RELATIONSHIP BETWEEN PLASMA LEVELS OF ATRIAL NATRIURETIC PEPTIDE AND CYCLIC GUANOSINE MONOPHOSPHATE IN PATIENTS WITH HEART DISEASES

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The relation of plasma levels of atrial natriuretic peptide (ANP) to those of cyclic 3', 5'-guanosine monophosphate (cGMP) was studied in 43 patients with various heart diseases. Plasma levels of both ANP and cGMP were significantly (p < 0.001) elevated in 34 patients with chronic heart diseases, and a significant positive correlation was observed between the two variables (r = 0.706, p < 0.01). Clinical improvement of congestive heart failure resulted in a concomitant decrease in plasma ANP and cGMP levels in 6 patients. In 3 patients with paroxysmal atrial fibrillation, plasma levels of ANP and cGMP increased markedly during arrhythmia. These results indicate that increased circulating ANP may stimulate cGMP production in target cells, which in turn raises plasma levels of cGMP in humans.

A growing body of evidence now indicates that atrial natriuretic peptide (ANP) is a circulating hormone in humans.1-6 Plasma levels of ANP are shown to increase in heart diseases such as paroxysmal atrial arrhythmias3,4 and congestive heart failure2,4-6. ANP has various biological actions, which may be mediated by an increase in cyclic 3', 5'-guanosine monophosphate (cGMP) in target cells.7-9 Intravenous injection of ANP into humans elevates plasma levels of cGMP.10 ANP fragments of different potencies exert a biological activity that corresponds to their capacities to enhance cGMP levels in vitro.11 If cGMP can serve as a marker for ANP action, plasma levels of cGMP may increase in patients with elevated plasma ANP levels. The present study was undertaken to prove this hypothesis by comparing plasma levels of ANP with those of cGMP in patients with heart diseases.

SUBJECTS AND METHODS

Subjects
Forty-three patients (29 men and 14 women) with various heart diseases, admitted to or attending the Hospital of the Institute for Adult Diseases, Asahi Life Foundation, were studied. The mean ± SD age of the patients was 66.3 ± 9.9 years. The patients were divided into three groups. Group 1 consisted of 34 patients with chronic heart diseases who were visiting our outpatient clinic. Their underlying heart diseases were: valvular heart diseases in 11 patients; old myocardial infarction in 9; primary myocardial

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diseases in 8; chronic atrial fibrillation without organic heart disease in 5; and excessive bradycardia in 1. Their functional states, judged by patients' physical activities, were: NYHA class12 I in 12 patients; class II in 15; and class III in 7. Eight patients were taking digitalis, 5 patients diuretics and 10 patients both. Plasma levels of ANP and cGMP were simultaneously examined in this group of patients. Group 2 consisted of 6 hospitalized patients with acute exacerbations of congestive heart failure (NYHA class III or IV). Their underlying heart diseases were: valvular heart diseases in 3 patients; old myocardial infarction in 2; and excessive bradycardia in the remainder. Changes in plasma levels of ANP and cGMP were examined after clinical improvement by treatment with catecholamines, digitalis or diuretics in this group of patients. Group 3 consisted of 2 inpatients and one outpatient with paroxysmal atrial fibrillation. One of the inpatients had an old myocardial infarction, while no organic heart disease was demonstrated in the other two patients (see legend to Fig. 3(B)). Changes in plasma levels of ANP and cGMP during paroxysms were studied in this group of patients. The patients received no medication at the time of blood sampling.

Normal values for plasma ANP levels were obtained from 25 age-matched (65.5 ± 6.5 years, mean ± SD) normotensive healthy subjects (18 men and 7 women). Normal values for plasma cGMP concentrations were obtained from 13 age-matched (63.7 ± 7.1 years, mean ± SD) normotensive healthy subjects (9 men and 4 women).

They consisted of medical staff and healthy subjects seeking a medical check-up.

Blood samples were collected from all study subjects on ad libitum water intake by venipuncture in the upright position after they were kept in the sitting position for 30 min, except for 3 patients with severe congestive heart failure (NYHA class IV) in group 2. Blood from these patients was taken in the semi-supine position. Blood was transferred to heparinized tubes (for ANP assay) or tubes containing EDTA (for cGMP assay). Plasma was quickly separated by centrifugation and stored at −20°C until assayed.

**ANP and cGMP assays**

Plasma concentrations of ANP were determined by a specific and sensitive radioimmunoassay after separation of ANP from plasma by means of affinity chromatography on anti-ANP-coupled agarose. The detail of the radioimmunoassay has been described previously.1 The recovery of ANP from plasma was 80.7 ± 1.0% (mean ± SEM, n = 37) and the sensitivity of the assay was 12.5 pg/ml. The coefficients of variation averaged 7.2% for intra-assay error and 11.1% for interassay error.

Plasma concentrations of cGMP were determined by radioimmunoassay using a commercial kit (Yamasa Shoyu Co. Ltd., Choshi, Japan).13 The sensitivity of the assay was 1.25 pmol/ml. The coefficients of variation averaged 3.9% for intra-assay error and 12.3% for interassay error.

