Mol Neurobiol (2007) 35:329–345
DOI 10.1007/s12035-007-0021-2

Heterotrimeric G Proteins and the Single-Transmembrane Domain IGF-II/M6P Receptor: Functional Interaction and Relevance to Cell Signaling

C. Hawkes · A. Amritraj · R. G. MacDonald · J. H. Jhamandas · S. Kar

Received: 17 January 2007 / Accepted: 2 April 2007 / Published online: 11 July 2007

Abstract The G protein-coupled receptor (GPCR) family represents the largest and most versatile group of cell surface receptors. Classical GPCR signaling constitutes ligand binding to a seven-transmembrane domain receptor, receptor interaction with a heterotrimeric G protein, and the subsequent activation or inhibition of downstream intracellular effectors to mediate a cellular response. However, recent reports on direct, receptor-independent G protein activation, G protein-independent signaling by GPCRs, and signaling of nonheptahelical receptors via trimeric G proteins have highlighted the intrinsic complexities of G protein signaling mechanisms. The insulin-like growth factor-II/mannose-6 phosphate (IGF-II/M6P) receptor is a single-transmembrane glycoprotein whose principal function is the intracellular transport of lysosomal enzymes. In addition, the receptor also mediates some biological effects in response to IGF-II binding in both neuronal and nonneuronal systems. Multidisciplinary efforts to elucidate the intracellular signaling pathways that underlie these effects have generated data to suggest that the IGF-II/M6P receptor might mediate transmembrane signaling via a G protein-coupled mechanism. The purpose of this review is to outline the characteristics of traditional and nontraditional GPCRs, to relate the IGF-II/M6P receptor's structure with its role in G protein-coupled signaling and to summarize evidence gathered over the years regarding the putative signaling of the IGF-II/M6P receptor mediated by a G protein.

Keywords G protein-coupled receptor · IGF-II/MGP · Heterotrimeric G protein

C. Hawkes · A. Amritraj · S. Kar
Department of Psychiatry, Centre for Alzheimer and Neurodegenerative Research, University of Alberta, Edmonton, AB, Canada T6G 2B7

R. G. MacDonald
Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA

J. H. Jhamandas · S. Kar
Department of Medicine (Neurology), Centre for Alzheimer and Neurodegenerative Research, University of Alberta, Edmonton, AB, Canada T6G 2B7

S. Kar (✉)
Alberta Centre for Prions and Protein Folding Diseases
Departments of Medicine (Neurology) and Psychiatry, University of Alberta,
Edmonton, AB, Canada T6G 2M8
e-mail: skar@ualberta.ca

Introduction

The family of classical G protein-coupled receptors (GPCRs) constitutes the largest class of cell-surface receptors with an essential role in almost all physiological processes in both mammalian and nonmammalian species. These receptors are recognized and bound by a diverse category of ligands, including odorants, taste ligands, light, metals, nucleotides, lipids, photons, ions, biogenic amines, amino acids, proteins, and peptides [1–5]. Current estimates indicate that more than 800 genes within the mammalian genome code for GPCRs, some of which still remained orphaned, with no known ligand or function. Given the association of GPCRs to a broad range of physiological functions, this family of receptors represents one of the most important drug targets for the pharmaceutical industry. Indeed, by some estimates, nearly 45% of the marketed drugs are known ligands of

approximately 30 members of the GPCR family [2, 6]. To fully exploit therapeutic potential further, considerable effort is being made to identify cognate ligands for the remaining GPCRs and to characterize their functional relevance in human physiology.

The main structural characteristic of GPCRs is a seven-transmembrane-helix core domain that is capped on the extracellular side by a ligand-binding domain and on the cytoplasmic side by a domain that interacts with heterotrimeric G proteins composed of α, β, and γ subunits. The Gα subunit binds to and hydrolyzes guanosine 5'-triphosphate (GTP), whereas the Gβγ subunits form an undissociable functional unit. In the resting state, the Gα subunit is bound to guanosine 5'-diphosphate (GDP) and is associated with the Gβγ subunit complex. Upon ligand binding, a conformational change is induced within the intracellular domains of the receptor, which promotes receptor interaction with the heterotrimeric G protein [1, 3, 4]. Subsequently, GDP on the Gα subunit is exchanged for GTP, triggering a conformational change in the heterotrimer that results in dissociation of the GTP-bound Gα subunit from the βγ subunits. The released G protein subunits then either positively or negatively regulate a diverse array of effector enzymes and ion channels, leading to a cellular response to the ligand-initiated signal [1, 2, 7]. It has now become clear that in addition to the GTP-bound Gα subunit, free βγ subunits can also bind to and regulate the activity of a great variety of effector molecules. Signaling is terminated after hydrolysis of GTP to GDP by intrinsic GTPase activity of the Gα subunit. The resulting GDP-bound inactive Gα subunit undergoes a conformational change that allows it to re-acquire affinity for the Gβγ subunits, and the heterotrimer thus returns to the basal state, ready to respond to a new cycle of receptor binding. Each receptor can activate a large number of G proteins before the signal is terminated by receptor internalization. All GPCRs use the same basic mechanism to act on a wide spectrum of signal transduction pathways in mediating a variety of cellular responses [3–8]. Interestingly, however, recent data obtained from cross-linking and fluorescence resonance studies indicate that dissociation of the G protein subunits may not be required for downstream signaling by the GPCR [5, 9].

The heterotrimeric G protein subunits, which interact with effector molecules to transduce extracellular signals into cellular responses, display significant homology in both their primary sequence and tertiary structure. To date, 27 distinct Gα subunits, 5 forms of Gβ subunits, and 14 forms of Gγ subunits have been cloned and characterized [2, 7]. The various permutations of Gα, Gβ, and Gγ subunit combinations provide a large diversity for signaling via heterotrimeric G proteins. G proteins are classified into four families, Gs, Gi/o, Gq, and $G_{12/13}$, based on the sequence homology of the α subunits and their associated effects on a selective effector [7, 10, 11]. In general, Gαs proteins activate, whereas Gαi proteins inhibit, adenylyl cyclase (AC) activity and modulate cAMP levels, although both families can also regulate src tyrosine kinases and phosphodiesterases. Stimulation of Gαq proteins, on the other hand, can activate phospholipase Cβ (PLCβ) and increase protein kinase C (PKC) activity, whereas $G_{12/13}$ proteins can mediate signals by activating Rho GTPases and proteins from the ezrin/radixin/moesin family [7, 12, 13].

In addition to the various Gα protein subunits, Gβγ dimers can also mediate signaling by regulating the activity of PLCβ, AC, potassium channels, Cav_2 calcium channels, and phosphoinositide 3'(PI-3)-kinase γ. Some recent evidence indicates that the Gβ subunit plays a critical role in determining the specificity of signaling induced by the Gβγ complex [3, 4, 7, 14]. It is also of interest to note that although most GPCRs are preferentially coupled to a certain subfamily of G proteins, they can activate other classes of G protein with reduced efficiency. Given the diversity of G protein subunits and downstream effector molecules, the pattern of responses of a cell/tissue to a particular GPCR can be complex. The specificity of the cellular response to GPCR stimulation depends on the G protein heterotrimers recognized by the receptor, specific effector molecules expressed in the cells/tissue, and the relative concentrations of the various components in the signaling pathways [3, 7, 14, 15]. Additionally, as the modulation of second messengers often results in measurable intracellular/biochemical changes such as alterations in cAMP or inositol 1,4,5-trisphosphate (IP_3) levels, it is possible to screen the specific receptor-G protein coupling using both functional and nonfunctional assays.

Characterization of GPCRs

Over the years, several methods, ranging from simple receptor ligand-binding assays to high-throughput technologies, have been developed to characterize receptor–protein interactions. More recently, the advancement of high-resolution visualization techniques has provided an additional tool to view the in vivo effects of agonist stimulation on GPCRs [1, 3, 5, 6, 16, 17]. Some of the basic procedures that are typically used for the characterization of several GPCRs are based on the ligand-induced activation of the Gα protein and its interaction with GTP analogues.

Receptor-binding Assays Some of the most straightforward procedures for determining a putative receptor-G protein interaction are receptor-binding assays performed in the presence of nonhydrolyzable GTP analogues such as GTPγS and Gpp(NH)p, which act to permanently activate the targeted G protein and thereby promote a reduction in affinity of ligand/receptor-binding. In this approach, tissue/

cells or plasma membrane preparations incubated with radiolabeled or fluorescently labeled ligands in the presence of increasing concentrations of nonhydrolyzable GTP analogues show a dose-dependent decrease in the binding of the ligand to the receptor if the receptor does indeed interact with a G protein. In addition, the general subclass of G protein associated with a given receptor can also be determined by examining ligand-binding profiles in the presence of different G protein-selective toxins, such as *Bordetella pertussis* toxin (PTX, which inhibits Gi proteins) and cholera toxin from *Vibrio cholerae* (CTX, which affects Gs proteins) [4, 16, 17]. More recently, the [^{35}S]GTPγS-binding assay has been used to measure the level of G protein activation after agonist occupation of a GPCR. In this assay, [^{35}S]γGTPS replaces endogenous GTP and binds to the Gα subunit after activation of the receptor to form a Gα-[^{35}S]γGTPS complex. Because the γ-thiophosphate bond is resistant to hydrolysis by the GTPase of Gα, the G protein is prevented from reforming as a heterotrimer, and thus, [^{35}S]γGTPS-labeled Gα subunits accumulate and can be measured by counting the amount of [^{35}S]-label incorporated [18–20]. A nonradioactive GTPγS-binding assay, using europium (Eu)-labeled GTP derivatives in which the amount of Eu-GTP remaining after filtration is quantified by time-resolved fluorometry, has also been developed for a variety of GPCRs [21].

Biochemical/structural Assays Another experimental approach that is used to investigate G protein–receptor coupling is the examination of their physical interaction. This can be done by performing co-immunoprecipitation experiments, in which the receptor protein is pulled down from the tissue homogenates using a receptor-specific antibody and then probed by Western blotting for an associated G protein [22–24]. Although these experiments are useful in recognizing the G protein subclass (e.g., Gs, Gi, Gi2, etc.) associated with a specific GPCR, this procedure cannot conclusively determine a direct physical interaction between the two proteins. To resolve this issue, fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer assays, based on the principle of resonance energy transfer between chromophores carried by two moieties/proteins residing within 100 Å of each other, have been developed [17, 25–27]. Both techniques have been successfully used to study GPCR oligomerization, as well as to demonstrate GPCR interactions with associated kinases and β-arrestins in living cells [13, 17, 28–30]. Although studies involving a direct demonstration of interaction of a GPCR with G proteins have not yet been published, GPCR-mediated dissociation of the Gα and Gβ subunits has been investigated using FRET [31, 32]. More recently, Hoffman et al. [33] have developed the use of fluorescein arsenical hairpin binder-based FRET assay, using a smaller fluorescent moiety that interferes less with normal GPCR functioning during imaging, to determine GPCR activation. The continued development of new technologies will undoubtedly improve the resolution and real-time examination of G protein–receptor interactions in living cells.

