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Regulation and therapeutic targeting of peptide-activated receptor guanylyl cyclases

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Abstract

Cyclic GMP is a ubiquitous second messenger that regulates a wide array of physiologic processes such as blood pressure, long bone growth, intestinal fluid secretion, phototransduction and lipolysis. Soluble and single-membrane-spanning enzymes called guanylyl cyclases (GC) synthesize cGMP. In humans, the latter group consists of GC-A, GC-B, GC-C, GC-E and GC-F, which are also known as NPR-A, NPR-B, StaR, Ret1-GC and Ret2-GC, respectively. Membrane GCs are activated by peptide ligands such as atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), guanylin, uroguanylin, heat stable enterotoxin and GC-activating proteins. Nesiritide and carperitide are clinically approved peptide-based drugs that activate GC-A. CD-NP is an experimental heart failure drug that primarily activates GC-B but also activates GC-A at high concentrations and is resistant to degradation. Inactivating mutations in GC-B cause acromesomelic dysplasia type Maroteaux dwarfism and chromosomal mutations that increase CNP concentrations are associated with Marfanoid-like skeletal overgrowth. Pump-based CNP infusions increase skeletal growth in a mouse model of the most common type of human dwarfism, which supports CNP/GC-B-based therapies for short stature diseases. Linaclotide is a peptide activator of GC-C that stimulates intestinal motility and is in late-stage clinical trials for the treatment of chronic constipation. This review discusses the discovery of cGMP, guanylyl cyclases, the general characteristics and therapeutic applications of GC-A, GC-B and GC-C, and emphasizes the regulation of transmembrane guanylyl cyclases by phosphorylation and ATP.

Keywords

Hypertension; Diarrhea; Vision; Heart failure; Glaucoma; cGMP

1. Introduction

Cyclic GMP was first purified and identified in rat urine in 1963 (Ashman et al., 1963), but guanylyl cyclases (GCs), the enzymes that catalyze the conversion of GTP into cGMP, were not discovered until 1969 (Hardman & Sutherland, 1969; Schultz et al., 1969; White & Aurbach, 1969). Unlike adenylyl cyclases, which were discovered as a result of

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investigations into the mechanism of glucagon action, guanylyl cyclases were discovered before any factor was known to activate these enzymes.

Initial experiments revealed distinct soluble and particulate GC isoforms (Kimura & Murad, 1974; Chrisman et al., 1975). Subsequent Nobel prize-winning studies determined that nitric oxide is the major ligand for soluble GC (Murad, 2006). Particulate GC diversity was determined in the late 1980s and early 1990s when individual family members were purified and cloned. The first mammalian membrane-spanning GC molecularly cloned was GC-A (Fig. 1) (Chinkers et al., 1989; Lowe et al., 1989). GC-A is activated by atrial natriuretic peptide (ANP), the natriuretic factor in atrial extracts that was originally described by de Bold et al. (1981) (Fig. 2). Matsuo et al. purified ANP and a second cardiac peptide called, B-type natriuretic peptide (BNP) that also activates GC-A (Sudoh et al., 1988). GC-B was identified by homology cloning a year later (M. S. Chang et al., 1989; Schulz et al., 1989). It was initially suggested to be the receptor for BNP but was later shown to preferentially bind the third and final member of the natriuretic peptide family, called C-type natriuretic peptide (CNP) (Koller et al., 1991). A decoy receptor, called natriuretic peptide receptor-C (NPR-C) was the first natriuretic peptide receptor cloned (Fuller et al., 1988), but NPR-C is not a cyclase and primarily clears natriuretic peptides from the circulation (Matsukawa et al., 1999). GC-C was cloned in 1990 and shown to bind heat stable enterotoxin (ST) (Schulz et al., 1990; de Sauvage et al., 1991). Later, guanylin and uroguanylin were identified as endogenous intestinal peptides that activate GC-C (Currie et al., 1992; Hamra et al., 1993). Individual cDNAs for two retinal GCs known as Ret-GC-1 or GC-E and Ret-GC-2 or GC-F were identified in the 1990s (Shyjan et al., 1992; Yang et al., 1995). Later, two small cytoplasmic, calcium-binding guanylyl cyclase activating proteins (GCAPs) 1 and 2 were shown to activate the retinal cyclases in the presence of low but not high calcium concentrations (Palczewski et al., 2004). No extracellular-binding peptides have been shown to activate GC-E or GC-F. GC-D and GC-G were identified in rodents by molecular cloning (Fulle et al., 1995; Schulz et al., 1998), but these cyclases are pseudogenes in humans (Manning et al., 2002).

2. Overview of mammalian transmembrane guanylyl cyclases

2.1. General topology and extracellular domain

The common structural and regulatory features of the human guanylyl cyclases will be initially described then the individual receptors and their activators will be discussed in more detail in subsequent sections.

Human membrane GCs are encoded by a single polypeptide chain of about 1050 amino acids. The basic topology consists of an extracellular ligand-binding domain, a single membrane spanning region, and intracellular kinase homology, dimerization and guanylyl cyclase domains. The minimal catalytic unit is a homodimer. The approximate 500 residue extracellular region is the least conserved domain, consistent with ligand-directed evolution of this portion of the receptors (Schoenfeld et al., 1995). The extracellular domains contain multiple intramolecular disulfide bonds and are glycosylated on asparagine residues (Koch et al., 1994; Fenrick et al., 1997; Ghanekar et al., 2004; Hasegawa et al., 1999, 2005; Miyagi

et al., 2000). The role of glycosylation in ligand binding is controversial but most data indicate that it is required for proper folding but is not directly involved in ligand binding.

2.2. Transmembrane domain

A transmembrane domain of 21 to 25 hydrophobic amino acids bisects the extracellular and intracellular portions of these receptors. Amino acid identity in this region is low and no activating or inactivating mutations have been identified in this domain (Potter, 2005).

2.3. Kinase homology domain

The kinase homology domain (KHD) contains about 250 residues, displays similarity to protein kinases and is essential for transducing ligand binding activation signals from the extracellular to catalytic domains. Receptors lacking KHDs exhibit maximal cyclase activities that are unresponsive to ligand, which suggest that the KHDs repress guanylyl cyclase activity in the absence of ligand (Chinkers & Garbers, 1989; Koller et al., 1992; Deshmane et al., 1997). Prolonged exposure of GC-A and GC-C to ligands causes a shift from high to low affinity binding and this affinity shift is not observed in receptors lacking the KHD (Jewett et al., 1993; Deshmane et al., 1997).

