Structure and activity of OK-GC: a kidney receptor guanylate cyclase activated by guanylin peptides

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Guanylin and uroguanylin have two intramolecular disulfide bonds, but lymphoguanylin has only one disulfide. A synthetic form of lymphoguanylin also activates T84 and OK cell GCs. This family of guanylin peptides is similar in structure and biological activity to heat-stable enterotoxin (ST) peptides secreted by strains of enteric microorganisms that cause secretory diarrhea (16). The active guanylin peptides are found from COOH termini of longer, biologically inactive prohormones (6, 11, 22, 48).

Guanylin, uroguanylin, and ST peptides stimulate the transepithelial secretion of chloride and bicarbonate anions when administered into the apical (mucosal) reservoirs of Ussing chambers containing mounted segments of intestinal mucosa (4, 18, 25, 31). These peptides also increase the short-circuit current of a model T84 cell epithelium in a luminal pH-dependent fashion by stimulating electrogenic chloride secretion (4, 20, 21). Physiological roles for the endogenous guanylin peptides are postulated to include the regulation of salt and water secretion during digestion, as well as neutralization of HCl in the duodenum and of organic acids derived from enteric bacteria in the large intestine (20, 25). Control of these intestinal functions by guanylin peptides is mediated via intracellular cGMP through activation of cGMP-dependent protein kinase II and/or cAMP-dependent protein kinase II with subsequent phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR) protein (15, 25, 40). CFTR and guanylin receptor GCs are localized together in apical plasma membranes of target enterocytes (12, 32). An intestinal receptor GC has been identified by molecular cloning of cDNAs encoding a membrane protein that binds 125I-labeled ST (41), and this protein also is activated by guanylin peptides and Escherichia coli ST (4, 11, 21, 41). When this GC gene is impaired in transgenic mice, a marked reduction in intestinal secretory responses of suckling mice was observed (37, 42). However, ~10% of specific 125I-ST binding to receptor sites on intestinal membranes still remained in GC-C-knockout animals (37). This suggests that an additional gene or multiple genes encoding receptors for guanylin peptides and ST exist in the mouse genome.

Bacterial STs were the first peptides shown to activate receptor-like GC signaling molecules in mammalian models, thus stimulating intestinal secretion via cGMP and causing secretory (i.e., traveler’s) diarrhea (9, 23). Our subsequent finding that E. coli ST also

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stimulates receptor GCs found on OK (opossum kidney) and PTK-2 (potoroo kidney) cell lines as well as in the opossum kidney revealed a broader physiological role for this class of cell surface receptors (13). "ST receptors" also were found in other epithelia in addition to the receptors localized to brush-border membranes (BBM) of epithelial cells lining the intestinal tract and within renal tubules (8, 14, 30, 47). The renal "ST receptor" predicted the existence of endogenous ST-like peptides that activate this enzyme, which led to the isolation of uroguanylin from urine (7, 21, 26). Uroguanylin is the major bioactive peptide found in urine, which contains very small amounts of bioactive guanylin (7, 21, 26, 38). Thus uroguanylin may regulate the enzymatic activity of these tubular receptors in vivo. Uroguanylin or ST stimulates urinary sodium, potassium, and water excretion in both the isolated perfused rat kidney and in the mouse in vivo (10, 17, 35).

Guanylin has less natriuretic and diuretic potency compared with either uroguanylin or ST, but guanylin does elicit a marked kaliuresis in the rat kidney in vitro (10). Uroguanylin mRNA is most abundant in the small intestine compared with guanylin mRNA levels, which peak in the large intestine of rats and mice (2, 36, 46). Uroguanylin and prouroguanylin are also found in the circulation, and the gastrointestinal (GI) tract may be a source of these peptides (6, 38). A natriuretic factor such as uroguanylin was postulated to exist in the digestive system as an explanation for the greater sodium excretion that occurs following a high-salt meal (33). The release of a natriuretic hormone like uroguanylin from the GI tract into the plasma in response to dietary NaCl may help explain the sustained increase in urinary sodium excretion that occurs following a high-salt meal (33).

