Effects of Intravenously Administered β-Human Atrial Natriuretic Polypeptide in Humans

Hiroshi Itoh, Kazuwa Nakao, Masashi Mukoyama, Shozo Shiono, Narito Morii, Akira Sugawara, Takayuki Yamada, Yoshihiko Saito, Hiroshi Arai, Kiminori Hosoda, Yoshikazu Kambayashi, Ken Inouye, and Hiroo Imura

SUMMARY β-Human atrial natriuretic polypeptide (β-hANP) is an antiparallel dimer of α-human ANP (α-hANP) that was isolated from human aatria. Using synthetic β-hANP and a radioimmunoassay for α-hANP that also detects β-hANP, we have previously demonstrated that β-hANP is converted into α-hANP in human plasma in vitro. In the present study, we compared the effects of Intravenous administration of β-hANP (100 µg) to five normal human volunteers with those of an equimolar administration of α-hANP (50 µg) to the same subjects, and we also investigated the possible mechanisms of actions of β-hANP. Although the administration of α-hANP caused a significant decrease in blood pressure with a reactional increase of heart rate, β-hANP elicited minimal change of blood pressure. In contrast, β-hANP exerted more potent and longer lasting diuretic and natriuretic activities than did α-hANP. Net changes in urine volume and sodium excretion induced by β-hANP (579 ± 65 ml, 56.0 ± 9.9 mEq) were significantly greater than those elicited by α-hANP (396 ± 50 ml, 34.7 ± 4.9 mEq; p<0.05, respectively). The administration of β-hANP revealed a longer retention of the ANP-like immunoreactivity level in plasma, compared with that of α-hANP. High performance gel permeation chromatography coupled with the radioimmunoassay revealed that β-hANP (Mr = 6000) was also converted into α-hANP (Mr = 3000) in human plasma in vivo. The demonstrated conversion of β-hANP into α-hANP could be relevant to the observed effects of β-hANP in humans. (Hypertension 11: 697-702, 1988)

KEY WORDS atrial natriuretic factor • blood pressure • heart rate • diuresis • natriuresis • glomerular filtration rate

The epochal discovery by de Bold et al.1 that rat atrial extracts possess potent diuretic and natriuretic properties led to the identification of multiple forms of atrial natriuretic polypeptide (ANP), also known as atrial natriuretic factor (ANF), with high and low molecular weights.2-4 Growing evidence now indicates that the heart is a novel endocrine organ involved in the regulation of body fluid and blood pressure control. From the human heart, three distinct molecular forms of ANP, α-, β-, and γ-human ANP (α-hANP, β-hANP, and γ-hANP, respectively) have been isolated.2-5 α-hANP (human ANF-[99-126]) is composed of 28 amino acids with an intramolecular disulfide linkage; γ-hANP (human ANF-[1-126]) 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) possesses a remarkable structure of an antiparallel dimer of α-hANP with intermolecular disulfide bridges. Such a dimeric form of α-ANP has not been found in other mammals.

Using high performance gel permeation chromatography (HP-GPC) and reverse-phase high performance liquid chromatography (RP-HPLC) coupled with specific radioimmunoassay (RIA) for ANP, we and others have demonstrated that considerable amounts of β-hANP exist along with γ-ANP and α-hANP.6-7 Recently, we also demonstrated the augmented synthesis of β-hANP in the failing heart.8 In contrast, we have reported that the major circulating form of ANP secreted through the coronary sinus from the heart as a hormone is α-hANP.9-11 Miyata et al.12 have reported the presence of β-hANP in human plasma, and we have also detected β-hANP in plasma from patients with some cardiovascular disorders (unpublished observation, 1988). These results suggest that β-hANP is also...
a circulating hormone in humans under certain physiological or pathophysiological conditions.

Based on these findings, interest naturally has been focused on the possible hormonal actions of β-hANP. Kangawa et al. reported that β-hANP isolated from human atria possesses diuretic and natriuretic activities with a slower onset and a longer duration than α-hANP and γ-hANP. β-hANP has been synthesized and demonstrated to exhibit a slower onset and a longer duration of diuretic and natriuretic actions in anesthetized rats, compared with α-hANP. However, the mechanisms involved in the manifestation of actions of β-hANP and the hormonal actions of β-hANP in humans are currently unknown. Since the presence of β-hANP has been confirmed only in humans, possible actions of β-hANP and the importance of β-hANP as a hormone should be clarified in humans.

Recently, we demonstrated that β-hANP (M_r = 6000) is converted into α-hANP (M_r = 3000) in human plasma in vitro, and we suggested that the conversion of β-hANP into α-hANP would be relevant to the biological properties. In the present study, using synthetic β-hANP, we examined the effects of β-hANP administered to humans and investigated the possible mechanisms of actions of this unique peptide in vivo.