Functional Assays Another way in which receptor–G protein interactions may be measured is to assess the functional outcome of receptor stimulation/antagonism using second messenger or reporter gene assays. For example, intracellular cAMP levels, which are modulated by Gi/Gs proteins, can be measured using a variety of methods including anti-cAMP antibody-mediated competition assays, forskolin-stimulated agonism/antagonism assays, reporter gene assay systems, time-resolved fluorescence, and amplified luminescence proximity assays [13, 34–40]. Other assays used to measure GPCR function include commercially available radioligand kits, which detect changes in IP_3 production after GPCR-initiated activation of PLC activity, as well as melanophore-based pigment dispersion/aggregation assays, which reflect the activation of AC or PLC [41]. Alterations in the flux of intracellular calcium, as measured by Ca^{2+}-sensitive fluorescent dyes, bioluminescent indicators, or reporter gene assays, can also be used as a GPCR screening technique [13, 42, 43]. Clearly, the choice of appropriate functional GPCR assay will depend on considerations of tissue, G protein type, feasibility, and scale of detection.

Novel Regulators of G Protein Signaling

Although receptor coupling to trimeric G proteins is a central event in GPCR-mediated signaling, recent data obtained using a variety of experimental approaches have provided a glimpse into the inherent complexity of G protein signaling by demonstrating that (1) G proteins can be directly activated without receptor involvement and (2) nonclassical GPCRs can mediate signaling via trimeric G proteins [2, 3, 15, 44, 45]. Two general types of direct G protein regulators have been identified that either accelerate the GTPase activity of the Gα subunit or alter G protein signaling by influencing nucleotide exchange or G protein subunit interactions. Thus, these proteins provide alternative binding partners for G protein subunits and enable them to serve functions independent of GPCRs, such as the regulation of cell polarity and cell division [45–47]. On the other hand, a number of studies have indicated that nonclassical GPCRs can also mediate some of their biological effects via possible activation of heterotrimeric G proteins. These include receptor tyrosine kinases, which mediate the actions of certain growth factors

such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) as well as receptors for atrial natriuretic peptide, integrins, and insulin-like growth factor-II (IGF-II). A number of recent reviews have highlighted the paradigms by which tyrosine kinase receptors can evoke cellular effects via activation of G proteins [2, 15, 44, 48, 49], but very little information is currently available on the implication of an interaction between the IGF-II receptor and a G protein. This review aims to provide an overview of our current knowledge about the IGF-II receptor and its possible significance in regulating neuronal function in the central nervous system (CNS) by activating a trimeric G protein.

Insulin-like Growth Factor-II Receptor

The IGF-II receptor, which is identical to the cation-independent mannose 6-phosphate (M6P) receptor (hereafter referred to as the IGF-II/M6P receptor), is a multifunctional single pass transmembrane glycoprotein with no intrinsic tyrosine kinase activity (Fig. 1). The receptor binds IGF-II with higher affinity than IGF-I and does not bind insulin [50–54]. It also interacts, via distinct sites, with various M6P-containing ligands, such as transforming growth factor-β (TGF-β) [55], leukemia inhibitory factor (LIF) [56], proliferin [57], thyroglobulin [58], *Chlamydia pneumoniae* [59], and *Listeria monocytogenes* [60], as well as the non-M6P-containing retinoic acid [61]. Accumulated evidence suggests that the IGF-II/M6P receptor is expressed in most cell types, where it plays a role in lysosomal enzyme trafficking, clearance and/or activation of a variety of growth factors, and endocytosis-mediated degradation of IGF-II. In addition, a growing body of evidence supports a role for this receptor in transmembrane signaling in both neuronal and nonneuronal systems possibly via activation of heterotrimeric G protein, although this remains controversial [51–54].

Structurally, the human IGF-II/M6P receptor is composed of four distinct domains: a 40-residue amino-terminal signal sequence, an extracytoplasmic domain of 2,264 residues, a single, 23-residue transmembrane region, and a carboxy-terminal cytoplasmic tail of 164 residues (Fig. 1). The extracytoplasmic domain, i.e., the receptor region that protrudes into the extracellular space or the lumen of vesicles and intracellular organelles, is composed of 15 repeating segments, which share 14–38% sequence identity [50–54, 62, 63] (see Fig. 1). The cytoplasmic domain contains four regions that are substrates for various protein kinases including PKC, cAMP-dependent protein kinase, and casein kinases I and II [50, 51, 53, 54]. The receptor may exist as a dimer under both in vitro and in vivo conditions, and dimerization has been shown to alter the kinetics of IGF-II/M6P receptor internalization at the cell surface [64–66]. A truncated form of the receptor lacking the intracellular and transmembrane domains has also been identified in bovine serum and in the serum, urine, and amniotic fluid of rats and humans [51, 67–70].

The truncated receptor represents the product of proteolytic cleavage of the receptor's extracytoplasmic domain, which is subsequently released from the cell surface as a result of normal receptor turnover [69, 71]. Although high levels of the truncated receptor can interfere with IGF-II action by sequestering IGF-II away from the IGF-I receptor [72–74], it is not known whether the concentrations achieved physiologically have significant biological function.

IGF-II/M6P Receptor Ligand-binding Properties The IGF-II/M6P receptor binds three general categories of ligands at two distinct sites—IGFs, M6P-containing molecules and retinoic acid [50–54]. Two high-affinity M6P-binding sites are localized to repeats 1–3 and 7–11 within the extracytoplasmic region, whereas a third lower-affinity M6P recognition site within receptor domain 5 has recently been identified [75] (Fig. 1). In mammals, IGF-II binds the receptor at a primary site localized to the amino-terminal portion of extracytoplasmic domain 11, although sequence elements within domain 13 have been suggested to contribute an approximately five- to tenfold enhancement to the binding affinity of the receptor for IGF-II [76–81]. IGF-II and M6P-containing peptides are able to bind simultaneously to the IGF-II/M6P receptor, and binding of one ligand has been shown to reciprocally modulate receptor affinity for the other [82–85].

IGF-II/M6P Receptor Distribution The IGF-II/M6P receptor plays a critical role in prenatal tissue growth. It is therefore not surprising that receptor expression is developmentally regulated, with high-prenatal levels preceding a sharp postnatal decline, which is less acute in humans than in other mammals [86–91]. In adult, the receptor is ubiquitously expressed in various tissues including the heart, lung, muscle, intestine, kidney, liver, spleen, thymus, testis, ovary, and brain [92–94]. Within the CNS, IGF-II/M6P receptor mRNA and/or protein has been detected across all major regions of the brain and spinal cord, with particular enrichment in cortical areas, hippocampus, hypothalamus, and cerebellum as well as in certain brainstem and spinal motor nuclei [89, 95–100]. In general, high receptor immunoreactivity has also been found in the striatum, deep cortical layers, pyramidal and granule cell layers of the hippocampus, thalamic nuclei, cerebellar Purkinje cells, and in motor neurons of the brainstem and spinal cord [100]. Although IGF-II/M6P receptor expression has not been detected on microglia or astrocytes in the adult rat brain,

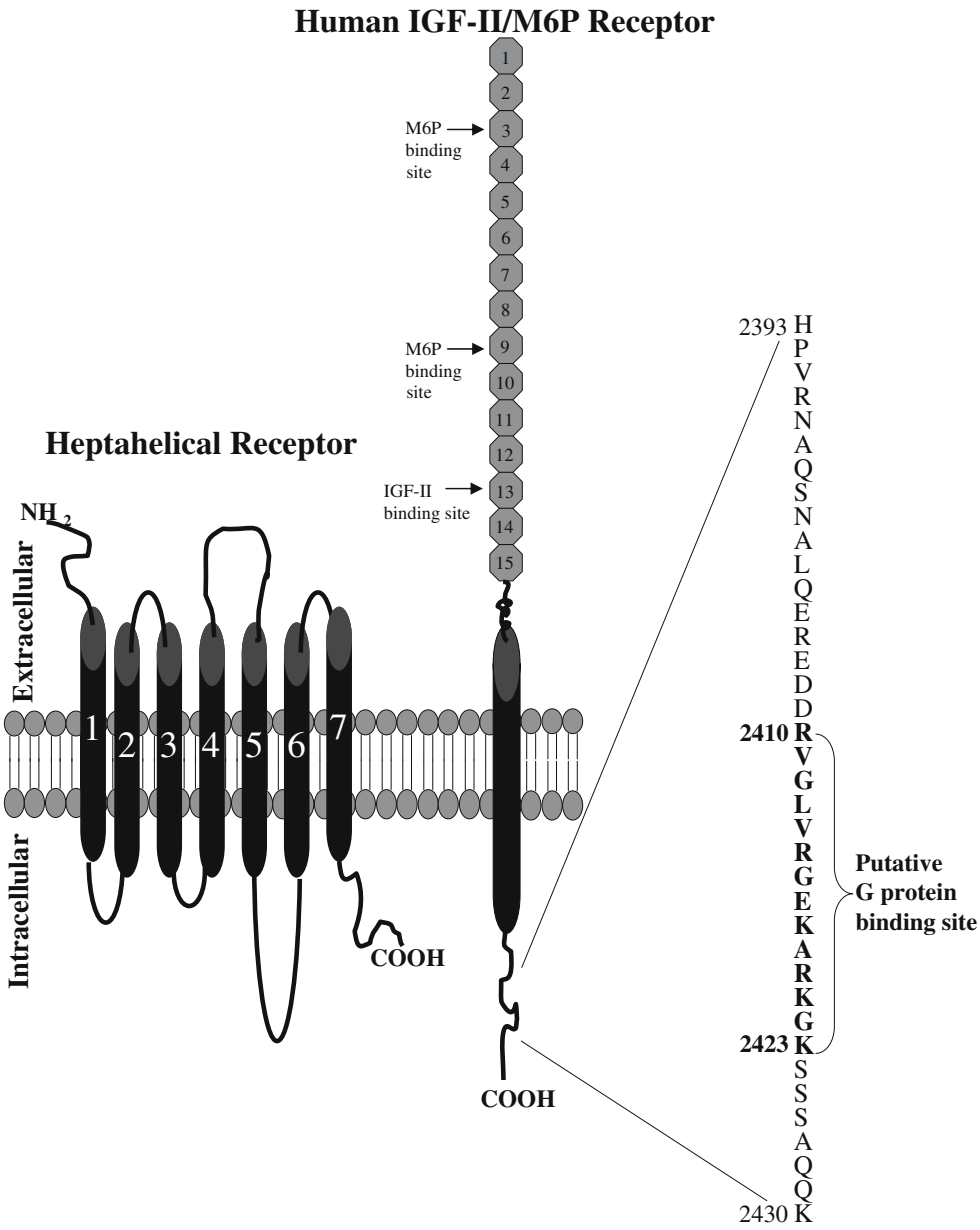

Fig. 1 Diagram of the structures of a heptahelical G protein-coupled receptor and the IGF-II/M6P receptor. Traditional G protein-coupled receptors have a transmembrane core composed of seven membrane-spanning domains (*black cylinders*) capped on the extracellular side by a ligand-binding domain and on the cytoplasmic side by a domain that interacts with heterotrimeric G proteins composed of α, β, and γ subunits. By contrast, the IGF-II/M6P receptor is a type I single-transmembrane glycoprotein consisting of four structural domains, including an amino-terminal signal sequence, which is not shown because it is subsequently cleaved during the formation of the mature receptor. The large extracytoplasmic domain is made up of 15 structurally homologous repeat domains, indicated as *grey octagons*. The receptor has a single-transmembrane domain and a carboxy-terminal cytoplasmic tail. G protein binding to the IGF-II/M6P receptor is believed to occur within a 14-amino-acid segment (RVGLVRGEKARKGK) within the cytoplasmic tail, which does not have a kinase domain

receptor immunoreactivity has been reported on glial cells of the adult mouse brain under normal conditions [100, 101]. Additionally, receptor levels are differentially altered in response to ischemic [102, 103], electrolytic [104], and chemical [105, 106] brain trauma, suggesting the receptor might have a role in neurodegeneration or regeneration after brain lesion/trauma.