The sequence identity of KHDs to protein kinase domains is low (Potter, 2005). No guanylyl cyclase KHD has greater than 25% identity with proteins that have demonstrated phosphotransferase activity. GC-A contains a GXGXXXG sequence, which is similar to the GXGXXXG ATP binding motif found in subdomain I of most protein kinases. Subdomain I is the least similar between guanylyl cyclases and known protein kinases. Studies by Sharma et al. indicated that mutations to this region severely inhibited the activation of GC-A and GC-B (Duda et al., 1993a, 1993b). However, we demonstrated that these mutations reduced the phosphorylation state of GC-B (Potter & Hunter, 1998a). More importantly, two independent groups found that the mutation of the GXG motif to AXA in GC-A had no effect on activation of GC-A (Koller et al., 1993; Antos & Potter, 2007). Furthermore, GC-C, GC-E and GC-F are also activated by ATP and they completely lack this Gly-rich region and show no similarity with protein kinases in subdomain I (Fig. 3). Hence, the majority of data indicate that the GXGXXXG motif is not required for ATP-dependent activation of GC-A or GC-B.

The highest degree of homology between guanylyl cyclase KHDs and protein kinase catalytic domains is in subdomains VI, VII, VIII and IX. However, the KHDs are missing the glutamate in subdomain VI that serves as the catalytic base in known protein kinases. Thus, the KHDs are either devoid of protein kinase activity or have evolved a unique mechanism for phosphate transfer. As described below, GC-E was shown to possess intrinsic protein kinase activity, but other researchers failed to demonstrate autophosphorylation of GC-A (see below) (Larose et al., 1992; Wong et al., 1995; Aparicio & Applebury, 1996). Alanine substitutions for highly conserved residues within the KHD of GC-A resulted in receptor mutants that were incompletely glycosylated and not phosphorylated, which suggests that proper processing of the KHD is required for normal maturation and function of GC-A (Koller et al., 1993).

2.4. Dimerization domain

A 50–70 residue stretch that separates the kinase homology and guanylyl cyclase domains is called the hinge or dimerization domain. This region is conserved between all five human transmembrane guanylyl cyclases and is almost invariant within the natriuretic peptide and retinal subfamilies (Potter, 2005). Primary amino acid sequence suggests that it forms an amphipathic α helix, a domain known to mediate protein–protein interactions. Chinkers and Wilson (1992) showed that this region is sufficient to mediate dimerization and Thompson and Garbers (1995) found that deletion of the dimerization domain abolishes the catalytic activity of soluble intracellular versions of GC-A (Wilson & Chinkers, 1995). Dominant mutations in the dimerization domain of GC-E constitutively activate this receptor, which leads to autosomal dominant cone-rod dystrophy (Ramamurthy et al., 2001; Smith et al., 2007). However, recent mutagenesis data challenge the coiled-coil structure of the dimerization domain of GC-A and GC-C and suggest that the primary role of this domain is to suppress cyclase activity in the absence of ligand (Saha et al., 2009).

2.5. Guanylyl cyclase domain

The minimal catalytic unit of a particulate guanylyl cyclase is a homodimer. No structural data are available for mammalian guanylyl cyclase domains but the structure of an active homodimeric GC catalytic domain from cyanobacteria and an inactive heterodimeric GC catalytic domain from green algae were solved and indicate that the catalytic unit is highly related to the catalytic domain of adenylyl cyclase (Rauch et al., 2008; Winger et al., 2008). Both guanylyl and adenylyl cyclases are members of the class three purine nucleotidyl cyclase family (Linder & Schultz, 2002). Each guanylyl cyclase domain contains a central seven-stranded β sheet surrounded by several α helices. The dimer has a wreath-like structure. The central cleft is formed at the dimer interface and contains two symmetric active sites. Each monomer provides residues that are critical for catalysis. Each active site in the guanylyl cyclase from cyanobacteria binds GTP and ATP with similar affinities. Catalytic specificity of the cyanobacterial form appears to result from increased turnover of GTP compared to ATP (Rauch et al., 2008; Winger et al., 2008). Mutagenesis studies converting human GC-E and rat soluble guanylyl cyclase to adenylyl cyclases are consistent with mammalian guanylyl cyclases adopting similar structures and catalytic mechanisms as observed in guanylyl cyclases from lower organisms (Sunahara et al., 1998; Tucker et al., 1998). Positive cooperative kinetics was obtained for all membrane guanylyl cyclases when measured in the presence of non-ionic detergent and Mn^{2+} GTP as substrate, which is consistent with the two catalytic site structural models. However, linear kinetics were observed when activities were measured under biologic conditions containing Mg^{2+} GTP, natural peptide ligand and no detergent, consistent with a model employing a single active site and a regulatory site (Antos & Potter, 2007).

2.6. Regulation by phosphorylation

The KHDs of GC-A, GC-B and GC-E are highly phosphorylated and the C-terminal tail of GC-C is phosphorylated. Five serine and two threonine phosphorylation sites were identified in rat GC-A and five of these sites were identified in human GC-A (Fig. 3) (Potter & Hunter, 1998b; Schroter et al., 2010; Yoder et al., 2010). Four serine and two threonine

phosphorylation sites were identified in rat and human GC-B (Potter & Hunter, 1998a; Yoder et al., 2010). Two and four phosphorylated serines were identified in bovine and murine GC-E, respectively (Bereta et al., 2010). Five of the seven total sites are conserved in GC-A and GC-B. Although the phosphorylation sites in GC-E are in the same general location as those in GC-A and GC-B, only two sites (S530 and S533) align with sites in GC-B and only one site aligns with sites in GC-A. The phosphorylated region is highly conserved between GC-A and GC-B, but only displays limited similarity with the same region of GC-E and has essentially no similarity with GC-C (Fig. 3).

GC-A and GC-B are maximally phosphorylated in the absence of natriuretic peptides and prolonged exposure of receptors to natriuretic peptides in whole cells or exposure to receptors in crude membranes to protein phosphatase 2A causes reductions in receptor phosphate content that is correlated with losses in natriuretic peptide-dependent but not detergent-dependent, guanylyl cyclase activity (Potter & Garbers, 1992; Koller et al., 1993; Potter, 1998; Joubert et al., 2001). The ANP-dependent dephosphorylation results primarily from reduced phosphorylation not increased dephosphorylation (Joubert et al., 2001). Receptors containing individual alanine substitutions for phosphorylation sites are less responsive to natriuretic peptides and mutation of four or more sites to alanine resulted in hormonally unresponsive receptors. In contrast, receptors containing glutamate substitutions for the same phosphorylation sites were activated by natriuretic peptides, although to a much lower extent than the wild type receptors (Potter & Hunter, 1998a, 1998b, 1999; Bryan & Potter, 2002). The glutamate substituted version of GC-A is resistant to ANP-dependent inactivation in membrane or whole cell preparations, which supports desensitization by dephosphorylation model (Potter & Hunter, 1999). PKA inhibitors were reported to block the desensitization of GC-A to ANP (Muller et al., 2006).

