Synthesis of Atrial Natriuretic Polypeptide in Human Failing Hearts

Evidence for Altered Processing of Atrial Natriuretic Polypeptide Precursor and Augmented Synthesis of β-human ANP

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Abstract

To elucidate the synthesis of atrial natriuretic polypeptide (ANP) in the failing heart, 20 human right auricles obtained at cardiovascular surgery were studied. The concentration of α-human ANP-like immunoreactivity (α-hANP-LI) in human right auricles ranged from 13.8 to 593.5 μg/g, and the tissue α-hANP-LI concentration in severe congestive heart failure (CHF) (New York Heart Association [NYHA] functional class III and class IV) (235.4±57.2 μg/g) was much higher than that in mild CHF (NYHA class I and class II) (52.5±15.6 μg/g). Atrial α-hANP-LI levels were significantly correlated with plasma concentrations of α-hANP-LI in these patients ($r = 0.84$, $P < 0.01$).

High performance gel permeation chromatography and reverse phase high performance liquid chromatography coupled with radioimmunoassay for ANP revealed that the α-hANP-LI in the human auricle consisted of three major components of ANP, γ-human ANP (γ-hANP), β-human ANP (β-hANP) and α-human ANP (α-hANP). Comparing percentages of γ-hANP, β-hANP, and α-hANP in α-hANP-LI in severe CHF with those in mild CHF, the predominant component of α-hANP-LI was γ-hANP in mild CHF, whereas β-hANP and/or α-hANP were prevailing in severe CHF and, especially, β-hANP was markedly increased in human failing hearts.

These results demonstrate that the total ANP concentration in the atrium of the human heart is increased in severe CHF and that the increase of ANP in the human failing heart is mainly due to the increase of small molecular weight forms of ANP, β-hANP, and α-hANP, especially β-hANP, and indicate that the processing of ANP precursor, or γ-hANP, in the human failing heart differs from that in the normal heart, suggesting that the failing heart augments synthesis and secretion of ANP as one of its own compensatory responses.

Introduction

The discovery of potent diuretic, natriuretic, and vasorelaxant activities in extracts of rat atria by de Bold et al. (1) led to the isolation of multiple forms of natriuretic polypeptides with high and low molecular weights (2–9). These polypeptides are now called atrial natriuretic polypeptide (ANP)[1] and implicated in the body fluid and cardiovascular control (10–13). From human atria obtained at autopsy three distinct molecular forms of ANP, γ-human ANP (γ-hANP), β-human ANP (β-hANP), and α-human ANP (α-hANP), were isolated by Matsuo and his colleagues (3, 4). α-hANP comprises 28 amino acids with an intramolecular disulfide linkage; γ-hANP is composed of 126 amino acids, carrying the 28-amino acid sequence of α-hANP at its carboxy terminus; and β-hANP (56 amino acids) is an antiparallel dimer of α-hANP with intermolecular disulfide bridges (3, 4). Several lines of studies including ours using high performance gel permeation chromatography (HP-GPC) and reverse phase high performance liquid chromatography (RP-HPLC) coupled with RIA for ANP revealed that ANP is stored in secretory granules of atrial cardiocytes as the 126-amino acid precursor, γ-ANP, and only a little amount of α-ANP was detectable in the atrium, especially in animals (4, 5, 14–16). It has also been reported that the secretory and circulating form of ANP is a small molecular weight form of 28-amino acid peptide, α-ANP (16–25). Thus, the heart is now regarded as an endocrine organ secreting ANP as well as a pump organ, and the relationship between endocrine and hemodynamic functions of the heart attracts interests of many investigators (10, 11, 26–33). Accumulating evidence indicates that plasma ANP levels are elevated in congestive heart failure (CHF) in relation to the severity of the heart failure (18, 26–31) and that this elevation of the plasma ANP level is mainly due to the increased ANP secretion from the failing heart (18). These findings indicate that the ANP-secreting function is augmented in CHF, the failing state of hemodynamic function. This augmentation of ANP-secreting function in the failing heart presents a striking contrast to the hypofunction of the endocrine organ such as insulin-deficiency state in secondary diabetes due to pancreatic diseases. However, little is known about the biosynthesis and processing of ANP precursor, or γ-hANP, in the failing heart at present. In the present study, we have studied ANP concentrations and molecular forms of ANP in 20 human right auricles obtained at cardiovascular surgery using HP-GPC and RP-HPLC coupled with RIA for ANP.

Methods

Subjects

20 patients with heart diseases (15 men and 5 women, 49±3 yr) who underwent cardiovascular operations were studied. Among them eight

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1. *Abbreviations used in this paper:* ANP, atrial natriuretic polypeptide; CHF, congestive heart failure; NYHA, New York Heart Association; RP, reverse-phase; SHR, spontaneously hypertensive rats.