IGF-II/M6P Receptor Functions The majority of IGF-II/M6P receptors are located intracellularly, where they function principally in the trafficking of M6P-containing lysosomal enzymes from the trans-Golgi network (TGN) to the endosomal-lysosomal system. After receptor binding to soluble lysosomal enzymes within the Golgi complex, clathrin-associated proteins bind to an acidic-cluster-dileucine amino acid motif within the cytosolic tail of the IGF-II/M6P receptor, and the receptor–enzyme complex is carried to early endosomes via clathrin-coated vesicles [53, 107–110]. Previously, the interaction between clathrin adaptor protein 1 (AP-1), ADP-ribosylation factor, and the IGF-II/M6P receptor was thought to mediate clathrin-coat assembly on vesicles budding from the TGN [53, 111–113]. However, several recent studies have indicated that members of the clathrin-associated Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding (GGA) protein family, rather than AP-1 alone, participate in IGF-II/M6P receptor movement out of the Golgi [108, 114–119]. Receptor-enzyme complexes dissociate within the acidic pH of the endosomes, after which receptors are either recycled back to the TGN or trafficked up to the plasma membrane. Cell surface receptors

(~10% of the total receptor population) mediate the binding and internalization of a number of extracellular growth factors and peptides, including IGF-II, proliferin, LIF, renin, and EGF, for their subsequent degradation. IGF-II/M6P receptor-mediated internalization is also believed to facilitate activation of the latent TGF- β to the active factor [55, 120, 121], which regulates differentiation and growth of many cell types.

Latent pro-TGF-β, which bears an M6P-tagged N-linked oligosaccharide side chain, is secreted from cells and stored in the extracellular matrix as an inactive precursor, which is activated after cleavage by proteases such as plasmin [55, 120, 121]. The ability of plasminogen and the urokinase-type plasminogen activator receptor (uPAR) to bind the IGF-II/M6P receptor at regions distinct from its M6P-binding sites supports a putative model in which binding of urokinase plasminogen activator to uPAR complexed to the IGF-II/M6P receptor facilitates conversion of plasminogen to plasmin, which in turn proteolytically activates the receptor-bound TGF-β precursor [108, 122].

The importance of IGF-II/M6P receptor-mediated regulation of extracellular IGF-II concentrations is underscored by gene targeting studies that have shown that deletion of the IGF-II/M6P receptor gene results in excess IGF-II levels, organomegaly, and perinatal death, which can be rescued when expressed in an IGF-II or IGF-I receptor-deficient background [123, 124]. A number of studies have also reported that expression of the IGF-II/M6P receptor can stimulate apoptosis or suppress proliferation and tumorigenesis in cultured cell model systems using SW 48 colorectal cancer cells [125], JEG-3 choriocarcinoma cells [126], and PC-3 prostate cancer cells [127]. Accordingly, decreased IGF-II/M6P levels provoke increased proliferation in vitro or enhanced tumorigenesis upon injection of the altered cells into animals [128–131]. The mechanisms by which the IGF-II/M6P receptor acts to suppress cell growth and inhibit apoptosis, both in the universal sense and as they apply to individual types of cells, are still not entirely clear. It is possible that both major ligand-binding functions of the receptor—IGF-II binding and phosphomannosyl ligand binding—may contribute to the receptor's growth-suppressive activity. An important recent example illustrating this problem can be found in the elegant study of Li and Sahagian [132] that showed that expression of the IGF-II/M6P receptor in receptor-deficient 66c14 mouse mammary tumor cells blocked the ability of the cells to proliferate and form tumors in mice, despite having no apparent effect on the in vitro rate of proliferation or invasiveness. It was unclear whether this effect was due to enhanced internalization of IGF-II, increased activation of TGF-β, or decreased secretion of lysosomal enzymes in the IGF-II/M6P receptor-expressing cells.

Several studies suggest that in addition to its trafficking role, the IGF-II/M6P receptor also mediates transmembrane signaling after IGF-II binding, including increased amino acid uptake in muscle cells [133], glycogen synthesis in hepatoma cells [134], exocytosis of insulin from pancreatic cells [135], cell proliferation in K562 erythroleukemia cells [136], increased gene expression in spermatocytes [137], motility of human rhabdomyosarcoma cells [138], migration of human extravillous trophoblasts [139], stimulation of Na^+/H^+ exchange and inositol triphosphate production in canine kidney cells [140], calcium influx (but not cell proliferation) in primed BALB/c3T3 fibroblast cells [141–143], increased choline acetyltransferase (ChAT) activity in septal cultured neurons [144], and potentiation of acetylcholine (ACh) release from the adult rat hippocampus [145]. Receptor specificity in most cases was confirmed by the use of IGF-II analogues, receptor antibodies that mimic/block IGF-II effects, or by evaluating biological effects in a cellular model system lacking IGF-I receptors [135, 136, 139, 145].

Despite the evidence that a variety of cellular/biological effects are being mediated by the IGF-II/M6P receptor, not much is known about the intracellular mechanisms by which the receptor regulates such effects. The single–transmembrane domain structure of the receptor and the lack of a kinase domain in its cytoplasmic tail suggest that components of its downstream signaling pathways likely differ from those of the IGF-I and insulin receptors, which usually mediate their effects by triggering intrinsic tyrosine kinase activity that interfaces with an array of intracellular signaling cascades, such as mitogen-activated protein (MAP) kinase and PI-3-kinase pathways. Evidence demonstrating that the effects of IGF-II are PTX sensitive and are mediated either by a decrease in AC activity [139, 146], increased IP_3 production [140], or stimulation of PKC activity [147] has raised the intriguing possibility that the IGF-II/M6P receptor might mediate signaling via a G protein-coupled mechanism. Although this suggestion has been met with some skepticism in the past, the identification of other single-transmembrane domain receptors coupling to G proteins, together with a growing number of recent studies on IGF-II in both neuronal and nonneuronal systems, indicate that IGF-II/M6P receptor–G protein coupling may constitute a genuine physiologically important interaction.

IGF-II/M6P Receptor–G Protein Interaction

Over the last two decades, a variety of experimental approaches under both in vivo and in vitro conditions have indicated that the single-transmembrane domain IGF-II/

M6P receptor may be associated either directly or indirectly with a heterotrimeric G protein (Table 1). Evidence for such an interaction was first suggested in 1987 by Ikuo Nishimoto and colleagues [142, 148, 149] when they reported that IGF-II-induced Ca^{2+} influx in competent BALB/ c3T3 cells primed with EGF was inhibited by PTX in a concentration-dependent manner and that [^{125}I] IGF-II binding was inhibited in the presence of GTPγS. Low concentrations of IGF-I and insulin did not alter Ca^{2+} levels, suggesting that this effect was possibly mediated via the IGF-II/M6P receptor. The authors further strengthened the notion of an IGF-II/M6P receptor–G protein interaction with their finding that IGF-II attenuated PTX-catalyzed ADP ribosylation in mouse BALB/c3T3 fibroblast cell membrane, an effect that was significantly attenuated when IGF-II was washed out, but mimicked by aluminum fluoride (i.e., 10-mM NaF+100-μM $AlCl_3$) and GTPγS [150]. Additionally, when phospholipid vesicles were reconstituted with purified rat IGF-II/M6P receptor and Gi_2 protein, there was a resulting decrease in [^{125}I]IGF-II binding in the presence of GTPγS; moreover, IGF-II facilitated [^{35}S]GTPγS binding to Gi_2 in a PTX-sensitive manner. The specificity of the IGF-II/M6P receptor was indicated by the continued effectiveness of IGF-II in IGF-I receptor-deficient membranes and by the attenuation of IGF-II-induced effects in IGF-II/M6P receptor-free membranes, as well as in the presence of an anti-IGF-II/M6P receptor blocking antibody [150]. It was further demonstrated that the stimulatory effect of IGF-II on [^{35}S]GTPγS binding and GTPase activity in phospholipid vesicles expressing the human IGF-II/M6P receptor and Gi proteins can be regulated by M6P, although binding of M6P-bearing ligand to the receptor itself is not altered by GTPγS [151] (Table 1).

By comparing the primary sequence of the human IGF-II/M6P receptor with that of known GPCRs and mastoparan, a wasp venom peptide belonging to the activators of G protein signaling protein (AGS) family [152], Nishimoto and colleagues [153, 154] have identified a 14-residue sequence (Arg^{2410}-Lys^{2423}) within the cytoplasmic region of the IGF-II/M6P receptor that shares structural homology with both the venom peptide and the terminal portion of the third cytoplasmic loop of GPCRs. When the synthesized peptide sequence (RVGLVRGEKARKGK) was tested in an aqueous system, it was found to stimulate [^{35}S]GTPγS binding to $Gα_{i2}$, induce GTPase activity, and increase GDP release, all of which were sensitive to both PTX treatment and Mg^{2+} concentrations. Furthermore, in phospholipid vesicles reconstituted with the human IGF-II/M6P receptor and $Gα_{i2}$, administration of an antibody raised against the 14-residue sequence attenuated the IGF-II-induced stimulation of [^{35}S]GTPγS binding to soluble $Gα_{i2}$ proteins [154–156]. To examine this effect under more physiological conditions, COS cells stably expressing constitutively activated Gαi proteins were transfected with human IGF-II/M6P receptor cDNA. In these cells, IGF-II significantly impaired CTX- or forskolin-stimulated AC activity, whereas no effect was observed in cells transfected with empty vector, or in cells expressing truncated IGF-II/M6P recep-

