Brain (BNP) and atrial natriuretic peptides (ANP) have been identified which may represent endogenous agonists of kidney receptor subtypes. Quantitative in vitro autoradiography was used to investigate the regional distribution of receptor subpopulations and the competitive inhibition of \(^{125}\)I porcine BNP\(_{1-26}\), \(^{125}\)I rat \(\alpha\)-ANP\(_{1-28}\), and \(^{125}\)I des\([Gln^{*}, Ser^{*}]\) porcine BNP\(_{1-26}\) renal binding sites. Specific, high affinity binding \((K_d = 0.2-1.37 \text{ nM range})\) was localized to glomeruli, inner medulla, interlobar and arcuate arteries, vasa recta bundles, and smooth muscle in the renal pelvis. \(^{125}\)I pBNP\(_{1-26}\) competed for the same sites as \(^{125}\)I ANP\(_{1-28}\), but displayed a lower potency and was less selective for nonclearance sites. Clearance binding sites were discriminated by competitive inhibition with \(^{125}\)I C-ANP\(_{4-23}\) and comprised some 65% of glomerular sites as well as the vast majority of sites in the renal pelvis. Nonclearance sites predominated in the inner medulla and intrarenal arteries. C-terminal changes in amino acid sequence induced a significant loss of inhibitory potency. Immunohistochemical studies identified a distinct population of BNP-like immunoreactive renal nerve fibers, associated with intra-renal arteries. Circulating natriuretic peptides and BNP sequences derived from renal nerves may influence renal function by interacting with specific receptor subpopulations in the kidney.

Cardiac myocytes synthesize and secrete the hormone atrial natriuretic peptide (proANP\(_{99-126}\) or \(\alpha\)-ANP\(_{1-28}\)) which has natriuretic, diuretic, and hypotensive properties and is considered to have a role in the regulation of body fluid and electrolyte balance. The renal actions of the peptide are mediated, at least in part, by an interaction with specific receptors. The receptor subtypes mediate glomerular filtration and sodium reabsorption. Molecular cloning and affinity cross-linking studies have identified at least three natriuretic peptide receptor subtypes, the first of which (A-ANP) is a monomeric, 120-140 kDa molecular mass protein that incorporates a guanylate cyclase domain and is sensitive to C-terminal modification of the \(\alpha\)-ANP\(_{1-28}\) sequence for binding and activity. It is distinct from another guanylate cyclase-linked, 115-kDa molecular mass, \((\beta\)-ANP\) receptor which responds preferentially to porcine brain natriuretic peptide (pBNP\(_{1-20}\)) when expressed in COS-7 cells. The third subtype (C-ANP) is a dimeric protein composed of two disulfide-linked subunits, with a molecular mass of 60-70 kDa. This receptor is not coupled to guanylate cyclase and displays an indiscriminate affinity for a variety of analogues, including ANP\(_{5-25}\), D-amino acid substituted or ring-deleted sequences such as des\([Gln^{*}, Ser^{*}]\) porcine BNP\(_{1-26}\) (C-ANP\(_{4-23}\)) (8-13). The C-ANP\(_{4-23}\) analogue is ineffective in eliciting diuretic and natriuretic responses in the isolated, perfused kidney (10) and unlike ANP\(_{5-25}\), is considered to be completely selective in discriminating rat C-ANP receptors (13). In contrast to guanylate cyclase-linked receptors, renal C-ANP receptors may be influenced by body fluid volume (14, 15) and are thought to have a clearance function (10), modulating the plasma concentration of ANP\(_{1-28}\) (12). The possibility of ANP receptors being coupled to other secondary messenger systems cannot be excluded, however, as the interaction of C-ANP\(_{4-23}\) with ANP receptors has been found to inhibit adenylate cyclase activity in rat brain, aorta, and adrenal cortical membranes (16). Apart from ANP\(_{1-28}\), several other natriuretic peptide sequences have now been identified, which may represent additional endogenous agonists of receptor subtypes. The distinct brain natriuretic peptide (BNP) gene is expressed in both the heart and brain and encodes a peptide sequence with significant homology to ANP\(_{1-28}\). Unlike ANP, however, the posttranslational processing of BNP seems to be species dependent, with a number of different molecular forms having been identified in rat, pig, and human tissues, including BNP\(_{1-45}\) and the N terminally extended sequences BNP\(_{1-52}\) and BNP\(_{1-46}\) (17-20). In addition to its sequence homology with ANP\(_{1-28}\), pBNP\(_{1-26}\) possesses natriuretic and diuretic properties, stimulates guanylate cyclase activity, and displays a similar distribution of renal binding sites, suggesting that the two peptides may act via common receptors (19, 21-24). As well as being a circulating natriuretic peptide (25), the presence of BNP-like immunoreactivity in rat autonomic andafferent neurons (26) raises the possibility of BNP-containing nerves supplying peripheral tissues and suggests that BNP may also function as a local neuromodulator. Natriuretic peptides occur in tissues other than the heart and brain, and in the kidney for example, ANP-like immunoreactivity has been localized to cells in renal tubules and collecting ducts and identified in urine (27-29). The distinct 32 amino acid peptide, urodilatin (proANP\(_{99-126}\)) appears to be the main form of ANP immunoreactivity present in urine and is an N terminally extended form of ANP\(_{1-28}\), which also displays natriuretic, diuretic, and vasodilatory actions and has a high affinity for guanylate cyclase-linked A-ANP receptors (30). A
further natriuretic peptide, designated C-type natriuretic peptide or CNP, has recently been identified and although structurally distinct from both ANP and BNP, it also exerts similar pharmacological effects when injected into anesthetized rats (31).

