Mechanisms Involved in the Response to Prolonged Infusion of Atrial Natriuretic Factor in Patients With Chronic Heart Failure

Thomas Münzel, MD; Helmut Drexler, MD; Jürgen Holtz, MD; Sabine Kurtz, BS; and Hanjörg Just, MD

We examined the mechanisms involved in the cardiovascular and renal response to prolonged infusion of atrial natriuretic factor (ANF) in patients with chronic heart failure. ANF infusion was titrated to produce a 30% decrease in pulmonary capillary wedge pressure or a 20% increase in cardiac output, and this dose (average, 75±4 ng/kg/min) was then administered for 20 hours. The short-term response to ANF included significant reductions in central filling pressures, increases in cardiac output, modest increases in diuresis and glomerular filtration rates, significant reduction in plasma aldosterone levels, and a 3.6-fold increase in plasma cyclic GMP levels. During prolonged infusion, plasma cGMP levels and cardiac output gradually returned to baseline. Similarly, the initially increased diuretic effects were completely abolished during prolonged ANF infusion, although plasma α-hANF levels remained consistently elevated above baseline values (control, 198±38; titration, 2,760±596; 20 hours, 3,499±659 pg/ml). Four hours after beginning the ANF infusion, marked increases in hematocrit levels were noted (42.5±1.0% versus 45.3±1.4%, control and infusion, respectively, p<0.05); during this time, no change in total plasma protein concentration occurred, indicating extravascular shift of fluid and plasma proteins. No evidence was noted for activation of vasoconstrictor hormones during prolonged ANF infusion, although mean arterial pressure was significantly reduced throughout the infusion period. Plasma pro-ANF (31-67) levels, determined as a marker for endogenous ANF secretion, were significantly suppressed as were the reductions of central filling pressures. After ANF discontinuation, heart rate and pulmonary capillary wedge pressure increased significantly above baseline values without evidence for sympathetic stimulation. We conclude that 1) prolonged infusion of ANF causes only transient increases in plasma cGMP levels but a sustained reduction of the cardiac release of ANF and that 2) the beneficial hemodynamic effects of ANF, that is, unloading of the ventricles, may be associated with or, in part, may be secondary to a shift of plasma constituents into the extravascular space. The latter may limit the therapeutic potential of ANF for long-term treatment. (Circulation 1991;83:191-201)

Short-term administration of atrial natriuretic factor (ANF) has improved cardiac performance in patients with chronic heart failure by reducing preload and afterload\(^1\)-\(^3\); however, its effects during prolonged administration remain to be established. In addition to having vasodilator properties (effects restricted to arteries and arterioles)\(^4\)-\(^6\) and diuretic properties,\(^7\) ANF has inhibitor properties of the renin-angiotensin-aldosterone system and the sympathoadrenal axis,\(^8\)-\(^11\) which are features that limit the activation of neurohumoral counterregulatory mechanisms during prolonged vasodilator and diuretic therapy. However, chronically elevated plasma levels seem to reduce responsiveness to the effects of exogenous ANF in patients with chronic heart failure,\(^1\),\(^2\) for example, due to receptor down-regulation,\(^12\),\(^13\) which is a well-documented phenomenon noted with the use of other peptide hormones.\(^14\) Furthermore, experimental studies indicate that ANF causes substantial shifts of fluid\(^15\)-\(^17\) into the third space. These findings call into question the usefulness of ANF in the treatment of chronic heart failure.

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failure, a disease characterized by chronic fluid overload and edema formation. Therefore, the present study was designed to determine the cardiovascular and renal efficacy of ANF during a 20-hour infusion period in patients with moderate-to-severe chronic heart failure. In an attempt to elucidate the mechanisms involved in this response, we measured neurohumoral parameters, such as norepinephrine, epinephrine, aldosterone, plasma renin activity, and plasma cGMP levels. In addition, measurements of the pro-ANF fragment provided a means to characterize the endogenous secretion of ANF during administration of the biologically active 99-126 peptide. Relative changes within fluid compartments were assessed by serial determination of hematocrit levels and total plasma protein concentrations.