PMA-dependent activation of protein kinase C also causes the dephosphorylation and inhibition of GC-A and GC-B and the protein kinase C inhibitor, GF-109203X, blocks the PMA-dependent dephosphorylation and inhibition (Potter & Garbers, 1994; Potter & Hunter, 2000). Unlike the natriuretic peptide-dependent dephosphorylation process, PKC activation causes increased phosphorylation of one site and decreased phosphorylation of another site (Potter & Hunter, 2000). Consistent with the increased phosphorylation of GC-A, purified PKC was shown to phosphorylate GC-A in vitro (Duda & Sharma, 1990; Larose et al., 1992). However, neither the sites of phosphorylation nor the effect of phosphorylation on the activity of the receptor was reported. Agents or conditions that elevate intracellular calcium concentrations cause GC-B dephosphorylation in a manner associated with dephosphorylation of all sites (Potthast et al., 2004; Abbey-Hosch et al., 2005). PMA exposure increased the EC_{50} and K_m of GC-B eight- and three-fold, respectively, whereas calcium ionophore, ionomycin, primarily decreased V_{max} (Abbey-Hosch et al., 2005). A recent study reported that the calcineurin inhibitor, cyclosporin, blocks calcium-dependent dephosphorylation of GC-A (Fortin & De Lean, 2006). In contrast, we were unable to block calcium-dependent inhibition of GC-B with calcineurin inhibitors or dominant negative constructs in various cell lines (Robinson et al., unpublished data).

The Kuhn group identified a new GC-A phosphorylation site (S487) and used multiple reaction monitoring to demonstrate that phosphorylation of this residue increases

approximately 9-fold in response to ANP exposure. They found that a mutant form of GC-A containing a glutamate substitution for S487 did not desensitize in response to ANP (Schroter et al., 2010). Working separately, we also identified this site in rat and human forms of GC-A. However, we found that GC-A mutants containing alanines or glutamates at this position desensitized similarly to the wild type receptor (Yoder et al., 2010).

The sites that are phosphorylated in GC-A, GC-B and GC-E are not conserved in GC-C and GC-C was not basally phosphorylated when isolated from 293 cells (Vaandrager et al., 1993b). However, GC-C was basally phosphorylated on serines when isolated from Caco2 cells, but the level of receptor phosphorylation was unchanged in cells exposed to ligand (Ghanekar et al., 2003). Unlike GC-A and GC-B that are dephosphorylated and inhibited in response to PKC activation, phorbol ester exposure increases the phosphate content and maximum activation of GC-C by ST (J. K. Crane & Shanks, 1996). Mutation of Ser-1029, a PKC consensus site (SYK), to Ala abolished the ability of PMA to activate GC-C, consistent with PKC-dependent phosphorylation of GC-C at Ser-1029 (Wada et al., 1996).

GC-E is the only guanylyl cyclase that has been shown to possess phosphotransferase activity. GC-E purified from bovine retina was cross-linked to 8-N₃(α -³²P)ATP in a manner that was competitive with ATP but not GTP. Interestingly, the binding of the adenine nucleotide was greater in the absence than in the presence of magnesium (Aparicio & Applebury, 1996). This same purified GC-E preparation contained a protein kinase activity that phosphorylated exogenous substrates as well as serines on GC-E. The kinase activity was dependent on magnesium. However, the stoichiometry of phosphate to receptor resulting from an in vitro kinase assay was low (0.05), suggesting that only a small portion of the receptor preparation was active or that low concentrations of a separate protein kinase contaminated the preparation. Recently, Bereta et al. (2010) identified two phosphorylation sites in the KHD of bovine GC-E and four phosphorylated serines in mouse GC-E by mass spectrometry (Fig. 3). Unlike GC-A and GC-B, mutation of the phosphorylation sites in GC-E to either alanine or glutamate failed to block cyclase activation by guanylyl cyclase activating proteins (Bereta et al., 2010). Mutation of the putative magnesium binding residues in GC-E yielded dephosphorylated receptors, which was interpreted to support an autophosphorylating activity of GC-E. However, magnesium binding to the wild type receptor and reduced magnesium binding to the mutant receptor were not demonstrated (Bereta et al., 2010). As described above, multiple charge to alanine mutations in the KHD of GC-A, including a residue homologous to the putative magnesium binding site in GC-E, caused incomplete glycosylation and phosphorylation of GC-A (Koller et al., 1993).

2.7. Regulation by ATP

ATP modulates guanylyl cyclase activity of human GC-A, GC-B, GC-C and GC-E in broken cell preparations through at least three mechanisms (Kurose et al., 1987; Gazzano et al., 1991a; Yamazaki et al., 2003). First, ATP is the source of the phosphate (substrate) that is covalently attached to the receptors, and the addition of ATP to membranes or purified preparations increases the phosphorylation state GC-A, GC-B and GC-E (Aparicio & Applebury, 1996; Foster & Garbers, 1998; Joubert et al., 2001; Abbey-Hosch et al., 2005). Secondly, ATP is an allosteric activator of GC-A, GC-B, GC-C and GC-E. Thirdly, at high

concentrations, ATP competes for GTP binding at the catalytic site. To distinguish the allosteric effects from the phosphorylation effects, the ATP analog AMPPNP was used because it has an N instead of an O connecting the β and γ phosphates and is not a substrate for protein kinases. To emphasize the kinase-dependent effects, ATP- γ -S was used because thiophosphorylated proteins are resistant to dephosphorylation. GC-A is more highly phosphorylated when ATP- γ -S is included in the preparation and preincubation of membranes with ATP- γ -S generates a stable and active form of the enzyme (C. H. Chang et al., 1990; Foster & Garbers, 1998). These observations are consistent with the rank order of adenine nucleotide activators of GC-A being ATP- γ -S > ATP > AMPPNP $\gg$ AMP (C. H. Chang et al., 1990; Chinkers et al., 1991; Gazzano et al., 1991b; Kurose et al., 1987). Phosphorylation-independent activation of GC-A by adenine nucleotides was first demonstrated by showing that ATP and AMPPNP are equivalent activators of a mutant form of GC-A containing glutamate substitutions for the first six identified phosphorylation sites (Potter & Hunter, 1999).

It is important to keep in mind that older studies on adenine nucleotide regulation of GC-A were conducted before the critical role of phosphorylation on enzymatic activity was appreciated. Hence, phosphatase inhibitors were not included in receptor preparations and ATP or ATP- γ -S, not AMPPNP, was used as the activating adenine nucleotide. Hence, the effect of ATP on receptor phosphorylation was exaggerated in these studies (C. H. Chang et al., 1990; Chinkers et al., 1991; Gazzano et al., 1991b; Kurose et al., 1987; Marala et al., 1991).