α-ANP, as is therefore one of several homologous peptides that belong to a family of endogenous natriuretic peptides derived either by distinct gene expression or alternative post-translational processing, and as with other peptide families there appears to be a multiplicity of receptors as well as peptides. Few studies have examined both the localization and binding characteristics of receptor subpopulations for ANP and ANP ligands in the kidney as a whole, and the relative affinity of these binding sites for peptide isomers is uncertain. In the present study, we used the technique of quantitative in vitro autoradiography to investigate the distribution of subpopulations of 125I- BPNI, 125I-rANP, binding sites in four regions of the rat kidney and examined the relative potency of endogenous peptides to compete with these ligands. The ring-deleted analogue C-ANP-23 was used to selectively identify the clearance (C-ANP) receptor subpopulation, and the possible presence of ANP-like immunoreactivity in renal nerves was investigated using immunohistochemical techniques and a specific antiserum raised to BPNI-26.

EXPERIMENTAL PROCEDURES

In Vitro Autoradiography—Kidneys were rapidly removed from exsanguinated male Wistar rats (200–300 g; n = 8), surrounded in moistening medium (Tissue Tek, Miles Inc.) and immediately frozen in melting dichlorodifluoromethane (Arcton-12, ICI) suspended in liquid nitrogen. Cryostat sections (10 μm thick) were cut at -25 °C and thaw-mounted on gelatin-chrom-alum-coated glass slides. After drying overnight at 4 °C, the sections were preincubated at room temperature for 10 min in 50 mM phosphate buffer, pH 7.2, containing 120 mM NaCl and 40 mM/liter bacitracin, and then incubated in fresh buffer containing 0.5% bovine serum albumin (Fraction V) and 250 pM [3H]-iodo-tyrosyl porcine BPNI (100 pM BPNI, or [3H]-iodo-tyrosyl rat α-ANP (100 pM iso-ANP, or α-ANP) (specific activity ≥2,000 Ci/mmol, 74 TBq/mmol) for 15 min at room temperature. The slides were then quickly rinsed in phosphate buffer and distilled water at 4 °C and dried under a stream of cold air. These incubation conditions were previously found to give the highest specific/nonspecific binding ratio for 125I-rANP, binding, with equilibrium being achieved within 15 min of applying the ligand to sections of rat kidney. The integrity of the ligand following application to tissue sections was assessed by reapplying the incubation medium and ligand to fresh sections, under identical conditions, and using quantitative image analysis to compare the subsequent autoradiographic images.