Table I. Profiles of 20 Patients

Case	Age	Sex	Disease	NYHA class
1	61	M	IHD	I
2	34	M	ASD	I
3	64	M	IHD	I
4	2	F	T/F	I
5	47	M	IHD	I
6	24	M	ASD	I
7	59	M	IHD	I
8	64	M	IHD	I
9	46	M	AR	II
10	40	M	IHD	II
11	53	F	MR	II
12	45	M	IHD	II
13	54	M	MS + AS	III
14	56	F	MS	III
15	54	M	AR	III
16	59	M	MSR + AR	III
17	59	F	MSR + AR	III
18	46	F	MSR + TR	IV
19	51	M	MSR + TR	IV
20	68	M	IHD	IV

NYHA, New York Heart Association; IHD, ischemic heart disease; ASD, atrial septal defect; T/F, tetralogy of Fallot; AR, aortic regurgitation; ASR, aortic stenosis and regurgitation; MR, mitral regurgitation; MS, mitral stenosis; MSR, mitral stenosis and regurgitation; TR, tricuspid valve regurgitation.

patients suffered from ischemic heart disease (IHD) (six from old myocardial infarction and two from angina pectoris), nine from valvular heart diseases (VHD) and three from congenital heart diseases (CHD). According to the classification of New York Heart Association (NYHA) (34), eight patients were classified as functional class I, four as class II, five as class III, and three as class IV. None of the patients had evidence of renal failure nor systemic hypertension. Profiles of the patients studied are summarized in Table I. Informed consent was obtained from them and the study was approved by the ethical committee on human research of Kyoto University (No. 61-9).

Atrial tissues

Tissue samples (39 −623 mg, 218.1±38.1 mg, mean±SE) were obtained from almost the same apical portion of the right auricle when the venous cannula was inserted into the right auricle at cardiovascular surgery. The atrial tissues were immediately frozen in liquid nitrogen and stored at −70°C until extraction.

Tissue extraction

Tissue extraction was performed as previously reported (14). In brief, tissues were boiled for 5 min in 10 vol of 1 M acetic acid containing 20 mM HCl to abolish intrinsic proteolytic activity and then homogenized with a polytron homogenizer (Kinematica GmbH Kriens, Luzern, Switzerland). The homogenate was centrifuged at 15,000 *g* for 30 min at 4°C and the supernatant was stored at −70°C until assayed.

To exclude the possibility that γ-hANP is converted into β-hANP and/or α-hANP in the extraction process of atrial tissues, especially failing atrial tissues and HP-GPC analysis, following experiments were performed.

Experiment I. Two atrial tissue samples (case 14 in NYHA class III and case 20 in NYHA class IV) were dissected into pieces (~ 20 mg) and two pieces of the atrial tissues from the same case were subjected to the tissue extraction separately. The molecular forms of ANP of two samples from the same atrial tissue were compared.

Experiment II. γ-hANP fraction was added to the extraction solution with atrial tissues from two patients studied in experiment I (21.7 μg of γ-hANP (5 μg of α-hANP-like immunoreactivity [α-hANP-LI]) to the atrial tissue from case 14 and 13.0 μg of γ-hANP (3 μg of α-hANP-LI) to that from case 20) and then subjected to the extraction and HP-GPC analysis. The gel chromatographic pattern of γ-hANP-added extract was compared with that of the corresponding atrial tissue.

Plasma samples

Blood was withdrawn from the antecubital vein at a recumbent position in the operation room before anesthesia. Blood samples were transferred to chilled siliconized disposable glass tubes containing aprotinin (1,000 kallikrein inactivator units/ml) (Ohkura Pharmaceutical, Kyoto, Japan) and EDTA (1 mg/ml), then immediately placed on ice and promptly centrifuged at 4°C. An aliquot of plasma was immediately frozen at −20°C and thawed only once at the time of extraction.

Plasma extraction

Extraction of ANP from plasma was performed using a Sep-Pak C_{18} cartridge (Waters Associates Inc., Milford, MA) as previously described (12, 17). Recoveries of 50 pg and 100 pg of α-hANP added to 1 ml plasma were 68 and 71%, respectively.

Radioimmunoassay for ANP

Measurement of tissue and plasma ANP levels were performed using the specific RIA for ANP (12, 17, 35). Antibodies were raised in New Zealand white rabbits immunized with synthetic α-hANP [17–28] (35). This RIA recognizes the C-terminal portion of α-hANP and detects α-hANP and α-hANP [17–28] equally on a molar basis (35). Cross-reactivities with β-hANP and γ-hANP in the RIA were 120 and 100% on a molar basis and those with α-hANP [1–6], α-hANP [8–22], and α-hANP [24–28] were 0.05, 0.2, and 0.09%, respectively. The final dilution of antiserum was 1:200,000 and the minimal detectable quantity was 1 pg/tube. The 50% binding intercept of the standard curve was 20 pg/tube. The RIA incubation mixture consisted of 100 μl of standard α-hANP or sample, 100 μl of the antiserum, 100 μl of ^{125}I-α-hANP, and 200 μl of the standard buffer of 0.1 M phosphate buffer, pH 7.0 containing 0.5% gelatin (Merck, Darmstadt, FRG), 1 mM NA_2EDTA, 0.2 mM cystine, 0.1% Triton X-100, and 0.01% merthiolate. The mixture was incubated for 48 h at 4°C. Bound and free ligands were separated by adding 1.0 ml of a suspension of dextran-coated charcoal consisting of 250 mg of Norit SX Plus (N.V. Norit-Vereeniging, The Netherlands) and 25 mg of Dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.05 M phosphate buffer (pH 7.4) containing 0.01% merthiolate. The intra- and interassay variations were 7.2 and 7.8%, respectively.

High performance gel permeation chromatography (HP-GPC)

HP-GPC was performed on a TSK-GEL G2000 SW (Toyo Soda, Tokyo, Japan) column (7.5 × 600 mm), eluted with 10 mM trifluoroacetic acid containing 0.2 M sodium chloride and 30% acetonitrile as a solvent as previously reported (14). The flow rate was 0.3 ml/min and the fraction volume was 0.36 ml. Calibration was done using a series of myoglobins of the peptide molecular weight calibration kit (Pharmacia Fine Chemicals), synthetic α-hANP, synthetic β-hANP and purified γ-hANP. The ANP level in each fraction was measured by the RIA.

