Possibility of Downregulation of Atrial Natriuretic Peptide Receptor Coupled to Guanylate Cyclase in Peripheral Vascular Beds of Patients With Chronic Severe Heart Failure

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Background. High levels of endogenous atrial natriuretic peptide (ANP) are thought to compensate the condition of patients with heart failure by reducing preload and afterload. However, recent reports have indicated that a high plasma ANP level is a prognostic predictor in patients with heart failure. Therefore, the role of endogenous ANP has not been clearly established in patients with heart failure.

Methods and Results. The plasma ANP and cGMP levels were determined in the femoral artery and the femoral vein of 97 patients with chronic congestive heart failure (CHF). The plasma ANP level decreased significantly, whereas the plasma cGMP levels increased significantly from the femoral artery to the femoral vein. Among patients with mild CHF (n=52), the plasma cGMP level correlated with the ANP level, and the calculated ANP extraction level also correlated with the calculated cGMP production in the peripheral circulation (r=0.70, p<0.001). In contrast, these correlations were not found in patients with severe CHF (n=45). Among these patients, the plasma cGMP levels seemed to reach a plateau despite high levels of plasma ANP, and the molar ratio of cGMP production to ANP extraction in the peripheral circulation was significantly lower than in patients with mild CHF (36.7±9.5 versus 183±17, p<0.001). In patients with acute severe CHF (n=9) and those with mild CHF, patients who were administered exogenous ANP, plasma cGMP levels increased in proportion to those of plasma ANP without saturation.

Conclusions. These results indicate that downregulation of ANP receptors coupled to guanylate cyclase may occur in the peripheral vascular beds of patients with chronic severe CHF. (Circulation 1993;87:70–75)

KEY WORDS • atrial natriuretic peptide • cGMP • circulation, peripheral • congestive heart failure

Various neurohumoral factors that increase in patients with heart failure are thought to play important roles in the pathogenesis of heart failure.1–3 Atrial natriuretic peptide (ANP) has been implicated in the regulation of water, electrolytes, blood volume, and blood pressure.4 Recent reports have demonstrated that ANP is produced and secreted not only from the atria but also from the ventricles of patients with heart failure.5–7 Excessive levels of plasma ANP have been reported in patients with increasing severity of heart failure. Although endogenous ANP is thought to compensate the condition of patients with heart failure by reducing preload and afterload, recent findings have indicated that a high plasma ANP concentration is a prognostic predictor in patients with heart failure.8,9 The effect of exogenous ANP infusion is markedly attenuated in patients with severe heart failure.10,11 It has also been reported that ANP receptors are downregulated under high concentrations of ANP.12–15 Hirooka et al10 have indicated that the forearm vasodilatory response by exogenous ANP is markedly attenuated in patients with heart failure. In contrast, some reports have demonstrated the vasodilatory effect of large doses of exogenous ANP in patients with heart failure.16

In our previous studies, we demonstrated the possibility of downregulation of ANP receptors coupled to guanylate cyclase in the pulmonary vascular beds of patients with severe heart failure.17 That study was extended here, where we explored the role of endogenous ANP in the peripheral circulation of patients with chronic heart failure. The intracellular second messenger of ANP is cGMP,18 and exogenously administered ANP elevates plasma cGMP, resulting in physiological responses such as vasodilation and natriuresis.19,20 Based on these facts, we used plasma cGMP concentrations as an index of the physiological effects of endogenous ANP in the peripheral circulation.

Methods

Patients

We studied 97 patients with chronic heart failure who were undergoing cardiac catheterization for clinically
indicated purposes. Informed consent was obtained from all patients for participation in the study, according to a protocol approved by the Committee on Human Investigation at our institution. There were 58 men and 39 women ranging in age from 17 to 78 years (mean, 57 years). Of these patients, 41 had suffered a myocardial infarction more than 3 months before the study, 22 had dilated cardiomyopathy, 20 had valvular heart disease, nine had hypertrophic cardiomyopathy, and five had hypertensive heart disease. Fifty-two patients were classified according to the standards of the New York Heart Association (NYHA) as functional class II, 27 patients as class III, and 20 patients as class IV. Patients were divided into two groups according to their NYHA class: group 1 (NYHA class II, n = 52) and group 2 (NYHA class III or IV, n = 45). Fifty-six patients were treated previously with digitalis, 46 with diuretics, and 43 with vasodilators. All drugs were discontinued at least 18 hours before the study.