ANP was originally shown to activate GC-A in the absence of ATP (Waldman et al., 1984; Winkquist et al., 1984). De Lean (1986) first demonstrated that ATP decreases ANP binding to GC-A. Shortly thereafter, Kurose et al. reported that ATP enhances ANP-dependent GC-A activity in membranes from various rat tissues (Kurose et al., 1987). The effect of ATP on basal activities was equivocal in this study. Subsequent investigations indicated that ATP increases cyclase activities measured in the presence or absence of ANP but reduced activities measured in the presence of Mn^{2+} GTP and nonionic detergent (C. H. Chang et al., 1990; Gazzano et al., 1991b). Since AMPPNP also increased activity, Kurose and coworkers originally suggested that ATP directly binds GC-A and causes it to undergo an allosteric change that increases its catalytic activity. Other groups also observed that ATP, and especially ATP- γ -S, activated GC-A in the presence and absence of ANP but concluded that ATP binds an unidentified GC-A activating protein because solubilized receptors, purified receptors or washed membranes were less responsive to ATP than unsolubilized, unpurified or unwashed preparations (C. H. Chang et al., 1990; Gazzano et al., 1991b). In retrospect, this loss of activity is best explained by receptor dephosphorylation that occurred during purification. Similar activation of membrane and highly purified preparations of GC-A by ANP and ATP provided evidence that ATP directly binds GC-A (Wong et al., 1995).

The vast majority of early studies showed that either ANP or ATP alone increased cyclase activity of GC-A but when added together, ANP and ATP resulted in synergistic receptor activation (C. H. Chang et al., 1990; Chinkers & Garbers, 1989; Gazzano et al., 1991b; Jewett et al., 1993; Kurose et al., 1987; Song et al., 1988). However, two later reports suggested that ATP is absolutely required for activation of GC-A and GC-B (Chinkers et al.,

1991; Marala et al., 1991). Based on these data, we proposed a model where ANP binding to the extracellular domain causes a conformational change in the KHD that facilitates ATP binding, which ultimately brings the catalytic domains together (Potter & Hunter, 2001). In support of this model, Joubert et al. (2005) observed that GC-A was photoaffinity labeled by an azido-biotin-conjugated form of ATP in membranes isolated from ANP exposed but not naive cells. The labeled construct lacked the cyclase domain, consistent with ATP binding to the KHD. However, unlike ATP, the azido compound inhibited GC-A. In a separate study, Burczynska et al. (2007) used UV radiation to cross-link 8-azido-ATP to a GC-A KHD purified from bacteria. Mass spectrometric methods determined that the ATP analog was covalently linked to the peptide, S⁶³¹SNC⁶³⁴V⁶³⁵VDGRC, at the numbered residues. A mutant receptor containing double Trp substitutions for C⁶³⁴V⁶³⁵ demonstrated reduced activation by ATP compared to the wild type receptor, although it was still activated two-fold. We also analyzed the role of this region by mutating the conserved ⁶³⁰KSS region to AAA and found this mutant receptor was synthesized but had no guanylyl cyclase activity, so we were unable to assess the contribution of this region to the ATP-dependent activation of GC-A (Antos & Potter, 2007). Based on a previous report identifying a critical residue in the KHD of GC-C (Bhandari et al., 2001), we mutated a conserved Lys (535) in subdomain II to alanine and found that this mutation blocked ATP-dependent activation of GC-A (Antos & Potter, 2007). Recently, Duda et al. (2010) reported that staurosporine, a competitive inhibitor of ATP binding to many protein kinases, substitutes for ATP in the activation of GC-A. In fact, staurosporine activated GC-A to similar levels as AMP-PNP in their assay. We repeated this experiment and observed activation with AMP-PNP, but failed to observe any stimulatory effect of staurosporine on the guanylyl cyclase activity of GC-A (Robinson & Potter, data not shown).

Based on the current literature, there are three putative intracellular ATP binding sites in GC-A: one in the KHD and the two sites in the catalytic domain. Despite well-planned studies, experiments on soluble intracellular GC-A constructs have not shed light on the domain responsible for the ATP-dependent activation. Joubert et al. (2007) observed that ATP inhibited cyclase activity of a GC-A construct containing only a dimerization and catalytic domain with an IC₅₀ of 3.3 mM, which is consistent with ATP competing for one of the GTP catalytic sites. Pattanaik et al. (2009) found that ATP inhibited soluble GC-A constructs containing either the whole intracellular domain or just the dimerization and catalytic domains, and unlike the full length receptor, inhibition was observed regardless of whether cyclase activity was measured in the presence of Mg²⁺ or Mn²⁺. Thus, normal ATP activation has not been observed in soluble intracellular versions of GCA, possibly because these soluble, truncated molecules have lost the normal repression that occurs in the absence of ligand.

We reported that initial rates of GC-A and GC-B cyclase activity were elevated 100-fold by ANP in 293 cell membranes in the absence of adenine nucleotides (Antos, Abbey-Hosch, Flora, & Potter, 2005). AMPPNP had no effect at very early activation times, but did increase activity at later times consistent with a model where ATP stabilizes the ANP activated form of the enzyme but is not required for activation. Washing the membranes to remove ATP did not reduce receptor activation. However, preparing membranes in the presence of phosphatase inhibitors, especially microcystin, and assaying activity in the

presence of 1 mM as opposed to 0.1 mM GTP concentrations increased natriuretic peptide dependent activation 100-fold (Antos et al., 2005). Similar ATP-independent activation of GC-A and GC-B endogenously expressed in various cells lines was observed (Antos & Potter, 2007). Furthermore, we found that the EC₅₀ for ATP and the magnitude of the activation were decreased when guanylyl cyclase activities were measured in the presence of 1 mM versus 0.1 mM GTP (Antos & Potter, 2007). We also found that AMPPNP dramatically reduced the apparent K_m while having little effect on the V_{max}, which differs from previous reports measuring the effect of ATP or ATP-γ-S on the cyclase activity of GC-A (Kurose et al., 1987; Gazzano et al., 1991b). However, as mentioned above, these older studies were conducted with adenine nucleotides that are substrates for kinases (ATP or ATP-γ-S) and on GC-A preparations lacking phosphatase inhibitors. Thus, we believe that they were primarily measuring effects of ATP on changes in the phosphorylation state of GC-A, which we have shown reduces the maximal velocity of the enzyme. The differences between our observations and those from other groups are likely explained by four experimental details: 1) preparing membranes in phosphatase inhibitors including microcystin, 2) measuring activity for short time periods, 3) measuring activity in presence of 1 mM GTP with only 1 mM free Mg²⁺ Cl₂, since increased Mg²⁺ increases protein phosphatase 2C activity and GC-A dephosphorylation (Bryan & Potter, 2002), and using AMPPNP, not ATP or ATP-γ-S as the stimulatory adenine nucleotide.