Reverse phase high performance liquid chromatography (RP-HPLC)

RP-HPLC was carried out on a TSK-GEL ODS 120T column (4.6 × 75 mm) (Toyo Soda, Tokyo, Japan) as previously reported (16, 36). A linear gradient in acetonitrile was applied as follows: at 5 min, 20 to

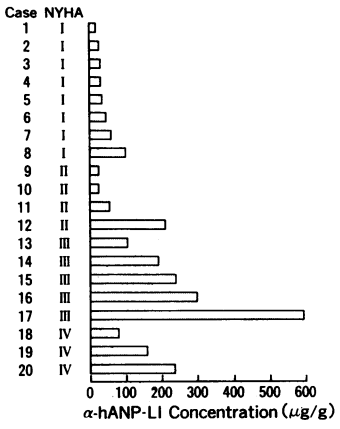

Figure 1. The relationship between α-hANP-LI concentrations in right auricles obtained at cardiovascular surgery from 18 patients with heart diseases and the severity of congestive heart failure. Cases are placed in order of the severity of congestive heart failure classified by the functional classification of NYHA.

50% in 50 min. The flow rate was 0.6 ml/min and the fraction volume was 0.3 ml. The retention times of α-hANP, β-hANP, and γ-hANP were 24.0, 28.5, and 43.0 min, respectively.

Peptides

α-hANP and γ-hANP were donated by Dr. H. Matsuo (Miyazaki Medical College, Miyazaki, Japan) (3, 4). β-hANP was generously supplied by Dr. K. Inouye (Shionogi Research Laboratories, Shionogi Co., Ltd., Osaka, Japan) (37). Fragments of ANP such as α-hANP [1–6], α-hANP [8–22] and α-hANP [24–28] were donated by Dr. Y. Kiso (Kyoto Pharmaceutical University, Kyoto, Japan) (38).

Statistical analysis

Data were expressed as means±SE. Statistical analysis was performed using paired or nonpaired Student's *t* test and Duncan's multiple range test (39) when appropriate. Logarithmic transformation of the data was done when appropriate (40). Linear regression analysis was used to determine correlations between results.

Results

α-hANP-LI concentrations in right auricles

Profiles of 20 patients and their α-hANP-LI concentrations in the right auricle were shown in Table I and Fig. 1. The α-hANP-LI concentration in the right auricle ranged from 13.8 to 593.5 μg/g and the mean value (±SE) was 125.7±30.7 μg/g. As shown in Fig. 1, the α-hANP-LI concentration in the atrium revealed a tendency to increase in relation to the severity of CHF. Mean values in four groups, that is NYHA class I, class II, class III, and class IV, were summarized in Table II. The α-hANP-LI concentration in severe CHF (class III and class IV) was significantly higher than that in mild CHF (class I and class II). No significant difference was observed in the α-hANP-LI concentration between class I and class II and between class III and class IV. The tissue concentration in class IV tended to be rather low than that in class III. As can be seen in Table I, patients with VHD were composed of two of class IV, five in class III, and two in class II. Patients with CHD were all in class I and patients with IHD consisted of five in class I, two in class II, and one in class IV. Thus, the grade of CHF in patients with VHD was more severe than those in patients with CHD and IHD in the present study. Atrial α-hANP-LI concentrations of patients with VHD. IHD and CHD were 191.4±58.3, 87.3±30.9, and 30.8±5.09 μg/g, respectively, being significantly higher in VHD than in CHD ($P < 0.05$) and tended to be higher in VHD than in IHD. Patients with CHD were the youngest three patients in the present study and all of them belonged to class I. Their ANP levels were lower than those in patients with VHD and IHD. There was no significant difference in the atrial α-hANP-LI concentration between the sexes. No significant correlation was observed between the tissue α-hANP-LI concentration and age.

Table II. Comparison of Concentrations of α-hANP-LI, γ-hANP, β-hANP, and α-hANP, and Percentage of Each ANP among Patients in NYHA Class I, Class II, Class III, and Class IV

NYHA class	α-hANP-LI concentration	γ-hANP μg/g	β-hANP μg/g	α-hANP μg/g	β-hANP and α-hANP μg/g
	μg/g	(%)	(%)	(%)	(%)
I	40.5±9.5	31.9±8.2	4.9±2.9	3.7±1.8	8.6±4.6
		(82.5±7.8)	(8.6±4.9)	(8.9±3.2)	(16.3±6.7)
II	76.7±44.4	34.4±10.0	8.2±6.3	34.2±30.3	42.3±36.6
		(66.5±13.6)	(6.8±2.7)	(26.8±12.0)	(33.5±13.8)
III	282.8±75.1‡	61.8±10.6	165.8±80.4*§	55.0±25.2‡‡	220.8±77.6‡§
		(31.0±9.3)‡§	(46.0±12.1)*‖	(23.0±10.8)	(69.0±9.3)‡
IV	156.3±37.2*	18.0±12.8¶	94.0±17.6*	44.4±10.2‡‡	138.3±27.7*
		(9.0±5.1)‡‖	(62.7±4.0)‡‖	(28.3±1.9)**	(91.0±5.1)‡‖
I or II	52.5±15.6	32.7±6.1	5.0±2.7	13.9±10.2	19.8±12.4
		(77.2±6.9)	(8.0±3.3)	(14.8±4.9)	(22.0±6.5)
III or IV	235.4±57.2**	45.4±11.9	138.9±55.8**	51.0±17.4‖‖	189.9±55.1**
		(22.8±7.7)§§	(52.3±8.8)§§	(25.0±7.3)	(77.3±7.7)§§