Materials and Methods

All components of this study were approved by the Ethical Committee on Human Research of Kyoto University (No. 61-9), and informed consent was obtained from each subject.

Preparation of β-hANP and α-hANP

β-hANP and α-hANP were synthesized by the solution method, as described previously. The homogeneity of β-hANP and α-hANP were confirmed by RP-HPLC and amino acid analysis. β-hANP and α-hANP were dissolved in 0.9% saline with 10% lactose, sterilized by passage through a 0.22-µm Millipore filter (Bedford, MA, USA), and stored at -20°C until used. The chemical nature and content of β-hANP and α-hANP in vials were verified by RP-HPLC and RIA.

Administration of Synthetic β-hANP and α-hANP to Humans

Five normal male volunteers, between 27 and 30 years old, were studied twice, once for the intravenous injection of β-hANP and once for that of α-hANP, in random order, with an interval of 1 week between injections. After an overnight fast, the subjects were kept recumbent from 0900 and two intravenous catheters (Medikit, Tokyo, Japan) were inserted for the administration of ANP and blood sampling, respectively. A constant infusion of physiological saline was given at a rate of 0.2 ml/kg/min throughout the examination. Urine was collected through a balloon catheter (16F, Terumo, Tokyo, Japan) placed in the bladder at 5-minute intervals during the study. After a 60- to 90-minute equilibration period during which urine flow became constant (3.5 ± 0.6 ml/min), equi-

molar β-hANP (100 µg) or α-hANP (50 µg) was injected as a bolus. Blood samples were obtained 15 minutes before, at Minute 0, and 1, 2.5, 5, 10, 20, 30, 45, 60, and 90 minutes after the injection. Blood pressure and heart rate were recorded continuously at 1-minute intervals with a noninvasive automatic device (Nippon Colin, Aichi, Japan).

Measurements of Renal Excretory Parameters

Sodium and potassium concentrations (mEq/L) in serum and urine were measured by the electrometric titration method (Hitachi 736, Hitachi, Tokyo, Japan). Serum and urine osmolarities (mosm/L) were determined by the freezing point depression (Auto Stat OM-6010, Kyotodaichikagaku, Kyoto, Japan). Urine volume was expressed as the urine flow rate (ml/min), and change in urine flow (ml/min) represented the urine flow rate after the administration of ANP minus the basal urine flow rate. Sodium and potassium excretion rates (expressed as µEq/min) were determined for each urine collection, and change in sodium excretion (µEq/min) was determined in the same way as urine flow. Endogenous creatinine clearance was calculated based on a standard formula as an index of glomerular filtration rate.

Determination of Plasma ANP-Like Immunoreactivity

Plasma was obtained following a method reported previously. The immunoreactivity was determined by the RIA that recognizes a common C-terminal sequence of α-hANP and α-rat ANP as reported previously. The cross-reactivity of β-hANP is 120% on a molar basis. The plasma ANP-like immunoreactivity (ANP-LI) was measured without extraction with 25 µl of plasma.

High Performance Gel Permeation Chromatography

Plasma obtained 1 minute and 30 minutes after the administration of β-hANP was extracted according to a method described previously with a Sep-Pak C_18 cartridge (Waters Associates, Milford, MA, USA). Extracted plasma and the β-hANP-containing solution in the vial, which we administered, were analyzed in HP-GPC on a TSK-GEL G2000 SW (Toyo Soda, Tokyo, Japan) column (7.5 × 600 mm), as described previously.

Statistical Analysis

Analysis of variance with repeated measures and subsequently with Duncan's test was used to determine significant changes during time-dependent multiple observations. A paired Student's t test was used to examine the significance of single comparisons of corresponding values in the same subjects. All values are expressed as means ± SEM; p values less than 0.05 were considered significant.

Results

Comparison of Effects of β-hANP and α-hANP on Blood Pressure and Renal Function

The administration of 50 µg of α-hANP caused a rapid fall in blood pressure and a concomitant increase
of heart rate. Significant decreases of systolic blood pressure and diastolic blood pressure persisted for 5 (−8 ± 1 mm Hg; p < 0.01 vs basal level) and 10 minutes (−2 ± 1 mm Hg; p < 0.02 vs basal level) after the administration, respectively. Heart rate remained significantly elevated for 10 minutes after the administration (+11 ± 3 beats/min; p < 0.02 vs basal level).

In contrast, the administration of equimolar β-hANP (100 μg) elicited no significant alteration in blood pressure or heart rate as compared with the baseline throughout the observation (Table 1).