This study investigates the renal mechanism of uroguanylin action by characterizing the structure and signaling activity of a membrane GC expressed in OK cells and in the opossum kidney. A 3,762-bp cDNA was isolated by molecular cloning and PCR utilizing OK cells and opossum kidney as sources of kidney mRNA/cDNA. This OK-GC cDNA contains an open-reading frame (ORF) encoding a 1,049-residue mature protein (ORF) encoding a 1,049-residue mature protein. This ORF encodes the uroguanylin receptor and portions of the 5'- and 3'-untranslated regions (UTR) (nucleotides 37–3415). The PCR product was verified as the cDNA encoding the opossum kidney receptor by nucleotide sequence analysis, before ligation into a mammalian expression vector pcDNA3.1/V5/His-TOPO (Invitrogen, San Diego, CA).

For transient transfection, COS-1 cells (1.35 × 10^6 cells in 150-mm dishes), maintained in DMEM with 10% FBS, 100 U/ml of penicillin, and 100 µg/ml of streptomycin, were...

**EXPERIMENTAL PROCEDURE**

RT-PCR amplification of a kidney uroguanylin receptor GC. Total RNA was extracted from various opossum tissues using the RNEasy Kit (Qiagen, Chatsworth, CA). Total RNA from opossum kidney (3–5 µg) was reverse transcribed with oligo(dT)18 primer using the Superscript II RNase H-Reverse Transcriptase kit (GIBCO-BRL; Life Technologies, Gaithersburg, MD). The cDNAs were then used for PCR reactions employing the protocol recommended in the instructions with the Superscript II kit. The PCR amplification reaction conditions were as follows: denaturation at 93°C for 5 min; then 30 cycles consisting of 93°C for 1 min, 59°C for 1 min and 72°C for 1.5 min, and then an extension period at 72°C for 10 min. A set of primers [GAAACCTCTCCGCCCAGAT and AC-G(A/T)ACTTAAAGGGAAGG] was used to amplify a segment of the opossum GC-C kinase-catalytic domain (nucleotides 2155–3130) mRNA/cDNA (8).

RACE-PCR. A 0.9-kb portion of an opossum kidney uroguanylin receptor GC (8) amplified by RT-PCR was extended in the 3' and 5' direction using rapid amplification of cDNA ends (RACE)-PCR. For 3'-RACE-PCR, total RNA was reverse transcribed as described above except that an oligo(dT)17 adapter (GACTCGAGTCGACATCGA) was used as the primer. The cDNAs were then amplified with a gene-specific primer (GAAACCTCTCCGCCCAGAT) and the adapter primer (GACTCGAGTCGACATCGA) using the conditions described above. The PCR product was cloned into pCR II, sequenced, and analyzed as previously described (36). For 5'-RACE-PCR, total RNA was reverse transcribed with a gene-specific primer (CAGTCAAACACCGAGAGTT or CTGATGGACACCTTTGAGAATGTTTCTGG) and the oligo(dT)17 adapter and adapter primer. The PCR product was then cloned into pCR II and sequenced.

Northern analysis of receptor mRNA expression. Total RNA (40 µg) from various opossum tissues were fractionated on a 1% agarose gel containing formaldehyde and then transferred to nylon membranes. The membranes were hybridized with a cDNA representing portions of the ligand-binding domain (nucleotides 130–631) and ligand-binding and kinase-homology domains (nucleotides 916–1810), which were labeled with [α-32P]dCTP by the random priming method (Boehringer Mannheim). A human β-actin cDNA (HHCl89; American Type Culture Collection, Rockville, MD) was also used in the hybridization to estimate RNA loading; however, this probe bound to two sizes of mRNA transcript (36). Total RNA from three different animals was examined on three separate blots, with the blots being hybridized twice with each probe.