Percentages of each ANP in α-hANP-LI in right auricles are shown in brackets. Values are mean±SE. * $P < 0.05$, ‡ $P < 0.01$ compared with values in NYHA class I group. § $P < 0.05$, ‖ $P < 0.01$ compared with values in NYHA class II group. ¶ $P < 0.05$ compared with values in NYHA class III group. ** $P < 0.05$, ‡‡ $P < 0.01$ compared with values in NYHA class I group using a logarithmic transformation of the data. ** $P < 0.05$, §§ $P < 0.01$ compared with values in NYHA class I or II group. ‖‖ $P < 0.01$ compared with values in NYHA class I or II group using a logarithmic transformation of the data.

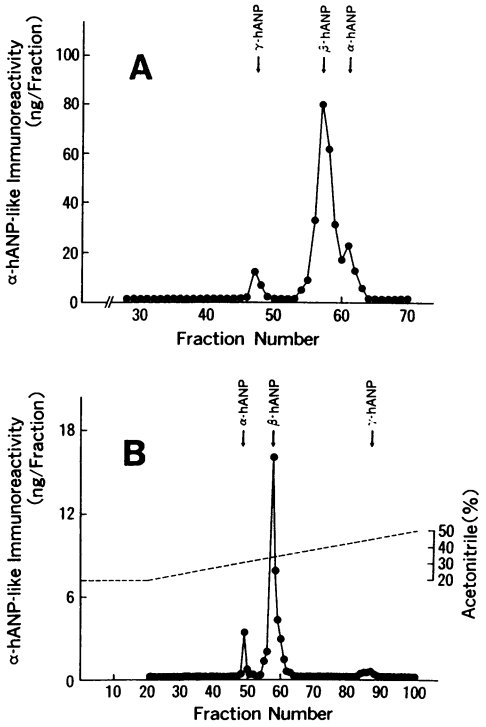

Figure 2. HP-GPC (*A*) and RP-HPLC (*B*) profiles of the same atrial extract. The α-hANP-LI level in each fraction was assayed by the RIA for α-ANP. The elution positions of α-hANP, β-hANP, and γ-hANP are indicated by the arrows.

Separation of α-hANP, β-hANP, and γ-hANP

To separate γ-hANP, β-hANP, and α-hANP in atrial extracts, HP-GPC and RP-HPLC analyses were carried out. Representative profiles of HP-GPC and RP-HPLC of the same atrial extract (case 14) were depicted in Fig. 2. As shown in Fig. 2 *A*, the gel chromatographic profile revealed that α-hANP-LI in the human auricle consisted of three major components with different molecular weights. The first peak eluted at an apparent molecular weight of γ-hANP, and the second and the third peaks emerged at elution positions of synthetic β-hANP and α-hANP, respectively. RP-HPLC analysis confirmed that the first, the second and the third components of α-hANP-LI in HP-GPC comigrated with γ-hANP, β-hANP, and α-hANP, respectively (Fig. 2 *B*). Therefore, we analyzed all of the other auricular tissues using HP-GPC coupled with RIA.

Table III. Recovery of γ hANP Added to Extraction Solution with Atrial Tissues during Extraction and HP-GPC

	Samples	α-hANP-LI μg		
		γ-hANP	β-hANP	α-hANP
Case 14	Tissue extract	7.80	17.0	2.40
	Δ α-hANP-LI in γ-hANP-added extract (5 μg of α hANP-LI)	4.45 (89%)	−0.10 (−2%)	0.25 (5%)
Case 20	Tissue extract	4.05	9.95	3.90
	Δ α-hANP-LI in γ hANP-added extract (3 μg of α-hANP-LI)	2.70 (90%)	0.06 (2%)	0.09 (3%)

Percentages in recoveries of γ-hANP are shown in parentheses.

Exclusion of nonspecific conversion of γ-hANP into β-hANP and/or α-hANP in the process of extraction and HP-GPC

Experiment I. Identical gel filtration profiles of ANP were observed between two pieces from the same atrial tissues (case 14 in class III and case 20 in class IV). These atrial tissues from patients with severe heart failure contained high amounts of β-hANP and α-hANP as shown in Table III.

Experiment II. Recoveries of γ-hANP added to the extraction solution with atrial tissues were given in Table III. No conversion of γ-hANP into β-hANP and/or α-hANP was observed during the extraction and HP-GPC.

Tissue levels of α-hANP, β-hANP and γ-hANP

HP-GPC profiles of auricular extracts of four patients with mild CHF (two in class I and two in class II) are shown in Fig. 3. As is clear in Fig. 3, the predominant component of α-hANP-LI was γ-hANP in all of the four cases, and β-hANP and/or α-hANP were seen as minor components. In contrast, HP-GPC profiles of four patients with severe CHF (two in class III and two in class IV) showed that γ-hANP was only a minor component, and that either β-hANP or α-hANP was the predominant component in these patients as shown in Fig. 4. Fig. 5 and Table II summarize the results of HP-GPC analyses in 20 patients. Peptide concentrations of γ-hANP, β-hANP, and α-hANP and the percentage of concentrations of γ-hANP, β-hANP, and α-hANP in the total ANP concentration in the right auricles are given in Fig. 5.