Table 1 Evidence supporting IGF-II/M6P receptor–G protein interaction

Cell type	Evidence of interaction with G protein	References
BALB/c3T3 cells	PTX-blocked IGF-II-mediated increase in Ca^{2+} mobilization PTX-catalyzed ADP ribosylation is abolished by IGF-II and mimicked by GTPγS GTPγS inhibition of [^{125}I]IGF-II binding	[142, 148–150]
Phospholipid vesicles expressing IGF-II/M6P receptor and Gi_2 protein	[^{35}S]GTPγS binding to Gi_2 is facilitated by IGF-II and is sensitive to PTX and an antibody against the 14 a.a. tail sequence GTPγS-sensitive decrease in [^{125}I]IGF-II binding	[150, 151, 154–156]
COS cells stably transferred with full length IGF-II/M6P receptor and $Gα_{12}$ protein	CTX and forskolin-stimulated increase in AC activity is impaired by administration of IGF-II	[157]
CHO cells	PTX-blocked IGF-II-medicated increase in Ca^{2+} influx	[159]
Rabbit chondrocytes	IGF-II-medicated increase in Ca^{2+} influx abolished in the presence of PTX	[160]
Bovine capillary endothelial cells	PTX treatment prevents Leu^{27}IGF-II and proliferin-induced chemotaxis	[161]
Mouse pancreatic β cells	IGF-II-medicated insulin exocytosis is sensitive to PTX	[135]
Human extravillous trophoblasts	Decreased AC activity and increased migration in the presence of IGF-II and Leu^{27}IGF-II	[139]
Rat hippocampal neurons and dissociated basal forebrain cholinergic neurons	[^{125}I]IGF-II binding inhibited in the presence of Gpp(NH)p, GTPγS, and PTX Co-immunoprecipation of IGF-II/M6P receptor with $Gi_{α2}$ protein; sensitive to PTX treatment Leu^{27}IGF-II-induced ACh release inhibited in the presence of PTX PTX-abolished reduction in whole cell current and increased depolarization induced by Leu^{27}IGF-II	[145]

tors without the Arg^{2410}-Lys^{2423} sequence [157]. Furthermore, the C-terminal Ser^{2424}-Ile^{2451} region of the IGF-II/M6P receptor was found to exhibit sequence similarity with a part of the pleckstrin homology domain of several proteins that bind the Gβγ dimer and inhibit its stimulatory action on AC activity [157]. These results, taken together, led Nishimoto and colleagues [156, 157] to postulate that the IGF-II/M6P receptor may couple directly to the $G\alpha_{i2}$ protein via the identified 14-residue sequence located in the cytoplasmic domain of the receptor (Fig. 1).

Interestingly, the interaction between the IGF-II/M6P receptor and the G protein was challenged in 1995 by a report from Körner et al. [158] in which they failed to replicate the coupling of the IGF-II/M6P receptor with an inhibitory G protein.

In their paradigm, IGF-II did not alter PTX-mediated [^{32}P]ADP ribosylation or GTPase activity in mouse L cells (L929, a mouse embryonic fibroblast line) transfected with wild-type or truncated human IGF-II/M6P receptor cDNA.

Furthermore, IGF-II had no effect on either GTPγS binding or GTPase activity of PTX-sensitive G proteins in phospholipid vesicles reconstituted with wild-type or truncated IGF-II/M6P receptors. In addition, IGF-II did not affect GTPγS binding in vesicles containing purified wild-type IGF-II/M6P receptor and monomeric Gαo or Gαi and βγ dimers. These results suggested that the human IGF-II/M6P receptor does not interact or couple to G proteins in mouse L cell membranes or in phospholipid vesicles [158]. Nevertheless, a number of reports over the past decade persistently suggest that IGF-II/M6P receptors expressed in various tissues across different species may couple to an inhibitory G protein. This is supported by experimental data showing that: (1) IGF-II/M6P receptor-mediated increases in Ca^{2+} influx in Chinese hamster ovary cells, as well as Ca^{2+} mobilization in articular rabbit chondrocytes, are both abolished in the presence of PTX [159, 160]; (2) chemotaxis of bovine capillary endothelial cells induced by the IGF-II analogue Leu^{27}IGF-II is mediated via an IGF-II/M6P receptor-dependent PTX-sensitive pathway [161]; (3) IGF-II, acting through the IGF-II/M6P receptor, promotes exocytosis of insulin from mouse pancreatic β cells via a PTX-sensitive, PKC-dependent pathway [135]; and (4) IGF-II/M6P receptor stimulation by IGF-II, Leu^{27}IGF-II, or a related analogue, QAYL-Leu^{27}IGF-II, decreases AC activity and promotes migration of human extravillous trophoblasts [139] (Table 1).

Although most of the results on IGF-II/M6P receptor signaling have been obtained from nonneuronal systems, an accumulating body of evidence indicates that IGF-II/M6P receptors expressed in the brain may also utilize G proteins to transduce extracellular signals across the plasma membrane. Earlier studies have shown that IGF-II/M6P receptors exhibit a distinct distributional profile from that of the IGF-I receptor in the adult rat brain and respond differentially to various pharmacological/surgical manipulations [104, 105, 110, 162], thus raising the possibility that this receptor may have a unique role in the normal maintenance and activity-dependent functioning of the adult brain. This is substantiated, in part, by our previous results that showed that IGF-I inhibits, whereas IGF-II potentiates, endogenous ACh release from the rat hippocampal formation [163, 164]. Tetrodotoxin, a sodium channel blocker, suppressed the effects of IGF-I but not those of IGF-II, suggesting that IGF-I acts indirectly via the release of other transmitters/modulators, whereas IGF-II may act directly on, or in close proximity to, cholinergic terminals [164, 165]. Our report of a widespread distribution of the IGF-II/M6P receptor throughout the brain, including its expression on cholinergic cell bodies and fibers, provided a neuroanatomical foundation to support this idea. However, in light of the evidence that high concentrations of IGF-II can bind to the IGF-I receptor, it was not initially apparent whether the effects of IGF-II on ACh release were mediated via activation of the IGF-I receptor or the IGF-II/M6P receptor. To address this issue, we used Leu^{27}IGF-II, an IGF-II analogue that binds rather selectively to the IGF-II/M6P receptor [143, 145, 166, 167], and demonstrated that this analogue can potentiate ACh release from adult rat hippocampal slices in a TTX-insensitive manner, as observed for IGF-II. Additionally, treatment with Leu^{27}IGF-II caused a reduction in whole-cell currents and depolarization in acutely dissociated rat cholinergic basal forebrain neurons [145].

To determine if these effects of IGF-II/M6P receptor stimulation were mediated via an interaction with a G protein, we first performed competition binding experiments in rat hippocampal membrane preparations and showed that (1) GTPγS and Gpp(NH)p inhibited [^{125}I]IGF-II interaction with its receptor and (2) the Gi-sensitive toxin PTX inhibited [^{125}I]IGF-II receptor binding. Subsequently, we demonstrated that the Giα protein, but not Gsα or Gqα proteins, co-immunoprecipitated with the IGF-II/M6P receptors and was sensitive to PTX treatment, thus suggesting an association between hippocampal IGF-II/M6P receptors and an inhibitory G protein. The functional relevance of this interaction was confirmed by our findings that pretreatment with PTX abolished Leu^{27}IGF-II-mediated ACh release, as well as the electrophysiological effects of this peptide in isolated cholinergic neurons [145]. Additionally, we showed that the Giα protein-IGF-II/M6P receptor coupling resulted in the activation of PKCα, possibly through either IGF-II/M6P receptor-mediated increase in IP_3 levels via PLC [168, 169] or by direct activation of PLC by the βγ subunits of the G protein [170, 171]. Subsequently, phospho-PKCα was found to phosphorylate and activate two of its downstream effectors, myristoylated alanine-rich C kinase substrate (MARCKS) and growth-associated protein-43 (GAP-43; see Fig. 2).

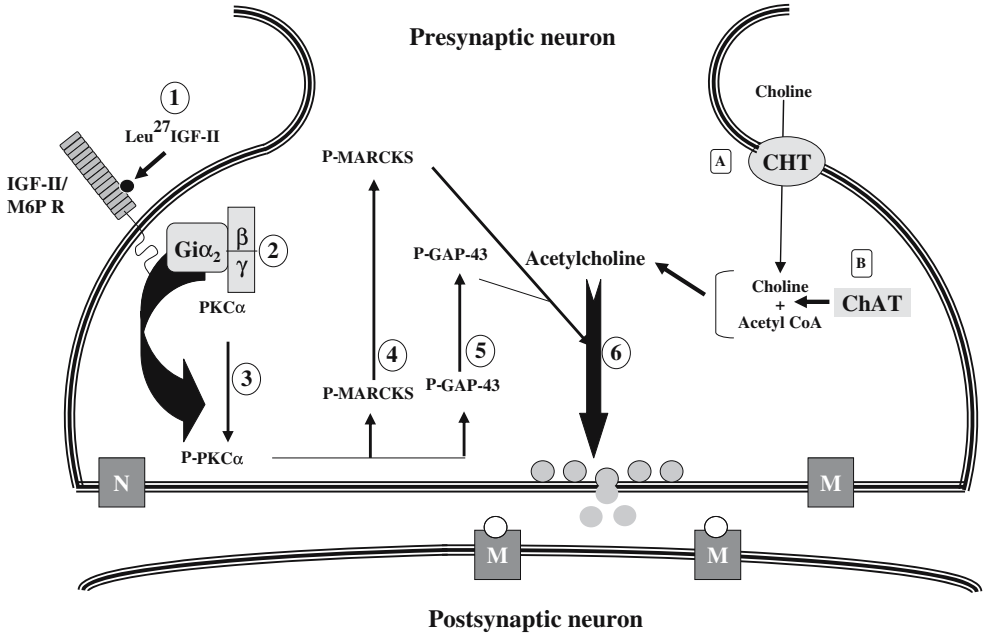

Fig. 2 Schematic representation of the events that are proposed to mediate Leu^{27}IGF-II-induced potentiation of ACh release from cholinergic terminals. Leu^{27}IGF-II binds IGF-II/M6P receptors located on cholinergic terminals (*1*). Activated IGF-II/M6P receptors couple to a Gi protein (*2*), which recruits cytosolic PKC to the plasma membrane for subsequent phosphorylation (*3*). Activated PKC phosphorylates membrane-associated MARCKS (*4*) and GAP-43 effectors (*5*), causing translocation of phospho-MARCKS into the cytoplasm and resulting in increased ACh release from synaptic terminals (*6*). Leu^{27}IGF-II-induced potentiation of ACh release is not associated with alterations in ACh synthesis, including high-affinity choline uptake (*A*) or ChAT enzyme activity (*B*). *CHT* High-affinity choline transporter, *M* muscarinic ACh receptor, *N* nicotinic ACh receptor

MARCKS and GAP-43 are well-established mediators of neurotransmitter/ACh release, as the phosphorylation and subsequent translocation of both molecules are important for actin cytoskeleton barrier breakdown at the active zone, as well as calcium/calmodulin-dependent kinase activation, respectively [172]. Collectively, these results suggest that IGF-II/M6P receptors expressed in the adult rat brain are coupled to an inhibitory G protein and that this association may be involved in mediating the effects of IGF-II on neurotransmission in the CNS (Table 1). However, it should be noted that there is still no tangible evidence to indicate whether the IGF-II/M6P receptor interacts directly or indirectly with a G protein. Certainly more work is needed to determine the nature of neuronal IGF-II/M6P receptor–G protein interaction and its relevance to the normal operation of the developing and adult brain.