Saturation binding studies were carried out by incubating consecutive sections with increasing concentrations of either 125I-BPNI, 125I-rANP, or 125I-rANP, respectively. Competitive inhibition of specific 125I-BPNI or 125I-rANP binding was examined in the presence of increasing concentrations (10–12–10/ M) of unlabeled α-ANP, BPNI, or rANP, respectively. The specificity of binding was further investigated by incubating sections together with an excess of (1 μM) unrelated peptide (vasoactive intestinal polypeptide, calcitonin gene-related peptide, gastrin, and angiotensin II).

Macroautoradiographic images were produced by exposing the dry labeled sections to Hyperfilm-H (Amersham International, United Kingdom) together with sections of 125I standards (American Radiolabeled Chemicals Inc., St. Louis), previous studies having demonstrated that 125I-labeled standards can be used for the calibration of [125I]-labeled ligands in quantitative in vitro autoradiography (32, 33). After exposure for 2–4 days at 4 °C the autoradiographic film was developed in filtered Kodak D-19 developer for 5 min at 20 °C and fixed in Amfix for 5 min at 20 °C.

Macrautoradiographs—A higher degree of anatomical resolution of binding sites was obtained in macroautoradiographic images, produced by applying liquid emulsion directly to labeled sections. Following exposure to Hyperfilm-H, the sections were fixed in Bouin's solution for 1 h, washed in phosphate buffer saline, rinsed in distilled water, dried under a stream of cold air, and dipped in liquid emulsion (Ilford K5) at 43 °C. After drying, the slides were stored in the dark in the 4 °C for 10–12 days. Development in Kodak D-19 developer for 2.5 min at 20 °C, fixed, stained with hematoxylin and eosin, and mounted in synthetic medium (DPX). Microautoradiographic images were analyzed using a transmitted light microscope.

Image Analysis and Quantification—Quantification of the film autoradiographic images was carried out by means of computer analysis (in an ISBPIX2000 Digitizing System, Kontron, Federal Republic of Germany). The density of binding (mmol) was determined for glomeruli, inner medulla, intra-renal (interlobar and arcuate) arteries, and renal pelvis. Measurements were made of 50–70 glomeruli, at least four intra-renal arteries, 15–20 areas of inner medulla, and three to four separate regions of renal pelvis in each labeled kidney section. Specific binding was determined by subtracting the nonspecific binding, obtained by incubating sections with an excess (1 μM) of the unlabeled peptide, from the total binding obtained by incubating sections with 250 pM 125I-BPNI, 125I-rANP, alone. Nonspecific binding was linearly related to the concentration of labeled peptide. Saturation and competitive binding data were analyzed by nonlinear curve-fitting using the programs GraphPAD INPLOT version 3.0 (GraphPAD Software, San Diego) and LIGAND (Elsevier-BIOSOFT, Cambridge, United Kingdom). A four parameter logistic function was used to describe the relationship between the percentage of 125I-BPNI, or 125I-rANP, bound and the concentration of unlabeled peptide in each of the competitive inhibition experiments. The inhibitory concentration which displayed 50% of specific binding (IC50) was calculated for each competing peptide and expressed as a geometric mean with 95% confidence limits, being derived from logarhythmically transformed data (34). Estimates of the apparent equilibrium dissociation constant (Kd) and in vitro potency (BR) were assessed using a Scatchard analysis of saturation binding data for 125I-BPNI, 125I-rANP, binding to glomeruli, inner medulla, intra-renal arteries, and renal pelvis in each animal.

Immunohistochemistry—An indirect immunofluorescence technique was employed to investigate the presence of natriuretic peptide immunoreactivity in the rat kidney, using antisera raised to BPNI (26) and rANP (35). Further antisera to synaptophysin, neuropeptide Y and calcitonin gene-related peptide (36) were also used to distinguish the renal innervation in general and identify peptide-containing nerve subpopulations. Kidney tissue was fixed by immersion in a modified Bouin's solution and cryostat sections immunostained as previously described (36). Briefly, sections were incubated in diluted (1/200–1/800) primary antisera overnight at 4 °C, rinsed in phosphate-buffered saline and then incubated in fluorescein isothiocyanate-labeled goat anti-rabbit IgG (1/100) for 1 h at room temperature. Controls included the omission of the primary antisera, displacement with preimmune serum, and application of preabsorbed antisera preabsorbed with synthetic peptide (1 nM to 10 μM). Antiser specificity for natriuretic peptides was also assessed using an enzyme-linked immunoasay system. Immunostained preparations were examined using an Olympus AH2 microscope equipped with epifluorescence illumination.