γ-hANP was detected in 19 of 20 cases. As shown in Table II, the mean percentages of γ-hANP in α-hANP-LI were 82.5% in class I, 66.5% in class II, 31.0% in class III, and 9.0% in class IV and showed the graded decrease in accordance with the severity of CHF. The percentage of γ-hANP in class IV was significantly smaller than those in class I ($P < 0.01$) and class II ($P < 0.05$). The percentage in class III was also significantly smaller than those in class I ($P < 0.01$) and class II ($P < 0.05$). No significant difference was observed in the percentage of γ-hANP between class I and class II and between class III and class IV, however, the γ-hANP concentration in class IV (18.0±12.8 μg/g) was significantly lower than that in class III (61.8±10.6 μg/g, $P < 0.05$), showing that γ-hANP is decreased in severe CHF (Table II).

β-hANP was not detected in four cases (three in class I and one in class II). Most cases in class I and class II showed low concentrations of β-hANP and the mean values (±SE) of β-hANP concentration were 4.9±2.9 μg/g in class I and 8.2±6.3 μg/g in class II. On the other hand, the β-hANP concentration was tremendously increased in class III (165.8±80.4 μg/g) and class IV (94.0±17.6 μg/g) (more than one order of magnitude higher than those in class I and class II). The mean percentage of β-hANP in α-hANP-LI reached ~ 50% in class III and more in class IV, although those in class I and class II were < 10% (Table II). Thus, the β-hANP concentration and the percentage of β-hANP in α-hANP-LI were markedly increased in severe CHF.

α-hANP was also detected in all of 20 cases. As shown in Table II, the α-hANP concentration in class I (3.7±1.8 μg/g) was similar to the β-hANP concentration. The α-hANP con-

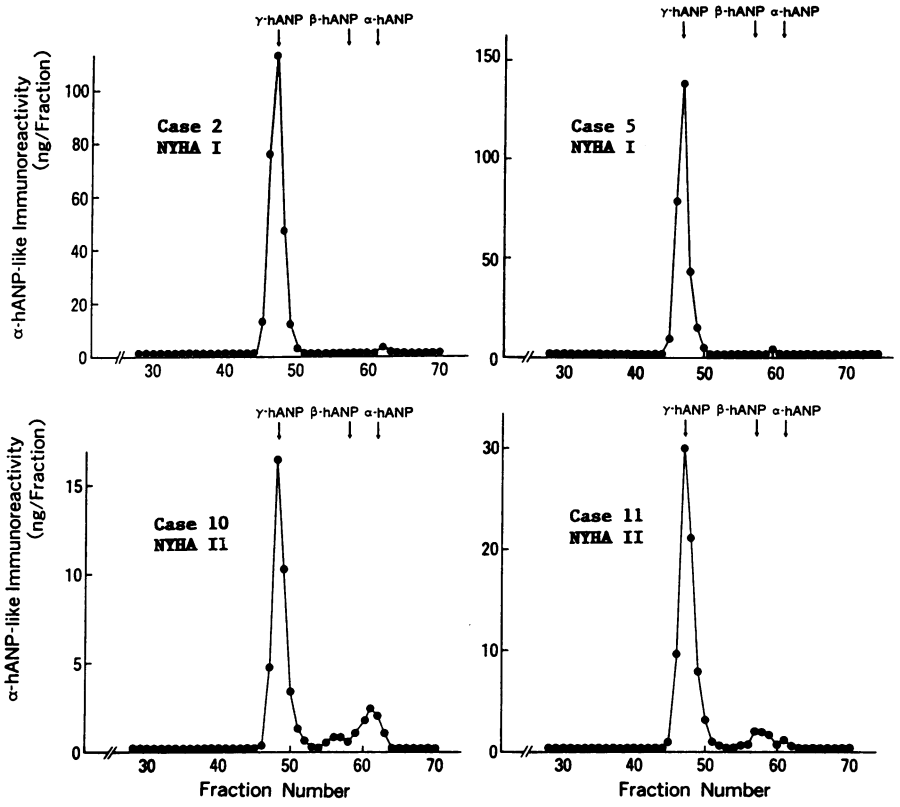

Figure 3. HP-GPC profiles of auricular extracts of four patients with mild CHF in the functional classification of NYHA class I and class II. Case 2: 34 yr, male, atrial septal defect in class I, case 5: 47 yr, male, ischemic heart disease in class I, case 10: 40 yr, male, ischemic heart disease in class II, and case 11: 53 yr, female, mitral regurgitation in class II.