Methods

Patients

Ten patients with chronic congestive heart failure were studied after giving written, informed consent. The experimental protocol was approved by the ethical committee of the University of Freiburg. The group comprised nine men and one woman, aged 45–67 years, who had an ejection fraction (determined by left ventricular angiography) of 30% or less (Table 1). Nine patients were in New York Heart Association (NYHA) functional class III, and one patient was in NYHA class II. Eight patients had ischemic cardiomyopathy, and two patients had idiopathic dilated cardiomyopathy. Patients with recent history of myocardial infarction, valvular heart disease, or recent acute decompensation were excluded. Four days before the study, therapy with angiotensin converting enzyme inhibitors was discontinued, and 2 days before the study, therapy with short-acting vasodilators and diuretics was stopped (Table 1). Anticoagulants, antiarrhythmic agents, and digitalis were continued throughout the study.

Table 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>NYHA class</th>
<th>Concomitant medication</th>
<th>Previous medication</th>
<th>Cardiothoracic ratio</th>
<th>EF (%)</th>
<th>PCP (mm Hg)</th>
<th>ANF (pg/ml)</th>
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<tr>
<td>1</td>
<td>54</td>
<td>M</td>
<td>IDC</td>
<td>III</td>
<td>Digoxin</td>
<td>Frusemide</td>
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<td>2</td>
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<td>M</td>
<td>CAD</td>
<td>III</td>
<td>Digoxin, warfarin</td>
<td>Captopril, frusemide</td>
<td>0.63</td>
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<tr>
<td>3</td>
<td>66</td>
<td>M</td>
<td>CAD</td>
<td>III</td>
<td>Digoxin</td>
<td>Nifedipine, ISM</td>
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<td>25</td>
<td>21</td>
<td>191</td>
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<tr>
<td>4</td>
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<td>III</td>
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<td>Captopril, frusemide</td>
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<td>Captopril, ISDN, frusemide</td>
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<td>20</td>
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<td>8</td>
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<td>III</td>
<td>Digoxin</td>
<td>ISMN, frusemide</td>
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<tr>
<td>9</td>
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<td>M</td>
<td>IDC</td>
<td>III</td>
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<td>Enalapril, ISMN</td>
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<td>52</td>
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<tr>
<td>Mean ±SEM</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>1.4</td>
<td>2.3</td>
<td>35</td>
</tr>
</tbody>
</table>

n=10 patients.

NYHA, New York Heart Association functional class; EF, left ventricular ejection fraction; PCP, pulmonary capillary wedge pressure; ANF, plasma levels of atrial natriuretic factor; IDC, idiopathic dilated cardiomyopathy; CAD, coronary artery disease; ISMN, isosorbide mononitrate; ISDN, isosorbide dinitrate.

Study Protocol

At 8:00 AM on the day of the study, a 7F Swan-Ganz thermodilution catheter was placed percutaneously from the basilic vein in the arm and was advanced into the pulmonary artery under fluoroscopic guidance. A peripheral line was inserted for infusion of α-hANF (99-126) (Bissendorf, Wedemark, FRG), and a bladder catheter was placed for urine sampling. Thereafter, a light breakfast was provided (one cup of tea and one sandwich). Additional light meals were provided immediately after hemodynamic measurements were obtained at 2:00 PM and 6:00 PM. Urine produced in an initial 60-minute collection period was discarded. At 10:00 AM, a 3.5-hour urine collection period was started, and it served as the control phase. Hemodynamic stability (<10% variation) was ensured by two consecutive measurements performed at 30-minute intervals (beginning at 1:30 PM). After baseline measurements were obtained (B1 and B2; see Figure 1), dose titration was started by infusing ANF at a rate of 20 ng/kg/min. The infusion rate was doubled every 15 minutes to achieve a 30% reduction in pulmonary capillary wedge pressure or a 20% increase in cardiac index. After achieving the desired hemodynamic response, the infusion rate (average, 75±4 ng/kg/min) was maintained for 20 hours. Hemodynamic measurements were repeated 4, 8, 12, 16, and 20 hours after starting the ANF infusion. Thereafter, the drug was discontinued, and repeated hemodynamic measurements were obtained 4 hours later. Blood samples were drawn from the right atrium for analyzing plasma α-hANF (99-126) and pro-hANF (31-67) levels, plasma cGMP concentrations, hematocrit levels, plasma protein levels, electrolyte levels, aldosterone levels, catecholamine levels, and plasma renin activity as indicated in Figure 1. Urine volume, sodium level, and potassium level were measured.
after the titration period and every 4 hours, with hemodynamic measurements and blood sampling. Urine volume and sodium and potassium loss during each collection period was subsequently replaced by infusing sodium and potassium chloride. Oral fluid intake was allowed but was restricted to a maximum of 1.5 l to account for fluid losses by perspiratio sensibilis and insensibilis during the 28-hour protocol.