Other Non-GPCRs and G Protein Interaction

It has become apparent over the last decade that certain tyrosine kinase receptors such as the EGF receptor, the IGF-I receptor, and the insulin receptor may also interact with G proteins to mediate some of their pleiotropic effects [2, 15, 44, 48, 49] (Table 2). The EGF receptor consists of an extracellular ligand-binding domain, a single-transmembrane domain, and a cytosolic domain that encompasses its tyrosine kinase activity.

It has been reported that EGF-induced increases in intracellular Ca^{2+} in hepatocytes are mediated by EGF receptor stimulation of a G protein that modulates PLC activity [173–175].

Additionally, activation of the EGF receptor in cardiac myocytes, parotid glands, and pancreatic cells has been shown to enhance cAMP levels by stimulating AC via a stimulatory G protein [44, 176]. Studies designed to unravel the mechanism(s) involved in EGF receptor-mediated activation of Gs have shown that a 13-amino-acid sequence (Arg^{646}-Arg^{658}) in the cytosolic, juxtamembrane region of the EGF receptor is sufficient to activate the G protein. However, this peptide did not stimulate GTP binding to Gi but increased the GTPase activity of this protein [44, 177].

Unlike the EGF receptor, the insulin and IGF-I receptors consist of two extracellular α chains that are connected to each other by sulfhydryl bridges and to two β chains that traverse the membrane. Although these receptors usually mediate their actions by intrinsic tyrosine kinase activity, some earlier reports indicated that insulin-dependent lipolysis and inhibition of glucose oxidation in adipocytes may be mediated by activation of a Gαi protein [178, 179]. There is also evidence that insulin receptor-mediated upregulation of GLUT4 transporters in NIH 3T3 adipocytes may depend on $G\alpha_{q/11}$ proteins, whereas in rat fibroblasts,

Table 2 Other nonheptahelical receptors that interact with G proteins

Nonheptahelical receptor	Associated G protein	References
EGF receptor	Gs	[173–177]
Insulin receptor	Gi/o, $Gq/_{11}$	[178–180, 183]
IGF-I receptor	Gi, Gβγ	[151, 160, 184–186]
Kainate receptor	Gi/o	[187]
AMPA receptor	Gi	[188–191]
PDGF receptor	Gi	[192, 193]
FGF receptor	Gs	[194]
Thrombospondin receptor	Go, Gs	[195, 196]
Zona pellucida glycoprotein receptor	Gi/o	[197, 198]
C-type natriuretic peptide receptor	Gi	[199–202]
T cell receptor	$Gq/_{11}$	[203–206]

Note that some receptor–G protein interactions, including that of the thrombospondin receptor, are believed to be indirectly mediated.

the receptor can stimulate Gs proteins [44, 180]. However, more recent work on the mechanism of cross talk between insulin signaling through its intrinsic tyrosine kinase and potential activation of a G protein-regulated pathway has revealed some details of the process. Analysis of insulin action in cells from transgenic mice having inducible antisense RNA to Giα2 showed that knockdown of Giα2 expression impaired insulin action on a variety of processes [181]. It was found that this effect was due to a Giα2-mediated increase in activity of protein tyrosine phosphatase 1B, which interfered with insulin signaling by dephosphorylation of insulin receptor substrate-1 [181, 182]. Thus, it does not appear that the point of cross talk between the pathways in this case involves direct interaction between the insulin receptor and Giα2. This conclusion is supported by another study in human umbilical vein endothelial cells, in which insulin stimulated cyclic GMP production in a pertussis toxin-sensitive manner [183]. This action of insulin appeared to require extracellular signal-regulated kinase (ERK1/2) phosphorylation, suggesting signaling through the Ras–MEK–ERK cascade. Finally, direct interaction between the insulin receptor and Giα2 could not be shown by co-immunoprecipitation [183], thus indicating that many questions remain to be addressed in relation to the interaction between the insulin receptor and the G protein-regulated signaling pathway.

Similar to insulin, a number of experimental paradigms have shown that IGF-I-induced increases in Ca^{2+} influx and DNA synthesis in BALB/c-3T3 cells, as well as activation of MAP kinase in neuronal cells, are sensitive to PTX treatment [151, 160, 184, 185]. Furthermore, there is evidence that activation of the IGF-I receptor can lead to an inhibition of AC via a Gαi2 protein and activation of ERKs through Gβγ subunits in human smooth muscle cells [186]. Although these results suggest the existence of putative alternative mechanisms for insulin and IGF-I receptor signaling, the physiological relevance of these pathways remains to be established.

Within the nervous system, nonheptahelical receptors such as the glutamatergic kainate [187] and α-amino-3-hydroxy-5-methyisoxazole-4-proprionate (AMPA) receptors [188] have also been shown to interact with G proteins. Activation of the kainate receptor, a member of the cation-permeable ionotropic glutamate receptors, has been shown to inhibit γ-aminobutyric acid (GABA) release from the hippocampus in a PTX-sensitive manner. Additionally, the binding profile of [^{3}H](2S,4R)-4-methylglutamate, a kainate receptor-selective agonist, is shifted to the right in the presence of Gpp(NH)p and PTX, but is not affected by CTX in rat hippocampal membranes, thus suggesting a possible association between the kainate receptor and a Gαi/o protein [187]. There is also evidence that the AMPA receptor, another member of the ionotropic glutamate receptor family, may regulate cellular mechanisms by interacting with a G protein. This is supported by experimental data that have shown that (1) AMPA receptor activation reduced PTX-mediated ADP ribosylation of the Gαi protein and inhibited forskolin-stimulated AC activity in isolated membranes and intact cortical cultured neurons [188]; (2) in retinal ganglion cells, AMPA-induced suppression of the cGMP-gated current is blocked by PTX, but not by GTPγS, suggesting that AMPA receptor activation in these cells leads to the inhibition of cationic channels gated by cGMP through PTX-sensitive G-protein [189]; (3) a Gi/o protein inhibitor *N*-ethylmaleimide attenuates the AMPA receptor-mediated presynaptic inhibition at cerebellar GABAergic synapses, whereas Gi/o-coupled receptor agonists baclofen (GABAB receptor agonist) and DCG-IV (mGlu receptor agonist) and the P/Q-type calcium channel blocker ω-agatoxin IVA markedly occluded the AMPA receptor-mediated inhibition of GABAergic transmission [190]; and (4) AMPA receptor-mediated inhibition of Ca^{2+} channels in rat auditory brainstem neurons can be abolished in presence of the nonhydrolyzable GTP analogue GTPγS [191]. These results suggest that AMPA receptors expressed at various central synapses may influence neurotransmitter release by interacting with heterotrimeric G proteins (Table 2).

A variety of other nonclassical GPCRs that have been shown to interact with G proteins include the PDGF [192, 193] and FGF receptors [194], the thrombospondin receptor

[195, 196], the zona pellucida glycoprotein receptor [197, 198], the C-type natriuretic peptide receptor (NPR-C) [199–202], and the T cell receptor [203–206] (see reviews [2, 44]; Table 2). It is of interest to note that NPR-C, like the IGF-II/M6P receptor, is a single-transmembrane protein that was originally thought to act as a "clearance receptor" for circulating atrial natriuretic peptide involved in cardiac development and natriuresis from the kidney. Subsequently, it was shown that activation of NPR-C can decrease AC activity in a variety of tissues via stimulation of a PTX-sensitive G protein [2, 207]. Although the significance of the NPR-C/G protein interaction remains to be fully defined, these results reinforce the notion that a single-transmembrane domain receptor can indeed interact with G proteins to mediate cell signal transduction.

Conclusion

The IGF-II/M6P receptor is a widely expressed, single-transmembrane domain, multifunctional protein that is involved primarily in lysosomal enzyme trafficking, peptide internalization, and degradation of a variety of molecules including IGF-II. A growing body of evidence, including several recent reports, suggests a role for the receptor in mediating signal transduction via coupling to an inhibitory G protein. Some of these effects include exocytosis of insulin from mouse pancreatic β cells, migration of human extravillous trophoblasts, and potentiation of ACh release from adult rat brain. However, more work is needed to better characterize the interaction between the IGF-II/M6P receptor and G protein, particularly with regard to whether the receptor–G protein interaction is direct, mediated by an intermediate protein or through the formation of a heterodimer receptor complex with a classical GPCR. Future research should also attempt to differentiate whether this receptor can couple selectively to Gi or Go protein in some systems to mediate specific effects to highlight the physiological significance of this interaction. Additionally, it would be of interest to determine whether the IGF-II/M6P receptor, apart from potentiating endogenous ACh release, can regulate the release of other neurotransmitters in the CNS by virtue of its interactions with G proteins. Nevertheless, the addition of the IGF-II/M6P receptor to the ever-growing list of putative non-seven-transmembrane receptors that can couple to G proteins, indicates that G protein-mediated signaling might not be limited to classical GPCRs. Moreover, the discovery that CNS metabotropic receptors, such as the muscarinic ACh and metabotropic glutamate receptors, can act on ion channels in a G protein-independent manner [208] has provided further evidence that classical GPCRs do not have to mediate signal transduction exclusively via heterotrimeric G proteins. Thus, the impetus may exist to redefine the term "G protein-coupled receptor" from a structural term referring specifically to heptahelical receptors to one of a more broad functional definition.

Acknowledgment We would like to thank Dr. G.B. Baker for his useful comments on this review. CH is a recipient of the Alzheimer Society studentship award, and SK is a recipient of a Canada Research Chair (CRC) in Neurodegenerative diseases and a Senior Scholar award from the AHFMR. JHJ is a CRC recipient in Alzheimer Research. This paper was supported by grants from the Natural Sciences and Engineering Research Council of Canada (SK), Alberta Heritage Foundation for Medical Research (AHFMR; SK), Canadian Institutes of Health Research (SK and JHJ), and National Institutes of Health grant CA91885 (RGM).