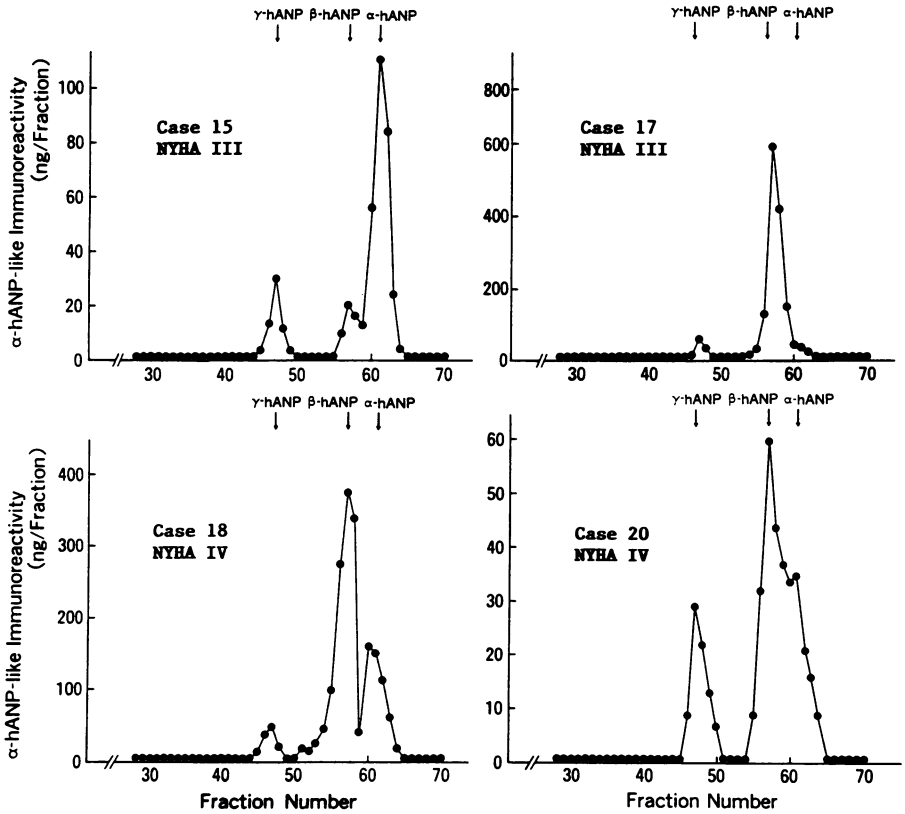

Figure 4. HP-GPC profiles of auricular extracts of four patients with severe CHF in the functional classification of NYHA class III and class IV. Case 15: 54 yr, male, aortic regurgitation in class III, case 17: 59 yr, female, mitral stenosis and regurgitation, and aortic regurgitation in class III, case 18: 46 yr, female, mitral stenosis and regurgitation, and tricuspid valve regurgitation in class IV, and case 20: 68 yr, male, ischemic heart disease.

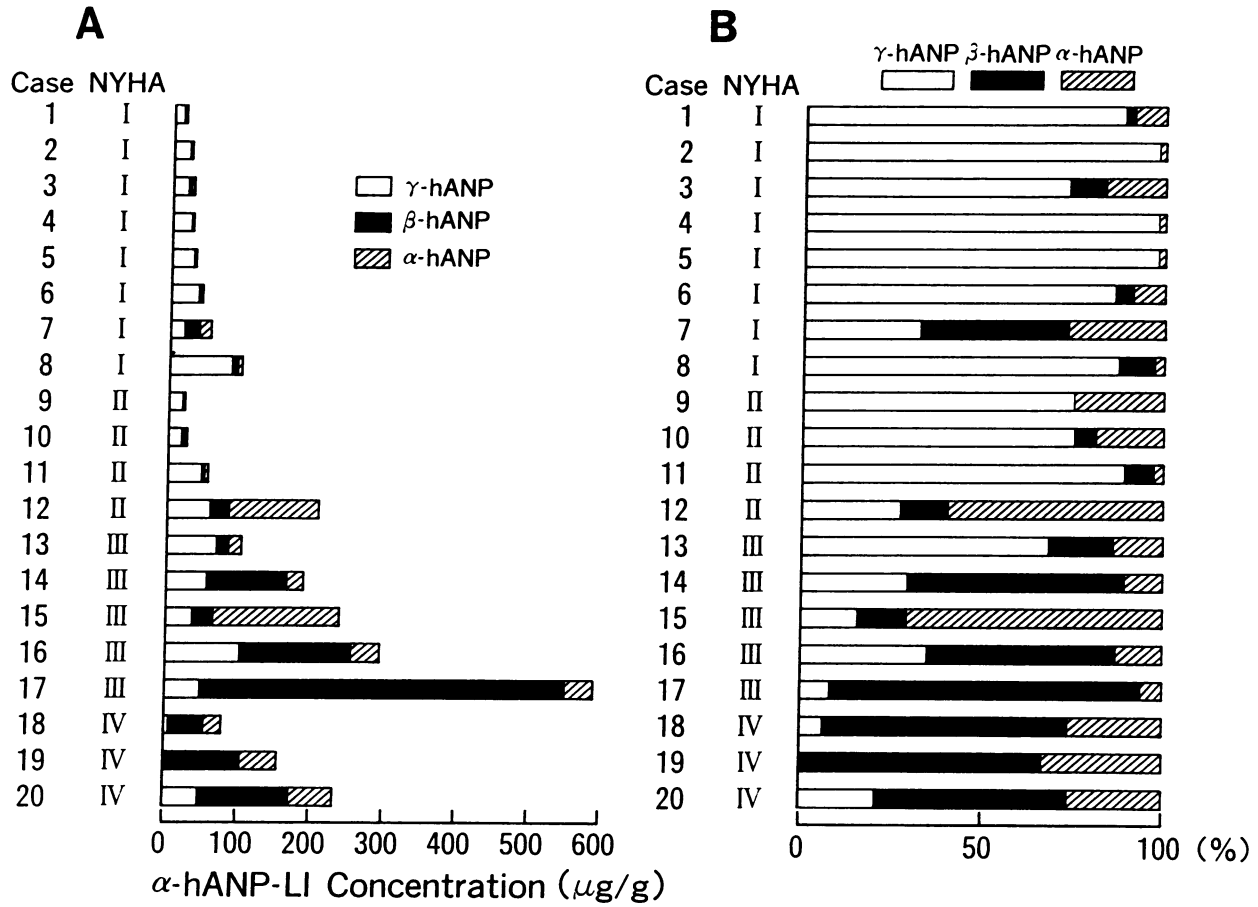

Figure 5. Schematic illustrations of tissue concentrations of γ-hANP, β-hANP and α-hANP (*A*) and percentages of concentrations of γ-hANP, β-hANP, and α-hANP in the total ANP concentration in right auricles from 20 patients (*B*). Percentages in α-hANP-LI and tissue concentrations of γ-hANP, β-hANP, and α-hANP are shown by the open column, closed column, and hatched column, respectively. Cases are placed in order of the severity of CHF classified by the functional classification of NYHA.