Hemodynamic Measurements
Systolic and diastolic blood pressures were determined by sphygmonanometry with an automated oscillome-tric technique (Dinamap, model 845 XT, Criticon Corp., Tampa, Fla.). The triple lumen Swan-Ganz catheter allowed the measurement of right atrial, mean pulmonary artery, and pulmonary capillary wedge pressures. Cardiac output was determined by the thermodilution technique. Measurements were performed in triplicate using ice-cold sodium chloride 0.9%. Heart rate was calculated from a continuously recorded electrocardiogram. Mean arterial pressure, cardiac index, stroke volume, and systemic vascular resistance were calculated using standard formulas.

Urinary Measurements
The urine volume was measured with graduated cylinders. Urine potassium and sodium excretion (meq/l) were determined by flame photometry and osmolality by freezing-point depression. Urine volume was expressed as urinary flow rate (ml/min). The glomerular filtration rate was calculated as endogenous creatinine clearance. Osmolar clearance and free water clearance were determined for each collection period expressed as milliliters per minute. Free water clearance was calculated as the difference between urinary volume and clearance of osmole per minute. Sodium and potassium excretion rates were calculated with standard formulas.

Hormonal Assay
Plasma catecholamines and plasma cGMP levels were determined as described previously.8 For renin and aldosterone determination, the blood was collected in prechilled tubes in which EDTA was used as an anticoagulant. The samples were kept cold and centrifuged at about 2,000g to recover the plasma. The samples were divided into aliquotes and were stored deep frozen until assayed. Intra-assay coefficients of variations were 5.4% and 6.5%, respectively, and the interassay coefficients of variations were 8.1% and 5.4%. Recoveries ranged from 100% to 107%.

Plasma Concentrations of α-hANF (1-28) and Pro-hANF (31-67)
Plasma α-hANF concentrations were measured as described previously with a rabbit antiserum (Amersham Buchler, Braunschweig, FRG) against synthetic α-hANP, after extraction according to the method of Lang et al23 and were expressed as picograms per milliliter of human α-hANF. The limit of detection was 12 pg/ml. With this assay, the plasma ANF of normal individuals (n=11) averages 14±2 pg/ml.24 The pro-hANF (31-67) concentrations were determined in extracted samples by a radioimmunoassay (Penninsula Laboratories, Belmont, Mass.) that recognizes the midportion of pro-hANF (1-126) with no cross-reactivity to α-hANF (99-126). The radioimmunoassays were performed in an assay buffer consisting of 19 mM monobasic and 81 mM dibasic sodium phosphate (pH 7.4), 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% NaN₃. To 12×75-mm polystyrene culture tubes were added 100 μl standard or unknown, 100 μl rehydrated antiserum, and the incubation mixture, and the incubations were continued at 4°C for an additional 24 hours. After the second 24-hour incubation was complete, 100 μl dilute goat anti-rabbit immunoglobulin serum and 100 μl dilute normal rabbit serum were added. The precipitations were
allowed to form for 2 hours at room temperature before 0.5 ml assay buffer was added. The precipitates were collected by centrifugation at 1,700g for 20 minutes. The supernatants were carefully removed by aspiration, and the pellets were counted for iodine 125 in a scintillation well gamma counter. The 50% intercept was 99 pg/tube. The plasma pro-hANF (31-67) levels of normal individuals (n=27) who were 52±4 years old averaged 312±28 pg/ml. Intra-assay and interassay coefficients of variation were 7% and 10%, respectively. The recovery rate was 83%.

Statistical Analysis

Intraindividual comparisons over time were evaluated by analysis of variance for repeated measurements. When the F test indicated significance, individual comparisons were made by the Student-Newman-Keuls test.25 Single comparisons were made by paired t test. Data are expressed as mean±SEM. A probability value less than 0.05 was considered significant.

