Receptor guanylyl cyclases.

S K Wong, D L Garbers

Research Article

Three different guanylyl cyclase cell receptors are known, but others will likely be discovered within the next few years. The general function of these receptors appear to relate to the regulation of fluid volume or fluid movement. New receptors, or possibly the currently known receptors, therefore, may be discovered in areas of the body where fluid volume regulation is important. Such fluids whose volume or composition might be regulated by guanylyl cyclase receptors include synovial fluid, uterine/oviductal luminal fluid, follicular fluid, aqueous humor, cerebral spinal fluid, seminiferous tubule luminal fluid, epididymal luminal fluid, seminal plasma, and airway luminal fluid. The function of the heterodimeric forms of guanylyl cyclase appear to relate to a primary regulation of nitric oxide (or similar molecules) concentrations, which are in turn regulated by a Ca2+/calmodulin-sensitive nitric oxide synthase.

Find the latest version:

https://jci.me/115862/pdf

Receptor Guanylyl Cyclases

Stephen K.-F. Wong and David L. Garbers
Department of Pharmacology, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Introduction

Cyclic GMP is an intracellular signaling molecule that regulates ion channels, cyclic AMP concentrations by virtue of its effects on selective phosphodiesterases, and protein kinases to alter cellular processes and behavior. It is known to act as a signaling molecule in many different cells and it likely serves as a second messenger in virtually all cells. The synthesis of cyclic GMP from GTP is catalyzed by a family of cell surface and cytoplasmic receptors known as guanylyl cyclases (GC),[1] each of which is markedly activated by the binding of specific ligands. A number of different guanylyl cyclase receptors have been isolated and characterized, but it is likely that other members of this family remain to be identified, which will even further expand the general physiological significance of these enzyme/receptors. This review discusses the general molecular structure of the known guanylyl cyclases, their ligands and regulatory molecules, and their potential or known physiological significance. Other recent reviews on the guanylyl cyclase family include (1–3).

Forms of guanylyl cyclase

In the mammal, there are now six different forms of guanylyl cyclase known: three are found associated with plasma membranes, one is soluble, and the fifth and sixth are apparent members of the heterodimeric part of the family whose cellular distribution remains to be determined. The guanylyl cyclases can be currently divided into two groups based on their general structure and cellular distribution. One major group, which associates with the particulate fractions of cell homogenates, contains a subgroup of cell surface receptor guanylyl cyclases (sea urchin spermatozoa, GC-A, GC-B, GC-C) that are activated by extracellular peptides, and appear to span the plasma membrane once. An intracellular protein kinase–like domain on the amino-terminal side of the cyclase catalytic region is a signature domain of this subgroup (Fig. 1). Intracellular proteins capable of binding Ca^{2+} have been shown to regulate a second subgroup of guanylyl cyclases found associated with the particulate fractions of mammalian retina, and the ciliary membranes of the protozoans *Tetrahymena pyriformis* and *Paramecium tetraurelia* (Table I). The primary amino acid sequence for a member of this subgroup is not yet known, and

it therefore remains possible that these forms will closely resemble known plasma membrane receptor forms.

The second major group of guanylyl cyclases appears to exist as heterodimers containing heme as a prosthetic group (Fig. 1), and are activated by vasodilatory agents such as nitroprusside and nitroglycerin (4). One endogenous physiological activator, known as endothelium-derived relaxation factor (EDRF) is thought to diffuse from endothelial cells to vascular smooth muscle cells where it binds to a heterodimeric form of guanylyl cyclase, resulting in its activation, elevations of cyclic GMP, and relaxation of smooth muscle (5, 6). Although EDRF may exist as multiple entities, one active component appears to be nitric oxide (7), a short-lived molecule generated by nitric oxide synthase in response to an increase in intracellular Ca^{2+} (8).

At least one of the heterodimeric forms ($\alpha_1\beta_1$) appears to be found in the cytoplasm of various tissues. This enzyme is composed of 82-kD (α_1) and 70-kD (β_1) subunits that appear to be linked by interchain disulfide bonds (9). Molecular cloning of the cDNA encoding the lung α_1 and β_1 subunits (see [3] for review) has revealed that the carboxyl-terminal regions are homologous to each other, and also to the catalytic domain of the membrane form of guanylyl cyclase (Fig. 1). A β_2-subunit (10), an apparent partner of an unknown α-subunit, contains a potential site for geranylgeranylation; its cellular localization remains to be determined. Recently, the cDNA of a second α-subunit (α_2) was cloned, but its normal partner also is not yet known (11).

Particulate forms of guanylyl cyclase

(a) Retinal guanylyl cyclases. Guanylyl cyclases with different functions are associated with various cells that exist in the retina (12, 13); at least one appears to play a key role in phototransduction and is associated with rod outer segments. It has been suggested that a decrease in intracellular concentrations of Ca^{2+} activates a protein called recoverin that binds to and activates this form of guanylyl cyclase (14, 15). The primary structure of the rod outer segment guanylyl cyclase has not yet been determined, and purification procedures have resulted in apparently different proteins. One form purified from bovine rod outer segments has an apparent molecular mass of 110–115 kD (16), similar to the particulate forms of guanylyl cyclase discussed later. The specific activity of the purified retinal enzyme (100–700 nmol cyclic GMP formed/min per mg protein), however, is quite low when compared with other particu-

Extracellular

Figure 1. Schematic model for plasma membrane and heterodimeric forms of guanylyl cyclases. The plasma membrane form of guanylyl cyclase contains an extracellular ligand-binding region, a single membrane-spanning domain, a protein kinase–like domain, and a catalytic domain at the carboxyl terminus. The heterodimeric form contains two distinct subunits that are linked by intermolecular disulfide bonds. This form also contains an associated heme prosthetic group.

late forms of guanylyl cyclase (17), and it is likely therefore that either much of the enzyme in the retinal preparation is not active, or that there are significant protein contaminants within the purified preparation. Like the plasma membrane receptor forms discussed below, it demonstrates positive cooperative kinetics with respect to MeGTP, suggesting that structural similarities may exist between the plasma membrane forms and this rod outer segment guanylyl cyclase. Horio and Murad (18) also have purified a second, apparently distinct guanylyl cyclase (M_r = 60 kD) from the particulate fraction of bovine rod outer segments. The activity of this form is activated by sodium nitroprusside, suggesting that its structure may resemble the heterodimeric forms of the enzyme. Whether or not the retinal guanylyl cyclases discussed above function in phototransduction has not been determined. The eye, for example, also contains plasma membrane receptor forms of guanylyl cyclase in the ciliary process, some of which may function to regulate intraocular pressure (19).

