Characterization of binding sites for amylin, calcitonin, and CGRP in primate kidney

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Characterization of binding sites for amylin, calcitonin, and CGRP in primate kidney. Am. J. Physiol. 274 (Renal Physiol. 43): F51–F62, 1998.—Analysis of receptor distributions for 125I-labeled amylin, 125I-labeled calcitonin, and 125I-labeled calcitonin gene-related peptide (CGRP) in Macaca fascicularis kidney by in vitro autoradiography revealed distinct patterns of binding for each peptide. 125I-rat amylin bound primarily to the cortex, being associated with the distal tubule, including apparent binding to the juxtaglomerular apparatus. 125I-salmon calcitonin displayed high-density binding in the cortex with low-density binding to the medulla. Emulsion autoradiogram indicated that binding was associated with both distal tubule and thick ascending limb of the loop of Henle. Intense binding was also found often over juxtaglomerular apparatus. 125I-rat CGRP-α exhibited low- to moderate-density binding to the inner medulla/papilla with high-density binding over small-, medium-, and large-caliber arteries. Weak binding to the glomerulus was also seen, but no binding was associated with cortical tubules. Competition binding studies, performed with each of the radioligands, revealed peptide specificity profiles for CGRP and calcitonin receptors that were similar to those described in rat. However, the monkey amylin receptors differed from those in rat, exhibiting relatively higher affinity for amylin but reduced affinity for CGRP peptides. These studies suggest potential roles for amylin, calcitonin, and CGRP in primate renal function.

Calcitonin (CT) receptors have been cloned from a number of species, including rat and human (30, 39), and classically have highest affinity for CTS with relatively weak interaction with the related peptides CGRP and amylin. We have termed these receptors C1 (42), and this forms the basis of the C1a and C1b nomenclature used for the CT receptor isoforms (39). CGRP receptors have high affinity for the CGRPs with only very weak interaction with any of the CTS (40). We have termed these receptors C2 to distinguish them from the C3-type amylin receptors described below. On the basis of pharmacological but not competition binding studies, division of CGRP receptors into two subtypes has been proposed: type 1, which is potently antagonized by CGRP-(8—37) and which has only weak interaction with linear CGRP analogs, or type 2, which has moderate interaction with linear analogs with poor antagonism by CGRP-(8—37) (14, 15). Nonetheless, neither type 1 nor type 2 receptors have significant affinity for salmon CT (sCT) and thus would fall into our C2 class of receptors. The C3-type amylin receptor, in the rat, has high affinity for both amylin and sCT, moderate to high affinity for the CGRPs, but only low affinity for rat or human CT (2, 41, 46).

Amylin shares ~50% and 18–33% sequence homology with the CGRPs and the CTS, respectively (Fig. 1). The three peptides form part of a related gene family (4) and exhibit a number of common primary and secondary structural features. These include a disulfide bridged loop of six or seven amino acids at the amino terminus, an amidated aromatic residue present at the carboxy terminus, and a region of predicted amphipathic α-helical secondary structure from residues 8–18 (residues 8–22 for sCT).

In concordance with the structural conservation among the peptides, amylin can induce CT-like actions in osteoclasts and CGRP-like actions in the vasculature (reviewed in Ref. 42). However, in both these actions, amylin is much weaker than either CT or CGRP and is thought to act via classic CT or CGRP receptors.

Recently, we identified high-affinity binding sites for amylin in the renal cortex of the rat and demonstrated cognate specificity of ligand interaction to the binding sites in brain (41, 46). Furthermore, low doses of amylin induce rises in plasma renin in both rats (46) and humans (11), with a corresponding hypertension in rats (25), consistent with the hypothesis that amylin may be a causative link between insulin resistance and hypertension in patients with syndrome X (11, 45).
Fig. 1. Amino acid alignment of calcitonin (CT), calcitonin gene-related peptide (CGRP), amylin, and analog amino acid sequences. Top: agonist peptides with amino acids in common with rat amylin boxed. Bottom: antagonist peptides with amino acids in common with salmon CT-(8—32) [sCT-(8—32)] boxed. Shaded region represents residues absent from full-length CT sequence. In both top and bottom, CT sequence has been gapped between amino acids 21 and 22 to allow alignment with amylin and CGRP sequences.

Fig. 2. Distribution studies. Ten- or twenty-micrometer sections were cut in a cryostat at 2°C, thaw mounted onto gelatin-coated slides, dehydrated, and then stored at −20°C until use (maximum of 4 wk).