Results

Effects of ANF Administration on Hemodynamics

During the titration period, pulmonary capillary wedge, right atrial, and mean pulmonary artery pressures fell significantly, whereas mean arterial pressure and heart rate did not change (Figure 2). Cardiac index increased and systemic vascular resistance decreased significantly at the highest titration dose. During prolonged infusion, mean arterial pressure declined significantly 4 hours after ANF initiation and remained reduced during long-term ANF infusion. The fall in mean arterial pressure was mainly due to a decrease in systemic vascular resistance because cardiac index returned to baseline at the 4-hour time point (Figure 3). The reduction in central filling pressures was maintained throughout the infusion period (Figure 2). The heart rate showed no significant changes despite the considerable decline in mean arterial pressure (Figure 3).

Plasma ANF, Pro-hANF (31-67), and Plasma cGMP Levels

ANF plasma concentrations increased 17-fold during the titration period (Figure 4). Four hours after the start of the ANF infusion, the ANF plasma values increased and then remained elevated throughout the entire infusion period. Despite a significant reduction in central filling pressures during the titration period, pro-hANF (31-67) levels did not change. Four hours after the beginning of the ANF infusion, pro-hANF (31-67) plasma levels declined significantly and remained decreased during prolonged infusion along with the reductions in central filling pressures. Plasma cGMP increased 3.6-fold during

Figure 2. Plots of hemodynamic responses to atrial natriuretic factor (ANF) infusion. Values are mean±SEM. *p<0.05 vs. baseline. B1 and B2 refer to the two baseline measurements. HR, heart rate; RAP, right atrial pressure; PCP, pulmonary capillary wedge pressure; SVR, systemic vascular resistance.
the titration period \( p<0.05 \) and then steadily declined during prolonged ANF infusion.

**Effects of ANF on Plasma Renin Activity and Aldosterone and Catecholamine Levels**

The infusion of ANF was associated with a significant inhibition of plasma aldosterone levels during the titration period but not during prolonged ANF infusion (Table 2). The plasma renin activity and the plasma norepinephrine and epinephrine levels did not change during ANF infusion.

**Hemodynamic and Hormonal Responses After Discontinuing ANF Infusion**

In eight patients, hemodynamics were measured 2–4 hours after discontinuation of ANF infusion. Pulmonary capillary wedge pressure and heart rate significantly increased after ANF withdrawal compared with control values before administration of ANF (pulmonary capillary wedge pressure, 21±3 versus 27±3 mm Hg; heart rate, 85±5 versus 95±5 beats/min; \( p<0.05 \) for both). Cardiac index (2.3±0.4 versus 2.6±0.21 l/min/m\(^2\)), stroke volume (55±5 versus 53±6 ml), systemic vascular resistance (1,754±141 versus 1,578±162 dynes·sec/cm\(^5\)), and mean arterial pressure (98±5 versus 103±3 mm Hg) were not significantly different from control values after discontinuation of ANF.

After ANF discontinuation, plasma ANF levels declined to 329±100 pg/ml, and pro-hANF (31-67) levels increased to 3,240±689 pg/ml, but plasma norepinephrine levels and plasma renin activity did not change.

**Effects of ANF Infusion on Renal Function and Electrolyte Excretion**

During titration, ANF caused significant changes only in glomerular filtration rate and in potassium excretion. By 4 hours after the start of ANF infusion, these parameters had returned to baseline. Thus, ANF elicited only short-lasting effects on diuresis and electrolyte excretion.

**Effects of ANF on Intravascular and Extravascular Fluid Distribution**

In patients with high pulmonary capillary wedge pressures (>22 mm Hg, \( n=5 \)) hematocrit levels did not change during the titration phase, whereas patients with lower filling pressures (16±2 mm Hg) responded with a marked increase by 7.2±1.1% \( (p<0.05) \).

Four hours after the start of ANF infusion, the hematocrit level increased in all patients and aver-
aged 6.5±2% (p<0.05) without a concomitant increase in total plasma protein content (Table 3). During prolonged infusion, the hematocrit level returned to baseline and was no longer significantly increased after 8 hours, whereas total plasma protein content fell steadily. To establish whether the decline in plasma protein concentration is due to protein loss by the kidney, the urinary protein excretion rate was determined in five patients (patients 6-10). The protein excretion rate did not change significantly during the entire ANF infusion period (control, 8.9±2.3 mg/dl/hr; titration, 9.6±4.1 mg/dl/hr; 8 hours, 8.7±2.8; 12 hours, 5.1±1.4; 16 hours, 8.20±3.1; 20 hours, 8.2±2.2 mg/dl/hr).