References

1. Oakley RH, Barak LS, Gagnon MG (2005) Real-time imaging of GPCR-mediated arrestin translocation as a strategy to evaluate receptor–protein interactions. In: George SR, O'Dowd BF (eds) G protein coupled receptor–protein interactions. Wiley, New Jersey, USA, pp 53–80
2. Landry Y, Niederhoffer N, Sick E, Gies JP (2006) Heptahelical and other G-protein-coupled receptors (GPCRs) signaling. Curr Med Chem 13:51–63
3. Oldham WM, Hamm HE (2006) Structural basis of function in heterotrimeric G proteins. Q Rev Biophys 39:117–166
4. Gudermann T, Schoneberg T, Schultz G (1997) Functional and structural complexity of signal transduction via G-protein-coupled receptors. Annu Rev Neurosci 20:399–427
5. Cabrera-Vera T, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE (2003) Insights into G protein structure, function, and regulation. Endocr Rev 24:765–781
6. Esbenshade TA (2006) G protein-coupled receptors as targets for drug discovery, in G protein–coupled receptors in drug discovery. In: Lundstrom KH, Chiu ML (eds) Taylor and Francis Group, LLC, Florida, USA, pp 15–36
7. Offermanns S (2003) G-proteins as transducers in transmembrane signalling. Prog Biophys Mol Biol 83:101–130
8. Robishaw JW, Berlot CH (2004) Translating G protein subunit diversity into functional specificity. Curr Opin Cell Biol 16:206–209
9. Levitzki A, Klein S (2002) G-protein subunit dissociation is not an integral part of G-protein action. ChemBioChem 3:815–818
10. Simon MI, Strathmann MP, Gautam N (1991) Diversity of G proteins in signal transduction. Science 252:802–808
11. Hamm HE (1998) The many faces of G protein signaling. J Biol Chem 273:669–672
12. Suzuki N, Nakamura S, Mano H, Kozasa T (2003) Gα12 activates RhoGTPase through tyrosine-phosphorylated leukemia-associated RhoGEF. Proc Natl Acad Sci USA 100:733–738
13. Warrior U, Gopalakrishnan S, Vanhauwe J, Burns D (2006) High throughput screening assays for G protein-coupled receptors, in G protein-coupled receptors in drug discovery. In: Lundstrom KH, Chiu ML (eds) Taylor and Francis Group, LLC, Florida, USA, pp 159–189
14. Hollmann MW, Strumper D, Herroeder S, Durieux ME (2005) Receptors, G proteins, and their interactions. Anesthesiology 103:1066–1078
15. Hur EM, Kim KT (2002) G protein-coupled receptor signalling and cross-talk: achieving rapidity and specificity. Cell Signal 14:397–405

16. Strange PG (1988) The use of radiochemicals for studying receptors. Crit Rep Appl Chem 24:56–93
17. Ramsay D, Milligan G (2005) Analysis of receptor–receptor interactions using time-resolved fluorescence resonance energy transfer. In: George SR, O'Dowd BF (eds) G protein coupled receptor–protein interactions. Wiley, New Jersey, USA, pp 19–36
18. Sim LJ, Selley DE, Childers SR (1997) Autoradiographic visualization in brain of receptor–G protein coupling using [^{35}S]GTPγS binding. Methods Mol Biol 83:117–132
19. Sovago J, Dupuis DS, Gulyas B, Hall H (2001) An overview on functional receptor autoradiography using [^{35}S]GTPγS. Brain Res Rev 38:149–164
20. Harrison C, Traynor JR (2003) The [^{35}S]GTPgammaS binding assay: approaches and applications in pharmacology. Life Sci 74:489–508
21. Frang H, Mukkala VM, Syysto R, Illikka P, Hurskainen P, Scheinin M, Hemmila I (2003) Nonradioactive GTP binding assay to monitor activation of G protein-coupled receptors. Assay Drug Dev Technol 1:261–273
22. Kalatskaya I, Schüssler S, Blaukat A, Müller-Ester W, Jochum M, Proud D, Faussner A (2004) Mutation of tyrosine in the conserved NP*XX*Y sequence leads to constitutive phosphorylation and internalization, but not signaling, of the human B_2 bradykinin receptor. J Biol Chem 30:31268–31276
23. Hall RA (2005) Co-immunoprecipitation as a strategy to evaluate receptor–receptor or receptor–protein interactions. In: George SR, O'Dowd BF (eds) G protein coupled receptor–protein interactions. Wiley, New Jersey, USA, pp 165–178
24. Chakrabarti S, Regec A, Gintzler AR (2005) Biochemical demonstration of mu-opioid receptor association with Gsα: enhancement following morphine exposure. Mol Brain Res 135:217–224
25. Mathis G (1995) Probing molecular interactions with homogenous techniques based on rare earth cryptates and fluorescence energy transfer. Clin Chem 41:1391–1397
26. Bazin H, Trinquet E, Mathis G (2002) Time resolved amplification of cryptate emission: a versatile technology to trace biomolecular interactions. J Biotechnol 82:233–250
27. Bertrand L, Parent S, Caron M, Legault M, Joly E, Angers S, Bouvier M, Brown M, Michael NP, Milligan G, Game S (2002) The BRET2/arrestin assay in stable recombinant cells: a platform to screen for compounds that interact with G protein-coupled receptors (GPCRs). J Recept Signal Transduct Res 22:533–541
28. van Rijn RM, Chazot PL, Shenton FC, Sansuk F, Bakker RA, Leurs R (2006) Oligomerization of recombinant and endogenously expressed human histamine H_4 receptors. Mol Pharmacol 70:604–615
29. Violin JD, Ren XR, Lefkowitz RJ (2006) G-protein-coupled receptor kinase specificity for beta-arrestin recruitment to the beta2-adrenergic receptor revealed by fluorescence resonance energy transfer. J Biol Chem 281:20577–20588
30. Bulenger S, Marullo S, Bouvier M (2005) Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. Trends Pharmacol Sci 26:131–137
31. Janetopoulos C, Jin T, Devreotes P (2001) Receptor-mediated activation of heterotrimeric G-proteins in living cells. Science 291:2408–2411
32. Pfleger KDG, Eidne KA (2005) Monitoring the formation of dynamic G-protein-coupled receptor–protein complexes in living cells. Biochem J 385:625–637
33. Hoffmann C, Gaietta G, Bunemann M, Adams SR, Oberdorff-Maass S, Behr B, Vilardaga J-P, Tsien RY, Ellisman MH, Lohse M (2005) A FlAsH-based FRET approach to determine G protein-coupled receptor activation in living cells. Nat Methods 2:171–176
34. Kariv II, Stevens ME, Beherns DL, Oldenburg KR (1999) High throughput quantitation of cAMP production mediated by activation of seven-transmembrane domain receptor. J Biomol Screen 4:27–32
35. Naylor LH (1999) Reporter gene technology: the future looks bright. Biochem Pharmacol 5:749–757
36. Prystay L, Gagne A, Kasila P, Yeh LA, Banks P (2001) Homogeneous cell-based fluorescence polarization assay for the direct detection of cAMP. J Biomol Screen 6:75–82
37. Golla R, Seethala R (2002) A homogenous enzyme fragment complementation cyclic AMP screen for GPCR agonists. J Biomol Screen 7:515–525
38. Gabriel D, Vernier M, Pfiefer MJ, Dasen B, Tenaillon L, Bouhelal R (2003) High throughput screening technologies for direct cyclic AMP measurement. Assay Drug Dev Technol 1:291–303
39. Liu B, Wu D (2004) Analysis of the coupling of G12/13 to G protein-coupled receptors using a luciferase reporter assay. Methods Mol Biol 237:145–149
40. Sturino CF, Lachance N, Boyd M, Berthelette C, Labelle M, Li L, Roy B, Scheigetz J, Tsou N, Brideau C, Cauchon E, Carriere MC, Denis D, Greig G, Kargman S, Lamontagne S, Mathieu MC, Sawyer N, Slipetz D, O'Neill G, Wang Z, Zamboni R, Metters KM, Young RN (2006) Identification of an indole series of prostaglandin D2 receptor antagonists. Bioorg Med Chem Lett 16:3043–3048
41. Chen WJ, Jayawickreme C, Watson C, Wolfe L, Holmes W, Ferris R, Armour S, Dallas W, Chen G, Boone L, Luther M, Kenakin T (1998) Recombinant human CXC-chemokine receptor-4 in melanophores are linked to Gi protein: seven transmembrane coreceptors for human immunodeficiency virus entry into cells. Mol Pharmacol 53:177–181
42. Niebauer RT, Gao ZG, Li B, Wess J, Jacobson KA (2005) Signaling of the Human P2Y(1) Receptor measured by a yeast growth assay with comparisons to assays of phospholipase C and calcium mobilization in 1321N1 human astrocytoma cells. Purinergic Signal 1:241–247
43. Christiansen B, Wellendorph P, Brauner-Osborne H (2006) Known regulators of nitric oxide synthase and arginase are agonists at the human G-protein-coupled receptor GPRC6A. Br J Pharmacol 147:855–863
44. Patel TB (2004) Single transmembrane spanning heterotrimeric g protein-coupled receptors and their signaling cascades. Pharmacol Rev 56:371–385
45. Cismowski MJ (2006) Non-receptor activators of heterotrimeric G-protein signaling (AGS proteins). Semin Cell Dev Biol 17:334–344
46. Blumer JB, Cismowski MJ, Sato M, Lanier SM (2005) AGS proteins: receptor-independent activators of G-protein signaling. Trends Pharmacol Sci 26:470–476
47. Sato M, Blumer JB, Simon V, Lanier SM (2006) Accessory proteins for G proteins: partners in signaling. Annu Rev Pharmacol Toxicol 46:151–187
48. Shah BH, Catt KJ (2004) GPCR-mediated transactivation of RTKs in the CNS: mechanisms and consequences. Trends Neurosci 27:48–53
49. Gavi S, Shumay E, Wang HY, Malbon CC (2006) G-protein-coupled receptors and tyrosine kinases: crossroads in cell signaling and regulation. Trends Endocrinol Metab 17:48–54
50. Kornfeld S (1992) Structure and function of the mannose 6-phosphate/insulin-like growth factor II receptors. Annu Rev Biochem 61:307–330
51. Dahms NM, Hancock MK (2002) P-type lectins. Biochim Biophys Acta 1572:317–340
52. Braulke T (1999) Type-2 IGF receptor: a multiple-ligand binding protein. Horm Metab Res 31:242–246

