Renal and vascular effects of C-type and atrial natriuretic peptides in humans

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1Service de Physiologie et d’Explorations Fonctionnelles, Hôpital Henri Mondor, 94000 Créteil; 2Service de Biochimie, Hôpital de la Pitié-Salpêtrière, 75013 Paris; and 3Institut Beaufour, 91952 Les Ulis, France

Pham, Isabelle, Said Sediame, Geneviève Maistre, Françoise Roudot-Thoraval, Pierre-Etienne Chabrier, Alain Carayon, and Serge Adnot. Renal and vascular effects of C-type and atrial natriuretic peptides in humans. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1457–R1464, 1997.—C-type natriuretic peptide (CNP) may affect renal and vascular functions differently from atrial natriuretic peptide (ANP). The objective of this study was to compare the renal and vascular actions of CNP to those of ANP in normal men. CNP or ANP (0.005, 0.01, and 0.05 µg·kg⁻¹·min⁻¹) were given by infusion to eight healthy volunteers. CNP caused dose-dependent increases in natriuresis (UNaᵥ) and in the fractional excretion of sodium (FE₈Naᵥ) with no effect on diuresis (UV), renal plasma flow, and glomerular filtration rate (GFR). Fraction of filtration (FF) increased only with the 0.05 µg·kg⁻¹·min⁻¹ CNP dose. ANP caused larger increases in UNaᵥ, FE₈Naᵥ, and FF than CNP and also increased UV at 0.01 and 0.05 µg·kg⁻¹·min⁻¹ and GFR at 0.05 µg·kg⁻¹·min⁻¹. Although the ANP and CNP infusions produced similar elevation in the respective peptides plasma levels, urinary and nephrogenous guanosine 3′,5′-cyclic monophosphate increased less in response to CNP than to ANP. Blood pressure, forearm blood flow, plasma renin activity, and aldosterone remained unaffected during the peptides infusion. Plasma ANP increased slightly during CNP infusion. Our data indicate a higher threshold of renal response to CNP than to ANP. In contrast to ANP, CNP probably may not act as an endocrine factor in humans.

renal function; vascular tone

C-TYPE NATRIURETIC PEPTIDE (CNP) belongs to the natriuretic peptide family, which also includes the atrial natriuretic (ANP) and the brain natriuretic peptide (BNP). Three different receptors for natriuretic peptides have been identified. ANP-A and ANP-B receptors (ANPR-A and -B) are coupled with the particulate guanylate cyclase, whereas the ANP-C receptor (ANPR-C) is not. Although ANPR-C plays a central role in ANP clearance, it may be linked to a second messenger signaling system and have other actions (19, 20). ANPR-A preferentially binds to ANP or BNP, whereas the selective ligand of ANPR-B appears to be CNP. CNP is produced by the central nervous system (29) and in smaller amounts by the kidneys (12, 22, 24, 32) and the vascular endothelial cells (28, 31). Production of both CNP and its receptor ANPR-B has been also demonstrated in the kidney (6), leading several investigators to speculate that CNP may act as an autocrine or paracrine factor in this organ (6, 12, 22, 24, 32).

Recent in vitro and in vivo studies have demonstrated biological effects of CNP that differ from those of ANP or BNP. CNP has been found to be a potent vasodilator but a weak natriuretic factor in various species of laboratory animals (9, 27). When infused in anesthetized dogs, CNP was more potent than ANP in causing hypotension but did not exhibit any natriuretic effect (9). In contrast, studies of isolated arterial rings from normal and spontaneously hypertensive rats found less relaxation with CNP than with ANP (33, 34). In normal volunteers receiving CNP as a continuous infusion, natriuresis either increased slightly or remained unchanged, and blood pressure did not vary (7, 16). Moreover, direct infusion of CNP into the brachial artery of normal subjects or patients with heart failure caused a smaller increase in forearm blood flow (FBF) than ANP (23).

Hence, the vascular and renal effects of CNP remain to be assessed in normal man, especially in reference to those of ANP (1). In the present study, we examined the effects of incremental rates of CNP infusion on renal excretory function, renal hemodynamics, FBF, and blood volume regulatory hormones in comparison with ANP at similar infusion rates in eight normal healthy subjects.

