Effects of Intravenously Administered C-type Natriuretic Peptide in Humans: Comparison with Atrial Natriuretic Peptide

Toshio Igaki, Hiroshi Itoh, Shin-ichi Suga, Norio Hama, Yoshihiro Ogawa, Yasato Komatsu, Jun Yamashita, Kentaro Doi, TaeHwa Chun, and Kazuwa Nakao

We have previously reported that C-type natriuretic peptide (CNP), the third member of the natriuretic peptide family, is produced in vascular endothelial cells and suggested that CNP might be a local regulator of vascular tone and growth. To evaluate the biological actions of CNP as compared with human atrial natriuretic peptide (hANP), we intravenously administered synthetic CNP (0.43 nmol/kg) and α-hANP (0.43 and 0.043 nmol/kg) to healthy humans. The experiments were done on different days in the same five healthy volunteers (31 ± 1 yr old). CNP injection caused a transient but significant decrease in both systolic and diastolic blood pressure (−4.3 ± 1.3, −4.1 ± 1.0 mmHg) with a significant increase in heart rate (+7.6 ± 2.6 bpm), and exerted significant diuretic and natriuretic activities (+130±80%, +160±100% over the basal level). These effects of CNP (0.43 nmol/kg) were comparable to, or less than, those of α-hANP (0.043 nmol/kg). CNP injection also significantly suppressed aldosterone secretion (22% reduction as compared with the basal level). Our results demonstrate that intravenously-administered CNP acts as a natriuretic peptide with less potency than ANP. (Hypertens Res 1998; 21: 7-13)

Key Words: C-type natriuretic peptide (CNP), atrial natriuretic peptide (ANP), blood pressure, natriuresis, guanosine 3',5'-cyclic monophosphate (cGMP)

Ever since the discovery of atrial natriuretic peptide (ANP) (1, 2) and brain natriuretic peptide (BNP) (3) in the mammalian heart and brain, ANP and BNP have been considered to comprise the natriuretic peptide family responsible for body fluid homeostasis and blood pressure control, acting both as cardiac hormones and as neuropeptides (4).

The third member of the natriuretic peptide family, C-type natriuretic peptide (CNP), was isolated from porcine brain (5). We have raised a specific antibody against CNP and have established a radioimmunoassay (RIA) for CNP (6). Using this RIA, we showed that CNP was produced mainly in the central nervous system, and demonstrated that the concentrations of CNP-like immunoreactivity (CNP-LI) in the human brain and cerebrospinal fluid are much higher than those of ANP and BNP (7, 8). On the other hand, no appreciable amount of CNP-LI was detected in the heart. These findings, together with evidence that CNP acts on the central nervous system (9), supported the hypothesis that CNP acts as a neuropeptide rather than a cardiac hormone. Recently, however, we found that CNP was synthezised and secreted by cultured endothelial cells (EC) (10). CNP secretion from EC was stimulated by various growth factors and cytokines, especially transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α) (11, 12). In contrast, CNP secretion was potently suppressed by insulin (13) and vascular endothelial growth factor (VEGF) (14). We further reported the in vivo gene expression of CNP in intact blood vessels (15) and the presence of CNP in human plasma (16). We also showed a marked increase in plasma CNP levels in patients with septic shock (16). In addition, CNP has been reported to inhibit the growth of vascular smooth muscle cells (SMC) (17, 18). These results together indicate the existence of a “vascular natriuretic peptide system (vascular NPS)” in which CNP induces relaxation and growth-inhibition of vascular smooth muscle cells. CNP has therefore been characterized as a novel endothelium-derived relaxing peptide (19-21).

In this study, to investigate the biological actions of CNP in humans we administered synthetic CNP (0.43 nmol/kg) to five healthy volunteers by in-
travenous bolus injection. Effects of CNP were compared with those of a-hANP, given at the one-tenth the dose of CNP (0.043 nmol/kg) and at the same dose as CNP (0.43 nmol/kg).

**Methods**

**Preparation of CNP and a-hANP**

CNP and a-hANP were synthesized by the solution method, as described previously (22). The homogeneities of CNP and a-hANP were confirmed by reverse-phase high-performance liquid chromatography (RP-HPLC) and amino acid analysis. CNP and a-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 use. The chemical nature and content of CNP and a-hANP in vials were verified by RP-HPLC and radioimmunoassay.

**Administration of Synthetic CNP and a-hANP to Humans**

We studied five healthy male volunteers 29 to 32 yr of age and given a normal diet (NaCl, 250 mEq/d; K, 80 mEq/d). Informed consent was obtained from all subjects before enrollment. The subjects were studied three times on different days, on which they were randomly assigned to receive 0.43 nmol/kg of CNP, 0.043 nmol/kg of a-hANP, or 0.43 nmol/kg of a-hANP by intravenous bolus injection. An interval of 1 wk was allowed between each injection. After an overnight fast, the subjects were kept supine for 1 h before injection of the peptide. Two intravenous catheters (Medikit, Tokyo, Japan) were inserted for injection of the peptide and blood sampling, respectively. A continuous infusion of physiological saline was given at a rate of 250 ml/h throughout the examination. Blood pressure and heart rate were continuously monitored with a noninvasive automatic device (Nippon Colin, Aichi, Japan), and urine and blood samples were collected according to the protocol shown in Fig. 1. The demographic characteristics of the subjects are shown in Table 1. In the same subject, there were no significant differences in any variable among the three experiments. However, since differences existed among the subjects, we calculated the percent increase (or decrease) as compared with the basal level to evaluate effects of the natriuretic peptides.