Adverse Effects

One patient developed angina 2 hours after the ANF-infusion was discontinued. Another patient with atrial fibrillation experienced a tachyarrhythmia 1 hour after cessation of ANF infusion. One patient with a history of frequent ventricular tachycardia developed a ventricular tachycardia (heart rate, 130 beats/min) 20 hours after starting the ANF infusion. All patients had uneventful recoveries.

Discussion

ANF in Heart Failure

In patients with chronic heart failure, ANF levels are usually elevated. In the present study, the mean

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**Table 2.** Plasma Hormone Levels Before and During Long-term Infusion of Atrial Natriuretic Factor

<table>
<thead>
<tr>
<th></th>
<th>B1</th>
<th>B2</th>
<th>p.T.</th>
<th>4 hr</th>
<th>8 hr</th>
<th>12 hr</th>
<th>16 hr</th>
<th>20 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>121±44</td>
<td>111±35</td>
<td>50*±14</td>
<td>81±23</td>
<td>131±29</td>
<td>95±35</td>
<td>99±18</td>
<td>73±20</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>5.08±2.3</td>
<td>5.04±2.5</td>
<td>4.02±2.1</td>
<td>5.97±2.6</td>
<td>5.14±2.0</td>
<td>4.26±1.5</td>
<td>5.48±2.1</td>
<td>5.98±3.1</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>587±8</td>
<td>590±97</td>
<td>549±53</td>
<td>422±39</td>
<td>459±59</td>
<td>375±54</td>
<td>480±80</td>
<td>488±80</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>154±27</td>
<td>167±44</td>
<td>126±24</td>
<td>113±18</td>
<td>120±17</td>
<td>101±13</td>
<td>197±12</td>
<td>145±24</td>
</tr>
</tbody>
</table>

Data are mean±SEM. n=10 patients.
B1 and B2, baseline measurements; p.T., post atrial natriuretic factor titration.

* p<0.05 vs. baseline.
ANF levels were 13 times higher than those of healthy individuals. The cardiocirculatory role of ANF in the setting of chronic heart failure and markedly elevated plasma ANF levels has been questioned; however, recent studies using monoclonal antibodies clearly demonstrate continued effects of elevated ANF levels, predominantly on preload. Several investigative teams have administered ANF and reported beneficial actions on hemodynamics, renal function, and neurohormonal parameters after short-term infusion in chronic heart failure. Although the renal effects of ANF in general were minimal in this setting, a therapeutic application was advocated, which, in turn, prompted the development of ANF-degradation inhibitors. We tested the therapeutic potential of ANF by analyzing the renal, hormonal, and cardiovascular effects of ANF during a 20-hour infusion. Unlike recent studies that administered arbitrary dosages of ANF, we used strict hemodynamic criteria for defining a dose, in particular, that dose that exerts significant reduction in preload or afterload. Of emphasis, the results of the present study should be viewed in the context of these prerequisites.

**Hemodynamic, Hormonal, and Renal Effects**

The hemodynamic effect of ANF in response to the highest titration dose included decreases in central filling pressures, systemic vascular resistance, and an increase in cardiac index, which is in keeping with previous studies. Four hours after ANF initiation, however, cardiac index returned to baseline. The return of cardiac index to control values may be, in part, due to a further fall in cardiac preload induced by the noticeable plasma contraction observed within the first 4 hours of ANF infusion. In contrast to the evanescent effects of ANF on cardiac output, the reductions in central filling pressure, mean arterial pressure, and systemic vascular resistance are maintained throughout the ANF infusion period.

During prolonged infusion, no counterregulatory changes were observed that involved the renin-angiotensin-aldosterone system and the sympathetic nervous system despite considerable reductions in mean arterial pressure. This may be explained either by an inhibitory action of ANF on hormone release or by the consequence of an overall improvement in systemic hemodynamics. Webster et al reported...
that nitroglycerin increased rather than decreased plasma norepinephrine, epinephrine, renin, and aldosterone levels in a similar patient population, whereas plasma ANF levels declined. Thus, our results suggest that the ability of ANF to inhibit the release of vasoconstrictor hormones is retained in patients with chronically elevated plasma ANF levels. Indeed, we recently demonstrated that the sympathetic inhibitory action of ANF persisted in a dog model of congestive heart failure even when vascular effects of ANF are blunted. 