SUBJECTS AND METHODS

Subjects. Eight male students with a mean age ± SD of 23.5 ± 1.5 yr (range: 21–26 yr), a mean weight of 70.6 ± 5.5 kg (range: 60–76 kg), and a mean body surface area of 1.86 ± 0.1 m² (range: 1.67–1.96 m²) gave their informed consent to the study, which was approved by the Ethics and Research Committee of the Henri Mondor Hospital. None of the subjects had clinical evidence of cardiac and renal diseases and none were on medication. Each subject took 2 g of salt orally per day as a supplement to a normal sodium diet 8 days before each test and on the test day.

Study protocols. The subjects were studied on three occasions, at intervals of 8 days. ANP, CNP, and saline were each tested on one of the 3 days, in random order. All studies were conducted in the morning, 2 h after a light breakfast. The subjects were weighed and then remained supine except during the urine collections. Catheters were inserted into a superficial vein of both forearms for infusions and blood sampling, respectively. A 15 ml/kg oral water load was given in 15 min, before the infusion was begun, and the subjects were asked to void their bladder. An equilibration period of 120 min was followed by a baseline phase composed of two 30-min urine collection periods; an infusion phase during which the subjects received either saline, ANP, or CNP; and a 30-min recovery phase. For the ANP and CNP infusions, sterile and pyrogenic preparations of crystallized synthetic human a-ANP fragment 1–28 or CNP-22 (Novabiochem, Lauffelfingen, Switzerland) were diluted in 50 ml normal saline to a 10 ng/ml concentration. ANP and CNP were...
infused at incremental doses of 0.005 µg·kg⁻¹·min⁻¹ during two periods of 30 min, 0.01 µg·kg⁻¹·min⁻¹ during 30 min, and 0.05 µg·kg⁻¹·min⁻¹ during 30 min.

Renal measurements. Glomerular filtration rate (GFR) and renal plasma flow (RPF), both corrected for body surface area, were assessed by inulin (C_in) and p-aminomethylurireate (PAH) steady-state clearances (C_PAH), respectively. Inulin (In) polyfructosan; Boehringer-Mannheim, Mannheim, Germany) and PAH (Laboratoires SERB, Paris, France) were given as a priming bolus of 40 and 10 mg/kg, respectively, and followed by an intravenous infusion designed to produce a stable plasma level after 120 min. At every 30-min interval throughout the study, venous blood and urine samples for sodium output were collected and the subjects were asked to drink 150 ml of water. Inulin and PAH concentrations were assessed using standard spectrophotometric methods. Values were calculated according to the following standard formulas: C_in (in ml·min⁻¹·1.73 m⁻²) = UV·[In]P/[(In]P×C_in), where UV is urine volume, brackets indicate concentration, and U and P indicate urine and plasma, respectively; C_PAH (in ml·min⁻¹·1.73 m⁻²) = UV·[PAH]P/[PAH]P; filtration fraction (in %) = C_in/C_PAH; sodium excretion (U NaV, in mmol/min) = [Na]UV; sodium fractional excretion (in %) = (∆NaV)/[Na]P; renal blood flow (RBF; in ml·min⁻¹·1.73 m⁻²) = RPF/((1 – hematocrit); and renal vascular resistance (in IU) = mean arterial pressure (MAP)/RBF.

Circulatory measurements. Heart rate and MAP were measured every 15 min using a Finapress automatic device. Determinations of FBF were performed by venous occlusion plethysmography during the baseline phase, the peptide infusions at each infusion rate, and the recovery period. The mercury-in-silicone rubber strain gauge was placed at each infusion rate, and the recovery period. The mercury-in-silicone rubber strain gauge was placed below the left elbow. The arm was elevated so that the proximal part of the forearm was ~10 cm above the anterior chest wall. FBF was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff on the arm at 40 mmHg. Blood flow to the hand was arrested by inflating a cuff around the wrist to suprasystolic pressures during determinations of FBF. FBF was expressed in milliliters per minute per 100 milliliters of forearm volume. Forearm vascular resistances were calculated by dividing the MAP by FBF.