Table 1 and Figure 1 show the renal responses to the administrations of β-hANP and α-hANP. The administration of α-hANP induced significant diuresis and natriuresis with an abrupt onset. Urine volume increased maximally (from 3.6 ± 0.3 to 15.6 ± 0.2 ml/min; p < 0.01), with a significant increase of osmolar clearance (from 6.5 ± 0.4 to 15.7 ± 1.9 ml/min; p < 0.01). Urinary sodium excretion increased maximally (from 635 ± 110 to 1650 ± 331 μEq/min; p < 0.01), with a significant increase of fractional excretion of sodium (from 2.8 ± 0.6 to 7.5 ± 1.8%; p < 0.01). Creatinine clearance increased significantly after the administration of α-hANP, but it was not significantly altered by the administration of β-hANP (see Table 1). Urinary potassium excretion also increased, but changes were only transient after administrations of α-hANP and β-hANP. Figure 1 shows the time courses of urinary output and urinary sodium excretion after the administrations of α-hANP and β-hANP. The administration of β-hANP exerted more

Table 1. Effects of Intravenous Administrations of β-hANP and α-hANP on Blood Pressure, Heart Rate, and Renal Function in Humans

<table>
<thead>
<tr>
<th>Variable</th>
<th>β-hANP (100 μg)</th>
<th>α-hANP (50 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>112 ± 5</td>
<td>114 ± 6</td>
</tr>
<tr>
<td>Maximal change</td>
<td>−1 ± 1*</td>
<td>−10 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>56 ± 4</td>
<td>57 ± 4</td>
</tr>
<tr>
<td>Maximal change</td>
<td>−2 ± 1†</td>
<td>−8 ± 2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>63 ± 2</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>Maximal change</td>
<td>+3 ± 2*</td>
<td>+21 ± 4</td>
</tr>
<tr>
<td>Change in urine flow (ml/min)</td>
<td>6.43 ± 0.73†</td>
<td>4.40 ± 0.56‡</td>
</tr>
<tr>
<td>Change in Na excretion (μEq/min)</td>
<td>623 ± 100‡</td>
<td>385 ± 55§</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>132.3 ± 12.2</td>
<td>119.5 ± 5.8</td>
</tr>
<tr>
<td>Maximal</td>
<td>162.2 ± 15.5</td>
<td>214.7 ± 15.9§</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 5). Ccr = endogenous creatinine clearance.

* p < 0.005, † p < 0.05, ‡ p < 0.02, compared with α-hANP values.

§ p < 0.01, compared with basal values.

Figure 1. Time course of urinary output (A) and urinary sodium excretion (B) after intravenous administrations of β-human ANP (β-hANP; ○) and α-human ANP (α-hANP; ●) to humans, showing cumulative changes in urine flow or sodium excretion. Values are expressed as means ± SEM (n = 5). Single (p < 0.05), double (p < 0.02), and triple asterisks (p < 0.01) indicate significant difference compared with the corresponding values after the administration of α-hANP.
prolonged actions in diuresis and natriuresis than did that of \( \alpha \)-hANP. Net changes of urine volume and urinary sodium excretion for 90 minutes were significantly greater after the administration of \( \beta \)-hANP (579 ± 65 ml, 56.0 ± 9.9 mEq) than were those seen after \( \alpha \)-hANP administration (396 ± 50 ml, 34.7 ± 4.9 mEq).

Plasma ANP-LI Level and Change in Molecular Form After Administrations of \( \beta \)-hANP and \( \alpha \)-hANP

Disappearance curves of exogenously administered \( \beta \)-hANP and \( \alpha \)-hANP in human circulation are depicted in Figure 2. The level of ANP-LI after the \( \alpha \)-hANP administration reached 680 ± 80 fmol/ml 2.5 minutes after the injection and rapidly decreased approximately 50% 5 minutes after the injection. The level of ANP-LI after the \( \beta \)-hANP administration reached 1290 ± 140 fmol/ml 2.5 minutes after the injection, and values were significantly higher than the corresponding values after the \( \alpha \)-hANP administration throughout the observation period, although the administered doses of \( \beta \)-hANP and \( \alpha \)-hANP were equimolar.

HP-GPC analyses coupled with the RIA were performed on the \( \beta \)-hANP solution in the vial and extracts from the plasma obtained 1 minute and 30 minutes after the injection of \( \beta \)-hANP. As shown in the left column of Figure 3, only one immunoreactive peak corresponding to \( \beta \)-hANP was detected in the \( \beta \)-hANP solution in the vial; however, in human plasma, in addition to the peak at the position of synthetic \( \beta \)-hANP, a second peak with ANP-LI emerged at the elution position of synthetic \( \alpha \)-hANP, as shown in the middle and right columns of Figure 3.