53. Hille-Rehfeld A (1995) Mannose 6-phosphate receptors in sorting and transport of lysosomal enzymes. Biochim Biophys Acta 1241:177–194
54. Hawkes C, Kar S (2004) The insulin-like growth factor-II/mannose-6-phosphate receptor: structure, distribution and function in the central nervous system. Brain Res Rev 44:117–140
55. Dennis PA, Rifkin DB (1991) Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 88:580–584
56. Blanchard F, Duplomb L, Raher S, Vusio P, Hoflack B, Jacques Y, Godard A (1999) Mannose 6-phosphate/insulin-like growth factor II receptor mediates internalization and degradation of leukemia inhibitory factor but not signal transduction. J Biol Chem 274:24685–24693
57. Lee S-J, Nathans D (1988) Proliferin secreted by cultured cells binds to mannose-6-phosphate receptors. J Biol Chem 263:3521–3527
58. Scheel G, Herzog V (1989) Mannose 6-phosphate receptor in porcine thyroid follicle cells. Localization and possible implications for the intracellular transport of thyroglobulin. Eur J Cell Biol 49:140–148
59. Puolakkainen M, Kuo C-C, Campbell LA (2005) *Chlamydia pneumoniae* uses the mannose 6-phosphate/insulin-like growth factor 2 receptor for infection of endothelial cells. Infect Immun 73:4620–4625
60. Gasanov U, Koina C, Beagley KW, Aitken RJ, Hansbro PM (2006) Identification of the insulin-like growth factor II receptor as a novel receptor for binding and invasion by *Listeria monocytogenes*. Infect Immun 74:566–577
61. Kang JX, Li Y, Leaf A (1997) Mannose-6-phosphate/insulin-like growth factor-II receptor is a receptor for retinoic acid. Proc Natl Acad Sci USA 94:13671–13676
62. Morgan DO, Edman JC, Standring DN, Fried VA, Smith MC, Roth RA, Rutter WJ (1987) Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 329:301–307
63. Oshima A, Nolan CM, Kyle JW, Grubb JH, Sly WS (1988) The human cation-independent mannose 6-phosphate receptor. Cloning and sequence of the full-length cDNA and expression of functional receptor in COS cells. J Biol Chem 263:2553–2562
64. Byrd JC, Park JH, Schaffer BS, Garmroudi F, MacDonald RG (2000) Dimerization of the insulin-like growth factor II/mannose 6-phosphate receptor. J Biol Chem 275:18647–18656
65. Hassan AB (2003) Keys to the hidden treasures of the mannose 6-phosphate/insulin-like growth factor 2 receptor. Am J Pathol 162:3–6
66. York SJ, Arneson LS, Gregory WT, Dahms NM, Kornfeld S (1999) The rate of internalization of the mannose 6-phosphate/insulin-like growth factor II receptor is enhanced by multivalent ligand binding. J Biol Chem 274:1164–1171
67. Costello M, Baxter RC, Scott CD (1999) Regulation of soluble insulin-like growth factor II/mannose 6-phosphate receptor in human serum: measurement by enzyme-linked immunosorbent assay. J Clin Endocrinol Metab 84:611–617
68. Kiess W, Greenstein LA, White RM, Lee L, Rechler MM, Nissley SP (1987) Type II insulin-like growth factor receptor is present in rat serum. Proc Natl Acad Sci USA 84:7720–7724
69. MacDonald RG, Tepper MA, Clairmont KB, Perregaux SB, Czech MP (1989) Serum form of the rat insulin-like growth factor II/mannose 6-phosphate receptor is truncated in the carboxy-terminal domain. J Biol Chem 264:3256–3261
70. Valenzano KJ, Remmler J, Lobel P (1995) Soluble insulin-like growth factor II/mannose 6-phosphate receptor carries multiple high molecular weight forms of insulin-like growth factor II in fetal bovine serum. J Biol Chem 270:16441–16448
71. Clairmont KB, Czech MP (1991) Extracellular release as the major degradative pathway of the insulin-like growth factor II/mannose 6-phosphate receptor. J Biol Chem 266:12131–12134
72. Zaina S, Squire S (1998) The soluble type 2 insulin-like growth factor (IGF-II) receptor reduces organ size by IGF-II-mediated and IGF-II-independent mechanisms. J Biol Chem 273:28610–28616
73. Scott CD, Weiss J (2000) Soluble insulin-like growth factor II/mannose 6-phosphate receptor inhibits DNA synthesis in insulin-like growth factor II sensitive cells. J Cell Physiol 182:62–68
74. Harper J, Burns JL, Foulstone EJ, Pignatelli M, Zaina S, Hassan AB (2006) Soluble IGF2 receptor rescues $Apc^{Min/+}$ intestinal adenoma progression induced by *Igf2* loss of imprinting. Cancer Res 66:1940–1948
75. Reddy ST, Chai W, Childs RA, Page JD, Feizi T, Dahms NM (2004) Identification of a low affinity mannose-6-phosphate-binding site in domain 5 or the cation-independent mannose-6-phosphate receptor. J Biol Chem 279:38658–38667
76. Dahms NM, Wick DA, Brzycki-Wessell MA (1994) The bovine mannose 6-phosphate/insulin-like growth factor II receptor. Localization of the insulin-like growth factor II binding site to domains 5-11. J Biol Chem 269:3802–3809
77. Schmidt B, Kiecke-Siemsen C, Waheed A, Braulke T, von Figura K (1995) Localization of the insulin-like growth factor II binding site to amino acids 1508-1566 in repeat 11 of the mannose 6-phosphate/insulin-like growth factor II receptor. J Biol Chem 270:14975–14982
78. Garmroudi F, Devi G, Slentz DH, Schaffer BS, MacDonald RG (1996) Truncated forms of the insulin-like growth factor II (IGF-II)/mannose 6-phosphate receptor encompassing the IGF-II binding site: characterization of a point mutation that abolishes IGF-II binding. Mol Endocrinol 10:642–651
79. Devi GR, Byrd JC, Slentz DH, MacDonald RG (1998) An insulin-like growth factor II (IGF-II) affinity-enhancing domain localized within extracytoplasmic repeat 13 of the IGF-II/mannose 6-phosphate receptor. Mol Endocrinol 12:1661–1672
80. Linnell J, Groeger G, Hassan AB (2001) Real time kinetics of insulin-like growth factor II (IGF-II) interaction with the IGF-II/mannose 6-phosphate receptor. The effects of domain 13 and pH. J Biol Chem 276:23986–23991
81. Brown J, Esnouf RM, Jones MA, Linnell J, Harlos K, Hassan AB, Jones EY (2002) Structure of a functional IGF2R fragment determined from the anomalous scattering of sulfur. EMBO J 21:1054–1062
82. Polychronakos C, Guyda HJ, Posner BI (1988) Mannose 6-phosphate increases the affinity of its cation-independent receptor for insulin-like growth factor II by displacing inhibitory endogenous ligands. Biochem Biophys Res Commun 157:632–638
83. Waheed A, Braulke T, Junghans U, von Figura K (1988) Mannose 6-phosphate/insulin like growth factor II receptor: the two types of ligands bind simultaneously to one receptor at different sites. Biochem Biophys Res Commun 152:1248–1254
84. MacDonald RG (1991) Mannose-6-phosphate enhances cross-linking efficiency between insulin-like growth factor-II (IGF-II) and IGF-II/mannose-6-phosphate receptors in membranes. Endocrinology 128:413–421
85. Nissley P, Kiess W (1991) Reciprocal modulation of binding of lysosomal enzymes and insulin-like growth factor-II (IGF-II) to the mannose 6-phosphate/IGF-II receptor. Adv Exp Med Biol 293:311–324
86. Sara V, Carlsson-Skwirut C (1988) The role of insulin-like growth factors in the regulation of brain development. Prog Brain Res 73:87–99

87. Senior P, Bryne S, Brammar W, Beck F (1990) Expression of the IGF-II/mannose-6-phosphate receptor mRNA and protein in the developing rat. Development 109:67–75
88. Valentino KL, Pham H, Ocrant I, Rosenfeld RG (1988) Distribution of insulin-like growth factor II receptor immunoreactivity in rat tissues. Endocrinology 122:2753–2763
89. Kar S, Chabot J-G, Quirion R (1993) Quantitative autoradiographic localization of [^{125}I]insulin-like growth factor I, [^{125}I] insulin-like growth factor II and [^{125}I]insulin receptor binding sites in developing and adult rat brain. J Comp Neurol 333:375–397
90. Nissley P, Kiess W, Sklar M (1993) Developmental expression of the IGF-II/mannose 6-phosphate receptor. Mol Reprod Dev 35:408–413
91. de Pablo F, de la Rosa EJ (1995) The developing CNS: a scenario for the action of proinsulin, insulin and insulin-like growth factors. Trends Neurosci 18:143–150
92. Sklar MM, Kiess W, Thomas C, Nissley SP (1989) Developmental expression of the tissue insulin-like growth factor-II/mannose 6-phosphate receptor in the rat. Measurement by quantitative immunoblotting. J Biol Chem 264:16733–16738
93. Funk B, Kessler U, Eisenmenger W, Hansmann A, Kolb HJ, Kiess W (1992) Expression of the insulin-like growth factor-II/mannose-6-phosphate receptor in multiple human tissues during fetal life and early infancy. J Clin Endocrinol Metab 75:424–431
94. Pfuender M, Sauerwein H, Funk B, Kessler U, Barenton B, Schwarz HP, Hoeflich A, Kiess W (1995) The insulin-like growth factor-II/mannose-6-phosphate receptor is present in fetal bovine tissues throughout gestation. Domest Anim Endocrinol 12:317–324
95. Hill JM, Lesniak MA, Kiess W, Nissley SP (1988) Radioimmunohistochemical localization of type II IGF receptors in rat brain. Peptides 9(suppl):181–187
96. Lesniak M, Hill J, Kiess W, Rojeski M, Pert C, Roth J (1988) Receptors for insulin-like growth factors I and II: autoradiographic localization in rat brain and comparison to receptors for insulin. Endocrinology 123:2089–2099
97. Smith M, Clemens J, Kerchner G, Mendelsohn L (1988) The insulin-like growth factor-II (IGF-II) receptor of rat brain: regional distribution visualized by autoradiography. Brain Res 445:241–246
98. Marinelli PW, Gianoulakis C, Kar S (2000) Effects of voluntary ethanol drinking on [^{125}I]insulin-like growth factor-I, [^{125}I] insulin-like growth factor-II and [^{125}I]insulin receptor binding in the mouse hippocampus and cerebellum. Neuroscience 98:687–695
99. Wilczak N, De Bleser P, Luiten P, Geerts A, Teelken A, De Keyser J (2000) Insulin-like growth factor II receptors in human brain and their absence in astrogliotic plaques in multiple sclerosis. Brain Res 863:282–288
100. Hawkes C, Kar S (2003) Insulin-like growth factor-II/mannose-6-phosphate receptor: widespread distribution in neurons of the central nervous system including those expressing cholinergic phenotype. J Comp Neurol 458:113–127
101. Konishi Y, Fushimi S, Shirabe T (2005) Immunohistochemical distribution of cation-dependent mannose 6-phosphate receptors in the mouse central nervous system: comparison with that of cation-independent mannose 6-phosphate receptors. Neurosci Lett 378:7–12
102. Lee WH, Clemens JA, Bondy CA (1992) Insulin-like growth factors in response to cerebral ischemia. Mol Cell Neurosci 3:36–43
103. Stephenson D, Rash K, Clemens J (1995) Increase in insulin-like growth factor II receptor within ischemic neurons following cerebral infarction. J Cereb Blood Flow Metab 15:1022–1031
104. Kar S, Baccichet A, Quirion R, Poirier J (1993) Entorhinal cortex lesion induces differential responses in [^{125}I]insulin-like growth factor I, [^{125}I]insulin-like growth factor II and [^{125}I] insulin receptor binding sites in the rat hippocampal formation. Neuroscience 55:69–80
105. Breese CR, D'costa A, Rollins YD, Adams C, Booze RM, Sonntag WE, Leonard S (1996) Expression of insulin-like growth factor-1 (IGF-1) and IGF-binding protein 2 (IGF-BP2) in the hippocampus following cytotoxic lesion of the dentate gyrus. J Comp Neurol 369:388–404
106. Kar S, Seto D, Dore S, Chabot J-G, Quirion R (1997) Systemic administration of kainic acid induces selective time dependent decrease in [^{125}I]insulin-like growth factor I, [^{125}I]insulin-like growth factor II and [^{125}I]insulin receptor binding sites in adult rat hippocampal formation. Neuroscience 80:1041–1055
107. Boker C, von Figura K, Hille-Rehfeld A (1997) The carboxy-terminal peptides of 46 kDa and 300 kDa mannose 6-phosphate receptors share partial sequence homology and contain information for sorting in the early endosomal pathway. J Cell Sci 110:1023–1032
108. Ghosh P, Dahms NM, Kornfeld S (2003) Mannose 6-phosphate receptors: new twists in the tale. Nat Rev Mol Cell Biol 4:202–212
109. Johnson KF, Kornfeld S (1992) A His-Leu-Leu sequence near the carboxyl terminus of the cytoplasmic domain of the cation-dependent mannose 6-phosphate receptor is necessary for the lysosomal enzyme sorting function. J Biol Chem 267:17110–17115
110. Lobel P, Fujimoto K, Ye RD, Griffiths G, Kornfeld S (1989) Mutations in the cytoplasmic domain of the 275 kd mannose 6-phosphate receptor differentially alter lysosomal enzyme sorting and endocytosis. Cell 57:787–796
111. Le Borgne R, Hoflack B (1998) Protein transport from the secretory to the endocytic pathway in mammalian cells. Biochem Biophys Acta 1404:195–209
112. Dell'Angelica EC, Payne GS (2001) Intracellular cycling of lysosomal enzyme receptors. Cytoplasmic tails' tales. Cell 106:395–398
113. Mullins C, Bonifacino JS (2001) The molecular machinery for lysosome biogenesis. BioEssays 23:333–343
114. Boman AL, Zhang C, Zhu X, Kahn RA (2000) A family of ADP-ribosylation factor effectors that can alter membrane transport through the trans-Golgi. Mol Biol Cell 11:1241–1255
115. Dell'Angelica EC, Puertollano R, Mullins C, Aguilar RC, Vargas JD, Hartnell LM, Bonifacino JS (2000) GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex. J Cell Biol 149:81–94
116. Hirst J, Lindsay MR, Robinson MS (2001) GGAs: roles of the different domains and comparison with AP-1 and clathrin. Mol Biol Cell 12:3573–3588
117. Puertollano R, Aguilar RC, Gorshkova I, Crouch RJ, Bonifacino JS (2001) Sorting of mannose 6-phosphate receptors mediated by the GGAs. Science 292:1712–1716
118. Takatsu H, Katoh Y, Shiba Y, Nakayama K (2001) Golgi-localizing, gamma-adaptin ear homology domain, ADP-ribosylation factor-binding (GGA) proteins interact with acidic dileucine sequences within the cytoplasmic domains of sorting receptors through their Vps27p/Hrs/STAM (VHS) domains. J Biol Chem 276:28541–28545
119. Zhu Y, Doray B, Poussu A, Lehto VP, Kornfeld S (2001) Binding of GGA2 to the lysosomal enzyme sorting motif of the mannose 6-phosphate receptor. Science 292:1716–1718
120. Ghahary A, Tredget EE, Mi L, Yang L (1999) Cellular response to latent TGF-beta1 is facilitated by insulin-like growth factor-II/mannose-6-phosphate receptors on MS-9 cells. Exp Cell Res 251:111–120
121. Villevalois-Cam L, Rescan C, Gilot D, Ezan F, Loyer P, Desbuquois B, Guguen-Guillouzo C, Baffet G (2003) The hepatocyte is a direct target for transforming-growth factor beta