ATP also increases ligand-stimulated guanylyl cyclase activity of GC-C (Gazzano et al., 1991a; Vaandrager et al., 1993a, 1993b), but the effect of ATP on basal activities was equivocal (Gazzano et al., 1991a; Vaandrager et al., 1993b). ATP increased maximal velocities of ST-stimulated GC-C without significant effects on the S_{0.5} (Gazzano et al., 1991a). Unlike GC-A, ST activation of GC-C has not been reported to depend on the presence of adenine nucleotide. The rank order of adenine nucleotide activators for GC-C is ATP-γ-S>ATP=AMPPNP>AMP (Gazzano et al., 1991a; Vaandrager et al., 1993b). Vaandrager et al. suggested that ATP-dependent stimulation does not result from a direct activation of the enzyme, but rather from the stabilization of an active state or an inhibition of the deactivation process (Vaandrager et al., 1993a,b, 1994). Additional studies by this group demonstrated that ATP increased activity of immunopurified GC-C, consistent with direct binding of ATP to the receptor (Vaandrager et al., 1993b). In the presence of Mn²⁺, adenine nucleotides activate and inhibit GC-C by regulating two unique sites (Parkinson et al., 1994). Additional kinetic studies suggested that ATP binding inhibits GC-C catalytic activity by occupying a GTP allosteric site (Parkinson & Waldman, 1996). The mutation of Lys-516 in the KHD of GC-C, which is homologous to Lys-535 in GC-A, reduced ligand-dependent activation of GC-C, and a monoclonal antibody against residues 491 to 568 that binds the wild type receptor in the absence but not presence of ATP failed to bind the K516A mutant form of GC-C, consistent with ATP causing a conformational change in the KHD of GC-C (Bhandari et al., 2001).

ATP has been reported to increase GC-E cyclase activity in the presence and absence of GCAPs. An early study found that ATP increased GC-E activity two-fold in bovine rod outer segments. The stimulatory effect was also observed with AMPPNP and was not synergistic with GCAP (Gorczyca et al., 1994). A separate study conducted in bovine rod outer segments found that ATP increased basal cyclase activity several fold and that AMPPNP and

ATP- γ -S were more potent than ATP (Aparicio & Applebury, 1996). Similar to GC-C, ATP was shown to block thermal inactivation of GC-E and this protective effect was not due to changes in phosphorylation because AMPPNP was more effective than ATP (Tucker et al., 1997). However, the same group also observed an additional slow stimulatory role of adenine nucleotides that was additive with the stimulatory effect of GCAP-2. A separate report found that preincubation with AMPPNP increased GCAP-2 activation of GC-E 10–13 fold compared to a 3–4 fold activation observed in membranes that were not preincubated with AMPPNP (Yamazaki et al., 2003). Thus, the majority of data indicate that ATP increases GC-E activity by a mechanism that is independent of phosphorylation and is additive, not synergistic, with GCAP stimulation. The lack of a significant phosphorylation contribution to the ATP-dependent activation of GC-E is consistent with GC-E phosphorylation not regulating activation by GCAPs (Bereta et al., 2010).

3. Guanylyl cyclase-A

3.1. Basic characteristics

GC-A is the best-characterized membrane guanylyl cyclase. The order of preference of natriuretic peptide dependent activation of GC-A is: ANP > BNP >> CNP (Bennett et al., 1991; Koller et al., 1991; Suga et al., 1992). The extracellular domain of GC-A contains three intramolecular disulfide bonds and is highly glycosylated on asparagine residues (Miyagi & Misono, 2000; Miyagi et al., 2000). GC-A binds ANP with a stoichiometry of 2:1 (Rondeau et al., 1995). Ligand binding does not increase receptor oligomerization but does bring the juxtamembrane regions of each monomer closer together (Labrecque et al., 2001). Crystal structure data indicate that the GC-A extracellular domains associate in a head-to-head or A-shaped manner and that each receptor monomer uses different residues to bind ANP (Ogawa et al., 2004). Structures from bound and unbound receptors indicate that ANP binding causes a counter clockwise rotation of the juxtamembrane region of the receptor (Ogawa et al., 2004), but how this motion is transmitted to the catalytic domains is not known. Down regulation of GC-A by prolonged ligand exposure is controversial. Some groups find that GC-A is rapidly internalized while other groups find that GC-A is not internalized at all (Vieira et al., 2001; Pandey, 2005). We recently found that ANP increases GC-A degradation in a variety of cell lines (Flora & Potter, 2010) and that the internalization rate of GC-A is dramatically affected by cellular environment, which may explain the disparate results of previous investigators (Dickey et al., submitted for publication).

3.2. Physiologic functions of GC-A

GC-A is a pleiotropic signaling molecule that stimulates many physiologic responses involving many tissues. GC-A is highly expressed in kidney, lung, adrenal, vasculature, brain, liver, endothelial, and adipose tissues (Bryan et al., 2006; Potter et al., 2006). It is expressed at lower but significant levels in the heart. Most physiologic functions of GC-A are associated with processes that reduce cardiac load. For instance, GC-A stimulates vasorelaxation, natriuresis, diuresis, endothelial permeability and inhibits the renin–angiotensin pathway. Considerable data regarding non-cardiovascular functions like lipolysis and immune cell functions have been reported as well (Ladetzki-Baehs et al., 2007;

Lafontan et al., 2008). A few reports suggest that GC-A selectively inhibits the proliferation of cancer cells (Vesely et al., 2004; Kong et al., 2008).

A great deal has been learned about the physiologic function of GC-A using knockout mice or mice selectively lacking expression or overexpressing GC-A in specific tissues. GC-A null animals exhibit cardiac hypertrophy, high blood pressure and ventricular fibrosis and are not responsive to ANP or BNP (Lopez et al., 1995; Oliver et al., 1997). GC-A null animals show diminished ability to compensate for pressure-induced heart failure, which is consistent with a primary role for GC-A in the compensation response associated with the initial stages of this disease (Knowles et al., 2001). Cre-lox mediated inactivation of GC-A in smooth muscle abolished the acute hypotensive effect of ANP but had no effect on chronic blood pressure (Holtwick et al., 2002). Data from a variety of under- and overexpression murine models indicates that GC-A exerts a direct inhibitory effect on cardiac growth (Kishimoto et al., 2001; Knowles et al., 2001; Holtwick et al., 2003). Mice lacking endothelial GC-A are slightly hypertensive and demonstrated a severely blunted hypovolemic response to ANP (Sabrane et al., 2005).