Binding data was then analyzed by analysis of variance using the GLIM program and differences were considered to be significant if the p value was less than 0.05. For saturation data, Kd and Bmax values were considered as two separate dependent variables.
whereas the four kidney regions and two labeled ligands were treated as within subject factors. In the case of competitive inhibition data, IC50 values for glomeruli, inner medulla, and intra-renal artery were used as three separate dependent variables and the competing peptides and labeled ligands as within subject factors. The residuals from the analysis of variance were examined for normal distribution using the Shapiro Wilk's test (37) and for equal variances in the different groups using the Schweder test (38). Because so many significance tests were performed on each set of data, a Bonferroni correction (39) was used to reduce the probability of differences being considered significant due to chance.

RESULTS

Localization of Renal Binding Sites—Specific 125I-pBNP1-28 and 125I-rANP1-28-binding sites were identified in macroautoradiograms and localized to glomeruli throughout the cortex, inner medulla, intra-renal arteries, bands in the inner stripe of the outer medulla corresponding to vasa recta bundles and to the wall of the renal pelvis (Fig. 2). Autoradiographic images of sections through the hilar region of the kidney also provided evidence of binding to the muscle coat in the ureter and to brown adipose tissue. Binding sites were further resolved in emulsion-dipped autoradiograms in which silver grains were observed overlying the capillary network of glomeruli, the media of interlobar, arcuate, and cortical radial arteries, and non-vascular smooth muscle in the renal pelvis (Fig. 3). The cellular localization of binding sites in the inner medulla could not be established, however, as silver grains were distributed diffusely throughout this region. The localization of binding sites was similar for both ligands, but displayed regional differences in the relative density and competitive inhibition of binding by unlabeled natriuretic peptide sequences. Coincubation of either ligand with an excess (1 μM) of unlabeled pBNP1-28, rANP1-28, rBNP1-28, iso-rANP1-46, or urodilatin completely inhibited the binding to all renal structures, whereas a differential inhibition was observed in the presence of 1 μM C-ANP1-23 (Figs. 2 and 3). Unlabeled C-ANP1-23 greatly reduced 125I-pBNP1-28 binding in glomeruli and completely inhibited binding to renal pelvis smooth muscle but had no apparent effect in other areas (Figs. 2 and 3). The ring-deleted analogue also significantly inhibited 125I-rANP1-28 binding in both the glomeruli and renal pelvis, but to a lesser extent than that observed with labeled 125I-pBNP1-28 and did not inhibit binding to the intra-renal arteries, inner medulla, or inner stripe of the outer medulla (Fig. 2). Binding sites were specific for natriuretic peptides and were not inhibited in the presence of unrelated peptides, vasoactive intestinal polypeptide, calcitonin gene-related peptide, gastrin, and angiotensin II. Reapplication of either ligand to fresh kidney sections produced autoradiographic images which were indistinguishable from those obtained previously, suggesting that there was no significant degradation of the ligand when incubated with tissue sections.