centration in severe CHF (class III and class IV) was significantly higher than that in mild CHF (class I and class II). The α-hANP concentration in class IV was significantly higher than that in class I ($P < 0.01$) and its percentage in α-hANP-LI was significantly larger than that in class I ($P < 0.05$) (Table II). The α-hANP concentration in class III was also significantly higher than that in class I ($P < 0.01$) (Table II).

The mean values of the summed percentages of β-hANP and α-hANP in α-hANP-LI were 16.3% in class I, 33.5% in class II, 69.0% in class III, and 91.0% in class IV (Table II). Thus, the percentage of β-hANP and α-hANP showed a graded rise in parallel with the severity of CHF. The summed concentration of β-hANP and α-hANP in class III was significantly higher than those in class I ($P < 0.01$) and class II ($P < 0.05$) and the concentration in class IV was also significantly higher than that in class I ($P < 0.05$).

Correlations among concentrations of α-hANP-LI, γ-hANP, β-hANP, and α-hANP were studied. There were significantly positive correlations between concentrations of α-hANP-LI and β-hANP ($r = 0.93$, $P < 0.001$) or α-hANP ($r = 0.48$, $P < 0.05$), but no significant correlation was observed between α-hANP-LI and γ-hANP. A highly significant correlation was seen between α-hANP-LI and low molecular weight forms of ANP (β-hANP and α-hANP) ($r = 0.98$, $P < 0.001$). On the other hand, no significant correlations were observed between γ-hANP and β-hANP ($r = 0.16$), between γ-hANP and α-hANP ($r = 0.14$) and between β-hANP and α-hANP ($r = 0.16$).

Correlation between plasma and atrial levels of ANP

The plasma α-hANP-LI concentration in patients in NYHA class III or class IV (261±24.8 pg/ml) was significantly higher than that in NYHA class I (108±24.3 pg/ml) ($P < 0.05$). The plasma α-hANP-LI concentration was significantly correlated with the α-hANP-LI concentration in the right auricle ($r = 0.84$, $P < 0.01$) (Fig. 6).

Discussion

Since the discovery of ANP from the atrium, the heart is regarded as an endocrine organ secreting ANP as well as a pump organ (10, 11, 16, 26–30). Growing evidence indicates, however, that the endocrine function, that is, ANP secretion from the heart, is augmented in CHF, a failing state of the pump function (18, 26–31). This study demonstrates that the total α-hANP-LI concentration in the right auricle is increased in severe CHF and that the increase in the total ANP concentration is due to the rise of β-hANP and/or α-hANP, especially

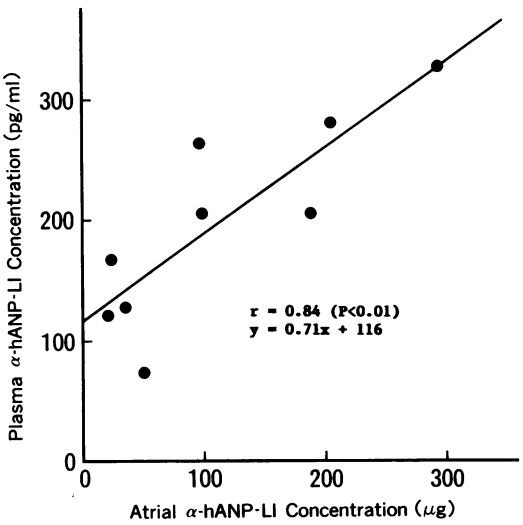

Figure 6. Significant positive correlation between the α-hANP-LI concentration in the right auricular tissue and the plasma α-hANP-LI concentration obtained at cardiovascular surgery from patients with heart diseases ($r = 0.84$, $P < 0.01$).

β-hANP, in human failing hearts. Thus, the posttranslational processing of γ-hANP to β-hANP and/or α-hANP in the human heart alters in accordance with the severity of CHF. The increased ANP synthesis and secretion in the atrium contrast with the decrease in the ventricular function in CHF.

