Differential Hormonal Profiles of Adrenomedullin and Proadrenomedullin N-Terminal 20 Peptide in Patients with Heart Failure and Effect of Treatment on Their Plasma Levels

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Summary

Background: Adrenomedullin (AM) is a potent vasodilatory peptide discovered in human pheochromocytoma tissue. Proadrenomedullin N-terminal 20 peptide (PAMP) processed from an AM precursor is also a novel hypotensive peptide which inhibits catecholamine secretion from sympathetic nerve endings.

Hypothesis: The present study sought to examine the relationships between the two peptides and other clinical parameters by measuring the plasma AM and PAMP concentrations in 98 patients with heart failure.

Methods: In all, 98 patients [65 men and 33 women, aged 58.2 ± 11.0 years, mean $\pm$ standard deviation (SD)] with heart failure and 26 healthy volunteers (12 men and 14 women, aged 54.1 ± 8.6 years) were examined in this study. Heart failure was secondary to previous myocardial infarction in 58 patients, valvular disease in 28, cardiomyopathy in 9, and congenital heart disease in 3. All patients were classified into two groups of class I or II (Group 1) and class III or IV (Group 2) according to the New York Heart Association (NYHA) functional classification.

Results: Both plasma AM and PAMP concentrations in the patients were significantly higher than those in healthy volunteers. In addition, plasma AM and PAMP concentrations in patients in class III or IV of New York Heart Association (NYHA) classification were significantly higher than those in NYHA class I or II. The elevated plasma concentrations of these peptides in patients in NYHA class III or IV significant-

ly decreased in response to the treatment for 7 days. There was a significant correlation between plasma AM and PAMP, though the plasma concentration of PAMP was one-fifth to one-seventh of that of AM in patients and controls. The plasma AM concentration correlated significantly with the plasma concentrations of atrial and brain natriuretic peptides, epinephrine, and right atrial pressure, whereas such a relationship was not noted for the plasma PAMP concentration.

Conclusions: Judging from the difference in not only the biological actions but also the hormonal profiles between AM and PAMP, they may differentially modulate the cardiovascular system in patients with heart failure, although they are processed from the same precursor.

Key words: adrenomedullin, proadrenomedullin N-terminal 20 peptide, natriuretic peptides, heart failure

Introduction

Adrenomedullin (AM) is a potent vasodilator peptide which was isolated from human pheochromocytoma tissue.¹ A cDNA cloning of the AM precursor revealed that pre-proAM is a 185-amino-acids peptide containing the AM sequence in its C-terminal.¹ In addition, another bioactive peptide was found to be processed from the N-terminal portion of proAM, and this peptide was named proadrenomedullin N-terminal 20 peptide (PAMP).¹ Intravenous administration of synthetic AM exerts a potent and long-lasting hypotensive effect on anesthetized rats.² It is interesting that synthetic PAMP has also been found to elicit a rapid and strong hypotensive activity in anesthetized rats when injected intravenously.¹ In contrast to the direct vasodilator activity of AM, PAMP is thought to lower blood pressure through the inhibition of catecholamine secretion from sympathetic nerve ending.³ We demonstrated that both AM and PAMP circulate in human blood at considerable concentrations and that these peptides are present in several human tissues such as adrenal medulla, lung, kidney, aorta, cardiac atrium, and ventricle where pre-proAM mRNA was found to be abundant.^{4,5} These findings imply that both peptides participate in the regulation of the car-

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Received: May 1, 1998

Accepted with revision: September 17, 1998

diovascular system. In this study, the plasma concentrations of AM and PAMP were measured in patients with heart failure secondary to various heart diseases, and we examined their relationships with other humoral and hemodynamic parameters in an effort to determine the pathophysiologic roles of these peptides in patients with heart failure. The results presented in this paper suggest a modulation of the cardiovascular system by this novel dual system of AM and PAMP in heart failure.