The membranes were prehybridized for 1–3 h at 68°C with QuickHyb solution (Stratagene, La Jolla, CA), then hybridized for a further 14–16 h at 68°C with 1 × 10^6 cpm of labeled cDNA per milliliter of QuickHyb solution. The blots were washed twice with 2× SSC/0.1% SDS at room temperature followed by a 5- to 15-min wash with 0.2× SSC/0.1% SDS at 60°C. Blots were exposed to film at −80°C with an intensifyer screen from 3–72 h.

OK-GC expression. Specific primers were designed to amplify the entire ORF of the opossum kidney uroguanylin receptor and portions of the 3'- and 5'-untranslated regions (UTR) (nucleotides 37–3415). The PCR product was verified as the cDNA encoding the opossum kidney receptor by nucleotide sequence analysis, before ligation into a mammalian expression vector pcDNA3.1/V5/His-TOPO (Invitrogen, San Diego, CA).
transfected with 22.5 µg of the expression construct (pcDNA3.1-OK-GC) or with vector alone (pcDNA3.1) using the Fugene transfection reagent following the manufacturer’s instruction (Boehringer Mannheim). The cells were harvested 24 h after transfection and reseeded into 24-well plates (~10,000 cells/well) for cGMP accumulation assay. Cells were allowed to grow a further 72 h before bioassay for cGMP accumulation in response to guanylin (10 µM), uroguanylin (10 µM), and E. coli ST13 (1 µM).

For stable expression, HEK-293 cells were transfected with 5 µg of pcDNA3.1-OK-GC per 100-mm dish using the Fugene transfection reagent. The cells were maintained in MEM Earle’s base with 10% FBS, 1 mM nonessential amino acid, 1 mM pyruvate, 100 U/ml of penicillin, and 100 µg/ml of streptomycin. On the 4th day, the medium was supplemented with 400 µg/ml of Geneticin (G418, Gibco-BRL) to select for neomycin-resistant clones. The G418 selection was continued until isolated resistant colonies could be identified and expanded for determining OK-GC expression by cGMP accumulation bioassay (4, 21).

cGMP accumulation bioassay. Transiently transfected COS-1 cells, stably transfected HEK-293 cells or OK-E cells in 24-well plates were stimulated with 1 µM synthetic E. coli ST13 or 10 µM of synthetic opossum guanylin or uroguanylin (4, 21). In brief, these peptides were diluted in 150 mM DMEM containing 20 mM HEPES, pH 7.4, and 1 mM IBMX. The cells were washed with 200 µl DMEM before the addition of the peptides or media alone. The cells were incubated at 37°C for 40 min. After incubation, the media was removed for later radioimmunoassay (RIA), the cells washed with 200 µl PBS, and 200 µl of 3.3% perchloric acid was added to each well to stop the reaction and extract the cGMP from the cells. The extract was adjusted to pH 7 with 10 N KOH before 50 µl of the supernatant was used to measure cGMP by RIA.

Preparation of membranes. BBM preparations were made from opossum small intestinal mucosa, kidney medulla, and kidney cortex (1, 14). The tissues were homogenized for 1 min on ice with an Ultra-Turrax (Tekmar, Cincinnati, OH) in a solution containing 300 mM mannitol, 12 mM Tris·HCl, pH 7.4, 5 mM EGTA, 1 µM aprotinin, 1 µM leupeptin, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1 µM pepstatin A (7.5 mg tissue). The homogenate was centrifuged at 2,450 g. To the supernatant, 10.5 ml of ice-cold 12 mM MgSO4 was added, and the solution was stirred constantly on ice for 15 min. The homogenate was centrifuged at 31,000 g, and the pellet was resuspended in 15 ml of medium containing 20% glycerol, 300 mM mannitol, 1 mM MgSO4, 1 µM aprotinin, 1 µM leupeptin, 0.1 mM PMSF, 0.1 µM pepstatin A, and 50 mM Tris, pH 7.5, and stored frozen at –80°C.

