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AUGMENTED SYNTHESIS OF β-HUMAN ATRIAL NATRIURETIC POLYPEPTIDE IN HUMAN FAILING HEARTS

Akira Sugawara[1], Kazuwa Nakao[1,2,*], Narito Morii[1], Takayuki Yamada[1], Hiroshi Itoh[1], Shozo Shiono[1], Yoshihiko Saito[1], Masashi Mukoyama[1], Hiroshi Arai[1], Kazunobu Nishimura[3], Kenji Obata[4], Hirofumi Yasue[4], Toshihiko Ban[3] and Hiroo Imura[1]

1. Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto 606,

2. Radioisotope Research Center, Kyoto University, Kyoto 606,

3. Department of Cardiovascular Surgery, Kyoto University School of Medicine, Kyoto 606,

4. Division of Cardiology, Kumamoto University School of Medicine, Kumamoto 860, Japan

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To elucidate the synthesis of atrial natriuretic polypeptide (ANP) in the failing heart, eighteen 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 or IV) was much higher than those in mild CHF of NYHA class I and class II. The α-hANP-LI in the human auricle consisted of 3 major components of ANP, γ-human ANP (γ-hANP), β-human ANP (β-hANP) and α-human ANP (α-hANP). The predominant component of α-hANP-LI was γ-hANP in the mild CHF, whereas β-hANP and/or α-hANP were prevailing in the severe CHF and, especially, β-hANP was markedly increased in human failing hearts.

From human atria obtained at autopsy three distinct molecular forms of atrial natriuretic polypeptide (ANP), γ-human ANP (γ-hANP), β-human ANP (β-hANP) and α-human ANP (α-hANP), were isolated by Matsuo

*Address correspondence to: Kazuwa Nakao, M.D., Ph.D., Second Division, Department of Medicine, Kyoto University School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan.

Abbreviations: ANP, atrial natriuretic polypeptide; α-, β- and γ-hANP, α-, β- and γ-human ANP; α- and γ-rANP, α- and γ- rat ANP; -LI, -like immunoreactivity; NYHA, New York Heart Association; CHF, congestive heart failure; HP-GPC, high performance gel permeation chromatography; RP-HPLC, reverse phase high performance liquid chromatography; RIA, radioimmunoassay; SE, standard error.

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and his colleagues (1,2). 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 radioimmunoassay (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 (3-5). 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 (5-8). Accumulating evidence indicates that plasma ANP levels are elevated in congestive heart failure (CHF) in relation to the severity of the heart failure (6,7,9-11) and that this elevation of the plasma ANP level is mainly due to the increased ANP secretion from the failing heart (9). These findings indicate that the ANP-secreting function is augmented in CHF, but little is known about the biosynthesis and processing of ANP precursor, γ-hANP, in the failing heart at present. In the present study, we have studied ANP concentrations and molecular forms of ANP in 18 human right auricles obtained at cardiovascular surgery using HP-GPC and RP-HPLC coupled with RIA for ANP.

MATERIALS AND METHODS

Subjects

Eighteen patients with heart diseases (13 men and 5 women, 48 ± 5 yrs) who underwent cardiovascular operations were studied. 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 extraction and radioimmunoassay (RIA) for ANP

Tissue samples (39 mg-623 mg, 226.8 ± 41.1 mg, mean ± SE) were obtained from almost the same apical portion of the right auricle. Tissue extraction was performed as previously reported (3). Measurement of tissue ANP levels were performed using the specific RIA for ANP (6,7,12). Cross-reactivities with β-hANP and γ-rat ANP (γ-rANP) in this RIA were 120 % and 100 % on a molar basis.

High performance gel permeation chromatography (HP-GPC)

HP-GPC was performed on a TSK-GEL G2000 SW (Toyo Soda, Tokyo, Japan) column (7.5 x 600 mm) previously reported (3).

Reverse phase high performance liquid chromatography (RP-HPLC)

RP-HPLC was carried out on a TSK-GEL ODS 120T column (4.6 x 75 mm) (Toyo Soda, Tokyo, Japan) as previously reported (5,13).

