma ANF in severe congestive heart failure, whereas α-ANF represented 39±3%. In marked contrast, no β-ANF was detectable in normal human plasma.

Fig 1B is a representative gel filtration chromatogram from a normal subject. Total immunoreactive plasma ANF was 11.6 pg/mL. Gel filtration demonstrated peak activity at fraction 12 but none at fraction 7, indicating that β-ANF was not present in plasma from normal subjects.

Fig 1C is a representative gel filtration chromatogram of a patient with severe congestive heart failure. Total ANF was 316 pg/mL, and gel filtration indicated the principal form to be β-ANF (66% of total) with a smaller percentage (33%) migrating to the α-ANF calibration position.

Fig 2A demonstrates the relationship of absolute β-ANF to absolute total ANF, and Fig 2B demonstrates the relationship of β-ANF to left ventricular ejection fraction of congestive heart failure patients. Increases in absolute β-ANF correlated significantly with increases in absolute total ANF and decreases in left ventricular ejection fraction.

Incubation of β-ANF in plasma and inactive tissue demonstrated that 60-minute incubation of β-ANF did not produce any change of peak area of β-ANF.
Table 4. Conversion of β-ANF Activity in Cardiac Tissue and Plasma

<table>
<thead>
<tr>
<th>β-ANF area (mm²)</th>
<th>0 Minutes</th>
<th>60 Minutes</th>
<th>Δ</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active tissue</td>
<td>39±2</td>
<td>27±1*</td>
<td>-12±1†</td>
<td>-30±2†</td>
</tr>
<tr>
<td>Inactive tissue</td>
<td>35±3</td>
<td>35±3</td>
<td>0.3±0.5</td>
<td>0.5±1.3</td>
</tr>
<tr>
<td>Plasma</td>
<td>43±5</td>
<td>44±6</td>
<td>1.5±1.3</td>
<td>3±3</td>
</tr>
</tbody>
</table>

ANF indicates atrial natriuretic factor. Values are mean±SEM. n=5.
*P<.05 vs 0 minutes, †P<.05 vs baseline value.

In contrast, peak area of β-ANF was markedly decreased at 60 minutes in active tissue, and the peak area of a fragment smaller than α-ANF was increased. Table 4 reports the changes of peak area of β-ANF in active tissue (n=5) and demonstrates that the peak area of β-ANF was 39±2 mm² at 0 minutes (baseline) and 27±1 mm² after 60-minute incubation with 1537-ANF. This is a decrease of 30±2% (P<.05) from the 0-minute value. In contrast, β-ANF was not converted in inactive tissue (35±3 mm² at 0 minutes and 35±3 mm² at 60 minutes) or in plasma (43±5 mm² at 0 minutes and 44±6 mm² at 60 minutes). Studies also revealed that α-ANF was 100% converted to a smaller fragment in active tissue but not in plasma.

Discussion

The present study demonstrates that β-ANF is present in high plasma concentrations and is the principal circulating form of ANF in patients with severe congestive heart failure. In marked contrast, β-ANF is not present in plasma of normal humans. This study also reveals that the percent of β-ANF increases as total ANF (α- and β-ANF) increases. Furthermore, the magnitude of plasma β-ANF elevation also increases with progressive ventricular dysfunction. Last, β-ANF appears to be degraded to a peptide fragment smaller than α-ANF in cardiac tissue but not in plasma.

Studies have demonstrated that plasma ANF is elevated in congestive heart failure9 and that this elevation is secondary to increased synthesis and secretion of ANF from the failing heart.11,28-30 Investigations also have reported that ANF elevation in congestive heart failure correlates positively with the functional class of congestive heart failure.10 The current study importantly confirms the elevation of plasma ANF in severe congestive heart failure. The current investigation also extends previous reports to establish that the principal form of increased endogenous ANF in severe congestive heart failure is the antiparallel dimer β-ANF. This study therefore complements the elegant work of Sugawara and colleagues,20 which reported that the increase in total ANF tissue concentration in the atria of the human failing heart is mainly due to an increase in tissue β-ANF. While these previous studies did not determine if β-ANF was increased in the circulation, the present study clearly establishes an important role for β-ANF as a circulating hormone in humans with severe congestive heart failure.

The present study may have important functional implications in neurohumoral and renal regulation in congestive heart failure. Severe congestive heart failure is characterized by marked sodium retention and activation of the RAAS in association with marked arterial vasoconstriction. While previous studies in experimental acute congestive heart failure have suggested that increased endogenous ANF has important biological activity to preserve sodium excretion and partially inhibit the RAAS despite arterial hypotension,12 a renal resistance to synthetic α-ANF is a hallmark of severe congestive heart failure.13,14,31 This resistance is mediated by multiple mechanisms, including receptor down-regulation, the opposing actions of angiotensin II, and increased degradation of ANF.15-17 With regard to β-ANF in vitro, reports have documented that β-ANF may have a reduced capacity to stimulate the production of cGMP, which is the second messenger for ANF.21 One could speculate that an additional potential mechanism for the renal, endocrine, and vascular adaptations in chronic congestive heart failure may be related to the release of an altered form of ANF that has reduced biological action.

The degradation of β-ANF has not been investigated. In the processing of β-ANF, it is speculated that β-ANF may be a postprocessing product of mature α-ANF in tissue of the failing heart. Such processing could be related to alterations in energy production, which favors dimerization as well as altered energy use or excitation-coupling mechanisms. The positive correlation between the magnitude of β-ANF presence in plasma with the severity of ventricular dysfunction provides circumstantial support for this concept. An alternative mechanism could be that β-ANF is the primary mature product of pro-hormone, as is α-ANF, and both are rapidly degraded. Although previous reports20 suggest that β-ANF is derived from the heart, we cannot exclude that β-ANF is formed in the peripheral plasma of patients with congestive heart failure. The current study demonstrates that β-ANF may be degraded to a smaller-molecular-weight peptide and that this occurs only in active cardiac tissue and not in plasma.

In summary, the current study demonstrates that the principal form of circulating ANF in severe congestive heart failure in humans is β-ANF. This altered form, which is reported to have reduced cGMP stimulating actions, increases in parallel with the magnitude of ventricular dysfunction, thus having implications in the role of ANF in the pathophysiology of congestive heart failure.

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References


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