(b) Tetrahymena and paramecium guanylyl cyclase. Guanylyl cyclase from the ciliary membranes of the unicellular protozoans Tetrahymena pyriformis (20) and Paramecium tet-

Table I. Different Forms of Guanylyl Cyclases

Guanylyl cyclase form	Cellular localization	Regulatory molecules	References
Retinal GC	Particulate	Ca^{2+}/recoverin	(14, 15, 18)
Tetrahymena GC	Particulate	Ca^{2+}/calmodulin	(20, 22)
Paramecium GC	Particulate	Ca^{2+}/calmodulin	(21, 22)
Sea urchin GC	Particulate	Egg peptides, resact	(17, 23, 24)
GC-A	Particulate	ANP, ATP	(19, 25, 26, 46)
GC-B	Particulate	CNP, ATP	(28, 33)
GC-C	Particulate	Heat-stable enterotoxins, ATP	(30–32, 66)
$GC_{(\alpha 1,\beta 1)}$	Soluble	Nitric oxide Nitro vasodilators	(5, 9, 60, 70)
$GC_{(\alpha 2,\beta ?)}$	?	?	(11)
$GC_{(\alpha ?,\beta 2)}$	?	?	(10)

raurelia (21) are distinct from the retinal guanylyl cyclases in that an increase in Ca^{2+} to micromolar concentrations activates them. The guanylyl cyclase from the cilia of paramecium is involved in ciliary beating of the protozoa and it appears to be regulated by a voltage-gated inward Ca^{2+} current (21). Guanylyl cyclases from both of these protozoans are regulated by calmodulin-like Ca^{2+}-binding modulator proteins, and the nature of these regulatory proteins has been discussed in a previous review (22).

(c) Plasma membrane/receptor guanylyl cyclases. Sea urchin sperm guanylyl cyclase has been shown to be a cell surface receptor for a peptide (resact) secreted from sea urchin eggs (23). This was the first plasma membrane form of guanylyl cyclase purified to apparent homogeneity (17) and its mRNA subsequently cloned (24). The sea urchin cDNA was then used to identify and isolate a mammalian cDNA encoding a guanylyl cyclase (GC-A) (25, 26), the first mammalian plasma membrane form of the enzyme to be cloned. GC-A was subsequently shown to be a cell surface receptor for a heart-derived peptide, atrial natriuretic peptide (ANP) (25, 27) (Table II). Subsequently, the mRNA for other apparent plasma membrane forms of guanylyl cyclase were found (GC-B, GC-C) (28–32). GC-B appears to be a cell surface receptor for C-type natriuretic peptide (CNP) (33) while GC-C has been suggested to be a cell surface receptor for heat-stable enterotoxins (STa) secreted by bacteria (30) (see below). CNP has been reported to exist within the central nervous system but not in other tissues (34), yet GC-B is found in many peripheral tissues (see below), suggesting that CNP is not the natural ligand for GC-B, that CNP is a ligand only within the central nervous system, that a peripheral ligand remains to be discovered, or that CNP is actually expressed in peripheral tissues (perhaps by neurons) and is the natural ligand in all cases.

Structure of plasma membrane forms of guanylyl cyclase

The above guanylyl cyclases, including the sea urchin enzyme, share common structural features (Fig. 1). The molecular mass of the various receptors appears to range between 110 and 180 kD (see [1] for review), and each appears to contain a

Table II. Structures of Known or Potential Guanylyl Cyclase Receptor Ligands

Ligand	Structure
rat ANP	SLRRSSCFGGRIDRIGAQSGLGCNSFRYRR
rat BNP*	NSKMAHSSSCFGQKIDRIGAVSRLGCDGLRLF
rat CNP	GLSKGCFGLKLDRIGSMSGLGC
Guanylin	PNTCEICAYAACTGC
Sta	NTFYCCELCCNPACAGCY

* BNP, brain natriuretic peptide.

single membrane spanning domain based on hydropathic analysis. These receptor guanylyl cyclases contain an extracellular ligand binding region, a stretch of $\sim$ 20 hydrophobic amino acids that represents the putative transmembrane domain, an intracellular region that contains a protein kinase–like domain highly homologous to the catalytic domains of protein kinases, and a cyclase catalytic domain within the carboxyl terminal regions. The plasma membrane guanylyl cyclase receptors are therefore analogous to single membrane spanning growth factor receptors such as the endothelial growth factor (EGF) or platelet-derived growth factor (PDGF) receptors, which have a cysteine-rich, extracellular ligand binding and an intracellular protein kinase domain (reviewed in [35]).

(a) Ligand-binding domain. The extracellular ligand binding domains are the most divergent regions within the particulate forms of the guanylyl cyclase family. Within each guanylyl cyclase subtype, however, the region is highly conserved across the species (> 95% amino acid identity exists between the rat, human, and mouse GC-A [36]). Rat GC-A and rat GC-B are only 43% identical across the extracellular domain and these two forms are < 10% identical with rat GC-C (30). This parallels the differences in the structure of ligands that activate these enzymes (Table II). STa has no apparent ability to bind to GC-A or GC-B, while neither ANP nor CNP activates GC-C (30).

