Natriuretic Peptides in the Human Kidney
Kazuhito Totsume, Kazuhiro Takahashi, Osamu Murakami, Fumitoshi Satoh, Masahiko Sone, Takao Saito, Hironobu Sasano, Toraichi Mouri, Keishi Abe

Abstract We studied the presence of three natriuretic peptides—atrial natriuretic peptide (ANP), human brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP)—in the human kidney by radioimmunoassay and immunocytochemistry. Immunoreactive ANP, immunoreactive human BNP, and immunoreactive CNP concentrations in six kidneys were 0.12±0.07 (mean±SD), 0.23±0.08, and 0.37±0.07 pmol/g wet wt, respectively. Sephadex G-50 superfine column chromatography and reversed-phase high-performance liquid chromatography of kidney extracts revealed a broad peak of immunoreactive ANP comigrating with ANP-28 and urodilatin. Renal immunoreactive human BNP consisted of three components; the major component comigrated with human BNP-32. Renal immunoreactive CNP consisted of at least two components; the major component comigrated with CNP-22, and the minor component eluted in a position similar to that of authentic human CNP-53. Immunocytochemistry showed that immunoreactive human BNP was colocalized with immunoreactive ANP in the segments of distal tubules, whereas immunoreactive CNP was found predominantly in the proximal tubules. These findings indicate that these three natriuretic peptides are present in the human kidney and raise the possibility that they form a renal natriuretic peptide system that participates in the local regulation of sodium and water transport and renal circulation in the human kidney. (Hypertension. 1994;24:758-762.)

Key Words • natriuretic peptides • radioimmunoassay • immunocytochemistry • kidney • chromatography

Sinc atrial natriuretic peptide (ANP) was first described by de Bold et al., two other natriuretic peptides have been discovered: brain or B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). These peptides form a natriuretic peptide family. Both ANP and BNP have hypotensive, diuretic, and natriuretic activities. CNP has more potent hypotensive activity than ANP in dogs but less diuretic and natriuretic activities than ANP. Immunoreactive (IR-) ANP and BNP are found mainly in cardiac atrium, whereas CNP concentrations in cardiac tissue are very low or undetectable. High concentrations of IR-CNP are present in the human brain, adrenal glands, and adrenal tumors. CNP is also produced by vascular endothelial cells.

The kidney is one of the main target organs for natriuretic peptides. It has natriuretic peptide receptors predominantly in glomeruli and inner medullae. It has also been reported that the kidney itself produces ANP. However, there has been no report on the presence of BNP and CNP in the human kidney. In the present study, we investigated the presence of human BNP (hBNP) and CNP in the human kidney as well as ANP with specific radioimmunoassay (RIA) and immunocytochemistry. IR-ANP, IR-hBNP, and IR-CNP in the kidney were characterized by gel chromatography and reversed-phase high-performance liquid chromatography (HPLC).

Methods

Peptides
Synthetic hANP-28/α-hANP or hANP (99-126)/hANP (103-125), urodilatin [or hANP(95-126)], hBNP-32, hCNP-22, and hCNP-53 were purchased from Peptide Institute.

Tissues and Extraction Procedure
The study was approved by the ethics committee on human research of Tohoku University, and the procedures followed were in accordance with the guidelines of this committee. Kidney tissues (approximately 10×10×10 mm mass, mainly composed of the renal cortex) were obtained at autopsy from four male patients (40 to 70 years old, aortitis syndrome with renal cell carcinoma, acute myocardial infarction, lung cancer, and gastric cancer) and at nephrectomy from two patients (61 and 54 years old) with renal cell carcinoma (Table). A right cardiac atrium tissue was obtained at autopsy from a male patient (43 years old) with acute lymphocytic leukemia for preparation of γ-ANP and γ-hBNP as the calibration markers in gel chromatography. All tissues were stored at −80°C until extraction. For the immunocytochemical study, normal parts of the kidney were obtained at nephrectomy from four patients with renal cell carcinoma. The tissue was fixed in 95% (vol/vol) ethanol or 4% (vol/vol) formaldehyde solution, embedded in paraffin, cut into 4-μm-thick sections, and mounted on slides.

Tissues (0.5 to 1.0 g) were boiled for 10 minutes in 5 mL of 1 mol/L acetic acid and homogenized in 10 mL of 50% (vol/vol) methanol in 1 mol/L acetic acid. The homogenates were centrifuged at 24 000g for 60 minutes. The supernatant was separated and dried in an air stream. The resulting residues were dissolved in 1 mol/L acetic acid containing 0.5% (wt/vol) bovine serum albumin and were reextracted by Sep-Pak C18 cartridges (Waters Chromatography Division). The adsorbed peptides were eluted with 60% (vol/vol) ethanol or 4% (vol/vol) formaldehyde solution, embedded in paraffin, cut into 4-μm-thick sections, and mounted on slides.