Membranes were prepared from confluent T84 and OK cells, and from COS cells at 72 h posttransfection with pcDNA3.1-OK-GC or pcDNA3.1. The cells were washed four times with 10 ml ice-cold PBS and scraped into 1 ml of ice-cold lysis buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, 2 mM PMSF, 1 µg/ml leupeptin, and 1 µg/ml aprotinin). The cells were disrupted by sonication and centrifuged for 1 h at 4°C and 15,000 g. The cell pellet was suspended by homogenization in 250 µl of buffer (50 mM HEPES, pH 7.4, 10 µg/ml leupeptin, and 10 µg/ml aprotinin) and stored at –80°C.

Western analysis of receptor protein expression. Membrane protein concentrations were estimated using the Bradford protein assay (3). Fifty micrograms of kidney or intestinal BBMs and 150 µg of membranes from cultured cells were resuspended in sample buffer containing β-mercaptoethanol and boiled for 3 min prior to SDS-PAGE. The solubilized proteins (20 µl) were separated on a 7.5% acrylamide, 0.65% N,N′-methylenebisacrylamide gel and electrophoretically transferred to a polyvinylidene fluoride membrane (Immobilon; Millipore, Bedford, MD) in 50 mM Tris-glycine buffer, pH 8.5, containing 20% methanol. The membrane was washed initially with PBS/0.1% Tween 20, then blocked with blocking reagent for 1 h (Amersham Life Science, Buckinghamshire, UK). The membrane was rinsed with PBS/0.1% Tween 20 before incubation for 2.5 h at room temperature with the primary antiserum GCC-CTD (a polydonal antibody for the COOH-terminal domain of human GC-C) diluted 1:7,500 in PBS/0.1% Tween 20/0.2% bovine serum albumin. The membrane was washed three times with PBS/0.1% Tween 20, then incubated with a 1:5,000 dilution of a horseradish peroxidase-conjugated anti-rabbit antibody for 1 h at room temperature (Amersham). The membrane was washed further three times with PBS/0.1% Tween 20, and protein bands were detected using the enhanced chemiluminescence detection system (ECL; Amersham).

RESULTS

We used 3’- and 5’-RACE-PCR to extend the structure of a previously isolated cDNA that encodes part of the kinase homology and catalytic domains of a GC expressed in OK cells (8). By PCR, we have produced and cloned the OK-GC receptor cDNA of 3,762 bp that contains 76 bp of 5’-UTR, 3,195 bp of ORF, and 491 bp of 3’-UTR (Fig. 1). A consensus polyadenylation site, AAAAAA, is found at nucleotides 3734–3739 of this cDNA. The ORF encodes a 1,065-amino acid precursor protein containing a 16-residue putative signal peptide. The mature protein (OK-GC) is deduced to be 1,049 amino acids in length. OK-GC is similar to other membrane GC proteins in having an NH2-terminal, extracellular agonist-binding domain, a single lipo-philic transmembrane region and intracellular kinase homology, and GC catalytic domains (41). The predicted mature protein has 10 potential N-linked glycosylation sites and 8 cysteine residues in the 409-amino acid long extracellular domain. There are an additional 8 cysteine residues in the 619-amino acid long intracellular domain. The OK-GC protein appears to be a new member of the growing family of membrane receptors for guanylin peptides. OK-GC shares 72.4, 75.8, and 75.0% identity of primary structure with the BBM receptors for guanylin peptides. OK-GC shows 72.4, 75.8, and 75.0% identity of primary structure with the BBM receptors for guanylin peptides expressed in the rat, human, and pig intestine, respectively (Fig. 2; see Refs. 41, 43, 45). Consistent with the high conservation of primary structure between the catalytic domains of GCs is the similarity between the OK-GC catalytic region and guanylin receptors of rat, human, and porcine intestine, which share 92.2, 94.1, and 95.2% identity, respectively, with OK-GC (41, 43, 45). The most highly variable region of membrane receptor GCs occurs within the NH2-terminal ligand-binding domain of these proteins. The OK-GC protein shares only 55.4–58.6% identity in this domain compared with the intestinal receptors for guanylin peptides previously identified in other mammals (41, 43, 45). The intestinal guanylin/uroguanylin receptors of other mammalian species are more closely related than this, because the extracellular ligand-binding region of an intestinal receptor of rats shares 70.1 and 69.9% iden-
tity with a corresponding domain in the intestinal
receptors of human and pig intestine, respectively (41,
43, 45). Thus OK-GC may represent a distinct subtype
of receptor GC within the emerging family of cell-
surface receptor signaling molecules that interact with
and are controlled by the guanylin peptides.