Despite the above differences, there are common structural motifs. The region contains multiple cysteine residues (rat GC-A and GC-B contain six cysteine residues while rat GC-C has nine), and the positions of the cysteine residues relative to the putative transmembrane spanning region are highly conserved in GC-A, GC-B, and a third ANP-binding protein (ANP-C receptor) (37). Although it is not yet clear which of these residues form intracellular disulfide bonds, the cysteine residues are likely required for the proper folding of the ligand binding domain, as is the case with the EGF receptor, where ^{125}I-EGF is cross-linked to a region flanked by two cysteine-rich domains (38). With respect to the site of ligand binding, Duda and colleagues (39) have shown that a double mutation within the putative extracellular region results in a form of GC-A that contains enzyme activity but no ANP-binding activity. The mutations (Gln 338 to His 338, and Leu 364 to Pro 364) likely represented cloning artifacts, but when the mutant amino acids were converted to the "normal" ones, it was found that a single mutation or deletion at position 364 destroyed apparent ANP binding. The Leu to Pro mutation site is extracellular to the transmembrane domain and is to the amino-terminal side of two highly conserved cysteine residues.

The extracellular region of all members of the receptor-linked guanylyl cyclase family also contain potential N-linked glycosylation sites (six in rat GC-A, seven in rat GC-B, and eight in rat GC-C). Recent unpublished data of Vaandrager and Garbers using tunicamycin in cells expressing rat GC-C have suggested that all eight sites in GC-C are glycosylated.

(b) Transmembrane region. Except for a few minor variations of amino acid residues (5 out of 21), the transmembrane regions are very similar between GC-A and GC-B (28). The transmembrane region is more divergent in GC-C, where only 3 of the 21 amino acids are identical with GC-A or GC-B.

(c) Protein kinase–like domain. The protein kinase–like region spans $\sim$ 250 amino acid residues and is highly identical to the protein kinase domain of the PDGF receptor and of other protein kinases (24, 25). Of the 33 residues targeted by Hanks et al. (40) as conserved or invariant residues in protein kinases, the protein kinase–like region of GC-A contains 30 amino acid residues that correspond within the same relative positions (24). Despite this homology with the protein kinases, there is as yet no evidence for protein kinase activity associated with the guanylyl cyclase receptors. Deletion of this region in GC-A results in a receptor that binds ANP and continues to possess guanylyl cyclase activity, but the cyclase activity of the ANP receptor (GC-A) is no longer regulated by ANP, and it appears to be constitutively active, suggesting that the kinase-like region acts as a negative regulatory element (41).

(d) Cyclase catalytic domain. This region of the membrane forms of guanylyl cyclase are highly conserved between GC-A, GC-B, GC-C, sea urchin sperm guanylyl cyclase, and the COOH-terminal domain of the heterodimeric guanylyl cyclases. Thorpe and Morkin (42) have expressed a domain corresponding to the COOH-terminal 293 amino acids of GC-A in bacteria and obtained catalytic activity. Although the specific activity of the recombinant protein was low as compared to a full-length GC-A, this study provided direct evidence that the COOH-terminal domain contains the cyclase catalytic region, supporting previous work on deletion studies of the cytoplasmic region of GC-A (41). In addition, this region is also homologous with two internally homologous domains of adenylyl cyclase from bovine brain (43, for sequence comparison see [1]) and other types of adenylyl cyclases (44). In a recent study of Thorpe et al. (45), the catalytic domain of GC-A also was reported to function as a homodimer. When the catalytic region (primary amino acid sequence thought to be to the amino-terminal side of the catalytic domain and to the carboxyl side of the protein kinase domain also was included) was expressed as a deletion mutant devoid of the extracellular or protein kinase–like regions, enzyme activity was detected. When subsequently analyzed on gel filtration columns, the M_r was reported as a value double that of the monomer (45); it should be noted, however, that the protein actually migrated faster on gel filtration columns than bovine serum albumin, where the M_r would be lower than $\sim$ 68,000. Also, it was not determined whether or not monomer existed, and therefore it remains to be determined whether enzyme activity is dependent on monomer dimerization. Thompson and Garbers (unpublished) have performed similar studies and have found no evidence, as of yet, for a monomeric form, therefore still leaving open the possibility that a monomer, if found, will possess cyclase catalytic activity.

(e) Regulation of plasma membrane forms of guanylyl cyclase by adenine nucleotides. ATP is known to potentiate the ANP-induced activation of GC-A in membrane preparations

from various tissues (46). The EC_{50} for activation by ATP is around millimolar, which lies in the range of intracellular ATP concentrations. Therefore, changes in intracellular concentrations of ATP may directly modulate the activation of GC-A by ANP. With the recombinant GC-A expressed in Sf9 cells (47), activation of GC-A is dependent on the presence of both ATP and ANP, and neither significantly stimulates enzyme activity alone. ATP also potentiates the ligand-induced activation of GC-B (Potter and Garbers, unpublished) and of GC-C (48). The role of ATP, in terms of signal transduction, does not appear to be that of a substrate for transphosphorylation, since ANP activates the receptor guanylyl cyclase in the presence of nonhydrolyzable analogues such as AMP-PCP or AMP-PNP (46), although the activation is considerably less (25–50%) than that produced by ATP. The efficacy of ATP to mediate the ANP activation appears to be related to the degree of hydrophobicity at the γ-phosphate group, since both ATPγS and caged-ATP (ATP analogue in which a nitrophenyl group has been attached to the γ-phosphate of ATP) activate GC-A with greater efficacy than ATP (49).