Plasma hormone measurements. Blood samples were collected in polypropylene chilled tubes containing 10 mg EDTA, 5 mg trypsin inhibitor, 17.4 mg phenylmethylsulfonyl fluoride, and 0.1 mg aprotinin. All tubes were centrifuged at 3,500 g for 20 min and at 4°C. Plasma was stored at −80°C for subsequent analysis.

ANP and CNP radioimmunoassays were performed after extraction of 1–2 ml of plasma with Vycor glass (Corning Glassware). The antisera used for ANP (RAS 8798, rabbit anti-α-atrial natriuretic polypeptide; Peninsula Laboratories) exhibited a high cross-reactivity with human α-ANP (100%) and rat ANP (100%). The assay half-maximal inhibitory concentration (IC50) was ~25 pg. For CNP, the antibody (RAS 9030, rabbit anti-CNP-22 serum; Peninsula Laboratories) had 100% cross-reactivity with human, porcine, and rat CNP-22 and CNP-53 and exhibited no cross-reactivity with rat α-ANP and human α-ANP and BNP-32. Tyr-CNP-22 was radioiodinated with 125I at 2,000 Ci/mmol using the lactoperoxidase/hydrogen peroxide method. After incubation of the samples with the antisera and the 125I-CNP, the bound and the free fractions were separated with charcoal and the pellets were counted. The limit of detection was 2.5 pg/tube and the IC50 was 14.5 pg.

Plasma renin activity (PRA) was determined indirectly via the generation of angiotensin I (angiotensin I radioimmunoassay kit SB-REN-2; ORIS, Gif-sur-Yvette, France) and expressed as nanograms per milliliter per hour.

Plasma aldosterone was determined by use of a radioimmunoassay kit (SB-ALDO-2, ORIS) and expressed as picograms per milliliter.

Plasma and urinary guanosine 3’5’-cyclic monophosphate (cGMP) were measured with a commercial radioimmunoassay kit (TGR 255) radioimmunoassay kit, DuPont NEX-133. Net renal generation of cGMP, i.e., nephrogenous cGMP, was calculated as the difference cGMP, GFR = U_GMPV (pmol/min).

Statistical analysis. All data are expressed as means ± SD. Analysis of variance (ANOVA) for repeated measurements was used to study interactions between peptide and time. If a significant interaction was found, factorial ANOVA was performed to compare the effect of the two peptides at each time point. To compare the effects of infusion rates on the plasma peptides concentrations, an ANOVA model for repeated measurements and covariates (infusion rates) changing over trials (BMDP statistical software, 2 V program) was performed. P values <0.05 were considered significant.

RESULTS

No adverse effects occurred during any of the study. During the control study (administration of saline), diuresis decreased (F = 4.2; P < 0.01; Fig. 1), whereas urinary sodium concentration (F = 7.1; P < 0.001; Fig. 2), natriuresis (F = 7; P < 0.001; Fig. 2), and fractional excretion of sodium (F = 8.7; P < 0.001; Fig. 3) increased significantly with time. GFR, RPF, and filtration fraction remained unchanged (Table 1).

Infusion of ANP was associated with increases in diuresis (Fig. 1), natriuresis (Fig. 2), and fractional excretion of sodium (Fig. 3). Urinary sodium concentration did not increase with ANP compared with control (Fig. 2). The increases in diuresis, natriuresis, and fractional excretion of sodium were dose dependent
significant increases occurred for diuresis and natriuresis with the 0.01 and 0.05 µg·kg⁻¹·min⁻¹ infusion rates and for fractional excretion of sodium with the 0.005, 0.01, and 0.05 µg·kg⁻¹·min⁻¹ infusion rates. Kaliuresis remained unchanged during ANP infusion (not shown). GFR increased in response to the highest dose of ANP (0.05 µg·kg⁻¹·min⁻¹; F = 8.8; P < 0.001) and CNP (F = 8.2; P < 0.001). *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline infusion (control).