The expression of OK-GC mRNA was examined in
tissues from opossums using Northern assays with
OK-GC cDNAs as the hybridization probes (Fig. 3). An
~3.8-kb mRNA was observed in total RNA prepara-
tions isolated from the kidney, OK cells, urinary blad-
ner, adrenal gland, heart, and intestinal mucosa indicat-
ing that the 3,762-bp OK-GC cDNA clone that we
isolated represents most of the OK-GC mRNA. The
subclonal cell line, OK-E, and the wild-type OK cell line
both express high levels of OK-GC mRNA, as does the
renal cortex of opossums. Lower levels of OK-GC
mRNA were detected in the corticomedullary junction
and medullary regions of kidney. Other tissues with
relatively low levels of OK-GC mRNA are the urinary
bladder, adrenal gland, and both the ventricle and atria
of heart. In the GI tract, high levels of OK-GC mRNA
were observed in the mucosa of duodenum, jejunum,
and ileum of small intestine and in the cecum and
colon. OK-GC mRNA was not detected in the stomach
mucosa and skin. Thus OK-GC mRNA is highly ex-
pressed in the kidney cortex and intestinal mucosa, with mRNA transcripts occurring at lower levels in the other tissues.

The OK-GC cDNA was transfected into COS cells for transient expression of the OK-GC protein. We observed that COS cells transfected with the OK-GC cDNA became responsive to stimulation with *E. coli* ST yielding cGMP accumulation responses to this peptide agonist (Fig. 4A). However, COS-1 cells transfected with the plasmid vector alone had no detectable cGMP responses to ST stimulation (Fig. 4A). Thus, we sought to establish an OK-GC-transfected cell line using HEK-293 cells as a model system. An HEK-293-C6 clonal subline was subsequently isolated following transfec-
tion with the OK-GC cDNA, and these cells exhibited substantial cGMP accumulation responses to uroguanylin, guanylin, and *E. coli* ST synthetic peptides (Fig. 4B). These agonists increased cGMP by 3.6- to 4.3-fold, at 10 µM concentration in HEK-293-C6 cells. There were no significant differences in estimated potencies between the peptides. However, ST appeared more potent than uroguanylin and guanylin, as seen previously in T84 and OK cell lines (8, 21, 26). This level of cGMP response in the HEK-293-C6 cells is considerably lower than that observed for these peptides in the OK-E cells (Fig. 4C). Concentrations of 10 µM guanylin and uroguanylin and 1 µM ST increased cGMP levels in confluent OK-E cells by 460-, 491-, and 421-fold, respectively. However, the rank order of potencies of ST > uroguanylin > guanylin was similar between OK-E and HEK-293-C6 cell lines expressing the OK-GC receptors. All of these synthetic peptides are full agonists for activation of OK-GC receptors expressed in HEK-293-C6 cells or for activation of the native receptor GCs found in OK-E cells.

We further characterized the OK-GC receptor protein using Western blot assays with a polyclonal anti-
sera that interacts with a conserved portion (amino acids 1011–1073; Fig. 3) of the human intestinal receptor GC-C protein (expressed as a fusion protein with glutathione S-transferase) used to raise the GC-C-CTD polyclonal antibody. We found that the antibody recognized the OK-GC protein, which shares 73% identity with the human GC-C protein in this region. GC-C-CTD, a polyclonal antibody for the COOH-terminal domain of human GC-C. Single dots and double dots are GCG descriptions of relative similarity.