The requirement for an adenine nucleotide is analogous to the requirement of guanine nucleotides for the activation of adenylyl cyclase, except that in this system, hormone receptor increases the binding of GTP to a separate, downstream GTP-binding protein, G_s. The activated G_s in turn activates yet another protein, adenylyl cyclase, to produce the intracellular second messenger cyclic AMP (50). Moreover, ATP has been reported to decrease the binding of ANP to GC-A (51), which is highly reminiscent of the attenuation of agonist binding by GTP in GTP-binding protein-coupled receptors such as the β-adrenergic receptor (52). One major difference between the adenylyl cyclase and the guanylyl cyclase system is that the nonhydrolyzable analogues of GTP activate adenylyl cyclase through activation of G_s in the absence of hormone-liganded receptor, but ATP or its nonhydrolyzable analogues do not appear to activate guanylyl cyclase in the absence of ANP (47). This suggests that the ligand binding region and the ATP-binding component of guanylyl cyclase are tightly coupled in a functional sense.

The identity of the ATP-binding component has not been determined. The ability of GC-A to be activated by ATP and ANP is sensitive to a variety of metal ions (53), detergents, and washing procedures (54). Although these results have led to speculation that the ATP-binding regulatory component is a separate protein (54), there is as of yet no report of successful reconstitution to restore the ATP response of purified GC-A. The sequence homology between the protein kinase-like domain of GC-A and protein kinases has led Chinkers and Garbers (1) to propose that the kinase-like domain contains the ATP-binding regulatory site. This is supported by a recent study showing that ATP decreases the binding of ANP to a purified preparation of GC-A (51). Whether or not GC-A can signal independently of other molecule(s) is not known, since purified GC-A from several laboratories is not responsive to ANP/ATP (55, 56).

(f) Mechanism of desensitization. Ligand-induced dephosphorylation has been shown to be one mechanism of desensitization. The binding of resact to sea urchin sperm guanylyl cyclase causes a marked and transient increase in guanylyl cyclase activity, where the apparent desensitization appears closely linked to the state of phosphorylation of the enzyme (57). The phosphorylated form (160 kD) has fivefold higher enzyme activities than the dephosphorylated form (150 kD) (17). Resact causes a rapid dephosphorylation of the enzyme along with a large decrease in enzyme activity after an initial transient increase in activity (57). Dephosphorylation of the purified, phosphorylated sea urchin enzyme by protein phosphatases also causes a change in the kinetic behavior of the enzyme from positive cooperative to linear as a function of MeGTP (58). Given the high degree of sequence homology within the intracellular region between the sea urchin cyclase and other particulate guanylyl cyclases, it was speculated that the mammalian forms of guanylyl cyclase would display similar mechanisms of desensitization. In fact, Potter and Garbers (59) have recently presented preliminary data to show that this is the case for GC-A. GC-A stably expressed in cultured cells was shown to exist as a phosphoprotein under basal conditions. Treatment of cells with ANP resulted in a decrease in the phosphate content, as well as desensitization of GC-A to activation by ANP.

Proposed mechanisms of regulation of guanylyl cyclase

The requirement of coexpression of the α and β subunits of the heterodimeric guanylyl cyclase from lung, and the finding that the catalytic subunit of GC-A migrates as a dimer (42, 60) suggests a possible mechanism by which the particulate form of guanylyl cyclase is activated. In addition, expression of the recently identified α_2 subunit has shown that, while this subunit alone does not possess catalytic activity, sodium nitroprusside stimulates cells where both α_2 and β_1 subunits are expressed (11), (the activity of the $\alpha_2\beta_1$ heterodimer appears less than that of $\alpha_1\beta_1$). A dimeric state of the catalytic regions also may be required with adenylyl cyclase, where two putative catalytic domains homologous to the catalytic domain of guanylyl cyclase are present (43). Infection of Sf9 cells with recombinant baculovirus, encoding either the NH_2- or COOH-terminal half of adenylyl cyclase, does not result in demonstratable adenylyl cyclase activity. However, enzyme activity can be restored when cells are coinfected with recombinant viruses encoding both halves of the adenylyl cyclase (61). These data suggest that at least dimerization (higher ordered states may exist) of two catalytic domains is required for adenylyl cyclase or guanylyl cyclase activity. Together with the evidence that the protein kinase–like domain serves as a negative modulator of the enzyme, we propose a model for activation of GC-A as depicted in Fig. 2. In its resting state the enzyme either exists as a monomer or as a homodimer with negligible activity. Upon ANP binding, ATP alters the kinase-like domain such that the enzyme can dimerize, or be activated as the dimer. Dephosphorylation of the activated enzyme represents one mechanism of desensitization. The model awaits experimental testing, but is consistent with the mechanism of signaling of various protein kinase–linked cell surface receptors.

Physiological significance of the guanylyl cyclase receptors

GC-A. The mRNA for GC-A has been detected by in situ hybridization in kidney, adrenal cortex, adrenal medulla, endocardial endothelial cells, the vasa vasorum, and the cerebellum of the monkey (62). cDNA clones for GC-A have been isolated from tissues such as placenta, brain, heart, kidney, liver, and testis (25, 26, 36), and cross-linking studies suggest the presence of the protein in testis, brain, adrenal cortex, smooth muscle, and retina (reviewed in [3]). The apparent molecular weight of GC-A in the rat is 130,000 while that of GC-B is
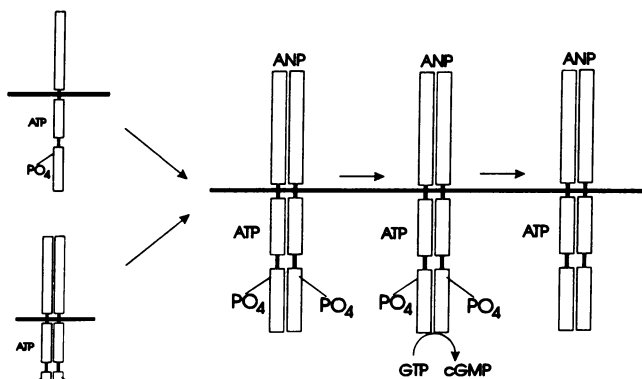
Figure 2. A model for regulation of GC-A by ANP, ATP, and phosphorylation. GC-A exists either as a phosphorylated monomer or dimer (possibly a higher ordered structure) in its native state and binds ATP. GC-A is activated by the binding of the extracellular ligand ANP which induces dimerization (or higher ordered structure) or causes a conformational change of a preexistent dimer. GC-A is subsequently dephosphorylated as a mechanism of desensitization.