3.3. ANP, Anaritide and Carperitide

All natriuretic peptides are synthesized as preprohormones that are processed to smaller mature forms containing an obligate C-terminal 17-amino acid disulfide ring structure (Fig. 2). Mature human ANP is a 28 amino acid disulfide-linked peptide released from atrial granules. Atrial wall stretch resulting from increased intravascular volume causes the release of ANP into the circulation. Genetic ablation of ANP results in hypertensive mice with cardiac hypertrophy (John et al., 1995, 1996).

Synthetic forms of ANP have been examined as potential treatments for several diseases. The peptide lacking the first three residues of ANP is called Anaritide. This peptide causes natriuresis and diuresis in healthy volunteers (Cody et al., 1986). The effects of Anaritide were diminished in patients with congestive heart failure. Blunted effects of the full-length form of ANP known as Carperitide were also observed in patients with congestive heart failure (Saito et al., 1987). However, statistically significant increases in renal function were observed in patients receiving Anaritide in later studies (Fifer et al., 1990). Carperitide was approved for the treatment of acute decompensated heart failure in Japan in 1995. Carperitide and Anaritide were also examined as potential therapeutic treatments for several renal diseases but various clinical trials revealed that they did not improve patient outcome (Allgren et al., 1997; Kurnik et al., 1998; Lewis et al., 2000; Rahman et al., 1994; Sward et al., 2005).

3.4. BNP and nesiritide

The mature 32-residue form of human BNP is released in greatest concentrations from the ventricles of the heart as a result of increased chamber stretch accompanying volume overload. It is transcriptionally regulated and is not stored in granules in the ventricles. Human serum BNP concentrations in healthy adults are about an order of magnitude lower than ANP concentrations. Disruption of BNP increases ventricular fibrosis but does not affect blood pressure in mice (Tamura et al., 2000).

Serum BNP levels increased in proportion to the severity of heart failure (Ruskoaho, 2003). The high dynamic range and consistency of response make serum BNP concentrations an ideal biomarker for cardiac stress. Millions of point-of-care BNP measurements are conducted every year to predict heart failure severity in cardiovascular patients worldwide (Ruskoaho, 2003).

Nesiritide is human BNP synthesized recombinantly in *E. coli*. Based on positive results from the Vasodilation in the Management of Acute Congestive Heart Failure (VMAC) trial, nesiritide, marketed as Natrecor, was approved for the treatment of acute decompensated heart failure in the United States in 2001 (Investigators, 2002). However after impressive initial sales, the utility and safety of nesiritide were questioned. The most devastating reports were those from the Sackner-Bernstein group that used meta-analysis to conclude that nesiritide worsened renal function and increased the likelihood of death (Sackner-Bernstein et al., 2005; Sackner-Bernstein et al., 2005). The FUSION (Follow-Up Serial Infusion of Nesiritide) I and II trials were specifically designed to investigate the potential adverse renal effects of nesiritide in patients with advanced heart failure. In contrast to Sackner-Bernstein and colleagues, they found no evidence of decreased renal function or early death, but also found little clinical benefit of the drug (Yancy, 2004; Yancy et al., 2008).

3.5. CD-NP, B-CDNP and CDNP-B

Research focusing on the development of designer natriuretic peptides that maintain the beneficial renal effects in the absence of negative hypotensive effects is progressing. One approach to improve the clinical profile of natriuretic peptide-based drugs is to generate molecules that are more susceptible to proteolysis in the vasculature and more resistant to proteolysis in the kidney, which would decrease vasorelaxation leading to hypotension and increase fluid and salt excretion, respectively. One such molecule is CD-NP, a chimeric designer peptide consisting of full length CNP fused to the carboxyl tail Dendroaspis natriuretic peptide (DNP) (Fig. 2). DNP is an ANP homolog purified from the venom of the green mamba that activates GC-A. Initial studies in canines suggest that CD-NP retains the beneficial renal effects of BNP while being substantially less hypotensive (Lisy et al., 2008). Biochemical studies indicated that CD-NP binds GC-B about ten-fold less tightly and GC-A about two-hundred fold more tightly than CNP (Dickey et al., 2008). Interestingly, the increased binding affinity of CD-NP for GC-A was not observed with the rat receptor due to its increased ability to be activated by high concentrations of CNP. Recent studies have shown that CD-NP is much less susceptible to proteolytic degradation compared to ANP, BNP or CNP (Dickey and Potter, submitted). Reduced degradation of a 12-residue C-terminally extended form of ANP identified in patients with familiar atrial fibrillation, suggests that increasing the C-terminal tails of natriuretic peptides may be a general mechanism to reduce proteolytic degradation (Dickey et al., 2009). Whether reduced in vitro degradation of CD-NP translates into increased half-life in vivo is not known. To further increase the potency of CD-NP for GC-A, we converted two different triplet sequences within the CNP ring to their corresponding residues in BNP (Fig. 3) (Dickey et al., 2008). Both variants demonstrated increased affinity and full agonist activity for GC-A, whereas one (B-CDNP) was as potent as any GC-A activator known. Similar single amino acid

mutations increased potency of BNP for GC-B (Dickey et al., 2010). The physiologic properties of these designer natriuretic peptides are under investigation.

4. Guanylyl cyclase-B

4.1. Basic characteristics

GC-B has a topology similar to GC-A. The order of preference of GC-B for natriuretic peptide is: $CNP \gg ANP = BNP$ (Bennett et al., 1991; Koller et al., 1991; Suga et al., 1992). Like GC-A, it contains three intramolecular disulfide bonds and is highly glycosylated on asparagine residues (Fenrick et al., 1996, 1997). Forced dimerization causing reduced distances between the juxtamembrane regions of the two GC-B monomers leads to constitutive activation (Langenickel et al., 2004). Molecular modeling based on ANP bound GC-A, suggests that CNP binds to GC-B in an asymmetric manner with a stoichiometry of 1:2 (He et al., 2006; Yoder et al., 2008). GC-B is abundantly expressed in the brain, lung, bone, heart and ovary tissue (Chrisman et al., 1993; Nagase et al., 1997; Schulz et al., 1989). It is also expressed at relatively high levels in fibroblast and vascular smooth muscle cells. GC-B is expressed at higher concentrations in homogenized heart tissue than GC-A but the cellular expression pattern of GC-B in this tissue is unclear (Dickey et al., 2007). Studies on the down regulation of GC-B have not been reported, but it has been suggested that mutations in GC-B that lead to dwarfism cause inappropriate receptor trafficking (Hume et al., 2009).