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Concentrations of Natriuretic Peptides in Human Kidney

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>IR-ANP (fmol/tube)</th>
<th>IR-hBNP (fmol/tube)</th>
<th>IR-CNP (fmol/tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65/Male</td>
<td>Renal cell carcinoma with aortitis</td>
<td>0.032</td>
<td>0.14</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>40/Male</td>
<td>Acute myocardial infarction</td>
<td>0.080</td>
<td>0.17</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>70/Male</td>
<td>Lung cancer</td>
<td>0.235</td>
<td>0.33</td>
<td>0.38</td>
</tr>
<tr>
<td>4</td>
<td>69/Male</td>
<td>Gastric cancer</td>
<td>0.169</td>
<td>0.33</td>
<td>0.41</td>
</tr>
<tr>
<td>5*</td>
<td>61/Male</td>
<td>Renal cell carcinoma</td>
<td>0.084</td>
<td>0.16</td>
<td>0.35</td>
</tr>
<tr>
<td>6*</td>
<td>54/Female</td>
<td>Renal cell carcinoma</td>
<td>0.145</td>
<td>0.22</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Mean±SD 0.12±0.07 0.23±0.08 0.37±0.07

IR indicates immunoreactive; ANP, atrial natriuretic peptide; hBNP, human brain natriuretic peptide; and CNP, C-type natriuretic peptide. Tissue concentrations (picomoles per gram wet weight) of the three natriuretic peptides were measured by respective radiomunoassays after extraction by Sep-Pak C18 cartridges as described in "Methods."

*Normal parts of the kidney were obtained at surgery; Cases 1 through 4 were autopsy cases.

**Immunocytochemistry**

Light-microscope immunocytochemistry was carried out using a peroxidase-antiperoxidase immune complex method. Deparaffinized sections were incubated in 0.3% (vol/vol) hydrogen peroxide for 40 minutes, washed in 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4), and incubated in 5% (vol/vol) normal swine serum for 30 minutes to block nonspecific staining. Sections were washed extensively in 0.01 mol/L PBS (pH 7.4) between the procedures. Next, the sections were incubated with the primary antisera (diluted at 1:1000 to 1:1500 in 0.01 mol/L PBS containing 0.1% [wt/vol] bovine serum albumin, pH 7.4) for 20 hours at 4°C, followed by swine anti-rabbit IgG (1:40) (Sigma Chemical Co) for 30 minutes at room temperature and peroxidase-antiperoxidase (1:80) (Dakopatts) for 30 minutes. The primary antisera were the same as those used in the respective RIAs. The sections were incubated with 3,3’-diaminobenzidine tetrahydrochloride for 7 minutes. Finally, the slides were counterstained with methyl green, washed, dehydrated, and mounted in neutral mounting medium.

Three adjacent serial sections were stained as follows: the first section was stained with anti-ANP antisera (dilution, 1:400), the middle section with anti-hBNP antisera (1:200), and the third section with anti-CNP antisera (1:400). Non-immunized rabbit serum was used instead of antisera as a negative control.

**Statistical Analysis**

Data are expressed as mean±SD unless indicated otherwise. Statistical analysis was performed by one-way ANOVA followed by Wilcoxon’s test. A value of P<.05 was considered to be significant.

**Results**

Fig 1 shows standard curves of ANP, hBNP, and CNP and serial twofold dilution curves of the kidney extract. Serial
twofold dilution curves were parallel to the respective standard curves of ANP-28, hBNP-32, and CNP-22. Tissue IR-ANP, IR-hBNP, and IR-CNP concentrations in the six kidneys were 0.12±0.07, 0.23±0.08, and 0.37±0.07 pmol/g wet wt, respectively (Table). IR-CNP concentrations were significantly higher than those of IR-ANP (P<.01) and IR-hBNP (P<.05), but no significant difference was noted between IR-ANP and IR-hBNP concentrations (P>.05).

Chromatographic patterns of ANP, hBNP, and CNP in three cases examined were similar, and the results in one representative case (case 5) are shown in Figs 2 and 3. Sephadex G-50 superfine column chromatography of the normal part of the kidney showed a broad peak of IR-ANP comigrating with hANP and urodilatin, three major peaks of IR-hBNP, and two major peaks of IR-CNP (Fig 2). The first peak of IR-hBNP comigrated with γ-hBNP, the second peak eluted at the position between γ-hBNP and hBNP-32, and the largest peak eluted at a position similar to that of hBNP-32. A minor IR-CNP peak comigrated with authentic hCNP-53, and a major IR-CNP peak comigrated with authentic CNP-22.