$\sim$ 135,000–140,000 (Takada and Garbers, unpublished) allowing one to distinguish between the two proteins based on size. Dr. M. Takada in our laboratory has now used antibodies produced to synthetic peptides specific to GC-A or GC-B to determine a partial tissue distribution of these receptors (Takada, M., and D. L. Garbers, not published). Based on analysis by Western blotting of various rat tissues, GC-B is highly expressed in the pituitary gland and in lung, and GC-A is highly expressed in the adrenal gland and kidney. Other works from our laboratory, however, have shown expression of GC-B in many different tissues (Chrisman, Schulz, Potter, Welsh, and Garbers, unpublished). ANP, the ligand which activates GC-A, appears to be produced mainly in the heart, but the finding of GC-A in many different tissues, including the testis (36) and retina (13) suggests local regulation by ANP, or closely analogous peptides in tissues outside the vasculature, kidney, or adrenal gland, where atrial-derived ANP is believed to exert its principal effects (reviewed by Brenner et al. [2]). ANP or ANP-like peptides have been reported in other tissues (reviewed in [2]), an example being the eye, where GC-A exists (13) and where intravitreous injection of ANP causes a decrease in intraocular pressure (19).

GC-B. Although recent studies suggest that this is the receptor for CNP, a peptide suggested as existing only in the central nervous system (34), GC-B is found in many different cells including fibroblasts (T. D. Chrisman, M. Takada, and D. L. Garbers, unpublished observations). GC-B mRNA has been reported in brain and adrenal medulla based on in situ hybridization in the monkey (62), and cDNA clones have been isolated from brain, placental, pituitary, and atrial cDNA libraries (28, 29). If CNP is restricted to the central nervous system as has been suggested (34), then another peptide ligand for GC-B seems likely to exist. At the moment, the function of GC-B is unclear. Even the cellular localization of CNP, one of the putative ligands is still unknown, although it has been suggested that the peptide is a neurotransmitter (34).

GC-C. This receptor appears to be principally found in the gut mucosa where it exists on the apical membrane (63). However, it can be found in other tissues as well. For example, the

binding of STa to GC-C has been observed in rat liver (64), where the number of binding sites for STa appears to be four-fold greater in 1-d-old than in adult rats. GC-C mRNA levels also are markedly increased in the regenerating rat liver (S. Schulz and D. L. Garbers, unpublished). In the North American opossum, heat-stable enterotoxin binding has been observed in epithelial cells throughout the body (65). Whether or not the protein which binds the STa is GC-C in these cases is not known, but the size of the binding protein (M_r = 140,000) from opossum kidney is consistent with its being GC-C (65). It is tempting to suggest that the function of GC-C in the intestine is to regulate fluid secretion since the heat-stable enterotoxins bind to GC-C, elevate cyclic GMP, and cause an acute secretory diarrhea (66). It can be speculated, then, that an unknown endogenous ligand in a similar manner would regulate fluid secretion by virtue of its effects on GC-C. Recently, a 15-amino acid peptide homologous to STa has been isolated from rat jejunum (67). The peptide (guanylin) elevates cyclic GMP concentrations and displaces the binding of STa from a human colon carcinoma-derived cell line (T84). Whether or not this peptide binds to and activates GC-C remains to be determined, since the presence of other STa receptors has been suggested (63). There are also reasons to speculate that the cystic fibrosis transmembrane regulator (CFTR) is a molecular target of cyclic GMP action, recent data (68) suggest that cyclic GMP activates the cyclic AMP–dependent protein kinase, which in turn phosphorylates and activates CFTR, at least in T84 colonic cells (69).

Heterodimeric. There are four different subunits for heterodimeric forms of guanylyl cyclase known (α_1, α_2, β_1, β_2); the cellular distribution of these forms remain to be firmly established. Based on the polymerase chain reaction or the isolation of cDNA clones, mRNA for the various subunits is found in virtually all tissues, but Northern analysis suggests that α_1 is expressed principally in lung (60) while β_2 is expressed principally in kidney and liver (10).

The soluble form of the enzyme ($\alpha_1\beta_1$) appears to be regulated by nitric oxide or related molecules. The current model for such regulation is that the enzyme contains heme as a prosthetic group, and that the nitric oxide, in binding to the heme moiety, causes a marked activation of the enzyme (reviewed by Ignarro [70]). The data that support this hypothesis are: (*a*) upon purification of the soluble form of the enzyme under neutral pH conditions, heme is found associated with the enzyme. This form of the enzyme can be stimulated by nitroprusside and similar agents. (*b*) The addition of weak acid causes dissociation of the heme group from guanylyl cyclase, and nitroprusside and similar agents now fail to stimulate. (*c*) The addition of protoporphrin IX to the heme-depleted enzyme restores the stimulation by nitroprusside.

Nitric oxide is generated from nitric oxide synthase, an enzyme that exists in multiple forms, some of which are inducible, and others which are constitutively regulated by Ca^{2+}/calmodulin (70). The Ca^{2+}-regulated, or closely related forms, appear to exist throughout the body, and may explain the plethora of data in the older literature showing that almost all agents tested that mobilize Ca^{2+} also elevate cyclic GMP (reviewed in [71]).