4.2. Physiologic functions of GC-B

The most obvious function of GC-B is to stimulate endochondral ossification, which leads to long bone growth (Yasoda et al., 1998). Inactivation of GC-B in two unique mouse models caused dwarfism, and in one model female sterility was observed (Tamura et al., 2004; Tsuji & Kunieda, 2005). Inactivating mutations in cGMP-dependent protein kinase II also produce dwarfed mice or rats, suggesting that mammalian long bone growth requires a $CNP > GC-B > PKGII$ signal transduction pathway (Chikuda et al., 2004; Pfeifer et al., 1996). Mice expressing one wild type and one inactive GC-B allele were significantly shorter than the wild-type animals. Human homozygous GC-B loss of function mutations cause acromesomelic dysplasia, type Maroteaux, a rare form of disproportional dwarfism (Bartels et al., 2004). No reports indicate that these patients are sterile, but like the “knock-out” mice, individuals with one normal and one abnormal allele display normal limb proportions but are statistically shorter than the average person from their respective populations (Olney et al., 2006). In mice, GC-B and cGMP-dependent protein kinase-I (PKG I) are essential for sensory axon bifurcation in the spinal cord of mice (Schmidt et al., 2007). Whether the same scenario exists in humans or other mammals is not known. Regarding CNP and GC-B in reproduction, a recent report suggest that CNP secretion from follicle granulosa cells maintains oocytes in meiotic arrest by activating GC-B in adjacent cumulus cells (Zhang et al., 2010).

4.3. CNP

CNP exists in 22 and 53 amino acid forms that lack residues that are carboxyl to the disulfide ring (Fig. 3) (Koller et al., 1991; Suga et al., 1992). Neither the short or long forms

of CNP are significantly stored in granules. The longer form is primarily found in tissues, whereas the short form is most often found in fluids (Minamino et al., 1991; Togashi et al., 1992). CNP does not circulate at high levels, rather it signals in a paracrine manner. At physiologic or pathologic concentrations CNP is not natriuretic despite its name.

Transgenic overexpression of CNP causes elongated bones in mice (Yasoda et al., 2004). Furthermore, chromosomal translocations that increase CNP concentrations are associated with Marfanoid like skeletal overgrowth in humans (Bocciardi et al., 2007; Moncla et al., 2007). Genome-wide association studies found that elevated CNP expression was associated with increased height in northern European populations (Estrada et al., 2009). Mice lacking the natriuretic peptide clearance receptor have longer than normal bones (Jaubert et al., 1999; Matsukawa et al., 1999) and a meta-analysis of genome-wide stature studies identified an association with NPR-C and stature (Soranzo et al., 2009), which is consistent with increased or decreased degradation of CNP by NPR-C modulating human height. Recently, CNP infusions were shown to increase long bone growth in a murine model of fibroblast growth factor receptor-3-dependent dwarfism, which represents the most common form of human dwarfism (Yasoda et al., 2009). Together, these data indicate that 1) GC-B inactivation causes dwarfism, 2) that GC-B over activation causes Marfanoid-like skeletal overgrowth, and 3) that CNP rescues long bone growth in a murine model of the most common form of human dwarfism. Identifying small molecule inhibitors and activators of GC-B may lead to a new class of drugs for skeletal diseases.

Glaucoma is a leading cause of blindness that results from progressive optic neuropathy and retinopathy caused by increased intraocular pressure. CNP and GC-B are the most highly expressed natriuretic peptide and guanylyl cyclase-linked natriuretic peptide receptors in the retina. CNP has been shown to reduce intraocular pressure for hours to days and to protect retinal ganglion cells from apoptotic damage (Fernandez-Durango et al., 1999; Ma et al.; Takashima et al., 1998). Eye drops containing lens permeable and degradation resistant forms of CNP represent a promising therapeutic approach for the treatment of glaucoma.

5. Guanylyl cyclase-C

5.1. Basic characteristics of GC-C

GC-C has similar domain architecture to GC-A and GC-B but contains an additional 60-residue carboxyl terminal extension that renders the receptor detergent insoluble. Deletion of this extension yields a GC-C variant that is unresponsive to ST (Wada et al., 1996). GC-C contains four intramolecular disulfide bonds (Hasegawa et al., 2005) and is glycosylated on eight extracellular asparagines (Hasegawa et al., 1999). GC-C exists as a higher ordered structure; dimeric (Vaandrager et al., 1993b) or trimeric forms have been reported (Vaandrager et al., 1994). Cyclase activity of soluble intracellular fragments synthesized in bacteria was higher in trimeric as opposed to dimeric complexes (Vijayachandra et al., 2000). Ligand binding does not increase oligomerization of GC-C (Rudner et al., 1995).

Down regulation of GC-C has been studied in several cell systems. Prolonged exposure of T84 cells to ST dramatically reduced ST-dependent GC-C guanylyl cyclase activity in a manner that was not explained by GC-C down regulation (Bakre & Visweswariah, 1997;

Urbanski et al., 1995). The V_{max} of the cyclase was decreased when measured in the presence of Mg^{2+} GTP or Mn^{2+} GTP (Bakre et al., 2000). The cellular desensitization mechanism was independent from the ATP-sensitive in vitro inactivation mechanism described by Vaandrager et al. and was only observed in Caco2 cells that endogenously express GC-C but not in 293 cells exogenously expressing GC-C (Bakre et al., 2000; Vaandrager et al., 1993b). Prolonged ST exposure caused greater losses in ligand-dependent cyclase activities than were explained by GC-C degradation in the Caco2 cells (Ghanekar et al., 2003). In contrast to the T84 cells, prolonged ST exposure downregulated GC-C in Caco2 cells. However, only the completely glycosylated form of GC-C was degraded in response to prolonged exposure to ST and removal of ST was associated with increased levels of the completely glycosylated form of GC-C and increased responsiveness to ST (Ghanekar et al., 2003).

5.2. Physiologic functions of GC-C

GC-C is primarily found highly concentrated at the apical membrane of epithelial cells throughout the intestine. Activation of GC-C by ST, guanylin or uroguanylin leads to intracellular cGMP elevations, PKGII-dependent phosphorylation of the cystic fibrosis transmembrane regulator and increased Cl^{-} secretion in the gut. Physiologic activation of GC-C prevents dehydration and obstruction of the intestine. Pathologic overactivation of GC-C by ST causes continuous Cl^{-} and water secretion into the intestine, which causes severe diarrhea that can lead to death. Mice lacking functional GC-C are resistant to ST infection and have increased proliferation of intestinal epithelial cells (Li et al., 2007; Mann et al., 1997; Schulz et al., 1997). In a clinical setting, GC-C is a marker for tumors of colon origin (Carrithers et al., 1994) and has been suggested to be a therapeutic target for colon cancer due to its ability to inhibit the proliferation of colon epithelial cells (Pitari et al., 2001, 2003; Shailubhai et al., 2000).