Infusion of CNP also caused increases in natriuresis (Fig. 2) and fractional excretion of sodium (Fig. 3), with no change in diuresis (Fig. 1) or kaliuresis (not shown). As natriuresis increased with no change in diuresis, urinary sodium concentration increased by 38 ± 5.1 mmol/ml in response to the highest CNP infusion rate, whereas only a 16 ± 2.6 mmol/ml increase was measured during saline infusion (P < 0.001). GFR, RPF, and renal vascular resistance remained unchanged during CNP infusion (Table 1). However, the filtration fraction increased in response to the highest CNP infusion rate (F = 2.8; P < 0.05) (Table 1). The effects of CNP on natriuresis (F = 3.1; P < 0.01), fractional excretion of sodium (F = 3.8; P < 0.01), and filtration fraction (F = 2.2; P < 0.05) were significantly smaller than those induced by ANP (Figs. 2 and 3 and Table 1).

Blood pressure, heart rate, FBF, and forearm vascular resistance were unaffected by ANP or CNP infusions (Table 2).

Plasma ANP (Fig. 4), PRA, and aldosterone (Table 3) remained unchanged during the control study (Fig. 5). Plasma CNP remained at the limit of detection (<0.5 pg/ml). Nor were there any changes in plasma and urinary cGMP concentrations or in nephrogenous cGMP (Fig. 6). During ANP infusion, plasma ANP increased 2- to 10-fold depending on the ANP infusion rate (Fig. 4), whereas plasma CNP remained at the limit of detection (Fig. 5). ANP infusion was associated with concomitant dose-dependent increases in levels of plasma, urinary, and nephrogenous cGMP (Fig. 6). Infusion of ANP also had no effect on PRA and plasma aldosterone, although there was a trend toward a decrease in PRA (Table 3). During CNP infusion, plasma CNP increased in a dose-dependent fashion (Fig. 5). To compare changes in plasma ANP and CNP levels during the peptide infusions, we compared the magnitudes of ANP and CNP level increases at increasing infusion rates, taking into account the difference in the molar infusion rate between ANP and CNP due to the different molecular weights of these two peptides. The plasma level increases produced by the peptide infusions were not significantly different between ANP and CNP, as shown in Fig. 7. However, due to the lower molecular weight of

![Fig. 2. Effects of incremental infusion rates of ANP and CNP on urinary sodium concentration ([Na]U; A) and natriuresis (U NaV; B). Interactions were not significant for ANP (F = 2.2; NS) and CNP (F = 1.5; NS) on urinary sodium concentrations (see RESULTS). Natriuresis increased during infusions of ANP (F = 8.8; P < 0.001) and CNP (F = 8.2; P < 0.001). *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline infusion (control).](image)

![Fig. 3. Effects of incremental infusion rates of ANP and CNP on fractional excretion of sodium (FENa). Two-way ANOVA for repeated measurements showed significant effects of ANP (F = 7.7; P < 0.001) and CNP (F = 8.1; P < 0.001). *P < 0.05, **P < 0.01 vs. saline infusion (control).](image)
CNP compared with ANP, at a given infusion rate the molar concentration of CNP was higher during CNP infusion than the molar concentration of ANP during ANP infusion. Infusion of CNP induced a 1.5-fold increase in urinary cGMP with no change in plasma nucleotide concentration. Nephrogenous cGMP increased significantly in response to the highest CNP nucleotide concentration. Nephrogenous cGMP in- 
crease in urinary cGMP with no change in plasma 

excretion of sodium, urinary cGMP, and nephrogenous cGMP with no changes in blood pressure and FBF. ANP infusion induced similar changes with, however, a greater effect on renal function. Although the increases in circulating ANP and CNP levels produced by the peptide infusions were of similar magnitude, the thresholds of renal response for CNP was higher than for ANP. Differences in the renal effects of exogenous ANP infusion in humans according to the dose have been reported. Whereas high doses of ANP induced natriuresis and altered renal hemodynamics, lower doses selectively increased natriuresis but had no effect on renal hemodynamic parameters (2, 3, 13, 15, 25). In our study, infusion of ANP at the rate of 0.01 µg·kg⁻¹·min⁻¹, providing a three- to sixfold increase in plasma ANP concentration, resulted in increases in diuresis, natriuresis, and sodium fractional excretion with no change in GFR or RPF. Infusion of ANP at the highest rate of 0.05 µg·kg⁻¹·min⁻¹, providing a 10-fold increase in 

DISCUSSION

We found that infusion of CNP in normal subjects was associated with increases in natriuresis, fractional 