Nine patients with acute severe heart failure (NYHA class IV) and high plasma ANP levels were compared with those with chronic severe heart failure. Six of these nine patients suffered from recurrent acute myocardial infarction, and the remaining three had valvular heart disease. All nine patients were subsequently assessed to have progressed acutely from NYHA functional class II to class IV.

Study Protocol

Patients were premedicated with an oral dose of diazepam (5 mg) and left to rest in bed in the supine position for at least 20 minutes. Right-sided cardiac catheterization was performed using a 7F Swan-Ganz catheter, and a 7F pigtail catheter was used for left-sided cardiac catheterization. The heart rate was monitored by an electrocardiogram, and blood samples for measuring plasma ANP and cGMP concentrations were drawn at the same time from the right femoral artery and the right femoral vein before the administration of contrast medium. Cardiac output was determined by the thermodilution method immediately after blood sampling.

In nine of the patients with acute severe heart failure (NYHA class IV) whose plasma ANP level in the femoral vein was elevated, the plasma cGMP in the femoral vein level was also measured to compare it with the plasma ANP and cGMP levels in chronic severe heart failure. In addition, to evaluate the response of the plasma cGMP levels after the introduction of exogenous ANP, ANP was infused intravenously from the antecubital vein in seven NYHA class I or II patients and in four NYHA class III patients in increasing doses of 10, 20, 50, 100, and 200 µg/kg·min⁻¹ until the systolic blood pressure decreased to below 100 mm Hg for 15 minutes at each dose. Blood samples were drawn from the femoral vein of these patients before and after ANP infusion, and the cGMP and ANP levels were determined at the various concentrations of ANP.

Measurement of Plasma ANP and cGMP

Samples for the assay of plasma ANP concentrations were transferred to chilled disposable tubes containing aprotinin (500 kallikrein inactivator units/ml) and ethylenediaminetetra-acetic acid (1 mg/ml). The blood samples were immediately placed on ice and centrifuged at 4°C. Aliquots of plasma were measured by radioimmunoassay as previously reported. The intra- and interassay coefficients of variance were 6.3% (n = 6) and 9.6% (n = 6), respectively. Plasma concentrations of cGMP were determined by radioimmunoassay with a commercial kit (Yamasa Shoyu Co. Ltd., Choshi, Japan). The minimal detectable concentration of this assay was 0.5 pmol/ml. The intra-assay and interassay coefficients of variance were 2.4% (n = 6) and 8.0% (n = 6), respectively.

Calculations

Whereas the cardiac index and mean arterial blood pressure were calculated from the standard formulas, the systemic vascular resistance was calculated as:

\[
\text{Systemic vascular resistance} = \frac{\text{Mean arterial pressure} - \text{Right atrial pressure}}{\text{Cardiac output}} \times 80 \quad \text{(dyne} \cdot \text{sec} \cdot \text{cm}^{-5})
\]

For the index for the amount of ANP extracted by the peripheral circulation, we used the formula:

\[
\text{Femoral artery} - \text{Femoral vein} \times \frac{\text{ANP}}{\text{Cardiac output}} \times \left(1 - \frac{\text{Hematocrit}}{100}\right) \quad \text{(ng/min)}
\]

The index for the amount of cGMP produced by the peripheral circulation was determined with the formula:

\[
\text{Femoral vein} - \text{Femoral artery} \times \frac{\text{cGMP}}{\text{Cardiac output}} \times \left(1 - \frac{\text{Hematocrit}}{100}\right) \quad \text{(nmol/min)}
\]

Statistical Analysis

All results were expressed as mean ± SEM. The least-squares method was used for linear regression analysis. Statistical comparisons were made with the paired or unpaired Student's t test. A value of p < 0.05 was considered statistically significant.

Results

The hemodynamic data of the patients are shown in Table 1. The cardiac index was significantly lower in group 2 (NYHA class III or IV) than in group 1 (NYHA class II) (2.12±0.06 versus 2.60±0.06 l/min/m², p<0.001). On the other hand, the pulmonary capillary wedge pressure was significantly higher in group 2 than in group 1 (22.0±1.0 versus 9.1±0.5 mm Hg, p<0.001). In addition, the systemic vascular resistance was also significantly higher in group 2 than in group 1 (2,160±92 versus 1,890±80 dyne·sec·cm⁻⁵, p<0.05).