5.3. Heat-stable enterotoxin, guanylin, uroguanylin and Linaclotide

STs from pathologic strains of *E. coli* were the first natural ligands shown to activate membrane guanylyl cyclases (Fig. 2) (Field et al., 1978; Hughes et al., 1978). The target of these peptides is GC-C. Hence, it is also called the heat-stable enterotoxin receptor or STaR. The first endogenous ligand of GC-C was identified in 1992 (Currie et al., 1992). The mature form of the peptide called guanylin is a 15 amino acid peptide that contains two intramolecular disulfide bonds. A second 19 amino acid endogenous GC-C ligand with conserved disulfide bonds to guanylin was purified from urine and named uroguanylin (Hamra et al., 1993). Human guanylin and uroguanylin are about 50% identical. Reduced pH markedly increases affinity and reduces the EC_{50} of uroguanylin but not guanylin for GC-C (Hamra et al., 1997), which suggests that the guanylin peptides may have unique actions in different regions of the intestine. ST contains three disulfide bonds and its affinity for GC-C is 10 and 100 times higher than the affinity of uroguanylin and guanylin, respectively, which only have two disulfide bonds. The EC_{50} of activation of GC-C is much higher (μM) than the K_d for binding (nM), which may result from changing affinity states of GC-C for ligand or possibly by the physiologic and low temperatures used to conduct the activation and binding studies, respectively (Crane et al., 1992; Deshmane et al., 1995). Mice lacking functional guanylin display increased colonic epithelial cell proliferation

(Steinbrecher et al., 2002). In contrast, mice lacking uroguanylin display decreased ability to excrete an enteral NaCl load as well as salt-independent hypertension (Lorenz et al., 2003). Hence, one report suggests that uroguanylin is an antihypertensive factor in mice that signals in a GC-C-independent manner. Whether uroguanylin regulates enteral sodium in humans or other mammals is not known.

Linaclotide is a 13 residue truncated homolog of ST that contains two amino acid substitutions that maintain maximal potency while improving resistance to proteolytic degradation (Fig. 2) (Bharucha & Waldman). Research in humans and rodents indicates that Linaclotide increases intestinal transit time by stimulating intestinal chloride secretion. In addition, Linaclotide possesses anti-nociceptive properties that reduce visceral hypersensitivity and the number of abdominal contractions that occurs in response to colorectal distensions (Eutamene et al., 2010). A Phase IIa, placebo-controlled, two-week clinical trial found that Linaclotide improved frequency of spontaneous bowel movements and decreased abdominal pain in patients with irritable bowel syndrome and constipation (Johnston et al., 2009, 2010).

6. Conclusions

All human receptor guanylyl cyclases contain a large glycosylated extracellular domain, transmembrane region and intracellular kinase homology, dimerization and guanylyl cyclase domains. GC-A, GC-B and GC-C are activated by unique disulfide containing peptides that interact with the extracellular domain. The KHDs of GC-A and GC-B are highly phosphorylated and phosphorylation is required for peptide-dependent activation of these receptors. GC-E is also phosphorylated but phosphorylation is not required for activation of this receptor by GCAPs. ATP activates human receptor guanylyl cyclases through a phosphorylation-independent allosteric mechanism of undefined nature. In broken cell preparations, ATP also increases the activity of GC-A and GC-B by maintaining receptor phosphorylation status. All three receptors are drug targets. Some drugs are approved, some are in clinical trials, and some are in development. Natural activators of each receptor were tested for therapeutic effects and Carperitide and Nesiritide were approved for clinical administration. A variant of ST called Linaclotide is in late stage clinical trials for the treatment of irritable bowel syndrome and constipation. Current efforts are focusing on chimeric peptides that modify receptor selectivity and/or decrease peptide degradation. An unrealized pharmacologic opportunity is the identification of ATP-like small molecule activators of transmembrane guanylyl cyclases.

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Abbreviations

ANP	atrial natriuretic peptide
BNP	B-type natriuretic peptide

CNP	C-type natriuretic peptide
cGMP	cyclic guanosine monophosphate
GC-A	guanylyl cyclase-A
GC-B	guanylyl cyclase-B
GC-C	guanylyl cyclase-C
GCAP	guanylyl cyclase activating protein
NPR-C	natriuretic peptide receptor-C
ST	heat stable enterotoxin

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Fig. 1.

Schematic of human transmembrane guanylyl cyclases and their ligands. The structure and function of each cyclase is discussed in the text. Similarity of extracellular domain color represents primary amino acid sequence identity. The blue “P” indicates known phosphorylation sites. Abbreviations are: ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; GC-A, guanylyl cyclase-A; GCAPs, guanylyl cyclase activating proteins; GC-B, guanylyl cyclase-B; GC-C, guanylyl cyclase-C; GC-E, guanylyl cyclase-E; GC-F, guanylyl cyclase-F; Gn, guanylin; ST, heat-stable enterotoxin; Uro, uroguanylin.

Fig. 2.

Structure of natural and designer natriuretic peptides and peptides that activate GC-C. All sequences are human unless otherwise noted. Grey boxes indicate identical amino acids. Lighter grey boxes indicate structurally conserved amino acids. Red residues indicate substitutions that reduce degradation of ST. Dark black lines represent disulfide bonds. The triple disulfides over ST apply to ST and Linacлотide. The double disulfide bonds below uroguanylin apply to guanylin and uroguanylin. Abbreviations are: ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; DNP, Dendroaspis natriuretic peptide; ST, heat stable enterotoxin from *E. coli*.

HUMAN-GC-A	S	L	E	R	H	L	R	S	A	G	S	R	L	T	L	S	G	R	G	S	N	Y	G	S	L	L	T
RAT-GC-A	S	L	E	R	H	L	R	S	A	G	S	R	L	T	L	S	G	R	G	S	N	Y	G	S	L	L	T
HUMAN-GC-B	N	S	E	R	Y	H	K	G	A	G	S	R	L	T	L	S	L	R	G	S	S	Y	G	S	L	M	T
RAT-GC-B	N	S	D	R	Y	H	K	G	A	G	S	R	L	T	L	S	L	R	G	S	S	Y	G	S	L	M	T
MOUSE-GC-E	P	P	G	G	S	S	R	K	V	V	Q	G	S	R	S	S	L	A	T	R	S	A	S	D	I	R	S
COW-GC-E	P	H	G	G	N	S	R	K	V	A	Q	G	S	R	T	S	L	A	A	R	S	I	S	D	V	R	S
HUMAN-GC-C	N	E	T	N	H	V	S	L	K	I	D	D	D	K	R	R	D	T	I	Q	R	L	R	Q	C	K	Y

Fig. 3.

Phosphorylation sites in mammalian transmembrane guanylyl cyclases. Red S or T indicates phosphorylation sites that were chemically verified. Black background indicates identical residues in four or more cyclases. Grey background indicates similar amino acids in four or more cyclases. The alignment was initially made with Clustal W2 and then adjusted by hand to maximize alignment of charged residues.