Fig. 3. Binding studies were performed as previously described for 125I-rat amylin (−70 pM) (41, 46) or 125I-sCT (100 pM) (38) and 125I-rat CGRP (−70 pM) (40). Briefly, sections were thawed and preincubated at ambient temperature (20–22°C) for a total of 15 min by transferring through three chambers of incubation buffer (amylin buffer: 20 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, pH 7.4, containing 100 mM NaCl, 1 mg/ml BSA, and 0.5 mg/ml bacitracin; sCT and CGRP buffer: 100 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.4, containing 2 mg/ml BSA and 0.5 mg/ml bacitracin). Sections were then incubated for 60 min in incubation buffer containing either 125I-rat amylin, 125I-sCT, 125I-rat CGRP (10 pM) or 125I-rat CGRP (10 pM). Nonspecific binding was measured in the presence of 10−6 M homologous unlabeled peptide. After incubation, sections were washed four times for 1 min in ice-cold incubation buffer without BSA or bacitracin before being dipped in ice-cold deionized water and dried under a stream of air. Sections were exposed to Agfa's CP3B X-ray film for 14–28 days before development. After exposure, sections were stained with hematoxylin and eosin to aid in anatomic localization of binding sites. For emulsion autoradiographic studies, sections were incubated as described above, except that, after washing, the sections were placed into 2.5% glutaraldehyde in phosphate-buffered saline (PBS; [in mM] 140 NaCl, 2 KCl, 1 KH2PO4, 8 Na2HPO4, pH 7.4, at 4°C for 30–60 min. The sections were then washed for 1 min in PBS at 22°C, dipped into distilled deionized water, and allowed to dry. Before emulsion dipping, the sections were defatted in graded alcohol and xylene. After defatting, slides were prewarmed to ~42°C and then dipped for 1–2 s into LM-1 liquid emulsion at 42°C (Amersham) diluted 1:1 with distilled deionized water. Excess emulsion was allowed to drain from the slides, which were subsequently dried for 4–5 h at 25°C and >80% humidity. The dried emulsion-covered sections were exposed.
in a light-tight box with dehydrated silica gel for 1–3 mo, at
4°C, before subsequent development, according to the manu-
facturer’s specifications. To aid in anatomic localization of
binding sites, sections were subsequently stained with hema-
toxin and eosin.

Competition binding studies. Serial 20-µm transverse sec-
tions were cut and incubated, as described above, with 125I-rat
amylin, 125I-sCT, or 125I-rat CGRP-α containing increasing
concentrations of unlabeled peptide. Serial sections to those
used for competitive binding at each concentration examined
were taken for measurement of total binding to account for
changes in binding densities with different levels of section.
As such, binding is expressed as a percentage of total binding
at each level of section. Three sections were analyzed for each
concentration of peptide. To enable quantitation of the autora-
diographs, a series of 125I-labeled radioactivity standards
were included in each cassette (40). After development, the
autoradiographs were analyzed by computerized densitometry,
using the personal computer-based microcomputer imaging
device system (Imaging Research, St. Catharines, ON,
Canada).

Dissociation constant (Kd) and maximum binding site
concentration (Bmax) values were determined by analysis of
homologous competition binding studies, using the program
LIGAND, as adapted for the personal computer (32). The
concentrations of nonhomologous ligands yielding 50% inhibi-
tion of radioligand binding were determined with the use of
SigmaPlot (Jandel Scientific, San Rafael, CA).

RESULTS

Receptor distribution. Analysis of receptor distribu-
tions, using dry-film autoradiography, demonstrated
high-density binding of both 125I-sCT and 125I-amylin to
the renal cortex (Fig. 2, A and C). 125I-amylin also demon-
strated low-density binding to the inner medulla (Fig. 2A). The binding of 125I-CGRP, in contrast to the other radioligands, was predomi-
nantly to vascular tissue, with low-level, diffuse bind-
ing seen throughout the inner medulla/papilla (Fig. 2E).