**Measurement of Plasma Hormone Levels**

Plasma a-hANP, BNP, and CNP levels were measured with our specific RIAs as reported previously (11, 15, 16). The minimal detectable values of CNP, a-hANP, and human BNP in each RIA were 1.0, 0.32, and 1.2 fmol/tube, respectively. The cross-reactivities of the RIA for CNP with a-hANP and human BNP were 0.2% and < 0.01%, on a molar basis, respectively. The cross-reactivities of the RIA for a-hANP and human BNP with CNP were both < 0.01%, on a molar basis. Plasma concentrations of guanosine 3',5'-cyclic monophosphate (cGMP) were measured by RIA after succinylation as described previously (23). Plasma renin activity and plasma aldosterone concentration were measured with commercially available kits, renin RIA beads (Dinabot Co., Tokyo), and aldosterone RIA kit II (Dinabot Co.), respectively.

**Other Biochemical Measurements**

Sodium, potassium, and chloride concentrations in serum and urine were measured with the ion electrode method (Hitachi 736, Hitachi Medical Co., Tokyo). Serum and urine creatinine concentrations were determined with Jaffe’s procedure (Hitachi 736).

---

**Table 1. Demographic Characteristics of the Subjects.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>Plasma concentration</td>
<td></td>
</tr>
<tr>
<td>PRA (ng/ml/h)</td>
<td>0.74 ± 0.07</td>
</tr>
<tr>
<td>PAC (pg/ml)</td>
<td>41.9 ± 3.8</td>
</tr>
<tr>
<td>ANP (fmol/ml)</td>
<td>6.0 ± 1.1</td>
</tr>
<tr>
<td>BNP (fmol/ml)</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>CNP (fmol/ml)</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>cGMP (pmol/ml)</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Urinalysis</td>
<td></td>
</tr>
<tr>
<td>cGMP excretion (nmol/30 min)</td>
<td>30.2 ± 4.3</td>
</tr>
<tr>
<td>Na excretion (mEq/30 min)</td>
<td>9.1 ± 1.8</td>
</tr>
<tr>
<td>K excretion (mEq/30 min)</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Cl excretion (mEq/30 min)</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>144 ± 14</td>
</tr>
</tbody>
</table>

n=5 (all male)
Statistical Analysis

All values are expressed as the means ± SE. Comparisons between values during the control period with those after the CNP with α-hANP injections were made by paired Student’s t-tests. When a p value was < 0.05, the difference was considered statistically significant.

Results

Effects of CNP and α-hANP on Plasma Levels of Natriuretic Peptides

The time courses of the plasma CNP and ANP levels after CNP or ANP injection are shown in Fig. 2. Plasma CNP and ANP levels promptly increased after bolus injection of the peptides, peaked (CNP 0.43 nmol/kg, 770 ± 93 fmol/ml; ANP 0.43 nmol/kg, 1,206 ± 155 fmol/ml) within 3 min, and returned to the basal level within 15 min. Figure 3 shows the plasma ANP and BNP levels after CNP injection. The plasma ANP and BNP levels significantly increased (ANP, from 6.9 ± 1.1 to 17.2 ± 2.6 fmol/ml; BNP, from 1.7 ± 0.4 to 4.1 ± 0.9 fmol/ml). The increase in the plasma ANP levels was, however, much lower than those after the α-hANP injections.

Effects of CNP and α-hANP on Plasma cGMP Levels and Urinary cGMP Excretion

The time course of the plasma cGMP level and the urinary cGMP excretion after CNP injection are shown in Fig. 4. The plasma cGMP level 15 min after CNP injection had increased significantly and was 2.1 times higher than the basal level (from 4.0 ± 1.1 to 8.4 ± 1.8 pmol/ml). The urinary cGMP excretion during the first 30 min after injection also had increased significantly and was 2.4 times higher than the basal level (from 30.7 ± 4.3 to 74.9 ± 13.4 nmol/30 min). There were also significant increases...
Effects of CNP and α-hANP on Blood Pressure and Heart Rate