After ANF discontinuation, a significant increase in capillary wedge pressure and heart rate occurred, without a change in arterial pressure and peripheral resistance. This pattern contrasts with hemodynamic changes reported after withdrawal of vasodilators, such as sodium nitroprusside, resulting in dramatic increases in mean arterial pressure and systemic vascular resistance. This rebound phenomenon was mainly related to the activation of vasoconstrictor hormones during vasodilator therapy. Such an activation of vasoconstrictor hormones did not occur with ANF. The hemodynamic changes after ANF discontinuation appear to be due to the disappearance of ANF effects on cardiac preload and may involve the discontinuation of vagal activation. The lack of a sustained effect on natriuresis and diuresis during prolonged infusion in our pa-

**FIGURE 6.** Bar graphs of renal excretory changes in response to short-term and prolonged atrial natriuretic factor (ANF) infusion. Urine samples were obtained at baseline (B), post ANF titration (p.T.), and every 4 hours during prolonged ANF infusion. Values are mean±SEM (n=9). *p<0.05 vs. baseline.

| TABLE 3. Changes in Hematocrit Level and Total Plasma Protein Content During Short-term and Prolonged Atrial Natriuretic Factor Infusion |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | B1              | B2              | p.T.            | 4 hr            | 8 hr            | 12 hr           | 16 hr           | 20 hr           |
| Hematocrit (%)  | 42.6±0.9        | 42.4±1.0        | 44.0±1.4        | 45.6±1.3*       | 44.0±1.6        | 43.2±1.6        | 42.6±1.7        | 41.4±1.3        |
| Total plasma    | 6.87±0.23       | 6.91±0.23       | 6.97±0.19       | 6.62±0.22       | 6.48±0.23*      | 6.36±0.27*      | 6.24±0.20*      | 6.00±0.15*†     |
| protein (g/dl)  |                 |                 |                 |                 |                 |                 |                 |                 |

Data are mean±SEM. n=10 patients.
B1 and B2, baseline measurements; p.T., post-atrial natriuretic factor titration.
*p<0.05 vs. baseline, †p<0.05 vs. 4-hour values.
tients is similar to observations obtained in healthy men\textsuperscript{22–24} and cannot be explained by a depletion of fluid and sodium because these losses were subsequently replaced. The short-lasting renal effects may be, in part, due to the concomitant decline in blood pressure because the diuretic and natriuretic action has been demonstrated to depend on renal perfusion pressure.\textsuperscript{35,36} However, Vierhapper and Nowotny\textsuperscript{34} showed that the transient renal effects of ANF persisted despite maintaining blood pressure by noradrenaline infusion. Thus, further studies are warranted to clarify the mechanism of the transient renal effects of ANF.

**Plasma cGMP Levels**

Plasma cGMP levels have been hypothesized to serve as biological markers for the action of ANF\textsuperscript{37–39} because cGMP levels changed in proportion to circulating plasma ANF concentrations.\textsuperscript{37,38} The vascular endothelium, as well as the vascular smooth muscle, possesses guanylate cyclase–coupled ANF receptors and may, therefore, contribute to the circulating plasma cGMP concentrations.\textsuperscript{40} We observed a transient increase of plasma cGMP during long-term ANF infusion, whereas mean arterial pressure and systemic vascular resistance remained decreased. Therefore, in the face of sustained hemodynamic effects of ANF long-term infusion, the transient changes in plasma cGMP may point to endothelial origin of the plasma cGMP rather than vascular smooth muscle cells. Thus, it remains to be established whether or not variations of plasma cGMP can reliably serve as a marker for the biological actions of ANF during long-term administration.