Microscopic analysis of the binding distributions,
using emulsion autoradiography, indicated no apparent
specific binding of 125I-amylin to the inner medulla.
However, as with other parts of the kidney, a number of
isolated cells exhibited nondisplaceable 125I-amylin
binding (Fig. 3, A and B). 125I-amylin binding was also absent from most of the transitional zone between
medulla and cortex. In the cortex, amylin binding was
to tubules (Fig. 3, C–E). The tighter nuclear spacing of
tubular cells that bound amylin suggested that the
binding was primarily to distal tubule. However, the
morphology of the tissue did not allow us to discern
between distal tubule and cortical collecting tubule. No
binding to glomeruli or blood vessels in the cortex was
observed (Fig. 3, C–E), although low-density binding to
large renal arteries in the medulla was seen (Fig. 3G).
Likewise, much of the proximal tubule clearly had no
discernible binding of 125I-amylin (Fig. 3, C–E), al-
though again it was impossible to exclude binding to a
population of proximal tubule as a component of the
binding. The lack of binding to either the inner or outer
medulla suggested that neither the thick limb nor the
thin limb of the loop of Henle contained amylin binding
sites. Moderate- to high-density binding of 125I-amylin
was often found adjacent to the vascular pole of the
glomerulus, consistent with binding to the juxtagram-
erular apparatus (Fig. 3E).

Binding of 125I-sCT in the medulla was confined to
the thick ascending limb of the loop of Henle (Fig. 4A).
No binding was found over small-caliber vasculature,
thin limb of the loop of Henle, or collecting tubules. In
the cortex, as with the amylin binding, although it was
possible to exclude binding to much of the proximal
tuble (Fig. 4, C, E, G, and H), it was impossible to
totally exclude binding to some part of the proximal
tubule as a component of the observed binding. The
extent of tubular binding of 125I-sCT in the cortex was
greater than that found for amylin and probably
reflects binding of 125I-sCT to thick limb of the loop of
Henle, which is absent for 125I-amylin. As with amylin,
mmoderate- to high-density binding occurred to distal
tubule (Fig. 4, C, E, G, and H), although we could not
delineate early cortical collecting tubule from distal
tubule. In many instances, intense binding was found
in close association with the glomerulus, suggesting
binding to juxtagramerial apparatus (Fig. 4, G and
H). 125I-sCT did not bind to either glomerul (Fig. 4, C,
E, G, and H) or to renal blood vessels (Fig. 4E).

In contrast to amylin and CT binding, the highest
densities of 125I-CGRP binding occurred over arteries or
arterioles (Fig. 5, C–E) and included intense binding to
the smooth muscle layer in the larger vessels (Fig. 5D).
The large renal arteries likewise had high-density
binding (Fig. 5A). As seen macroscopically (Fig. 2E),
low-level, diffuse binding also occurred in the inner
depa/papilla. This binding appeared to be predomi-
nantly extratubular, consistent with binding to the
extensive capillary network in this region of the kidney
(Fig. 5G). Whether specific binding occurred to tubules
in this region was unclear. In the cortex, binding was
absent from tubules, being confined mainly to blood
vessels; however, weak binding was seen over glo-
meruli (Fig. 5, C and E).

Competition binding studies. The specificity of bind-
ing to CT, amylin, and CGRP receptors was assessed by
radioligand binding competition to horizontal sections
of monkey kidney.
125I-sCT. 125I-sCT bound to monkey renal cortex with high affinity, exhibiting a $K_d$ in competition binding studies of 5.88 ± 100 × 10^{-10} M and a $B_{max}$ of 113 ± 14 fmol/mg protein. The rank order of potency for agonist peptides in competing for binding was sCT > pCT > hCT >> rat amylin, whereas the CGRPαs essentially did not compete for binding in concentrations up to 10⁻⁶ M (Fig. 6A, Table 1). For antagonist peptides, the order of potency was AC-512 was the most potent, being almost one order of magnitude more potent than either sCT-(8—32) or AC-413. Human CGRPα-(8—37) was without effect at concentrations up to 10⁻⁶ M (Fig. 6B, Table 1).

125I-rat amylin. Analysis of competition of 125I-rat amylin binding to renal cortex by rat amylin indicated both high-affinity ($K_d$ 7.15 ± 0.77 × 10⁻¹¹ M) and low-affinity ($K_d$ 7.77 ± 0.12 × 10⁻⁹ M) binding sites, with $B_{max}$ values of 1.40 ± 0.19 and 525 ± 59 fmol/mg protein for each site, respectively. The relative potency of nonamylon agonist peptides in competition for 125I-rat amylin binding was sCT > pCT > hCT > hCGRPβ and rat CGRPα > hCGRPα (Fig. 7A, Table 1). For antagonist peptides, the order of potency was AC-512 > sCT-(8—32) > AC-413, whereas hCGRPα-(8—37) was ineffective in competition for binding at concentrations up to 10⁻⁶ M (Fig. 7B, Table 1).