Discussion

When injected intravenously into humans, \( \alpha \)-hANP is known to elicit prompt diuretic, natriuretic, and hypotensive actions,\(^{10,15}\) effects that were confirmed in the present study in normal volunteers. A rapid and vigorous output in urine volume and sodium occurred, and blood pressure fell, with a reciprocal rise in heart rate. The present study demonstrates that the intravenous bolus administration of synthetic \( \beta \)-hANP, an antiparallel dimer of \( \alpha \)-hANP, to normal human subjects elicited minimal change of blood pressure and heart rate, but, in contrast, exerted more potent diuretic and natriuretic actions that persisted longer than those of the equimolar dose of \( \alpha \)-hANP.

The effects on blood pressure and heart rate of \( \beta \)-hANP in humans are compatible with a recent report stating that \( \beta \)-hANP exhibits smooth muscle spasmyotic activity as potent as one third to one sixth of that of \( \alpha \)-hANP, in rat aorta contracted with norepinephrine or in chick rectum contracted with carbachol in vitro.\(^{13}\)

In the present study, \( \beta \)-hANP elicited more potent and sustained diuretic and natriuretic activities than did \( \alpha \)-hANP. In anesthetized rats, diuretic and natriuretic responses induced by \( \beta \)-hANP are reported to persist longer than those induced by \( \alpha \)-hANP.\(^{3,12,13}\) These findings are consistent with our human study. Several mechanisms responsible for the renal actions of ANP have been postulated, including increases in glomerular filtration rate, blood flow redistribution together with secondary impairment of tubular sodium reabsorption,\(^{16,17}\) and direct inhibitory actions on renal tubules.\(^{18}\) Since in the present study creatinine clearance did not increase significantly after the administration of \( \beta \)-hANP, the observed renal actions of \( \beta \)-hANP may not be ascribed to the alteration of glomerular filtration rate. The sites of actions in the kidney of \( \beta \)-hANP remain to be identified.

The rapid disappearance of exogenously administered \( \alpha \)-hANP in human circulation observed in the present study is compatible with our previous report, which found that plasma half-lives of \( \alpha \)-hANP for the fast and slow components are 1.7 and 11.0 minutes, respectively.\(^{19}\) The administration of equimolar \( \beta \)-hANP revealed a significantly higher plasma ANP-LI level compared with that for \( \alpha \)-hANP throughout the study. To elucidate the mechanism responsible for the longer retention of \( \beta \)-hANP in plasma, we have examined the change in molecular form of \( \beta \)-hANP. We previously reported that the level of ANP-LI in normal human plasma is about 10 pM, and volume loading with saline infusion at the rate of 0.2 ml/kg/min, the same rate used in the present study, causes a twofold increase of plasma ANP level.\(^{10}\) In the present study under the constant saline infusion, the endogenous ANP level 1 minute or 30 minutes after the administration therefore would be one or two orders of magnitude lower than the level of exogenously administered ANP.
in plasma. Thus, the immunoactive peak eluted at the elution position of α-hANP in HP-GPC analysis was considered to represent almost entirely α-hANP converted from exogenous β-hANP. The present study revealed that the conversion of β-hANP into α-hANP, which we reported in human plasma in vitro, also occurs in human circulation in vivo. The sustained higher plasma ANP-LI level after the administration of β-hANP can be partly ascribed to the observed conversion of β-hANP. Because the immunoactive peak corresponding to α-hANP was already observed 1 minute after the administration and the peak of β-hANP persisted 30 minutes after the administration, the conversion of β-hANP into α-hANP in vivo seems to begin just after the entrance of exogenous β-hANP into the circulation and continue for at least 30 minutes after the administration. The converted product from β-hANP in human plasma in vitro is demonstrated by amino acid analysis and amino-terminal sequencing to be authentic α-hANP with 28 amino acids, and α-hANP converted from β-hANP in the circulation in vivo retains the ability to bind to receptors from the bovine adrenal cortex (unpublished observation, 1988). These findings suggest that the demonstrated conversion of β-hANP into α-hANP would be relevant to the biological properties of β-hANP, that is, the diuretic and natriuretic activities with a longer duration compared with α-hANP. However, any conclusive remarks on the mechanisms of actions of β-hANP should be withheld until the interactions of β-hANP and receptors for ANP have been elucidated.

α-hANP has been suggested to be therapeutically effective as a vasodilator or a diuretic in patients with congestive heart failure. The preferential effect of β-hANP on renal function with longer activity demonstrated in the present study may be of clinical value, especially in patients to whom the reduction of blood pressure is undesirable.

Since β-hANP was first isolated from human atria obtained at autopsy and had a unique structure, whether β-hANP is a native form of ANP or not is a matter for argument. Recently, we have demonstrated that the tissue level of β-hANP is increased in the failing heart, although little β-hANP is detectable in the normal heart. These observations raise the possibility that β-hANP is a specific product of the failing heart. However, the exact mechanism of synthesis and secretion of this unique peptide must await further investigation.

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Effects of intravenously administered beta-human atrial natriuretic polypeptide in humans.
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