**Effects of α-hANF Infusion on Endogenous ANF Secretion**

To characterize the endogenous ANF secretion during ANF infusion, we measured the pro-hANF (31-67) immunoreactivity. The antibody recognizes the midportion of pro-ANF (1-126) without any cross-reactivity to α-hANF (99-126). The biologically active α-hANF is released from the heart after cleavage from pro-ANF (1-126).\textsuperscript{45} Several groups demonstrated that a 10,000 molecular weight peptide consistent with the whole N-terminus (amino acids 1-98) circulates in humans and animals and is cosecreted with the C-terminus (amino acids 99-126).\textsuperscript{18,20,42} Furthermore, a 4-kDa fragment of the N-terminus was demonstrated in human plasma, which is recognized by the antibody against pro-hANF (31-67).\textsuperscript{20} It was proposed that simultaneous measurement of pro-ANF fragments may help to assess cardiac secretory function during therapeutic administration of α-hANF.\textsuperscript{18–20} The baseline pro-ANF (31-67) levels were nine times higher than the levels in healthy controls, indicating an increased secretory activity of the overloaded heart.\textsuperscript{43} During short-term unloading of the heart during the titration period, N-terminal peptide concentrations remained unchanged, although central filling pressures declined significantly, probably reflecting the slower rate of pro-hANF (31-67) degradation compared with that of α-hANF. Alternatively, a slowly changing constitutive release of pro-hANF from the ventricle\textsuperscript{44} contributed to the circulating pro-hANF (31-67) levels in our patients. In contrast, 4 hours after the start of ANF administration, levels of the pro-hANF declined significantly (Figure 4) and remained suppressed as did the reduction in central filling pressure. Therefore, the present data indicate that the N-terminal fragment (31-67) may be a marker for long-term changes of endogenous ANF secretion during infusion of the biologically active peptide.

**Relative Changes Between Fluid Compartments**

The observed hematocrit changes during ANF infusion indicate that ANF caused a shift of fluid into the extravascular space similar to recent reports.\textsuperscript{1} This effect has been attributed to an ANF-induced venoconstriction,\textsuperscript{17,45} with subsequent increase in capillary hydraulic pressure together with an increase in capillary hydraulic conductivity,\textsuperscript{46} favoring net filtration of fluid into the extravascular compartment. Interestingly, hematocrit levels increased only in patients with filling pressures of more than 22 mm Hg, indicating that the degree of fluid extravasation in response to short-term ANF administration may depend on preexisting interstitial fluid pressure. Four hours after the start of ANF infusion, a marked increase in the hematocrit level was noted in every patient, despite fluid replacement, suggesting a shift of fluid into the extravascular space. This increase in the hematocrit level was accompanied by a decreased rate rather than increased plasma protein concentration (Table 3), suggesting a transcapillary shift of proteins into the extravascular space because renal protein excretion rate remained unaltered. During long-term infusion, plasma protein concentrations declined further and were accompanied by a return of the hematocrit level to baseline. Obviously, a time-dependent dissociation of fluid and plasma protein shift emerged, which cannot readily be explained by the present data. However, a similar pattern of hematocrit changes was observed during ANF long-term infusion in the conscious sheep.\textsuperscript{47} Moreover, the protein extravasation occurring during ANF infusion is consistent with recent experimental findings.\textsuperscript{48,49} Taken together, ANF seems to alter three important variables of Starling’s law of the capillaries, favoring extravasation of plasma constituents into the third space. Conceivably, edema formation in congestive heart failure and other conditions associated with abnormally high plasma ANF levels may be, in part, attributed to ANF.

**Limitations of the Study**

The lack of a controlled study design raises the possibility that the observed changes in hemodynamics and hormonal parameters may, in part, represent diurnal variations. Because we considered a placebo-controlled design unethical in this population of
severely ill patients, a 4-hour run-in period was performed, yielding stable baseline conditions. Furthermore, ambulatory monitoring of pulmonary artery pressure in patients with chronic heart failure revealed that, during the night, central filling pressures increase rather than decrease in most patients. Therefore, the reduction in filling pressure during the night in our patient group can be attributed to the administration of ANF.

Clinical Implications

Prolonged administration of ANF results in attenuation of renal and, in part, of hemodynamic effects and facilities protein shift into the extravascular space, which may limit the therapeutic potential. Of emphasis, high doses of ANF were necessary for achieving a defined hemodynamic effect. Although hemodynamic unloading has been achieved by ANF, the reduction in circulating volume occurred mainly by extravasation rather than renal excretion. It remains to be elucidated whether intermittent administration of ANF or only modest increases in plasma ANF (e.g., inhibition of ANF degradation) can avoid these adverse effects and, therefore, exert beneficial effects on hemodynamics, neurohumoral constrictor forces, and renal function in patients with chronic heart failure.

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References


**Key Words** • chronic heart failure • atrial natriuretic factor • pro-ANF • cyclic GMP
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