Methods

Study Subjects

In all, 98 patients [65 men and 33 women, aged 58.2 ± 11.0 years, mean $\pm$ standard deviation (SD)] with heart failure and 26 healthy volunteers (12 men and 14 women, aged 54.1 ± 8.6 years) were examined in this study. Heart failure was secondary to previous myocardial infarction in 58 patients, valvular disease in 28, cardiomyopathy in 9, and congenital heart disease in 3. All patients were classified into two groups of class I or II (Group 1) and class III or IV (Group 2) according to the New York Heart Association (NYHA) functional classification.⁶ These patients were on a diet containing 7 g per day of salt. As controls, 26 age- and gender-matched healthy subjects were randomly selected from those who underwent medical examination showing no abnormalities.

In another group of seven patients in NYHA class III or IV, who were admitted to our hospital suffering acute or subacute exacerbation of congestive heart failure, the serial changes of plasma AM and PAMP concentrations were examined before and during the treatment for 7 days. Their symptoms improved and pulmonary rales disappeared in all patients after treatment mainly with digitalis, diuretics, and restrictions in salt and water intake.

Patients with acute coronary syndrome were not included in this study, and all patients had normal renal function with serum creatinine levels $<130 \mu\text{mol/l}$. Informed consent for all procedures was obtained from all patients.

Cardiac Catheterization and Blood Sampling

Of the 98 patients with heart failure, 92 underwent cardiac catheterization. In 58 patients with previous myocardial infarction, cardiac catheterization was carried out at least 6 months after the onset of myocardial infarction. Right-sided cardiac catheterization was done with a 7F thermodilution catheter (Baxter Healthcare Corporation, Irvine, Calif.) and left ventricular contrast studies were carried out with 6F pigtail catheter (Mallinckrodt Medical Inc., St. Louis, Mo.). Cardiac output was measured by either the thermodilution or Fick's method, and left ventricular ejection fraction (LVEF) was calculated by the area-length method.

Blood samples were drawn from the antecubital vein early in the morning after overnight fast and in supine position, and transferred into tubes with 1 mg/ml of EDTA-2Na for measurements of aldosterone, renin activity, and catecholamines, and with both 1 mg/ml of EDTA-2Na and 500 kallikrein in-

hibitory units/ml of aprotinin for AM, PAMP, and atrial and brain natriuretic peptides (ANP and BNP). The blood samples were centrifuged at 4°C and the plasma was kept at -35°C until assayed.

Assay Procedures for AM, PAMP, and Other Humoral Factors

The radioimmunoassay (RIA) for human AM and PAMP (hAM and hPAMP) was performed as described previously.^{5,7} The cross reactivities of an anti-hAM(1-52)-NH₂ antiserum used in the RIA were 100% with hAM(1-52)-COOH, 100% with hAM(1-52)Gly-COOH, and $<2\%$ with hAM(13-52). The anti-hPAMP(1-20)-NH₂ antiserum used in this RIA had cross reactivities of 19.4% with hPAMP(9-20)-NH₂ and 3.6% with hPAMP(13-20)-NH₂. The intra- and interassay coefficients of variation for the hAM assay were 5.0 and 4.8%, and those for the hPAMP assay were 4.0 and 8.0%, respectively.

Plasma ANP and BNP levels were measured with a specific immunoradiometric assay (Shionoria ANP and BNP kits, Shionogi Co., Osaka, Japan).⁸ Plasma renin activity and plasma aldosterone concentrations were measured by standard RIAs. The plasma catecholamine levels were determined by means of high-pressure liquid chromatography combined with a fluorometric procedure.

Statistical Analysis

All data were expressed as means $\pm$ SD. Differences between two groups were analyzed using the unpaired *t*-test. Multiple comparisons were assessed by either one-way analysis of variance followed by the Scheffé's test or chi-square test. Linear regression analysis was used to assess the correlation between variables. A *p* value of <0.05 was considered statistically significant.