As shown in Fig. 5A, both systolic and diastolic blood pressures significantly decreased after CNP injection (maximal change, $-4.3 \pm 1.3$ and $-4.1 \pm 1.0$ mmHg, respectively), with a significant reactional increase in heart rate (maximal change, $+7.6 \pm 2.6$ bpm). These changes were observed during the first 5 min after injection. Figures 5B and 5C show the changes in blood pressure and heart rate after injection of α-hANP at one-tenth the dose of CNP and at the same dose as CNP. After injection of α-hANP at one-tenth the dose of CNP, the maximal changes in systolic and diastolic blood pressures and heart rate ($-7.0 \pm 2.6$ and $-4.5 \pm 2.6$ mmHg, $+12.9 \pm 1.8$ bpm) were similar to those after CNP injection. Maximal changes after the injection of α-hANP at the same dose as CNP ($-11.8 \pm 2.3$ and $-10.9 \pm 0.9$ mmHg, $+11.6 \pm 1.8$ bpm) were significantly greater than those after CNP injection. These effects on blood pressure and heart rate persisted for 15 min after ANP injection, whereas the effects of CNP disappeared within the 5 min after injection.

Effects of CNP and α-hANP on Aldosterone Secretion

As shown in Fig. 7, within 30 min after injection, plasma aldosterone levels were significantly and almost equally suppressed by CNP (78% of the basal level), α-hANP at one-tenth the dose of CNP, and α-hANP at the same dose as CNP. These suppressive effects of CNP and α-hANP on the plasma aldosterone level persisted for at least 90 min after injection.
We previously demonstrated that intravenous administration of α-hANP or BNP results in diuretic, natriuretic, and hypotensive effects in healthy humans (24–26). The present study demonstrates that intravenous administration of synthetic CNP (0.43 nmol/kg) is also associated with biological actions characteristic of a natriuretic peptide in healthy humans.

Interestingly, plasma ANP and BNP levels...
creased significantly after CNP injection in this study. Since the cross-reactivities of CNP in RIAs for ANP and BNP are < 0.01%, the increased plasma levels of ANP and BNP could not be cross-reactive CNP at high levels (highest level, 770 fmol/ml) in each RIA for ANP or BNP. We thus attribute the observed increases in plasma ANP and BNP levels to reduced metabolism of endogenous ANP and BNP, caused by the large amount of exogenous CNP. The rapid inactivation of natriuretic peptides is ascribed to enzymatic degradation and receptor-mediated internalization. The enzymatic degradation of natriuretic peptides is mediated mainly by neutral endopeptidase EC3.4.24.11 (NEP), which was first recognized to inactivate the 28-amino acid a-hANP (27). Recently, we demonstrated that CNP degradation is also caused by NEP (28). On the other hand, C receptor has been shown to serve as a specific clearance binding site for natriuretic peptides, including CNP (15, 28). Although there was no direct evidence in the present study, the excessive amount of exogenous CNP may have blocked C receptor, NEP, or both, resulting in reduced degradation of ANP and BNP. The observed increases in plasma ANP and BNP levels were, however, inadequate to cause significant hemodynamic and renal effects (22, 24). In another series of experiments, we intravenously injected into a healthy subject a much lower dose of a-hANP (0.43 pmol/kg), which is 1/1,000 the dose of CNP administered in the present study. After this low dose, the peak plasma ANP level was 53 fmol/ml, which was higher than that after CNP injection in the present study, and there was no change in blood pressure or heart rate. Therefore, we attribute the biological actions after CNP injection in this study to CNP itself.

In this study, the potencies of CNP were comparable to or less than those of a-hANP at one-tenth the dose of CNP. These results are compatible with the observed plasma and urinary cGMP responses to the injections of CNP and ANP. Differences in biological potency between CNP and ANP can be attributed to the natriuretic peptide receptor subtype distribution (ANP-A vs. ANP-B receptor), since past studies demonstrated that ANP-A receptor shows high affinity to ANP, whereas ANP-B receptor is specific to CNP (15).

Some studies have examined the effects of intravenously-administered CNP in humans (29, 30). They demonstrated no significant biological actions of CNP. In those studies, however, lower doses of CNP were administered by continuous infusion to achieve plasma CNP levels of about 60 fmol/ml, which are at least one order lower than those in our present study (770 fmol/ml). In agreement with previous studies, we showed that after intravenous administration in humans CNP is a less active natriuretic peptide than ANP. Since the circulating CNP level in humans is at most 10 fmol/ml (16), our study indicates that the biological significance of CNP as a circulating hormone is minimal. However, we have proposed that CNP may act as a local vascular factor involved in the regulation of vascular tone and growth (12, 20, 21). There is also a report that demonstrates the local production of CNP in the kidney (31). Given that the local concentration of CNP within blood vessels or the kidney is much higher than the circulating level, our results further suggest the potential importance of CNP as a local paracrine/autocrine regulator in humans.

In conclusion, our study demonstrated that, although less potent than ANP, intravenously-administered CNP has significant effects on blood pressure, renal functions, and aldosterone secretion in humans.

Acknowledgements
We thank Ms. H. Kito, Ms. M. Shida, Ms. K. Sasamoto, Ms. A. Takagoshi, Ms. T. Okumura and Ms. Y. Mori for their excellent secretarial work.

References