Table 2. Effects of ANP and CNP infusions on MAP, HR, FBF, and FVR

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>0.005</th>
<th>0.05</th>
<th>Recovery</th>
</tr>
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<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>81.4 ± 5.2</td>
<td>80.1 ± 6.8</td>
<td>78.2 ± 7.6</td>
<td>80.6 ± 6.9</td>
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<tr>
<td>ANP</td>
<td>81.6 ± 6.5</td>
<td>78.9 ± 6.3</td>
<td>77.6 ± 4.3</td>
<td>79.3 ± 5.2</td>
</tr>
<tr>
<td>CNP</td>
<td>79.6 ± 6.1</td>
<td>79.1 ± 3.9</td>
<td>78.6 ± 4.5</td>
<td>79.9 ± 3.8</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>56.7 ± 9.7</td>
<td>57.7 ± 7.1</td>
<td>57 ± 7.1</td>
<td>57 ± 7.1</td>
</tr>
<tr>
<td>ANP</td>
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<td>58.8 ± 8.8</td>
<td>56.1 ± 7.8</td>
</tr>
<tr>
<td>CNP</td>
<td>55.4 ± 7.8</td>
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<td>54.8 ± 5.5</td>
<td>55.9 ± 7.3</td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·100 ml⁻¹</td>
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<tr>
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<td>2.1 ± 1.1</td>
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</tr>
<tr>
<td>ANP</td>
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</tr>
<tr>
<td>CNP</td>
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<td>1.6 ± 0.7</td>
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<tr>
<td>FVR, IU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>55.1 ± 19.5</td>
<td>49.8 ± 19</td>
<td>47.5 ± 20</td>
<td>51.1 ± 20.5</td>
</tr>
<tr>
<td>ANP</td>
<td>67.7 ± 41</td>
<td>54.7 ± 25.1</td>
<td>56.2 ± 32.1</td>
<td>56 ± 22.2</td>
</tr>
<tr>
<td>CNP</td>
<td>49.9 ± 20.3</td>
<td>52.1 ± 19</td>
<td>55.4 ± 25.8</td>
<td>54.6 ± 18.7</td>
</tr>
</tbody>
</table>

Data are means ± SD. Infusion rates of ANP and CNP are expressed in µg·kg⁻¹·min⁻¹. For each period, the value is the mean of 2 (0.005, 0.01, 0.05, and recovery) or 4 (baseline) recordings or calculations. ANP and CNP had no significant effect on mean arterial pressure (MAP) (ANP: F = 0.2, NS; CNP: F = 0.3, NS), heart rate (HR) (ANP: F = 1.2, NS; CNP: F = 0.1, NS), forearm blood flow (FBF) (ANP: F = 1.6, NS; CNP: F = 0.7, NS), or forearm vascular resistance (FVR) (ANP: F = 1.1, NS; CNP: F = 0.6, NS).
plasma ANP concentration, markedly increased diuresis and natriuresis and additionally increased GFR. RPF remained unchanged and filtration fraction increased in response to 0.05 µg·kg⁻¹·min⁻¹ ANP. Infusion of CNP similarly increased natriuresis without altering diuresis and renal hemodynamics. At the highest infusion rate of 0.05 µg·kg⁻¹·min⁻¹, CNP also caused an increase in filtration fraction due to a slight, nonsignificant increase in GFR. However, the natriuretic response to CNP was only one-half as large as that to ANP at a similar infusion rate and was not associated with any change in diuresis. These results are consistent with previous studies showing weaker renal effects of CNP compared with ANP. In laboratory animals, a weak renal effect (8) or an antinatriuretic action of the peptide (16). In another study, Igaki et al. (16) failed to detect any effect of CNP on diuresis, hemodynamics (9, 27). In normal humans given CNP at infusion rates similar to those used in our study, Hunt et al. (17) observed that the natriuretic response to ~1 µg/kg CNP given by bolus injection in normal humans was less marked than that induced by similar doses of ANP. Taken in concert, these results suggest that CNP has a weaker natriuretic effect than ANP when administered intravenously in humans and that its natriuretic action occurs without important changes in diuresis. Consistent with this suggestion, urinary cGMP excretion increased to a greater extent in response to ANP than to CNP in our study. Infusion of ANP caused marked rises in both plasma cGMP concentration and nephrogenous cGMP, whereas CNP infusion did not increase plasma cGMP concentration and only produced a slight elevation in nephrogenous cGMP. Because CNP and ANP preferentially bind to ANPR-B and ANPR-A, respectively, this result may be interpreted as a predominance of ANPR-A over ANPR-B in the kidney. Support for this hypothesis can be found in a study in rats showing that binding to ANPR-B was not detectable in the rat kidney and that smaller amounts of cGMP were produced by isolated glomeruli in response to CNP than to ANP (5). However, the expression of ANPR-B has been demonstrated in the human kidney (6), and several studies have recently provided evidence for a