Saturation and Competitive Inhibition Studies—Binding experiments with increasing concentrations of labeled ligand and Scatchard analysis of equilibrium binding data demonstrated that renal 125I-pBNP1-28 and 125I-rANP1-28-binding sites were saturable and of high affinity (Figs. 4 and 5, Table I). The apparent equilibrium dissociation constant (Kd) for 125I-pBNP1-28-binding sites was greater than that for 125I-rANP1-28 in all regions of the kidney, although the difference was only found to be significant in the inner medulla (Table I). Binding sites for 125I-pBNP1-28 in the glomeruli, renal arteries, and renal pelvis displayed significantly higher affinity than those in the inner medulla, whereas no significant differences were detected when 125I-rANP1-28 was used as ligand. All of the kidney regions possessed a higher binding capacity for 125I-rANP1-28 than 125I-pBNP1-28, although the difference was not significant in the inner medulla. Glomerular-binding sites displayed the greatest capacity for both ligands. 125I-rANP1-28-binding sites in the intra-renal arteries and renal
peptide sequences to inhibit either 125I-pBNP1-26 or 125I-rANP1-28 (rANP1-28 in these regions (Table I). Differences in the relative potency of the various natriuretic ligands from glomerular-binding sites (pBNP1-26, rANP1-28, and urodilatin) inhibited 125I-pBNP1-26 binding to a similar extent, with no significant difference being found between IC50 values for sites in glomeruli, inner medulla, and intra-renal arteries. Rat-BNP1-32 exhibited a significantly higher potency than pBNP1-26, for 125I-pBNP1-26-binding sites, with a significant difference being found in glomeruli. Competitive binding curves for iso-rANP1-46 were shifted to the right (Fig. 6) and gave significantly greater IC50 values for the displacement of 125I-pBNP1-26 binding to glomeruli (p < 0.0001) and intra-renal arteries (p < 0.05) than those obtained with other endogenous natriuretic peptide sequences (Table II). When 125I-rANP1-28 was used as ligand and no significant differences were detected in the relative potency of unlabeled rANP1-28, pBNP1-26, urodilatin and rBNP1-28 for the inhibition of glomerular binding, whereas in the inner medulla and intra-renal arteries rANP1-28 displayed a significantly higher potency (Table II). In contrast, iso-rANP1-46 was significantly less potent than the other natriuretic sequences in inhibiting 125I-rANP1-28 binding in all three regions (Table I and II).

C-ANP4-23 was significantly less potent than either pBNP1-26 or rANP1-28 in inhibiting ligand binding and exhibited both regional and ligand-specific differences in the extent to which binding was inhibited (Figs. 6 and 7, Table II). Less than 5% of 125I-pBNP1-26 glomerular binding remained in the presence of 1 μM C-ANP4-23, whereas 34.6 ± 4.7% of specific 125I-rANP1-28 binding was not inhibited (Figs. 6 and 7). The binding of both ligands to non-vascular smooth muscle in the renal pelvis was completely displaced by an excess of C-ANP4-23. However, in marked contrast, C-ANP4-23 had no apparent effect on the binding of either ligand to sites in the inner medulla and intra-renal arteries (Figs. 6 and 7). Unrelated peptides (vasoactive intestinal polypeptide, calcitonin gene-related peptide, gastrin, and angiotensin II) also failed to display any competitive inhibitory activity.

**Immunohistochemistry**—Immunofluorescence staining of kidney sections indicated the presence of BNP-like immunoreactivity in renal nerves. Immunoreactivity was localized to a subpopulation of fine nerve fibers and fascicles, which were associated with renal arteries in hilar connective tissue, interlobar, arcuate, and cortical radial arteries and to a lesser extent with non-vascular smooth muscle in the renal pelvis (Fig. 8). BNP-like immunoreactive nerves were less numerous than those displaying immunoreactivity for the general neural marker synaptophysin and appeared to be distinct from var-

### Table I

<table>
<thead>
<tr>
<th>Region</th>
<th>125I-pBNP1-26</th>
<th>125I-rANP1-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli</td>
<td>0.832 ± 0.08*</td>
<td>0.355 ± 0.03</td>
</tr>
<tr>
<td>Inner medulla</td>
<td>1.370 ± 0.23</td>
<td>0.200 ± 0.02†</td>
</tr>
<tr>
<td>Intra-renal arteries</td>
<td>0.610 ± 0.08**</td>
<td>0.306 ± 0.06</td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>0.686 ± 0.23**</td>
<td>0.410 ± 0.06</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E. for eight rats and were derived from separate Scatchard analysis of saturation binding data for each animal, using the program GraphPAD. Significant difference (at the *0.05 and **0.0001 level) in the regional Kd and Bmax values compared to inner medulla, for each ligand. Significant difference (at the **0.0001 and ††0.0001) level between the two ligands in the regional Kd and Bmax values.
Renal Natriuretic Peptide-binding Sites