125I-human CGRP. Analysis of competition of 125I-human CGRP binding by rat CGRPα also indicated both a high-affinity ($K_d$ 1.07 ± 0.78 × 10⁻¹⁰ M; $B_{max}$ 6.93 ± 4.62 fmol/mg protein) and a low-affinity ($K_d$ 1.49 ± 0.79 × 10⁻⁷ M; $B_{max}$ 258 ± 74 fmol/mg protein) binding site. The relative potency of agonist peptides in competition for binding was rat CGRPα, hCGRPβ > hCGRPα >> rat amylin, whereas sCT, pCT, and hCT did not compete for binding in concentrations up to 10⁻⁶ M (Fig. 8A, Table 1). For antagonist peptides, hCGRPα-(8—37) was the most potent ligand, being two- to fourfold more potent than AC-413 and AC-512, respectively. sCT-(8—32), however, did not compete for binding, even at 10⁻⁶ M (Fig. 8B, Table 1).

**DISCUSSION**

In this study, we demonstrated specific, high-affinity binding sites for amylin, CT, and CGRP in M. fascicularis kidney. The distribution of binding sites in monkey was predominantly similar to those reported in the rat (23, 38, 44, 46). However, a number of anomalies were apparent.

The distribution of 125I-sCT binding sites paralleled that of the rat, in which binding was localized to cortical and medullary thick ascending limb of the loop of Henle and distal tubule (38). This also parallels the distribution of CT-responsive adenylyl cyclase in both rat and human nephrons (28, 33, 34). Physiologically, in rats, CT decreases urinary excretion of magnesium and calcium (5, 19, 20) primarily because of increased reabsorption in the thick ascending limb of the loop of Henle (19). In the loop of Henle, CT also increases reabsorption of potassium and to a lesser extent sodium and chloride (19). In the distal tubule, in addition to effects on calcium and magnesium, CT decreases fractional excretion of sodium, chloride, and total solutes and decreases secretion of potassium (18). Because the distributions of receptors in monkey and rat are essentially equivalent, it is likely that similar functions are performed by CT receptors in distal tubule and thick ascending limb of the loop of Henle in primates. An additional observation in the current study was the intense binding of 125I-sCT to structures consistent with juxtaglomerular apparatus. The juxtaglomerular apparatus is the principal source of circulating renin, and binding of CT to this structure may provide an explanation for the rise in plasma renin seen with peripherally administered sCT (9, 31, 47). Interestingly, sCT may elevate blood pressure acutely, in association with the rise in plasma renin activity (25), and, consequently, potential mediation of this effect by receptors on the juxtaglomerular apparatus needs to be considered.

The distribution of 125I-CGRP binding sites was also similar between rat and monkey, with moderate-to-high-density binding to the renal medulla. However, the rat also exhibited relatively high-level, punctate binding in the cortex, indicative of glomerular binding (46). Although glomerular binding was also seen with 125I-CGRP in the current study, the level of binding was relatively low. In rats, on the basis of the relative efficacy of adrenomedullin and CGRP in stimulating cAMP production in glomeruli and the lack of inhibition of the response by the CGRP antagonist CGRPα-(8—37),

**Table 1.** IC₅₀ values for nonhomologous peptides in competition for 125I-sCT, 125I-rat amylin, or 125I-labeled rat CGRPα binding to horizontal sections of monkey kidney