Summary

Three different guanylyl cyclase cell receptors are known, but others will likely be discovered within the next few years. The

general function of these receptors appear to relate to the regulation of fluid volume or fluid movement. New receptors, or possibly the currently known receptors, therefore, may be discovered in areas of the body where fluid volume regulation is important. Such fluids whose volume or composition might be regulated by guanylyl cyclase receptors include synovial fluid, uterine/oviductal luminal fluid, follicular fluid, aqueous humor, cerebral spinal fluid, seminferous tubule luminal fluid, epididymal luminal fluid, seminal plasma, and airway luminal fluid.

The function of the heterodimeric forms of guanylyl cyclase appear to relate to a primary regulation of nitric oxide (or similar molecules) concentrations, which are in turn regulated by a Ca^{2+}/calmodulin-sensitive nitric oxide synthase.

References

1. Chinkers, M., and D. L. Garbers. 1991. Signal transduction by guanylyl cyclases. *Annu. Rev. Biochem.* 60:553–575.

2. Brenner, B. M., B. J. Ballermann, M. E. Gunning, and M. L. Zeidel. 1990. Diverse biological actions of atrial natriuretic peptide. *Physiol. Rev.* 70:665–699.

3. Yuen, P. S. T., and D. L. Garbers. 1991. The guanylyl cyclase receptor family. *Annu. Rev. Neurosci.* 15:193–225.

4. Craven, P. A., and F. R. DeRubertis. 1978. Restoration of the responsiveness of purified guanylate cyclase to nitrosoguanidine, nitric oxide, and related activators by heme and hemeproteins. *J. Biol. Chem.* 253:8433–8443.

5. Gerzer, R., E. Bohme, F. Hoffman, and G. Schultz. 1981. Soluble guanylate cyclase purified from bovine lung contains heme and copper. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 132:71–74.

6. Palmer, R. M. J., A. G. Ferridge, and S. Moncada. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (Lond.)*. 327:524–526.

7. Furchgott, R. F. 1988. Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid activatable inhibitory factor from retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide. *In* Vasodilation: Vascular Smooth Muscle, Peptides, autonomic nerves and endothelium. P. M. Vanhoutte, editor. Raven Press Ltd., New York. pp. 401–414.

8. Bredt, D. S., and S. H. Synder. 1990. Isolation of nitric oxide synthetase, a calmoldulin-requiring enzyme. *Proc. Natl. Acad. Sci. USA.* 87:682–685.

9. Kamisaki, Y., S. Saheki, M. Nakane, J. A. Palmieri, T. Kuno, B. Y. Chang, S. Waldman, and F. Murad. 1986. Soluble guanylate cyclase from rat lung exists as a heterodimer. *J. Biol. Chem.* 261:7236–7241.

10. Yuen, P. S. T., L. R. Potter, and D. L. Garbers. 1990. A new form of guanylyl cyclase is preferentially expressed in rat kidney. *Biochemistry.* 29:10872–10878.

11. Harteneck, C., B. Wedel, D. Koesling, J. Malkewitz, E. Bohme, and G. Schultz. 1991. Molecular cloning and expression of a new α-subunit of soluble guanylyl cyclase. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 292:217–222.

12. Hayashi, F., and A. Yamazaki. 1991. Polymorphism in purified guanylate cyclase from vertebrate rod photoreceptors. *Proc. Natl. Acad. Sci. USA.* 88:4746–4750.

13. Kutty, R. K., R. T. Fletcher, G. J. Chader, and G. Krishna. 1992. Expression of guanylate cyclase-A mRNA in the rat retina: detection using polymerase chain reaction. *Biochem. Biophys. Res. Commun.* 182:851–857.

14. Dizhoor, A. M., A. Ray, S. Kumar, G. Niemi, M. Spencer, D. Brolley, K. A. Walsh, P. P. Philipov, J. B. Hurley, and L. Stryer. 1991. Recoverin: a calcium sensitive activator of retinal rod guanylate cyclase. *Science (Wash. DC).* 251:915–918.

15. Stryer, L. 1991. Visual excitation and recovery. *J. Biol. Chem.* 266:10711–10714.

16. Koch, K.-W. 1991. Purification and identification of photoreceptor guanylyl cyclase. *J. Biol. Chem.* 266:8634–8637.

17. Radany, E. W., R. Gerzer, and D. L. Garbers. 1983. Purification and characterization of particulate guanylate cyclase from sea urchin spermatozoa. *J. Biol. Chem.* 258:8346–8351.

18. Horio, Y., and F. Murad. 1991. Purification of guanylyl cyclase from rod outer segment. *Biochim. Biophys. Acta.* 1133:81–88.

19. Korenfeld, M. S., and B. Becker. 1989. Atrial natriuretic peptides: effects on intraocular pressure, cGMP, and aqueous flow. *Invest. Opthamol & Visual Sci.* 30:2385–2392.

20. Kakiuchi, S., K. Sobue, R. Yamazaki, S. Nagao, S. Umeki, Y. Nozawa, M. Yazawa, and K. Yagi. 1981. Ca^{2+}-dependent modulator proteins from tetrahymena pyriformis, sea anemone, and scallop abd guanylate cyclase activation. *J. Biol. Chem.* 256:19–22.

21. Schultz, J. E., T. Pohl, and S. Klumpp. 1986. Voltage-gated Ca^{2+} entry into *Paramecium* linked to intraciliary increase in cyclic GMP. *Nature (Lond.)*. 322:271–273.

22. Shulz, S., M. Chinkers, and D. L. Garbers. 1989. The guanylate cyclase/receptor family of proteins. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:2026–2035.