Fig. 2. Comparison of amino acid sequences deduced from OK-GC cDNA (OKGC) with human intestinal GC-C (HUGCC). The GCG program (Wisconsin University Genetics Computer Group v8.0) was used to deduce the amino acid sequence of the 3,762-bp OK-GC cDNA that was cloned from the opossum kidney cortex. This sequence was compared with the published sequence for the human GC-C protein and was found to share 75.8% identity over the entire ORF. Human GC-C protein (expressed as a fusion protein with glutathione S-transferase) used to raise the GC-C-CTD polyclonal antibody is indicated by the shaded region. OK-GC protein shares 73% identity with the human GC-C protein in this region. GCG-CTD, a polyclonal antibody for the COOH-terminal domain of human GC-C. Single dots and double dots are GCG descriptions of relative similarity.
protein from opossum kidney BBMs and opossum intestinal BBMs that react with this antisera. The immunoreactive proteins found in COS-1 cells expressing the OK-GC receptor activity have a 160-kDa band of a size similar to that found in the opossum kidney and intestinal BBMs and T84 and OK cell membranes. This antisera also reacts with a 140-kDa protein that is relatively abundant in OK and T84 cell membranes and opossum small intestinal BBM, with a much lower level seen in kidney BBM. The 160- and 140-kDa bands may represent differentially glycosylated forms of the OK-GC receptor protein as previously reported for human and rat membrane receptor GCs (39, 44). The 140-kDa band may be undetectable in the kidney medulla BBM and COS-1 cells transiently expressing OK-GC, because the amount of protein present may be below the detection level. The OK-GC protein expressed in COS-1 cells migrates on SDS-PAGE similarly to the mobilities of the native receptor proteins found in either of the cell culture models or in the guanylin/uroguanylin target tissues. The apparent protein level for OK-GC in the COS-1 cells is lower than the expression of OK-GC in either intestine or renal cortical BBM or the cultured cell models.

**DISCUSSION**

Our findings are consistent with the hypothesis that the OK-GC protein is a renal tubular receptor for uroguanylin and/or guanylin peptides, which participates in a cGMP signaling pathway for regulation of the urinary excretion of sodium, potassium, and water (10, 17, 35). OK-GC is the first kidney receptor for the guanylin family peptides to be defined at a molecular level. Prior to the identification of receptor GC signaling molecules for *E. coli* ST in the OK and PTK-2 cells and in the opossum kidney (13, 14, 30, 47), it was thought that ST-stimulated GCs were restricted to the intestinal mucosa (19). Thus identification of receptor activities for OK-GC in OK cells and opossum kidney (13, 14, 30, 47) opened a new field of inquiry that culminated recently with the discoveries of guanylin, uroguanylin, and lymphoguanylin peptides (4, 11, 21). These peptides activate OK-GC and may serve as endogenous agonists for this membrane receptor GC (11, 21). The OK cell line has functional properties like those of renal proximal tubules, and receptor autoradiography experiments with $^{125}$I-ST have clearly shown that specific binding sites for this uroguanylin-like radioligand are found in cells of the convoluted and straight portions of proximal tubules in opossum kidney (8, 14, 30). High levels of these $^{125}$I-ST-labeled receptors are also found in BBMs isolated from the kidney and intestine, indicating that this receptor GC is preferentially localized to apical plasma membranes of both kidney and intestinal target cells (14, 47). It is likely that OK-GC serves as a physiological receptor for uroguanylin, which is the major bioactive peptide found in opossum urine (21). However, guanylin was also isolated from opossum urine, indicating that this pep-
tide may influence renal function in vivo via cGMP (21). Active uroguanylin and inactive prouroguanylin peptides circulate in plasma, thus providing a source for the urinary forms of bioactive uroguanylin in opossum, human, and rat urine (7, 21, 26). The kidney also expresses uroguanylin and guanylin mRNAs, which offers the possibility that an intrarenal signaling pathway exists for the guanylin peptides as well as providing another potential source of uroguanylin in the urine (2, 8, 46). Physiologically, uroguanylin may regulate kidney function via an endocrine axis linking the intestine to the kidney and/or through an intrarenal paracrine mechanism. Both possibilities involve the activation of OK-GC as a key signaling molecule in renal tubular target cells that possess the GC-cGMP signal transduction machinery in opossum kidney (13, 14, 30, 47).