Plasma ANP concentration in the femoral artery correlated significantly with pulmonary capillary wedge pressure for both groups 1 and 2 (group 1: r=0.74, p<0.001; group 2: r=0.57, p<0.001; Figure 1). There was a positive correlation between the plasma cGMP concentration and ANP level in the femoral vein of group 1 (r=0.75, p<0.001; Figure 2). Moreover, in

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<th>Table 1. Hemodynamic Data</th>
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<td>HR (beats per minute)</td>
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<td>Group 1 (n=52)</td>
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HR, heart rate; MBP, mean blood pressure; CI, cardiac index; PCWP, pulmonary capillary wedge pressure; SVR, systemic vascular resistance; group 1: New York Heart Association (NYHA) class II; group 2: NYHA class III or IV.

*p<0.01, †p<0.05, ‡p<0.001 vs. group 1.
patients with acute severe heart failure, the plasma cGMP levels increased proportionally with plasma ANP levels without reaching saturation. In contrast, there was no correlation between the plasma cGMP concentration and the ANP level in the femoral vein for group 2 (chronic severe heart failure, Figure 2). In these patients, the levels of plasma cGMP appeared to reach a plateau. As shown in Figure 3, exogenous ANP administration elevated the plasma cGMP levels in proportion to the plasma ANP concentrations. In NYHA class I or II patients, plasma cGMP levels increased proportionally with plasma ANP despite its elevated levels. However, the slope of the linear regression line between the plasma cGMP and ANP levels in NYHA class III patients was about one half of the slope of that in NYHA class I or II patients.

The plasma ANP concentration decreased significantly from the femoral artery to the femoral vein in both groups 1 and 2 (group 1: 125.6±8.9 versus 94.6±6.5 pg/ml, \( p < 0.001 \); group 2: 436±42 versus 319±37 pg/ml, \( p < 0.001 \); Figure 4). In contrast, the plasma cGMP level increased significantly from the femoral artery to the femoral vein in both groups of patients (group 1: 5.3±0.34 versus 6.5±0.41 pmol/ml, \( p < 0.001 \); group 2: 10.8±0.6 versus 11.4±0.6 pmol/ml, \( p < 0.01 \); Figure 4).

In group 1, there was a significant positive correlation between the ANP extraction and cGMP production levels in the peripheral circulation \( (r=0.70, p < 0.001) \), Figure 5). In contrast, no significant correlation was found between these two parameters in group 2. Moreover, the majority of cases were found to be located at the right side of the linear regression line of group 1 (Figure 5). Because of this phenomenon, the molar ratio of cGMP production to ANP extraction in the peripheral circulation in group 2 was significantly lower than in group 1 \( (36.7±9.5 \text{ versus } 183±17, p < 0.001) \); Figure 6).

**Discussion**

It has been reported that plasma ANP levels become elevated as the severity of heart failure progresses.\(^{21-24}\) Endogenous ANP is thought to compensate the condition of patients with heart failure by reducing the preload and afterload. It is, however, very difficult to evaluate the clinical significance of endogenous ANP in patients with heart failure; thus, no such reports have yet been published. The intracellular second messenger of ANP is thought to be cGMP,\(^{19}\) although it is believed that some intracellular cGMP leaks out of target cells.\(^{25,26}\) Moreover, exogenously administered ANP elevates the plasma cGMP concentration, resulting in physiological effects such as natriuresis and vasodilation;\(^{19,20} \) therefore, in this study, we used the plasma content of ANP for group 1 patients \( (r=0.75, \ p < 0.001) \). cGMP concentration increased in proportion to the regression line of group I patients with acute severe heart failure. In group II, there was no correlation between plasma ANP and cGMP levels.
cGMP concentration as an index of the physiological effects of ANP. This is supported by previous studies that have shown a positive correlation between the plasma ANP and cGMP concentrations in patients with heart failure. We also showed that the plasma cGMP concentration correlated with the plasma ANP level in patients with mild heart failure (group I). In contrast, in patients with chronic severe heart failure (group II) there was a lack of correlation, with levels of cGMP reaching a plateau despite the elevated levels of ANP (Figure 2). However, there was a positive correlation between the plasma ANP and cGMP levels in patients with acute severe heart failure despite the elevated plasma ANP levels. These results suggest that cGMP production in cells that are targets for ANP such as peripheral vascular smooth muscle cells may display an attenuated function in patients with severe heart failure, whose levels of ANP continue to remain high. These results support the previous observation of Can-