23. Shimomura, H., L. J. Dangott, and D. L. Garbers. 1986. Covalent coupling of a resact analogue to guanylate cyclase. *J. Biol. Chem.* 261:15778–15782.

24. Singh, S., D. G. Lowe, D. S. Thorp, H. Rodriguez, W.-J. Kuang, L. J. Dangott, M. Chinkers, D. V. Goeddel, and D. L. Garbers. 1988. Membrane guanylate cyclase is a cell surface receptor with homology to protein kinases. *Nature (Lond.)*. 334:708–712.

25. Chinkers, M., D. L. Garbers, M.-S. Chang, D. G. Lowe, H. Chin, D. V. Goeddel, and S. Schulz. 1989. A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature* 338:78–83.

26. Lowe, D. G., M. S. Chang, R. Hellmiss, E. Chen, S. Singh, D. L. Garbers, and D. V. Goeddel. 1989. Human atrial natriuretic peptide receptor defines a new paradigm for second messenger signal transduction. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1377–1384.

27. de Bold, A. J. 1985. Atrial natruiuretic factor: a hormone produced by the heart. *Science (Wash. DC).* 230:767–770.

28. Schulz, S., S. Singh, R. A. Bellet, G. Singh, D. J. Tubb, H. Chin, and D. L. Garbers. 1989. The primary structure of a plasma membrane guanylate cyclase demonstrate diversity within this new receptor family. *Cell.* 58:1155–1162.

29. Chang, M. S., D. G. Lowe, M. Lewis, R. Hellmiss, E. Chen, and D. V. Goeddel. 1989. Differential activation of atrial and brain natriuretic peptides of two receptor guanylate cyclases. *Nature (Lond.)*. 341:68–72.

30. Schulz, S., C. K. Green, P. S. T. Yuen, and D. L. Garbers. 1990. Guanylyl cyclase is a heat-stable enterotoxin receptor. *Cell.* 63:941–948.

31. de Sauvage, F. J., T. R. Camerato, and D. V. Goeddel. 1991. Primary structure and functional expression of the human receptor for *Escherichia coli* heat-stable enterotoxin. *J. Biol. Chem.* 266:17912–17918.

32. Singh, S., G. Singh, J.-M. Meim, and R. Gerzer. 1991. Isolation and expression of a guanylate cyclase-coupled heat stable enterotoxin receptor cDNA from a human colonic cell line. *Biochem. Biophys. Res. Commun.* 179:1455–1463.

33. Koller, K. J., D. G. Lowe, G. L. Bennet, N. Minamino, K. Kangawa, H. Matsuo, and D. V. Goeddel. 1991. C-type natriuretic peptide (CNP) selectively activates the B natriuretic peptide receptor (ANPR-B). *Science (Wash. DC).* 252:120–123.

34. Arimura, J. J., N. Minamino, K. Kangawa, and H. Matsuo. 1991. Isolation and identification of C-type natriuretic peptide and chicken brain. *Biochem. Biophys. Res. Commun.* 174:142–148.

35. Ullrich, A., and J. Schlessinger. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 61:203–212.

36. Pandey, K. N., and S. Singh. 1990. Molecular cloning and expression of murine guanylate cyclase/atrial natriuretic receptor cDNA. *J. Biol. Chem.* 265:12342–12348.

37. Fuller, F., J. G. Porter, A. E. Arfsten, J. Miller, J. W. Schilling, R. M. Scarborough, J. A. Lewicki, and D. B. Schenk. 1988. Atrial natriuretic peptide clearance receptor. *J. Biol. Chem.* 263:9395–9401.

38. Lax, I., W. H. Burgess, F. Bellot, A. Ullrich, J. Schlessinger, and D. Givol. 1988. Location of a major receptor-binding domain for epidermal growth factor by affinity labeling. *Mol. Cell. Biol.* 8:1831–1834.

39. Duda, T., R. M. Goraczniak, and R. K. Sharma. 1991. Site-directed mutational analysis of a membrane guanylate cyclase cDNA reveals the atrial natriuretic factor signaling site. *Proc. Natl. Acad. Sci. USA.* 88:7882–7886.

40. Hanks, S. K., A. M. Quinn, and T. Hunter. 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science (Wash. DC).* 241:42–53.

41. Chinkers, M., and D. L. Garbers. 1989. The protein kinase domain of the ANP receptor is required for signaling. *Science (Wash. DC).* 245:1392–1394.

42. Thorpe, D., and E. Morkin. 1990. The carboxyl region contains the catalytic domain of the membrane form of guanylyl cyclase. *J. Biol. Chem.* 265:14717–14720.

43. Krupinski, J., F. Cousseu, H. A. Bakalyar, W.-J. Tang, P. G. Feinstein, K. Orth, C. Slaughter, R. R. Reed, and A. G. Gilman. 1989. Adenylyl cyclase amino acid sequence: possible channel- or transporter-like structure. *Science (Wash. DC).* 244:1558–1564.

44. Beuve, A., B. Boesten, M. Crasnier, A. Danchin, and F. O'Gara. 1990. Rhizobium meliloti adenylate cyclase is related to eucaryotic adenylate and guanylate cyclases. *J. Bacteriol.* 172:2614–2621.

45. Thorpe, D. S., S. Niu, and E. Morkins. 1991. Overexpression of dimeric guanylyl cyclase cores of an atrial natriureic peptide receptor. *Biochem. Biophys. Res. Commun.* 180:538–544.

46. Kurose, H., T. Inagami, and M. Ui. 1987. Participation pf adenosine 5'-triphosphate in the activation of membrane-bound guanylate cyclase by the atrial natriuretic factor. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 291:375–379.

47. Chinkers, M., S. Singh, and D. L. Garbers. 1991. Adenine nucleotides are required for activation of rat atrial natriuretic peptide receptor/guanylyl cyclase expressed in a baculovirus system. *J. Biol. Chem.* 266:4088–4039.