**Statistical analyses**

Significance of differences was calculated by Student's t-test for paired and unpaired data. For multiple comparisons, Bonferroni's correction was used. Regression analysis was performed by the method of least squares. Values are expressed as the mean ± SEM, unless otherwise specified.

**RESULTS**

Figure 1 shows plasma cGMP levels in relation to ANP levels in 34 patients with chronic heart diseases (group 1). Plasma ANP levels (163.0 ± 19.6 pg/ml) in these patients were significantly (p < 0.001) higher than in control subjects (40.9 ± 4.3 pg/ml). Plasma cGMP levels (7.98 ± 0.62 pmol/ml) also were statistically (p < 0.001) higher than in controls (3.46 ± 0.33 pmol/ml). In addition, a significant positive (r = 0.706,
Fig. 2. Plasma levels (mean ± SEM) of ANP and cGMP in group 1 patients subdivided by NYHA classes. Plasma ANP levels in patients with severe congestive heart failure were significantly higher than in those with less severe heart disease. Plasma cGMP levels in patients in NYHA class II and III were significantly higher than in those in class I and controls.

Fig. 3. Changes in plasma levels of ANP and cGMP after clinical improvement of congestive heart failure (A) and during paroxysmal atrial fibrillation (B). (A) Open and filled circles denote plasma levels before and after treatment, respectively. (B) Open and filled circles denote plasma levels during paroxysms and during sinus rhythm, respectively. Blood samples during paroxysms were collected 30 min and 60 min after the onset of arrhythmia in patient (1) and patient (2), respectively, while the onset time of paroxysm in patient (3) was not exactly determined. Patient (1) had an old myocardial infarction, while no organic heart disease was demonstrated in other two patients. Leader lines denote the regression line and its 95% confidence limits calculated from the results in group 1 patients (Fig. 1). Note that all 3 patients had significantly higher plasma cGMP relative to ANP levels during paroxysmal atrial fibrillation.

p < 0.01) correlation was observed between plasma levels of ANP and cGMP.

Figure 2 shows plasma ANP and cGMP levels in group 1 patients subdivided by NYHA classes. Patients with severe congestive heart failure had higher plasma ANP and cGMP levels when compared to those with less severe heart diseases.

Figure 3(A) shows changes in plasma levels of ANP and cGMP after clinical improvement of congestive heart failure in 6 patients (group 2). Plasma ANP and cGMP levels decreased concomitantly in all patients. Plasma ANP (113.9 ± 25.1 pg/ml) and cGMP levels (5.65 ± 1.17 pmol/ml) after treatment were significantly (p < 0.05) lower than pre-treatment levels (188.4 ± 35.4 pg/ml and 10.78 ± 2.44 pmol/ml, respectively).

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Figure 3(B) shows changes in plasma levels of ANP and cGMP during paroxysmal atrial fibrillation in 3 patients (group 3). Both plasma ANP and cGMP levels markedly rose during paroxysms in all patients. Plasma cGMP levels relative to ANP levels during paroxysms in these patients were higher than in patients with chronic heart diseases (group 1) shown in Fig. 1.

DISCUSSION

The present study shows that plasma ANP and cGMP levels are elevated in patients with chronic heart diseases depending upon the severity of the disease and that a significant positive correlation exists between these two variables. In addition, clinical improvement of congestive heart failure was associated with a decrease in plasma ANP and cGMP levels. In patients with paroxysmal atrial fibrillation, plasma levels of ANP and cGMP showed a concomitant rise during arrhythmia. All these results indicate a close relationship between plasma ANP and cGMP levels in clinical conditions.

ANP has been shown to stimulate guanylate cyclase in various tissues. Cyclic nucleotides in plasma may be in a dynamic equilibrium with their intracellular pools. Taken together, increased plasma cGMP levels in patients with congestive heart failure and in those with paroxysmal atrial fibrillation demonstrated in earlier reports and in the present study may be explained by an increase in plasma ANP levels in these pathologic states. Plasma cGMP levels, therefore, may be a sensitive marker of ANP secretion. Of note in this regard is that the majority of the patients whose plasma ANP levels exceeded 100 pg/ml had higher plasma cGMP concentrations than control subjects (see Fig. 1). The level may correspond to that required for ANP to exert its biological actions in this state.

Blunted natriuretic response to ANP injection has been shown in animal preparations of chronic volume overload. In patients with congestive heart failure, infusion of ANP appears to be ineffective in producing diuresis and natriuresis. One of the possible explanations for this phenomenon may be the secretion of an inactive form of ANP in congestive heart failure. However, the fact that α-hANP is a predominant circulating form in such patients may not be in accordance with this view. Another explanation may be the down-regulation by ANP of its own receptor coupled with guanylate cyclase. In fact, plasma cGMP relative to ANP levels were generally higher in patients with paroxysmal atrial fibrillation than in those with chronic heart diseases (Fig. 3(B)). Further studies may be necessary to clarify the mechanism of the desensitized response to ANP in congestive heart failure.

REFERENCES