The validity of the methods of analysis, especially the extraction procedure is critical in the present study. Therefore, we have designed the present study to minimize the nonspecific proteolytic degradation of γ-hANP, or ANP precursor, as carefully as possible. In the present study, we used atrial tissues obtained at the operation to avoid the postmortem degradation. Atrial tissues were immediately frozen in liquid nitrogen within a few seconds after removal of the atrial tissues and stored at −70°C until extraction. As for the tissue extraction procedure, the tissues were boiled for 5 min in 1 M acetic acid containing 20 mM HCl to avoid intrinsic proteolytic enzyme activity and then homogenized with a polytron homogenizer. This procedure was first used by Kangawa and Matsuo when they purified α-hANP, β-hANP, and γ-hANP from human atrial tissues (3, 4). We have also confirmed that their extraction procedure is valid using RP-HPLC coupled with RIA for α-hANP (14, 36). In general, the breakage and rejoining of a disulfide linkage in peptides is conceivable particularly at high pH. Kangawa and Matsuo also showed that synthetic α-hANP, despite its exposure to the conditions used for the extraction, did not undergo dimerization to β-hANP (4). We have also confirmed no conversion of α-hANP into β-hANP during the extraction process. After the tissue extraction, we carried out HP-GPC analysis. We have already demonstrated that γ-hANP is not converted into small molecular weight forms of ANP, β-hANP, and α-hANP, in such analytical procedures (12, 17). Moreover, we have further performed additional experiments to rule out the nonspecific proteolytic degradation in failing atrial tissues, and demonstrated no conversion of γ-hANP into β-hANP and/or α-hANP even in failing atrial tissues during the extraction and HP-GPC analysis. These findings indicate the validity of the methods of analysis used in the present study.

We and others previously reported that the predominant component of ANP in the rat heart is γ-rat ANP (14, 15), whereas β-hANP and α-hANP are present together with γ-hANP in human atrial tissues obtained at cardiac surgery or autopsy (4, 14, 15). However, our subsequent study revealed that the predominant molecular form of ANP in atria obtained at autopsy from patients without heart diseases, from foetuses and from premature infants is γ-hANP (41). These results suggest that the major storage form of ANP in the human normal heart is γ-hANP like in rats. The finding in the present study that γ-hANP is the predominant molecular form of ANP in cases of mild CHF in class I and class II supports the notion that the storage form of ANP in the normal heart is γ-ANP in both man and animals (14–16, 24, 32, 33).

Since the tissue ANP concentration is determined by the synthesis, storage and secretion of ANP, the increased ANP concentration in the atrium per se does not necessarily imply the increased ANP synthesis in the failing heart. However, the significant correlation between ANP concentrations in the atrium and in plasma observed in the present study and our previous observation showing the increased ANP secretion in CHF (18) indicate that the increased ANP concentration means the increase of ANP synthesis in the human failing heart. Our unpublished observation also shows that ANP messenger RNA levels in human failing hearts are increased.

In the present study, total ANP concentrations in atria in class IV tended to be rather low than those in class III (Fig. 1 and Table II). In addition, the γ-hANP concentration in the atrium in class IV was significantly lower than that in class III (Table II). These findings raise the possibility that the failure of ANP secretory function occurs in parallel with the decompensation of pump function in the severest stage of CHF. However, since only three cases in class IV were examined in the present study, the possible decompensation of ANP secretory function in class IV must await further clarification.

The increased ANP concentration in the human failing heart contrasts with the results observed in BIO 14.6 hamsters, a hereditary model of cardiomyopathy (42), and spontaneously hypertensive rats (SHR) (43–46) and SHR-stroke prone (43). These animal models show decreased ANP concentrations in the atrium and elevated plasma ANP levels (43). The discrepancy in the atrial ANP concentration between man and animals may be accounted for in part by the difference of ANP turnover in the heart.

The present study demonstrates that the increased concentration of ANP in the human failing heart is mainly due to the increase of low molecular weight forms of ANP, β-hANP, and α-hANP, especially β-hANP. β-hANP is an antiparallel dimer of α-hANP (4) and ANP possessing such a unique structure has been isolated only from the human atrium obtained at autopsy (3, 4, 15). The process of synthesis and secretion of β-hANP is not known at present, however, these results raise the possibility that β-hANP is a product specific to the human failing heart. The changes in energy production, energy utilization and exitation-contraction coupling are reported in heart failure (47). Such changes in cardiac metabolism may be involved in the augmentation of β-hANP synthesis in the human failing heart. Recently, we also demonstrated that β-hANP is rapidly converted into α-hANP in human plasma (48). These findings may provide clues to elucidate the synthesis, storage and secretion of β-hANP and its pathophysiological significance in CHF and other pathologic states.

What is the implication of augmented synthesis and secretion of ANP of the failing heart? Heart failure is the condition in which the heart is no longer able to pump an adequate supply of blood for the metabolic needs of the body, provided there is adequate venous return to the heart (49). When heart failure develops, compensatory mechanisms are utilized (47). Activations of the sympathetic nervous system and of the renin-angiotensin-aldosterone system are two major extracardiac compensatory mechanisms to maintain the normal cardiac output in heart failure (47). In addition to these extracardiac compensatory mechanisms, the heart possesses its own compensatory mechanisms such as autoregulation (Frank-Starling Law of the heart) and hypertrophy (47). Since ANP has diuretic, natriuretic and vasorelaxant activities (1–3, 10–12), ANP secretion from the heart is one of the compensatory mechanisms of the heart. The augmented synthesis and secretion of ANP demonstrated in the present study is a possible important compensatory response of the failing heart. As was expected, several lines of evidence including ours have demonstrated that the intravenous infusion of ANP giving rise to plasma ANP levels comparable to those in patients with severe CHF improves left ventricular function of CHF (31, 50, 51).

In summary, the total ANP concentration in the atrium of the human heart is increased in severe CHF and the increase of ANP in the human failing heart is mainly due to the increase of small molecular forms of ANP, β-hANP, and α-hANP, especially β-hANP. These findings indicate the processing of γ-hANP in the human failing heart differs from that in the normal heart and suggest that the failing heart augments synthesis and secretion of ANP as one of its own compensatory responses.

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