FIG. 5. Saturation analysis of specific $^{125}$I-rANP$_{1-28}$ binding to glomeruli, inner medulla, intra-renal arteries, and smooth muscle in renal pelvis. Nonspecific binding was determined by incubating sections in the presence of 1 $\mu$m rANP$_{1-28}$. Insets represent Scatchard plots of the specific binding data. Each point represents the mean $\pm$ S.E. from eight rats.

icose nerve fibers containing neuropeptide Y and calcitonin gene-related peptide immunoreactivity (data not shown). No neural immunostaining was detected with the antiserum to rANP$_{1-28}$ and neither antisera immunostained epithelial cells in renal tubules or collecting ducts. BNP-like immunostaining was abolished following liquid-phase preabsorption of diluted BNP antiserum with 1–10 $\mu$m synthetic pBNP$_{1-26}$ (Fig. 8) and the antiserum showed no cross-reactivity with rANP$_{1-28}$, either on tissue sections or in an enzyme-linked immunosorbent assay system.

**DISCUSSION**

Binding sites for $^{125}$I-pBNP$_{1-26}$ and $^{125}$I-rANP$_{1-28}$ were localized to specific regions of the rat kidney and characterized by saturation and competitive binding experiments, using six different natriuretic peptide sequences including the receptor selective analogue C-ANP$_{4-23}$. The binding of both ligands

FIG. 6. Competitive inhibition curves of specific $^{125}$I-pBNP$_{1-26}$ (250 pm) binding to glomeruli and intra-renal arteries. Each point represents the mean $\pm$ S.E. derived from six individual rats, expressed as the percentage of binding of $^{125}$I-pBNP$_{1-26}$ in the presence of increasing concentrations of unlabeled rANP$_{1-28}$ (■), pBNP$_{1-26}$ (●), rBNP$_{1-32}$ (△), iso-rANP$_{1-46}$ (■), and C-ANP$_{4-23}$ (○). Competitive inhibition of binding to the inner medulla produced identical inhibition curves to those shown for intra-renal arteries.

FIG. 7. Competitive inhibition curves of specific $^{125}$I-rANP$_{1-28}$ (250 pm) binding to glomeruli and intra-renal arteries. Each point represents the mean $\pm$ S.E. derived from six individual rats, expressed as the percentage binding of $^{125}$I-rANP$_{1-28}$ in the presence of unlabeled rANP$_{1-28}$ (■), pBNP$_{1-26}$ (●), rBNP$_{1-32}$ (△), iso-rANP$_{1-46}$ (■), and C-ANP$_{4-23}$ (○). Competitive inhibition of binding to inner medulla produced identical inhibition curves to those shown for intra-renal arteries.
Renal Natriuretic Peptide-binding Sites

Table II
Competitive inhibition of specific $^{125}$I-pBNP$_{1-26}$ and $^{125}$I-rANP$_{1-28}$ renal binding by unlabeled peptide sequences

IC$_{50}$ (nm) values derived from non-linear regression analysis of competition binding data expressed as the geometric mean and 95% confidence limits (c.l.) for six rats. The potency of each competing peptide was compared to that of pBNP$_{1-26}$ and rANP$_{1-28}$ for $^{125}$I-pBNP$_{1-26}$ and $^{125}$I-rANP$_{1-28}$-binding sites, respectively, and differences were considered significant at the *0.05, **0.01, ***0.001, and ****0.0001 level. ND, not determined.