Fig 3 shows reversed-phase HPLC of the kidney extract. A broad major peak of IR-ANP comigrated with ANP-28 and urodilatin, and additional broad minor peaks eluted later. A major peak of IR-hBNP comigrated with authentic hBNP-32, and an additional broad minor peak eluted later. A major IR-CNP peak comigrated with authentic CNP-22, and a minor peak comigrated with authentic hCNP-53. Both gel chromatography and HPLC showed that main molecular forms of renal IR-hBNP and IR-CNP were BNP-32 and CNP-22, respectively.

Similar findings in immunocytochemistry were obtained from four kidneys examined, and one representative case (case 5 in the Table) is shown in Fig 4. Immunocytochemical studies showed positive ANP and hBNP immunostaining in the distal tubular segments (Figs 4A and 4B), but no immunostaining was observed in glomeruli or the renal vasculature. The distribution of hBNP immunoreactive tubules was quite similar to that of ANP. Most tubules positively stained with anti-hBNP.
antiserum were also stained with anti-ANP antiserum. Positive CNP immunostaining was found predominantly in proximal tubules and weakly in distal tubules (Fig 4C). No immunostaining of CNP was observed in glomeruli or the renal vasculature. Nonimmunized rabbit serum instead of the antisera produced no immunostaining (Fig 4D).

**Discussion**

The present study has shown the presence of ANP, hBNP, and CNP in the human kidney. These natriuretic peptides were localized in the renal tubules, and hBNP was colocalized with ANP in the distal tubular segments. Chromatographic analysis clarified the composition of IR-ANP, IR-hBNP, and IR-CNP in the human kidney. Although a few reports describing the molecular form of IR-ANP in the rat kidney are available, no information is available on the molecular forms of the natriuretic peptides in the human kidney. To our knowledge, this is the first report on the presence of hBNP and CNP in the human kidney. The IR-hBNP concentrations of the kidney were comparable to those of the brain tissues although far less than those of the cardiac atrium. The IR-CNP concentrations were comparable to those of the human brain and adrenal tissues.

Gel chromatography and HPLC showed a broad peak comigrating with hANP and urodilatin. This IR-ANP peak may represent both α-hANP and urodilatin. Urodilatin, the N-terminal extended form of α-hANP, was originally isolated from human urine, and its production in the kidney was expected. The composition of IR-ANP in the human kidney is remarkably in contrast to that in the rat kidney reported by Sakamoto et al and Suzuki et al; rat renal IR-ANP consisted of a monocomponent in a low molecular weight form comigrating with α-ratANP in high-performance gel-permeation chromatography and HPLC. This difference may reflect the difference of the characteristics of the ANP antisera or the species specificity in the processing of pro-ANP in the kidney.

Chromatographic studies showed that the main molecular form of IR-hBNP in the human kidney was hBNP-32 and that of IR-CNP was CNP-22. Gel chromatography showed two additional peaks of IR-hBNP eluting earlier than hBNP. The one eluting soon after the void volume may represent γ-hBNP. Such higher molecular forms of IR-hBNP have also been observed in human brain. The material eluting in the position of CNP-53 was observed in the gel chromatography and HPLC, but its amount was much smaller than that of CNP-22. The composition of renal IR-CNP is different from the composition of IR-CNP in the central nervous system and the adrenal tumors in which human CNP-53-like component is predominant over CNP-22. In addition, the composition of renal IR-CNP of humans contrasts with that of rats reported by Suzuki et al; the major molecular form of rat renal CNP was CNP-53.

The presence of ANP-like substances in the kidney has been reported previously by other investigators, and the presence of mRNA for ANP has been shown. Furthermore, the presence of BNP and CNP mRNAs in the kidney has recently been reported. Immunocytochemistry showed that IR-hBNP was colocalized with ANP in the distal tubular segments and
that unlike ANP and hBNP, IR-CNP was present predominantly in proximal tubules. But weak immunostaining of CNP was also observed in the distal tubular segments. Therefore, we could not deny the possibility that not only ANP and hBNP but also CNP were colocalized in some distal tubules.

The intravenous infusion of CNP in dogs caused a greater blood pressure decrease than that of ANP, but its natriuretic and diuretic effects were not observed. Therefore, CNP in the kidney may modulate the renal circulation as a vasodilator rather than as a natriuretic factor.

In conclusion, human kidney contains all members of the natriuretic peptide family. These findings raise the possibility that ANP, hBNP, and CNP are locally produced by the kidney and form a renal natriuretic peptide system that participates in the regional regulation of water and sodium transport and renal circulation in the human kidney.

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References
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