Peptides

α-hANP was donated by Dr. H. Matsuo (Miyazaki Medial College, Miyazaki, Japan) (1). β-hANP was generously supplied by Dr. K. Inouye (Shionogi Research Laboratories, Shionogi Co., Ltd., Osaka, Japan)

(14). γ-rANP was purified from rat atrial tissues according to the method described by Kangawa et al. (15).

Statistical analysis

Data were expressed as means ± SE. Statistical analysis was performed using paired or non-paired Student's t test and Duncan's multiple range test (16) when appropriate.

RESULTS

α-hANP-LI concentrations in right auricles

Profiles of 18 patients and their α-hANP-like immunoreactivity (α-hANP-LI) concentrations in right auricles were summarized in Table I. The α-hANP-LI concentration in the right auricle ranged from 13.8 to 593.5 μg/g and the mean value (±SE) was 117.9 ± 34.4 μg/g. In the present study, only one case of NYHA class IV was examined, so cases of NYHA class III and IV were combined for statistical analyses. The atrial α-hANP-LI concentration in patients with severe CHF (New York Heart Association (NYHA) class III·IV) was 248.5 ± 76.7 μg/g

Table I

Profiles of 18 patients and ANP concentrations in right auricles

Case	Age	Sex	Disease	NYHA	α-hANP-LI concentration (μg/g)
1	61	M	IHD	I	13.8
2	34	M	ASD	I	22.9
3	64	M	IHD	I	26.2
4	2	F	T/F	I	29.2
5	47	M	IHD	I	35.2
6	24	M	ASD	I	40.3
7	59	M	IHD	I	57.6
8	64	M	IHD	I	98.5
9	46	M	AR	II	22.6
10	40	M	IHD	II	23.9
11	53	F	MR	II	52.0
12	45	M	IHD	II	208.3
13	54	M	MS+AS	III	100.4
14	56	F	MS	III	190.0
15	54	M	AR	III	234.2
16	59	M	MSR+AR	III	296.1
17	59	F	MSR+AR	III	593.5
18	46	F	MSR+TR	IV	77.0

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

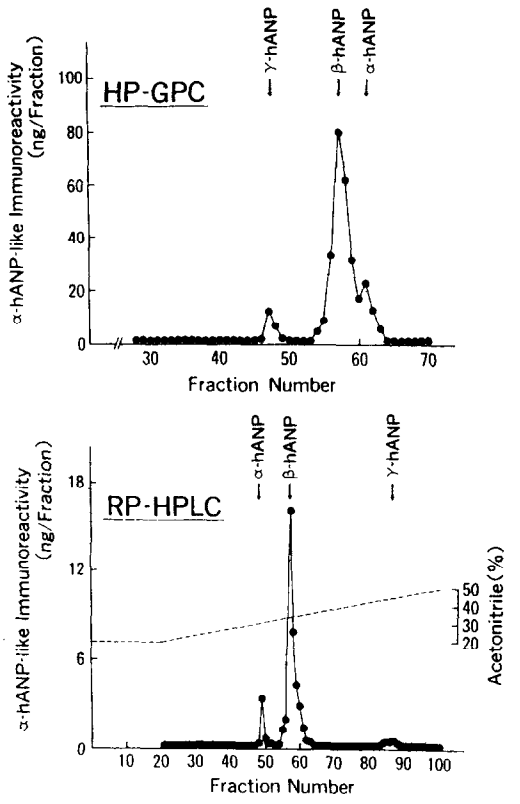

Figure 1. High performance gel permeation chromatographic (HP-GPC) and reverse phase high performance liquid chromatographic (RP-HPLC) profiles of the same atrial extract. The α-hANP-LI level in each fraction was assayed by the RIA for α-ANP. The elution positions of α-hANP, β-hANP and γ-hANP are indicated by the arrows.

which was much higher than those in NYHA class I (40.5 ± 9.5 $p<0.01$) and class II (76.7 ± 44.4, $p<0.05$).

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 (Figure 1). RP-HPLC analysis confirmed that the first, the second and the third components of α-hANP-LI in HP-GPC co-migrated with γ-hANP, β-hANP and α-hANP, respectively. Therefore, we analyzed all of the other auricular tissues using HP-GPC coupled with RIA.