The positive correlation between the pulmonary cap-
illary wedge pressure and plasma ANP concentration suggests that ANP release from the heart is regulated by the preload, as has been previously reported. The significant decrease in plasma ANP levels from the femoral artery to the femoral vein indicates the extraction of ANP from the peripheral circulation. In addition, this study shows a significant increase in the plasma cGMP concentration from the femoral artery to the femoral vein in patients with heart failure. These results suggest that whereas ANP is extracted from the circulation, cGMP is produced in plasma. Indeed, there was a significant positive correlation between the calculated ANP extraction level and cGMP production level in the peripheral circulation of patients with mild heart failure (group I). Bolli et al. have reported that exogenous ANP decreased forearm vascular resistance at concentrations within the upper normal range of ANP in humans. Accordingly, our data suggest that endoge-

Our studies also showed that in patients with severe heart failure, there was a lack of correlation between the plasma ANP and cGMP concentrations and between the ANP extraction and cGMP production levels in the peripheral circulation. Moreover, whereas the ANP extraction level in patients with severe heart failure was higher than in those with mild heart failure, cGMP production was significantly lower in patients with severe heart failure. Therefore, the ratio of cGMP production to ANP extraction in the peripheral circulation was significantly lower for patients with severe heart failure. In addition, the systemic vascular resistance was higher among patients with severe heart failure than among patients with mild heart failure. In this study, we also showed that the response of plasma cGMP to exogenous ANP infusion was attenuated in NYHA class III patients compared with those of NYHA.
class I or II. The infused ANP may be acting on sites other than the peripheral vascular beds such as the pulmonary vascular beds, which are rich in ANP receptors. These results suggest that there may be a down-regulation of ANP receptors that are coupled to guanylate cyclase in the vascular beds of patients with severe chronic heart failure. However, other possibilities such as upregulation of cGMP phosphodiesterase, potentiation of the cGMP effector system of protein kinase, and modification of cGMP egression cannot be excluded.32 Although endogenous ANP is thought to improve the condition of patients with heart failure, recent findings have indicated that elevated plasma levels of ANP are a prognostic predictor in patients with heart failure.6,9 Thus, in patients with severe heart failure, a high concentration of endogenous ANP may fail to exert a compensatory function in the peripheral circulation. This may be due to the downregulation of ANP receptors coupled to guanylate cyclase.

Recent studies using rodent models of heart failure have shown that after administration of monoclonal antibodies against ANP, there is an increase in the left ventricular end-diastolic pressure.33 In this study, we examined the relation between ANP extraction and cGMP production in the systemic vascular bed with respect to one of the physiological effects of ANP, that is, vasodilation. Our results indicate that in patients with mild heart failure, the increase in endogenous ANP levels may compensate for the heart failure by reducing the afterload. However, in patients with severe heart failure, where a high level of plasma ANP is sustained for an extended period of time, there may be a downregulation of ANP receptors in the peripheral vascular bed. Indeed, Schiffrin and St. Louis13 have also indicated that the mesenteric vascular ANP receptors are downregulated in deoxycorticosterone acetate–salt hypertensive rats with elevated levels of plasma ANP.

Study Limitations

In this study, we measured the arteriovenous differences in the plasma ANP and cGMP concentrations between the femoral artery and the femoral vein. However, the blood flow in the lower limbs was not measured. As a consequence, this may have led to inaccurate measurements of ANP extraction and cGMP production. If the ratio of blood flow of the lower limbs to cardiac output differs between patients with mild and severe heart failure, then the calculated ANP extraction and cGMP production rates cannot be simply compared between the two groups. However, the ratio of blood flow of the lower limbs to cardiac output is probably lower in patients with severe heart failure, thus leading to an overestimation of the production of ANP and cGMP compared with those with mild heart failure. Even if these criteria are considered, the cGMP production may be remarkably reduced in patients with severe heart failure. Moreover, the molar ratio of cGMP production to ANP extraction, which is independent of the lower-limb blood flow, was shown to be significantly lower in patients with severe heart failure.

Conclusions

Patients who suffer mild heart failure show an extraction of endogenous ANP and a concomitant production of cGMP in the peripheral circulation. It is therefore thought that endogenous ANP may result in vasodilation of the peripheral vasculature and reduction of the afterload. On the other hand, in patients with severe heart failure, where a high plasma concentration of ANP is chronically sustained, downregulation of ANP receptors may occur, resulting in the negation of the compensatory effects by ANP in the peripheral circulation.

References


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