Table 3. Effects of ANP and CNP infusions on PRA and plasma aldosterone

<table>
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<tr>
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<th>Baseline 1</th>
<th>Baseline 2</th>
<th>30 min 0.005</th>
<th>60 min 0.005</th>
<th>0.01</th>
<th>0.05</th>
<th>Recovery</th>
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<td></td>
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<tr>
<td>Control</td>
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<td>1.3 ± 0.4</td>
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<tr>
<td>ANP</td>
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<td>1.4 ± 0.4</td>
<td>1.4 ± 0.6</td>
<td>1.1 ± 0.5</td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.5</td>
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<tr>
<td>CNP</td>
<td>1.1 ± 0.5</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>27.1 ± 20.7</td>
<td>28.8 ± 24.7</td>
<td>24.0 ± 20.9</td>
<td>22.8 ± 20</td>
<td>23.8 ± 26.7</td>
<td>40.1 ± 26.3</td>
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<td>ANP</td>
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<tr>
<td>CNP</td>
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<td>51.9 ± 71.9</td>
<td>32.7 ± 36.3</td>
<td>62.7 ± 48.6</td>
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Values are means ± SD. Infusion rates ANP and CNP are expressed in µg·kg⁻¹·min⁻¹. Effects of ANP and CNP were not significant on plasma renin activity (PRA) (ANP: F = 1, NS; CNP: F = 0.7, NS) and plasma aldosterone (ANP: F = 0.4, NS; CNP: F = 1.2, NS).
local production of CNP in the proximal tubule either in rat or human kidney (12, 22, 24, 32). These results open up the possibility that CNP may preferentially act as a paracrine factor. If this is indeed the case, the weak renal response to exogenous CNP infusion in humans may not reflect the physiological importance of the peptide. The fact that CNP was not detectable in the plasma during basal conditions is consistent with this hypothesis. An alternative hypothesis is that the changes in renal parameters measured during infusion of CNP reflected activation of ANPR-A rather than ANPR-B by CNP and that weaker affinity of CNP compared with ANP for ANPR-A explained the weak renal response to CNP. Such an hypothesis is unlikely, because in vitro potency of CNP for cGMP production by ANPR-A is 300- to 700-fold lower than that of ANP and because the increase in nephrogenous cGMP in the present study was only 10-fold lower with CNP than with ANP. Another possibility is that CNP infusion, which caused a slight increase in plasma ANP concentration (probably due to CNP displacing ANP from clearance receptors), may have induced subsequent ANP-mediated activation of ANPR-A. Several investigators have also reported a slight increase in plasma ANP during exogenous CNP infusion (8, 9, 16). However, the plasma ANP peak during CNP infusion in our study remained lower than that obtained with the lowest ANP infusion rate, which had no natriuretic effect (43 vs. 65 pg/ml) in the same subjects. Therefore, it is unlikely that this mechanism contributed to the renal effects of CNP infusion in our normal subjects.