<table>
<thead>
<tr>
<th>Region</th>
<th>Competing peptide</th>
<th>$^{125}$I-pBNP$<em>{1-26}$ IC$</em>{50}$ nM (95% c.l.)</th>
<th>$^{125}$I-rANP$<em>{1-28}$ IC$</em>{50}$ nM (95% c.l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli</td>
<td>pBNP$_{1-26}$</td>
<td>0.62 (0.25–1.53)</td>
<td>1.90 (1.29–2.81)</td>
</tr>
<tr>
<td></td>
<td>rANP$_{1-28}$</td>
<td>0.51 (0.29–0.88)</td>
<td>2.47 (1.26–4.85)</td>
</tr>
<tr>
<td></td>
<td>Urodilatin</td>
<td>0.94 (0.75–1.17)</td>
<td>2.11 (1.49–3.15)</td>
</tr>
<tr>
<td></td>
<td>rBNP$_{3-22}$</td>
<td>1.65 (1.23–2.20)</td>
<td>3.57 (2.83–4.51)</td>
</tr>
<tr>
<td></td>
<td>Iso-rANP$_{4-46}$</td>
<td>6.37 (4.52–8.96)****</td>
<td>22.56 (15.81–32.11)****</td>
</tr>
<tr>
<td></td>
<td>C-ANP$_{1-21}$</td>
<td>18.55 (8.99–38.25)****</td>
<td>16.61 (5.39–51.20)****</td>
</tr>
<tr>
<td>Inner medulla</td>
<td>pBNP$_{1-26}$</td>
<td>0.90 (0.52–1.56)</td>
<td>8.12 (3.96–14.32)****</td>
</tr>
<tr>
<td></td>
<td>rANP$_{1-28}$</td>
<td>0.65 (0.27–1.54)</td>
<td>0.80 (0.21–2.98)</td>
</tr>
<tr>
<td></td>
<td>Urodilatin</td>
<td>ND</td>
<td>2.74 (1.54–4.87)</td>
</tr>
<tr>
<td></td>
<td>rBNP$_{3-22}$</td>
<td>ND</td>
<td>4.83 (2.96–7.88)***</td>
</tr>
<tr>
<td></td>
<td>Iso-rANP$_{4-46}$</td>
<td>ND</td>
<td>55.19 (14.84–205.3)***</td>
</tr>
<tr>
<td>Intra-renal arteries</td>
<td>pBNP$_{1-26}$</td>
<td>0.65 (0.20–2.11)</td>
<td>6.76 (3.70–12.35)*</td>
</tr>
<tr>
<td></td>
<td>rANP$_{1-28}$</td>
<td>0.69 (0.28–1.70)</td>
<td>0.80 (0.16–3.98)</td>
</tr>
<tr>
<td></td>
<td>Urodilatin</td>
<td>0.60 (0.14–2.56)</td>
<td>3.35 (2.04–5.50)</td>
</tr>
<tr>
<td></td>
<td>rBNP$_{3-22}$</td>
<td>1.65 (0.94–2.92)</td>
<td>4.01 (3.20–5.02)*</td>
</tr>
<tr>
<td></td>
<td>Iso-rANP$_{4-46}$</td>
<td>5.26 (3.94–17.02)*</td>
<td>54.69 (34.96–85.4)*</td>
</tr>
</tbody>
</table>

Fig. 8. Immunofluorescence micrographs showing synaptophysin and brain natriuretic peptide (BNP)-like immunoreactivity localized to renal nerve fibers in adjacent sections of rat kidney. Perivascular nerves associated with an arcuate artery (asterisk) display immunostaining for the general neuronal marker synaptophysin (a) and a subpopulation of nerve fibers contain BNP-like immunoreactivity (b). Perivascular nerve fibers (arrows) around cortical radial artery branches (asterisk) also exhibit BNP immunoreactivity (c) which is abolished by liquid-phase preabsorption of the diluted antisera with 1 μM pBNP$_{1-26}$ (d). G, glomerulus. Bar = 100 μm.