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

Figure 2 summarizes the results of HP-GPC analyses in 18 patients schematically. Table II shows the comparison of concentrations of

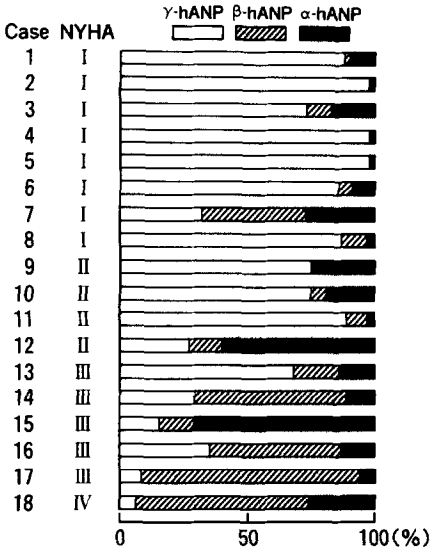

Figure 2. Schematic illustrations of percentages of concentrations of γ-hANP, β-hANP and α-hANP in total ANP concentration in right auricles from 18 patients. Percentages in α-hANP-LI of γ-hANP, β-hANP and α-hANP are shown by the open column, hatched column and closed column, respectively. Cases are placed in order of the severity of congestive heart failure classified by the functional classification of New York Heart Association (NYHA).

γ-hANP, β-hANP and α-hANP, and percentages of each ANP among patients in NYHA class I, class II and class III·IV. γ-hANP was detected in all of 18 cases. No significant difference was observed in the γ-hANP concentration among three groups. The percentage of γ-hANP in α-hANP-LI showed the graded decrease in accordance with the severity of CHF.

Table II

Comparison of concentrations of β-hANP and α-hANP, and percentage of each ANP among patients in NYHA class I, class II and class III·IV

NYHA class	γ-hANP μg/g (%)	β-hANP μg/g (%)	α-hANP μg/g (%)
I	31.9 ± 8.2 (82.5 ± 7 8)	4.9 ± 2.9 (8.6 ± 4.9)	3.7 ± 1.8 (8.9 ± 3.2)
II	34.4 ± 10.0 (66.5 ± 13.6)	8.2 ± 6.3 (6.8 ± 2.7)	34.2 ± 30.3 (26.8 ± 12.0)
III·IV	52.3 ± 13.6 (26.8 ± 9.5)** †	146.9 ± 75.8*† (49.7 ± 11.6)**††	49.1 ± 23.7¶ (23.5 ± 9.9)¶

Percentages of each ANP in α-hANP-LI in right auricles are shown in backets. Values are mean ± SE. * $p<0.05$, ** $p<0.01$ compared with corresponding values in NYHA class I group. †$p<0.05$, †† $p<0.01$ compared with corresponding values in NYHA class II group. ¶ $p<0.05$ compared with corresponding values in NYHA class I group using a logarithmic transformation of the data.

Most cases of NYHA class I and class II showed low concentrations of β-hANP. On the other hand, the β-hANP concentration was tremendously increased in NYHA class III IV (more than one order of magnitude higher than those in NYHA class I ($p<0.01$) and class II ($p<0.01$)). The mean percentage of β-hANP in α-hANP-LI reached approximately 50 % in NYHA class III·IV, although those in NYHA class I and class II were less than 10 %. α-hANP concentrations in NYHA class I and class II were similar to β-hANP concentrations. The α-hANP concentration in NYHA class III·IV was larger than those in NYHA class I and class II and its percentages in α-hANP-LI were about one fourth in NYHA class III·IV and less than 10 % in NYHA class I (Table II).

DISCUSSION

The present study demonstrates that the total α-hANP-LI concentration in the right auricle is increased in proportion to the severity of CHF and that the increase in the total ANP concentration is due to the rise of β-hANP and/or α-hANP, especially 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.

We and others previously reported that the predominant component of ANP in the rat heart is γ-rANP (3,4), whereas β-hANP and α-hANP are present together with γ-hANP in human atrial tissues obtained at cardiac surgery or autopsy (2-4). 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 (11). 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 of NYHA 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 (3-5,17,18).

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, especially β-hANP. 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. Recently, we also demonstrated that β-hANP is converted into α-hANP in human plasma (19). 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.

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