In our study, neither ANP nor CNP produced any changes in arterial pressure, heart rate, and FBF. There have been many reports that ANP induced only small changes on blood pressure when administered in low doses in laboratory animals (2, 15, 25) and normal humans (3, 14, 26). In contrast, studies in dogs have found that CNP induced a marked decrease in blood pressure due to decreases in venous return and cardiac output (9, 27). Systemic hypotension was more marked in response to infusion of CNP than to infusion of ANP (9). This hypotensive action of CNP is consistent with the potent venodilator effect of CNP in vitro (33). In contrast, studies performed in humans using either

Fig. 6. Changes in plasma cGMP concentrations (A), urinary cGMP excretion (U_cGMP; B), and renal cGMP production (nephrogenous cGMP; C) in response to saline (control) and to incremental infusion rates of ANP or CNP. Two-way ANOVA for repeated measurements showed significant increases in plasma (F = 4.8; P < 0.01), urinary (F = 17.3; P < 0.001), and nephrogenous cGMP (F = 11; P < 0.001) in response to ANP. Infusion of CNP increased only urinary (F = 4.1; P < 0.001) and nephrogenous (F = 6; P < 0.05) cGMP. *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline infusion (control).

Fig. 7. Correlation between changes in peptides plasma levels in response to the infusions according to the rate of infusion. ANP: open squares and dashed line, CNP: filled circles and solid line.
CNP infusion rates similar to ours or injection of a 1 µg/kg bolus failed to demonstrate any effect of CNP on blood pressure (7, 16, 17). In our study, it is unlikely that CNP infusion caused a decrease in venous return, because blood pressure, FBF, and vascular resistance remained unchanged. Thus our results do not support an important role for circulating CNP in the modulation of vascular tone in normal men. However, a high level of ANPR-B expression has been demonstrated for smooth muscle cells in culture (30), and CNP has been shown to relax isolated vascular preparations (33). It is therefore possible that CNP produced by endothelial cells also acts preferentially as a paracrine factor in arteries or veins.

Rapid degradation of CNP by clearance receptors or neutral endopeptidase may explain the modest vascular actions of CNP and its weaker renal effects compared with ANP (19, 21). ANP and CNP share the same pathways of degradation by neutral endopeptidase and clearance receptors. In vitro, evidence has been reported that CNP may be a better substrate than ANP or BNP for neutral endopeptidase with a faster rate of hydrolysis and a lower $K_m$ (18). In contrast, the affinity of CNP for the clearance receptor is three- to fivefold lower than that of ANP (30). In vivo, the half-life of CNP was slightly lower than that of ANP, consistent with faster degradation (16). Brandt et al. (4) recently reported that catabolism of CNP via the clearance receptor was predominant in the lungs, whereas degradation via neutral endopeptidase was the main pathway in the kidney and the peripheral vasculature. In our study, it is difficult to compare the degradation of ANP and CNP on the basis of plasma concentrations and infusion rates. When expressed in molar concentrations, the infusion-induced changes in ANP and CNP concentrations were similar in magnitude, suggesting that under our experimental conditions the two peptides were degraded at similar rates. Further experiments with a neutral endopeptidase inhibitor would be required to assess the exact role of the enzymatic pathway in CNP degradation.

ANP has been shown to decrease PRA and plasma aldosterone in normal humans and in heart failure patients (10). CNP is also a potent inhibitor of aldosterone production by adrenal gland in vitro (11). However, the in vivo effects of CNP on the renin-angiotensin system remain controversial. In one study, CNP had no effect on PRA but increased plasma aldosterone in anesthetized dogs (27). Other studies found that CNP either inhibited (16) or failed to affect plasma aldosterone in normal humans (7). These discrepancies may be due to differences in experimental conditions, species, and hemodynamics effects of CNP. In our study, neither ANP nor CNP exhibited any effect on the renin-angiotensin-aldosterone system, probably because of the low level of activation of this system due to the recumbent position and the sodium supplementation.

Perspectives

In summary, we found that CNP infused at rates ranging from 0.005 to 0.05 µg·kg$^{-1}$·min$^{-1}$ induced a natriuretic effect, with increases in fractional excretion of sodium, urinary cGMP, and nephrogenous cGMP in normal men. However, these effects were only two- to threefold lower than those produced by equivalent doses of ANP. Because circulating CNP levels are low in normal humans, these results suggest that CNP may differ from ANP in that it may act as a paracrine factor rather than as an endocrine factor. Inhibition of the peptide degradation by neutral endopeptidase blockade might potentiate both the endocrine and the paracrine effects of the peptide. Future studies using neutral endopeptidase inhibitors may provide additional information on the specific vascular and renal effects of CNP.

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