was completely inhibited by an excess of either pBNP$_{1-26}$ or rANP$_{1-28}$, indicating that the two peptides compete for the same population of renal binding sites. Regional variations in the distribution of binding sites and their relative affinity for selected peptide sequences suggests, however, that the binding is to a heterogeneous population of receptors. ANP sequences bind with high affinity to at least two glomerular receptor subpopulations in the cortex, which may be distinguished by their intrinsic guanylate cyclase activity and distinct affinity for ring-deleted analogues such as C-ANP$_{4-23}$ (10, 13, 15). The competitive inhibition of natriuretic peptide binding to sections in vitro indicates that some 65% of $^{125}$I-rANP$_{1-28}$ glomerular binding was to the clearance (C-ANP) receptor subtype, with the remaining 35% showing no detectable affinity for C-ANP$_{4-23}$. This corresponds closely with membrane-bind-
C-terminal modification of the natriuretic peptide sequence and lacks affinity for C-ANP4-23 (11, 15, 23, 40). Inner medulla binding sites also interact with pBNP1-26, but exhibit a higher equilibrium dissociation constant for the ligand and a lower potency, compared to rANP1-28, in inhibiting [125I]rANP1-28 binding. Low and high affinity [125I]pBNP1-26 binding sites have been identified in rat inner medullary membranes (22) and differences noted in the relative ability of pBNP1-26 and rANP1-28 to compete for [125I]rANP1-28 binding to rat papillae (23), suggesting that as in the glomeruli, pBNP1-26 is less selective for non-clearance receptors which predominate in the inner medulla. Similar regional variations in the relative affinity of binding sites for pBNP1-26 and rANP1-28 ligands have also been found in the rat brain (41, 42) and sympathetic ganglia (43), whereas in tissues from other mammals the two sequences display a similar affinity and appear equipotent in stimulating cGMP production (23, 44). Despite the homology which exists between mammalian BNP and ANP sequences there exist significant differences in the structure and post-translational processing of BNP in rat, pig, and man (17), and it has been considered that the apparent discrimination of rat-binding sites by pBNP1-26 and rANP1-28 may reflect species differences (23, 42). Like pBNP1-26, however, the endogenous rat sequence rBNP1-26 was also less potent than rANP1-28 in inhibiting [125I]rANP1-28 binding, indicating that the difference in affinity is not merely due to the use of a porcine ligand on rat tissue but may reflect species variations in the expression of receptor subtypes, as suggested from the screening of cDNA libraries for A-ANP and B-ANP receptor clones (7).

Cultured aortic vascular smooth muscle and endothelial cells display a heterogeneous population of ANP receptors (4, 5, 45), but the marked similarity between ligand binding in the inner medulla and intra-renal arteries and the lack of competitive inhibition by C-ANP4-23 indicates that non-clearance sites predominate in the interlobar and arcuate arteries and suggests that there may be a differential expression of receptor subtypes in different vascular beds. This is in accord with the finding that natriuretic peptides induce a selective relaxation of rat arcuate arteries and afferent arterioles at concentrations as low as 0.1–0.3 nM (46–48). The predominance of non-clearance sites is also consistent with the proposal that the direct vasodilatory action of ANP on renal blood vessels is mediated by an increase in guanylate cyclase activity (49).

Studies with truncated and ring-deleted analogues have demonstrated the significance of an intact ring structure and the C-terminal amino acids Phe3 and Arg7 in determining the activity and affinity of natriuretic sequences for receptor subtypes (50, 51). While the 17-amino acid ring structure of the ANP and BNP isomers is highly conserved significant structural heterogeneity occurs in the C-terminal region, and this is reflected in the rank order of potency for pBNP1-26, rBNP1-26, and iso-rANP1-26 compared to ANP1-28 and the equipotent N terminally extended isoform urodilatin. The iso-rANP1-26 sequence was first reported by Flynn and co-workers (17, 62) and is now known to differ by 5 C-terminal amino acids from the endogenous 8-kDa molecular mass cardiac peptide, designated as iso-rANP1-45 (53) or BNP1-45 (17). The low potency of iso-rANP1-45 in displacing renal binding underlines the significance of the C terminus in determining binding affinity.

Both ANP and BNP immunoreactivity has been localized to neurons in the brain and natriuretic peptides implicated in the central control of cardiovascular function, however, BNP-immunoreactive neurons appear to be more widely distributed, with immunostained neurons located in central autonomic and sensory ganglia (26, 54). Immunoactive nerve fibers were mainly associated with intrarenal arteries which also displayed a high density of binding sites, but the origin of these nerves has still to be established.

In conclusion, the present findings indicate that rat renal binding sites are heterogeneous, and subpopulations exhibit significant differences in regional distribution. Porcine BNP1-16 competes for the same binding sites as rANP1-28 but with a lower potency and is less selective for non-clearance sites which were discriminated by C-ANP4-23. C-terminal modification of naturally occurring rat cardiac peptides results in a significant loss of competitive inhibition. The localization of BNP immunoreactivity to a subpopulation of renal nerves suggests that renal function may be modulated by the local release of natriuretic peptide as well as by circulating cardiac sequences.

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REFERENCES

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