Although more than 10 years have elapsed since the initial report that opossum kidney responds to a uroguanylin-like peptide with increased cGMP (13), the general concept of uroguanylin/cGMP-mediated regulation of kidney function has been slow to be accepted as a physiological mechanism that applies to all mammals. In part, this may be associated with using the North American opossum, Didelphis virginiana, as an animal model and OK cells as a cell model for these studies (8, 13, 14, 30, 47). Following the isolation of cDNAs encoding the intestinal receptors for guanylin family peptides, it was reported that mRNAs encoding these receptors are not detected in rat kidney (41). This suggested that rat kidney was not a target for guanylin peptides. Clearly, the rat, mouse, and opossum kidneys...
all respond to treatment with either E. coli ST, uroguanylin, or guanylin with marked increases in urinary sodium, potassium, and water excretion indicating that uroguanylin/guanylin receptors are present in the kidneys of both major classes of extant mammals (10, 13, 14, 17, 30, 35, 47). If eutherian (placental, e.g., rat, mouse) and metatherian (marsupial, e.g., opossum) classes of mammals have functional receptors in the kidney that respond to guanylin family peptides, then it is quite clear that genes encoding these guanylin/uroguanylin receptors existed prior to the evolutionary divergence of placental and marsupial mammals from ancestral mammals that lived ~130 million years ago (24). Moreover, the guanylin and uroguanylin genes are also present in the genomes of both metatherian and eutherian mammals (4, 6, 21, 22, 48). Although certain differences between mammalian species may occur in some aspects of the uroguanylin-cGMP signaling pathway in the kidney, it is likely that the broader physiological features of this mechanism occur in the kidneys of all mammals. Thus we propose that the opossum kidney and cultured OK cell lines are useful experimental models that can provide new insights into the renal mechanism of action of uroguanylin and guanylin as novel regulatory peptides that influence kidney function via the intracellular second messenger, cGMP.

OK-GC may be a new member of an emerging family of receptor GCs that interact with guanylin peptides. The primary structure of the ligand-binding domain of OK-GC shares only 55.4–58.6% identity with the corresponding domain in the intestinal form of guanylin receptors in rats, humans, and pigs (41, 43, 45). In contrast, the ligand-binding domains of the intestinal receptors within these eutherian mammals share ~70% identity. This extracellular portion of the membrane GCs is the most highly variable domain, which accounts for the selectivity of these receptors for different peptide agonists. For example, the receptors for guanylin peptides do not interact with atriopeptins, and atriopeptin receptor GCs are not activated by the guanylin (5). OK-GC may be a second member of an emerging family of receptors for the guanylin peptides, in addition to the intestinal guanylin receptors that were identified previously (41, 43, 45). Other evidence supports the hypothesis that multiple genes exist in the mammalian genome that encode additional receptors for guanylin peptides. Transgenic mice with disabled genes for an intestinal receptor GC have no intestinal fluid secretion responses to E. coli ST in vivo, yet intestinal BBMs isolated from these guanylin receptor-knockout animals still retain specific binding sites for 125I-ST, a uroguanylin-like peptide agonist (37). These 125I-ST binding sites associated with intestinal BBMs are likely to represent another membrane receptor GC and physiological target for the guanylin peptides. Other molecular evidence supports the interpretation that at least two genes are present in the mammalian genome that encode receptors for the guanylin peptides. A partial cDNA encoding part of the kinase homology and catalytic domains of a membrane GC that is closely related to an intestinal form of guanylin receptor was isolated from the GI mucosa of rats (36). However, a 53-amino acid deletion in this receptor within the region linking the kinase homology and catalytic domain, along with several other differences in the primary structure of the deduced protein, suggests that this membrane GC expressed in the GI tract is derived from a novel gene. The cloning and molecular identification of OK-GC provides an opportunity to identify the second gene product that serves as a receptor for the guanylin peptides in either eutherian or metatherian species. Two different receptor GCs with different agonist selectivity properties, combined with the heterogeneity exhibited by the presence of guanylin, uroguanylin, and lymphoguanylin genes found in the opossum, provide for a physiologically complex signal transduction pathway for cGMP-mediated control of cell and organ function in this mammalian animal model (4, 11, 21).