Results

Clinical Characteristics of the Study Subjects

As shown in Table I, 71 patients were in Group 1 and 27 patients in Group 2. There were no significant differences in the age, gender, mean blood pressure, and serum creatinine levels among the two groups and the control group. All patients had received medications such as beta blockers, nitrates, calcium-channel blockers, angiotensin-converting enzyme inhibitors, diuretics, and digitalis. Cardiac index (CI) and LVEF in Group 2 were significantly reduced ($p < 0.05$) and the mean right atrial pressure (RAP) and pulmonary arterial wedge pressure (PAWP) in this group were significantly elevated ($p < 0.01$) compared with those in Group 1.

The Plasma Levels of AM, PAMP, and Other Humoral Factors

Figure 1 shows the plasma AM and PAMP levels in each group. The plasma AM levels in patients with heart failure ($8.3 \pm 3.2 \text{ pmol/l}$) was significantly higher ($p < 0.01$) than in the

TABLE I Clinical characteristics of the study groups

	Controls	Group 1	Group 2
No. of patients	26	71	27
Age (years)	54.1 ± 8.6	58.2 ± 11.1	58.1 ± 10.8
Male/female (n)	12/14	47/24	18/9
Mean BP (mmHg)	89.7 ± 13.7	87.4 ± 12.6	86.8 ± 10.9
Heart rate (beats/min)	68.7 ± 10.7	68.1 ± 10.5	74.1 ± 11.7 ^c
Urea nitrogen (mmol/l)	5.5 ± 1.3	6.2 ± 2.2	7.2 ± 2.6 ^a
Creatinine (μmol/l)	68.7 ± 12.3	68.9 ± 19.6	71.4 ± 16.6
CTR (%)	45.3 ± 3.4	50.8 ± 6.1 ^b	56.6 ± 7.7 ^{b,d}
CI (l/min/m ²)		2.34 ± 0.7	2.02 ± 0.7 ^c
RAP (mmHg)		5.7 ± 2.1	8.0 ± 4.2 ^d
PAWP (mmHg)		12.5 ± 3.6	16.6 ± 5.7 ^d
LVEF (%)		66.4 ± 13.9	57.1 ± 19.4 ^c

Values are mean ± standard deviation.

^ap < 0.05.

^bp < 0.01 vs. controls.

^cp < 0.05.

^dp < 0.01 vs. Group 1.

Abbreviations: BP = blood pressure, CTR = cardiothoracic ratio, CI = cardiac index, RAP = right atrial pressure, PAWP = pulmonary wedge pressure, LVEF = left ventricular ejection fraction.

controls (3.0 ± 0.9 pmol/l), and the plasma AM levels in Group 2 (9.5 ± 4.8 pmol/l) were significantly higher ($p < 0.05$) than in Group 1 (7.2 ± 2.1 pmol/l). Similarly, the plasma PAMP in the patients with congestive heart failure (1.8 ± 0.9 pmol/l) was significantly higher ($p < 0.01$) than in the controls (0.8 ± 0.4 pmol/l), and plasma PAMP increased progressively in proportion with the severity of the disease (Group 1, 1.5 ± 0.9 pmol/l; Group 2, 2.3 ± 0.7 pmol/l). In addition, a significant ($p < 0.01$) positive correlation was observed between the plasma concentrations of AM and PAMP (Fig. 2).

The plasma levels of other humoral factors in each group are shown in Table II. The plasma ANP concentration in Group 1 was higher than that in controls, though not significantly, and was significantly increased in Group 2 compared with controls. The plasma BNP concentration in patients increased similarly; however, the increment of BNP was greater than that of ANP. The plasma renin activity in Group 2 tended to be higher than that in Group 1, although there was no significant difference in plasma aldosterone levels between the two groups of patients. Plasma catecholamines in Group 2 slightly increased compared with those in Group 1.