<table>
<thead>
<tr>
<th>Peptide</th>
<th>125I-sCT</th>
<th>125I-rat amylin</th>
<th>125I-rat CGRPα</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCT</td>
<td>&lt;10⁻⁶ M</td>
<td>&gt;10⁻⁶ M</td>
<td></td>
</tr>
<tr>
<td>Pig CT</td>
<td>1.41 × 10⁻⁸ M</td>
<td>&lt;10⁻⁴ M</td>
<td>&gt;10⁻⁶ M</td>
</tr>
<tr>
<td>Human CT</td>
<td>3.17 × 10⁻⁸ M</td>
<td>&lt;10⁻⁸ M</td>
<td>&gt;10⁻⁶ M</td>
</tr>
<tr>
<td>Rat amylin</td>
<td>&gt;10⁻⁶ M</td>
<td>&gt;10⁻⁶ M</td>
<td>&gt;10⁻⁶ M</td>
</tr>
<tr>
<td>Rat CGRPα</td>
<td>&gt;10⁻⁶ M</td>
<td>1.43 × 10⁻⁸ M</td>
<td>&gt;10⁻⁶ M</td>
</tr>
<tr>
<td>Human CGRPα</td>
<td>&gt;10⁻⁶ M</td>
<td>2.1 × 10⁻⁷ M</td>
<td>&gt;10⁻⁹ M</td>
</tr>
<tr>
<td>Human CGRPβ</td>
<td>&gt;10⁻⁶ M</td>
<td>1.75 × 10⁻⁸ M</td>
<td>2.85 × 10⁻¹⁰ M</td>
</tr>
<tr>
<td>AC-512</td>
<td>3 × 10⁻⁶ M</td>
<td>1 × 10⁻¹⁰ M</td>
<td>9.61 × 10⁻⁶ M</td>
</tr>
<tr>
<td>AC-413</td>
<td>2.3 × 10⁻⁸ M</td>
<td>2 × 10⁻¹⁰ M</td>
<td>4.0 × 10⁻⁹ M</td>
</tr>
<tr>
<td>sCT-(8—32)</td>
<td>1.7 × 10⁻⁶ M</td>
<td>3 × 10⁻¹⁰ M</td>
<td>&gt;10⁻⁶ M</td>
</tr>
<tr>
<td>Human CGRPα-(8—37)</td>
<td>&gt;10⁻⁶ M</td>
<td>&gt;10⁻⁶ M</td>
<td>2.55 × 10⁻⁸ M</td>
</tr>
</tbody>
</table>

IC₅₀, 50% inhibitory concentration; CT, calcitonin; sCT, salmon calcitonin; CGRP, calcitonin gene-related peptide.
it has been suggested that the glomerular receptors are principally adrenomedullin receptors (17). As such, the low level of CGRP binding may represent a decreased affinity of these receptors for CGRP in monkey, although alternative explanations, including low levels of either adrenomedullin or CGRP receptors, are equally valid. In monkey, the highest levels of CGRP binding occurred over renal arteries and arterioles and are consistent with the potent action of CGRP as a renal vasodilator (1, 16, 21, 24), with consequent increases in renal blood flow (1, 43). Although the resolution of the autoradiographic procedure did not allow us to determine whether binding occurred to endothelium as well as to smooth muscle, physiological studies in the rat indicate that renal vasodilatation is dependent on release of endothelium-derived nitric oxide (21), and thus it is likely that receptors are present on endothelial cells.

CGRP-like immunoreactivity has been localized to both the cortex and medullopapillary portion of the human kidney (22), whereas renovascular innervation of CGRP-containing neurons is well described in other species (13, 29). Thus it is likely that CGRP-mediated actions in the kidney occur via local release of CGRP from peptidergic nerves.

The distribution of 125I-amylin binding sites in monkey presented as a subset of sites labeled with 125I-sCT and was localized to cortical tubule structures, which included at least the distal tubule. In this, it was consistent with binding to most other C3-type amylin receptors (2, 41) and probably reflects the high affinity of sCT for both C3-type amylin and C1-type CT receptors. This binding distribution, however, contrasts with that reported for amylin receptors in the rat, which indicated that binding was exclusively to proximal tubules, although not all proximal tubules bound 125I-amylin (23).

A potential explanation for the discrepancy in amylin binding distributions in rat and monkey is that, in monkey, the amylin binding represents lower-affinity binding to C1-type CT receptors, thus accounting for the parallelism in cortical distribution of CT and amylin binding, with no amylin binding observed in the medulla because of the relatively low density of CT receptors in this part of the kidney. However, we believe this is unlikely for the following reasons. In cells transfected with either rat or hCT receptor cDNAs, essentially no binding is seen using 125I-amylin at concentrations used in the current study (Ref. 26 and Sexton, unpublished data). Furthermore, in monkey brain, the pattern of amylin receptor distribution parallels that of the rat and, like the rat, forms a subset of sites labeled with 125I-sCT. In this tissue, many regions of high-density 125I-sCT binding remain devoid of amylin binding, indicating that amylin binding is not a consequence of low-affinity binding to C1-type CT receptors (Ref. 8 and G. Paxinos, S. Y. Chai, G. Christopoulos, X.-F. Huang, A. W. Toga, P. M. Sexton, unpublished observations). In addition, the specificity of amylin receptors in the brain is similar to that described in kidney in the current study (Ref. 26 and Sexton, unpublished observations), indicating that the renal and neuronal amylin receptors are equivalent and represent the monkey equivalent of rat C3-type amylin receptors.