Expression of the OK-GC mRNA in the intestinal mucosa at levels equivalent to those found in renal cortex suggests that this signaling protein may be the major receptor GC of both small and large intestine of opossums. Studies using receptor autoradiography and in vitro cGMP responses of isolated mucosa to the guanylin peptides with opossums as well as other mammals, and in birds and reptiles, has revealed that the guanylin/uroguanylin/ST receptors have their greatest concentration in the proximal small intestine, and receptor levels then decrease progressively along the longitudinal axis of the small and large intestine (27–29). Receptor autoradiography, in situ hybridization, and immunohistochemistry experiments are in general agreement that the guanylin peptide receptors also exhibit a gradient of concentration along the crypt-villus axis (27–29, 34, 39). The highest apparent level of receptors appears to be on the lower one-third of the villi of small intestinal cells and within the crypts of large intestine. It is likely that these receptors are involved in the tissue responses to this family of enteric peptides resulting in a paracrine influence of guanylin on the transepithelial secretion of chloride and bicarbonate anions during digestion (18, 25, 31). Thus OK-GC in the opossum may be a physiological counterpart to the receptor GC, GC-C, that has been identified by molecular cloning in the rat, human, and porcine intestine (41, 43, 45). Regulation of intestinal fluid secretion together with bicarbonate secretion-dependent neutralization of the highly acidic contents within the lumen of the duodenum and cecum are prominent physiological actions of this enteric paracrine mechanism and intracellular cGMP signaling pathway in the intestinal tract.

Cell-specific expression of the receptors for guanylin family peptides is also observed in the opossum kidney and testis with the highest levels found in cells comprising the pars recta and convoluted portions of the renal proximal tubules and in seminiferous tubules of testis (8, 14, 30). These high-density receptor sites in the nephron of the opossum kidney, as detected by receptor autoradiography and tissue cGMP response assays, are consistent with the high levels of OK-GC mRNA detected in the renal cortex compared with the mRNA
abundance found in corticomedullary and medullary regions of the kidney. These studies do not rule out the possibility of the existence of a separate and distinct receptor for the guanylin peptides that may also be expressed in renal tubules. Receptor autoradiography reveals a relatively high density of 125I-ST binding sites in the cells of the pars recta, which reside in the corticomedullary region of the kidney (8, 14, 30). The relatively low levels of OK-GC mRNA compared with the putative high receptor activity in this anatomical region is of interest. This apparent paradox could be explained by the expression of a receptor gene different from that of OK-GC in the corticomedullary region of the opossum kidney. Additional studies will be required to determine whether the receptor sites labeled by 125I-ST in vitro in various regions of the nephron can be quantitatively accounted for by the expression of the OK-GC receptor GC in opossum kidney.

In conclusion, OK-GC is the first kidney receptor GC that serves as a cell-surface receptor for guanylin peptides to be identified at a molecular level. Its expression in the kidney provides a key signaling molecule and physiological target for uroguanylin and/or guanylin acting as renal hormones in vivo. Uroguanylin and guanylin may serve to regulate the renal excretion of NaCl and water as a physiological means of maintaining salt and fluid balance in the body secondary to the consumption of meals containing excess salt. This postulated mechanism could have both an intestinal-to-kidney endocrine axis and/or an intrarenal paracrine signaling pathway, which ultimately influences the urinary excretion of sodium, potassium, and water via the intracellular second messenger, cGMP.

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