Correlations between Plasma Levels of AM or PAMP and Other Parameters

Table III shows coefficients for the correlations between plasma AM or PAMP concentrations and the other parameters. The plasma AM concentration correlated significantly

Fig. 1 Plasma concentrations of AM (A) and PAMP (B) in controls and patients with heart failure of New York Heart Association (NYHA) I or II (Group 1) and NYHA III or IV (Group 2). Values are means ± standard deviation. * $p < 0.01$ vs. controls, # $p < 0.05$ vs. Group 1. AM = adrenomedullin, PAMP = proadrenomedullin N-terminal 20 peptide.

($p < 0.01$) with the plasma concentrations of ANP, BNP, and epinephrine, right atrial pressure, and the cardiothoracic ratio. However, there were no significant correlations between the plasma PAMP concentration and these parameters except for the cardiothoracic ratio.

Plasma Levels of AM and PAMP before and after Treatment

Figure 3 shows the time course of plasma AM and PAMP levels during the treatment for exacerbated congestive heart failure. Both plasma AM and PAMP levels gradually but significantly decreased in response to the treatment.

Fig. 2 Relationship between the plasma concentrations of AM and PAMP in the study subjects. Abbreviations as in Figure 1.

TABLE II Plasma levels of other humoral factors in the study groups

	Controls	Group 1	Group 2
ANP (pmol/l)	4.4 ± 2.6	11.1 ± 9.3	18.9 ± 24.5 ^a
BNP (pmol/l)	3.0 ± 3.4	26.8 ± 27.3 ^b	62.6 ± 70.4 ^{a,c}
Renin activity (ng/l ⁻¹ ·s ⁻¹)		0.69 ± 1.08	1.21 ± 1.05
Aldosterone (pmol/l)		165.1 ± 122.1	163.9 ± 89.4
Norepinephrine (nmol/l)		1.62 ± 0.75	2.05 ± 1.08
Epinephrine (pmol/l)		191.2 ± 172.2	205.3 ± 82.3

Values are means ± standard deviation.

^a p < 0.01.

^b p < 0.05 vs. controls.

^c p < 0.01 vs. Group 1.

Abbreviations: ANP = atrial natriuretic peptide, BNP = brain natriuretic peptide.

Discussion

PAMP is a novel hypotensive peptide that is processed from the precursor peptide of AM, a potent vasodilator peptide isolated from tissue extracts of human pheochromocytoma.¹ Developments of specific RIAs revealed that AM and PAMP are similarly distributed in normal human tissues, such as the adrenal medulla, lung, aorta, kidney, cardiac atrium, and ventricle, in which a compatible expression of preproAM mRNA has been observed.^{4,5} These bioactive peptides were also found to circulate in human blood and we reported an increased plasma concentration of AM in patients with heart failure.⁹ In this study, not only the plasma AM but the PAMP concentration was progressively elevated in patients with heart failure in proportion to the severity of the disease. Similar elevation as well as the significant correlation between the plasma concentrations of the two peptides appears to be consistent with the fact that both peptides are processed from the same precursor.

This study showed that plasma AM has a significant relationship with plasma concentrations of ANP and BNP, and with right atrial pressure; however, such a relationship could not be found for plasma PAMP. It may also be interesting to note that the plasma concentration of PAMP was one-fifth to one-seventh of that of AM in patients as well as in controls. To date, information on the processing, secretion, and metabolism of these peptides is so limited that a wide range of possibilities should be considered as explanations for differences in these hormonal profiles of the two peptides. For example, Katoh *et al.* reported the secretion of AM and PAMP from cultured bovine adrenal medullary cells at a molar ratio of 2:1.^{10,11} In the meantime, much smaller amounts of PAMP are secreted from cultured human vascular endothelial cells which actively secrete AM.¹² Thus, the manner of processing or secretion of AM and PAMP seems to differ from tissue to

TABLE III Correlations between plasma concentration of AM or PAMP and other parameters