48. Gazzano, H., H. I. Wu, and S. A. Waldman. 1991. Activation of particu-

late guanylate cyclase by *Escherichia coli* heat-stable enterotoxin is regulated by adenine nucleotides. *Infect. Immun.* 59:1552–1557.

49. Chang, C.-H., B. Jiang, and J. G. Douglas. 1990. Caged ATP potentiates guanylate cyclase activity stimulated by atrial natriuretic factor in rat lung membranes. *Eur. J. Pharmacol.* 189:111–114.

50. Gilman, A. G. 1987. G-proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56:615–649.

51. Larose, L., N. McNicoll, H. Ong, and A. De Lean. 1991. Allosteric modulation of ATP of the bovine adrenal natrriuretic factor R_1 receptor functions. *Biochemistry.* 30:8990–8995.

52. Ross, E. M., M. E. Maguire, T. W. Sturgill, R. L. Biltonen, and A. G. Gilman. 1977. Relationship between the β-adrenergic receptor and adenylate cyclase. *J. Biol. Chem.* 252:5761–5775.

53. Chang, C.-H., B. Jiang, and J. G. Douglas. 1991. Calcium reveals different mechanisms of guanylate cyclase activation by atrial natriuretic factor and ATP in rat lung membranes. *Biochim. Biophys. Acta.* 1093:42–46.

54. Chang, C.-H., K. P. Kohse, B. Chang, M. Hirata, B. Jiang, J. E. Douglas, and F. Murad. 1990. Characterization of ATP-stimulated guanylate cyclase activation in rat lung membranes. *Biochim. Biophys. Acta.* 1052:159–165.

55. Kuno, T., J. W. Andresen, Y. Kamisaki, S. A. Waldmanm, L. Y. Chang, S. Saheki, D. C. Leitman, M. Nakane, and F. Murad. 1986. Co-purification of an atrial natriuretic factor receptor and particulate guanylate cyclase from rat lung. *J. Biol. Chem.* 261:5817–5823.

56. Takayanagi, R., T. Inagami, R. M. Snajdar, T. Imada, M. Tamura, and K. S. Misono. 1987. Two distinct forms of receptors for atrial natriuretic factor in bovine adrenocortical cells. *J. Biol. Chem.* 262:12104–12113.

57. Bentley, J. K., D. J. Tubb, and D. L. Garbers. 1986. Receptor-mediated activation of spermatozoan guanylate cyclase. *J. Biol. Chem.* 261:14859–14862.

58. Ramarao, C. S., and D. L. Garbers. 1988. Purification and properties of the phosphorylated form of guanylate cyclase. *J. Biol. Chem.* 263:1524–1529.

59. Potter, L. R., and D. L. Garbers. 1992. Dephosphorylation of the atrial natriuretic peptide receptor (GC-A) correlates with decreases in guanylyl cyclase activity. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 6:453A. (Abstr.)

60. Nakane, M., K. Arai, S. Saheki, T. Kuno, W. Buechler, and F. Murad. 1990. Molecular cloning and expression of cDNAs encoding for soluble guanylate cyclase from rat lung. *J. Biol. Chem.* 265:16841–16845.

61. Tang, W.-J., J. Krupinski, and A. G. Gilman. 1991. Expression and characterization of calmodulin-activated (type I) adenylylcyclase. *J. Biol. Chem.* 266:8595–8603.

62. Wilcox, J. N., A. Augustine, D. V. Goeddel, and D. G. Lowe. 1991. Differential regional expression of three natriuretic peptide receptor genes within primate tissues. *Mol. Cell. Biol.* 11:3454–3462.

63. Ivens, K., H. Gazzano, P. O'Hanley, and S. A. Waldman. 1990. Heterogeneity of intestinal receptors for *Escherichia coli* heat-stable enterotoxin. *Infect. Immun.* 58:1817–1820.

64. Laney, D. W., and M. B. Cohen. 1991. Binding of heat-stable enterotoxin to receptors in extraintestinal sites: developing rat liver. Digestive Disease Week (19–22 May, 1991) American Association for the Study of Liver Diseases. 1351. (Abstr.)

65. Krause, W. J., R. A. Freeman, and L. R. Forte. 1990. Autoradiographic demonstration of specific binding sites for *E. coli* enterotoxin in various epithelia of the North American opossum. *Cell Tissue Res.* 260:387–394.

66. Hughes, J. M., F. Murad, B. Chang, and R. L. Guerrant. 1978. Role of cyclic GMP in the action of heat-stable enterotoxin of *Escherichia coli. Nature (Lond.).* 271:755–756.

67. Currie, M. G., K. F. Fok, J. Kato, R. J. Moore, F. K. Hamra, K. L. Duffin, and C. E. Smith. 1992. Guanylin: an endogenous activator of intestinal guanylate cyclase. *Proc. Natl. Acad. Sci. USA.* 89:947–951.

68. Jiang, H. J., J. L. Colbran, S. H. Francis, and J. D. Corbin. 1992. Direct evidence for cross-activation of cGMP-dependent protein kinase by cAMP in pig coronary arteries. *J. Biol. Chem.* 267:1015–1019.

69. Forte, L. R., W. J. Krause, R. H. Freeman, S. H. Francis, and J. D. Corbin. 1991. *Pediatr. Pulmonology.* (Suppl. 6): 259. (Abstr. 135).

70. Ignarro, L. J. 1989. Heme-dependent activation of soluble guanylate cyclase by nitric oxide: regulation of enzyme activity by porphyrins and metalloporphyrins. *Semin. Hematol.* 26:63–76.

71. Goldberg, N. D., and M. K. Haddox. 1977. Cyclic GMP metabolism and involvement in biological regulation. *Annu. Rev. Biochem.* 46:823–896.