Although amylin receptors, distinct from C3-type amylin receptors, have been described that lack interaction with sCT (12, 27), it seems unlikely that these underlie the anomaly in rat renal amylin receptors, because equivalent receptor distributions were demonstrated by in vitro and in vivo techniques (23), and in vitro analysis of receptor specificity, in competition binding studies, revealed a similar profile to other C3-type amylin receptors, with amylin, CGRP, and sCT all exhibiting similar potency in inhibiting 125I-amylin...
binding (46). Nonetheless, the nature of the paradoxical lack of 125I-sCT binding to proximal tubule amylin receptors in the rat remains unclear. The apparent divergence in receptor distributions in monkey and rat suggests that renal amylin receptors may subserve different functions in the two species. Alternatively, the differences in distribution may be partly due to ontological differences in receptor expression. It has been suggested that rat cortical amylin receptors are initially expressed during a stage of extensive differentiation and organogenesis in the kidney and that amylin might have a mitogenic effect on proximal tubule cells during this phase of development (23). It is possible that the proximal tubule amylin receptors seen in the adult rat are carried over from this developmental role but are lost during maturation of monkey kidney to the adult state. No data are available on amylin receptor expression in developing monkey kidney.

The physiological role of amylin receptors in the distal tubule is unclear. The weak binding of amylin to smooth muscle of large renal arteries, however, is likely due to a low-affinity interaction of amylin with CGRP receptors. Studies on the renovascular action of amylin indicate that it is much weaker than CGRP and can be blocked by the CGRP antagonist CGRP-(8—37) (7), which is consistent with this hypothesis.

Analysis of receptor specificity in competition binding studies revealed specificity profiles for CT and CGRP receptors similar to the profile of rat receptors studied under similar conditions (38, 40). Although, on the basis of competition binding studies, division of CGRP receptors into type 1 or type 2 receptors has not been possible, most data indicate that at least the renal vascular receptor exhibits a type 1 phenotype (6, 7). Of note, however, was the potency of AC-512 in competing for 125I-CGRP binding. This peptide was only slightly weaker (~4-fold) than CGRP-(8—37) in competing for binding, whereas sCT-(8—32) was completely impotent. AC-512 differs from sCT-(8—32) in only three positions (Fig. 1), and the importance of the carboxy-terminal aromatic residue in binding (35) may indicate a significant role of the benzene ring structure in primate CGRP receptor recognition, at least for the inactive state receptor.
Little is known about the specificity of amylin receptors outside the rat. Our data indicate that there is considerable divergence in receptor specificity for monkey versus rat receptors. In particular, there is an apparent increase in the efficacy of CT peptides to compete for binding in the monkey along with a small decrease in the relative efficacy of CGRP peptides. Analysis of homologous competition studies indicated that the $^{125}$I-amylin binding could be divided into two components, a high-affinity state of $\sim 7 \times 10^{-11}$ M and a low-affinity state of $\sim 8 \times 10^{-9}$ M. Although it is possible that the low-affinity state reflects binding to CT receptors, the lack of $^{125}$I-amylin binding to high-density CT receptor sites in parts of the brain suggests that this is unlikely. A more probable explanation is that the binding reflects affinities for active (G protein coupled) and inactive (G protein uncoupled) states, with binding being primarily to inactive-state (low affinity) receptor. Indeed, in mouse $\alpha$-thyroid-stimulating hormone thyrotrhop cells, we demonstrated a direct effect of G protein coupling on amylin receptor affinity (36). As such, the differences in relative specificities between rat and monkey may, in part, reflect differences in the availability of G protein or other binding cofactors. As with the rat, the antagonist peptides sCT-(8—32) and peptide chimeras between sCT-(8—32) and amylin were potent competitors of amylin binding. However, CGRP-(8—37) failed to compete for binding in concentrations of up to 1 $\mu$M. Thus, unlike rat, in the monkey with CGRP1 receptors, the antagonist peptides sCT-(8—32) or AC-413 (46) and acts as a weak antagonist of amylin receptors (37). CGRP-(8—37) is unlikely to be an effective antagonist of amylin receptors in monkey and consequently may act as a more specific CGRP type 1 receptor antagonist.

In conclusion, these studies demonstrate the existence of high-affinity receptors for amylin, CT, and CGRP within the primate kidney. The distribution of receptor sites suggests that the peptides will exert distinct biological actions on renal vasculature, primarily mediated via CGRP receptors, and renal tubules, primarily mediated via amylin or CT receptors. Furthermore, the apparent divergence in receptor specificity between amylin receptors in monkey and rat indicates that caution should be taken in extrapolating on functional specificity of peptides between species.

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REFERENCES