		Correlation coefficient	
		AM	PAMP
Humoral parameters	ANP	0.42 ^b	0.04
	BNP	0.39 ^b	0.15
	Renin activity	0.11	0.06
	Aldosterone	0.003	0.10
	Norepinephrine	0.07	0.08
Hemodynamic parameters	Epinephrine	0.31 ^b	0.12
	MBP	0.10	0.16
	Heart rate	0.16	0.008
	LVEF	0.02	0.09
	CI	0.17	0.07
	RAP	0.31 ^b	0.02
	PAWP	0.08	0.06
Other clinical parameters	LVEDP	0.09	0.04
	Urea nitrogen	0.16	0.15
	Creatinine	0.07	0.08
	CTR	0.43 ^b	0.23 ^a

^a p < 0.05.

^b p < 0.01.

Abbreviations: AM = adrenomedullin, PAMP = proadrenomedullin N-terminal 20 peptide, MBP = mean blood pressure, LVEDP = left ventricular end-diastolic pressure. Other abbreviations as in Table I.

tissue. We previously showed that synthetic AM injected intravenously into rats rapidly disappeared from the blood,¹³ however, the plasma half life of PAMP is shorter than that of AM (unpublished observation), a finding that may partially explain the lower plasma concentration of PAMP.

In patients with heart failure, the renin-angiotensin system is activated to maintain the blood supply to the vital organs, but on the other hand it produces undesirable effects such as excessive vasoconstriction and the retention of fluid volume.¹⁴ As observed in this study, plasma concentrations of ANP and BNP are known to increase compensatingly to reduce expanded body fluid in patients with heart failure.^{15,16} We previ-

FIG. 3 Reduction of plasma AM and PAMP in response to the treatment. Values are means ± standard deviation. *p < 0.05, **p < 0.01 vs. Day 1. Abbreviations as in Figure 1.

ously reported that plasma AM is elevated in patients with impaired renal function¹⁷ and with primary aldosteronism,¹⁸ a secondary hypertension characterized by increased fluid volume. Judging from the fact that plasma AM correlates significantly with the plasma ANP and BNP concentrations and right atrial pressure, the increase in plasma AM seems to be associated with the retention of body fluid in patients with heart failure. Ebara *et al.* reported natriuretic action of AM concomitant with an increase in renal blood flow probably at the physiologic plasma level for AM in anesthetized dogs.¹⁹ Taken together with the reduction of plasma AM in response to the treatment in exacerbated heart failure, AM may have a defensive role in acting against further deterioration of heart failure through its potent vasodilator and natriuretic actions in patients with heart diseases.

Beside the renin-angiotensin system, the sympathetic nerve system also is known to have an active role in patients with heart failure by maintaining cardiac output and blood supply to the vital organs.¹⁴ In this study, the plasma concentrations of catecholamines in the NYHA III or IV group was slightly higher than those in the NYHA I or II group. It has been shown that PAMP, in contrast to AM, possesses no direct vasodilatory activity.²⁰ However, we previously reported the co-secretion of PAMP with catecholamines from cultured bovine adrenal medullary cells, and PAMP has been found to inhibit catecholamine release from these cells.^{10,21} Moreover, Shimosawa *et al.* showed that PAMP inhibits catecholamine release from sympathetic nerve ending in pithed rats.³ The increased plasma PAMP, which was reduced following the treatment, suggests that it also participates in the regulation of the cardiovascular system in patients with heart failure, presumably by regulating catecholamine secretion from the sympathetic nerve system.

Conclusion

Both plasma AM and PAMP levels were similarly elevated in patients with heart failure and decreased in response to the treatment in exacerbated heart failure. In addition to the differences in biological actions, hormonal profiles of AM and PAMP in the patients appeared to differ from each other, suggesting their differential pathophysiologic roles. Thus, the present findings suggest that a novel dual system of AM and PAMP modulates the cardiovascular system in patients with heart failure.

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