activation via the insulin-like growth factor II/mannose 6-phosphate receptor. J Hepatol 38:156–163
122. Godar S, Horejsi V, Weidle UH, Binder BR, Hansmann C, Stockinger H (1999) M6P/IGFII-receptor complexes urokinase receptor and plasminogen for activation of transforming growth factor-beta1. Eur J Immunol 29:1004–1013
123. Ludwig T, Eggenschwiler J, Fisher P, D'Ercole AJ, Davenport ML, Efstratiadis A (1996) Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in Igf2 and Igf1r null backgrounds. Dev Biol 177:517–535
124. D'Ercole AJ, Ye P, O'Kusky JR (2002) Mutant mouse models of insulin-like growth factor actions in the central nervous system. Neuropeptides 36:209–220
125. Souza RF, Wang S, Thakar M, Smolinski KN, Yin J, Zou T-T, Kong D, Abraham JM, Toretsky JA, Meltzer SJ (1999) Expression of the wild-type insulin-like growth factor II receptor gene suppresses growth and causes cell death in colorectal carcinoma cells. Oncogene 18:4063–4068
126. O'Gorman DB, Weiss J, Hettiaratchi A, Firth SM, Scott CD (2002) Insulin-like growth factor-II/mannose 6-phosphate receptor overexpression reduces growth of choriocarcinoma cells in vitro and in vivo. Endocrinology 143:4287–4294
127. Schaffer BS, Lin M-F, Byrd JC, Park JHY, MacDonald RG (2003) Opposing roles for the insulin-like growth factor (IGF)-II and mannose 6-phosphate (Man-6-P) binding activities of the IGF-II/Man-6-P receptor in the growth of prostate cancer cells. Endocrinology 144:955–966
128. O'Gorman DB, Costello M, Weiss J, Firth SM, Scott CD (1999) Decreased insulin-like growth factor-II/mannose 6-phosphate receptor expression enhances tumorigenicity in JEG-3 cells. Cancer Res 59:5692–5694
129. Chen Z, Ge Y, Landman N, Kang JX (2002) Decreased expression of the mannose 6-phosphate/insulin-like growth factor-II receptor promotes growth of human breast cancer cells. BMC Cancer 2:18
130. Chen Z, Ge Y, Kang JX (2004) Down-regulation of the M6P/IGF-II receptor increases cell proliferation and reduces apoptosis in neonatal rat cardiac myocytes. BMC Cell Biol 5:15
131. Osipo C, Dorman S, Frankfater A (2001) Loss of insulin-like growth factor II receptor expression promotes growth in cancer by increasing intracellular signaling from both IGF-I and insulin receptors. Exp Cell Res 264:388–396
132. Li J, Sahagian GG (2004) Demonstration of tumor suppression by mannose 6-phosphate/insulin-like growth factor 2 receptor. Oncogene 23:9359–9368
133. Shimizu M, Webster C, Morgan D, Blau H, Roth R (1986) Insulin and insulin-like growth factor receptors and responses in cultured human muscle cells. Am J Physiol 215:E611–E615
134. Hari J, Pierce S, Morgan D, Sara V, Smith M, Roth R (1987) The receptor for insulin-like growth factor-II mediates an insulin-like response. EMBO J 6:3367–3371
135. Zhang Q, Tally M, Larsson O, Kennedy R, Huang L, Hall K, Berggren P-O (1997) Insulin-like growth factor-II signaling through the insulin-like growth factor-II/mannose 6-phosphate receptor promotes exocytosis of insulin-secreting cells. Proc Natl Acad Sci USA 94:6232–6236
136. Tally M, Li CH, Hall K (1987) IGF-2 stimulated growth mediated by the somatomedin type 2 receptor. Biochem Biophys Res Commun 148:811–816
137. Tsuruta JK, Eddy EM, O'Brien DA (2000) Insulin-like growth factor-II/cation-independent mannose 6-phosphate receptor mediates paracrine interactions during spermatogonial development. Biol Reprod 63:1006–1013
138. Minniti CP, Kohn EC, Grubb JH, Sly WS, Oh Y, Muller HL, Rosenfeld RG, Helman LJ (1992) The insulin-like growth factor II (IGF-II)/mannose 6-phosphate receptor mediates IGF-II-induced motility in human rhabdomyosarcoma cells. J Biol Chem 267: 9000–9004
139. McKinnon T, Chakraborty C, Gleeson LM, Chidiac P, Lala PK (2001) Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. J Clin Endocrinol Metab 86:3665–3674
140. Roff CF, Wozniak RW, Blenis J, Wang JL (1983) The effect of mannose 6-phosphate on the turnover of cell surface glycosaminoglycans. Exp Cell Res 144:333–344
141. Kojima I, Nishimoto I, Iiri T, Ogata E, Rosenfeld RG (1988) Evidence that type II insulin-like growth factor receptor is coupled to calcium gating system. Biochem Biophys Res Commun 154:9–19
142. Matsunaga H, Nishimoto I, Kojima I, Yamashita N, Kurokawa K, Ogata E (1988) Activation of a calcium-permeable cation channel by insulin-like growth factor-II in BALB/c 3T3 cells. Am J Physiol 255:C442–C446
143. Sakano K, Enjoh T, Numata F, Fujiwara H, Marumoto Y, Higashihashi N, Sato Y, Perdue JF, Fujita-Yamaguchi Y (1991) The design, expression, and characterization of human insulin-like growth factor II (IGF-II) mutants specific for either the IGF-II/cation-independent mannose 6-phosphate receptor or IGF-I receptor. J Biol Chem 266:20626–20635
144. Konishi T, Takahashi K, Chui D-H, Rosenfeld R, Himeno M, Tabira T (1994) Insulin-like growth factor II promotes in vitro cholinergic development of mouse septal neurons: comparison with the effects of insulin-like growth factor I. Brain Res 649:53–61
145. Hawkes C, Jhamandas JH, Harris KH, Fu W, MacDonald RG, Kar S (2006) Single transmembrane domain insulin-like growth factor-II/mannose-6-phosphate receptor regulates central cholinergic function by activating a G-protein-sensitive, protein kinase C-dependent pathway. J Neurosci 26:585–596
146. Morrione A, Valentinis B, Xu SQ, Yumet G, Louvi A, Efstratiadis A, Baserga R (1997) Insulin-like growth factor II stimulates cell proliferation through the insulin receptor. Proc Natl Acad Sci USA 94:3777–3782
147. Zhang Q, Berggren PO, Tally M (1997) Glucose increases both the plasma membrane number and phosphorylation of insulin-like growth factor-II/mannose-6-phosphate receptors. J Biol Chem 272:23702–23706
148. Nishimoto I, Hata Y, Ogata E, Kojima I (1987) Insulin-like growth factor II stimulates calcium influx in competent BALB/c 3T3 cells primed with epidermal growth factor. Characteristics of calcium influx and involvement of GTP-binding protein. J Biol Chem 262:12120–12126
149. Kojima I, Nishimoto I, Iiri T, Ogata E, Rosenfeld RG (1988) Evidence that type II insulin-like growth factor receptor is coupled to calcium gating system. Biochem Biophys Res Commun 154:9–19
150. Nishimoto I, Murayama Y, Katada T, Ui M, Ogata E (1989) Possible direct linkage of insulin-like growth factor-II receptor with guanine nucleotide-binding proteins. J Biol Chem 264:14029–14038
151. Murayama Y, Okamoto T, Ogata E, Asano T, Iiri T, Katada T, Ui M, Grubb JH, Sly WS, Nishimoto I (1990) Distinctive regulation of the functional linkage between the human cation-independent mannose 6-phosphate receptor and GTP-binding proteins by insulin-like growth factor II and mannose 6-phosphate. J Biol Chem 265:17456–17462
152. Higashijima T, Burnier J, Ross EM (1990) Regulation of Gi and Go by mastoparan, related amphiphilic peptides and hydrophobic amines: mechanisms and structural determinants of activity. J Biol Chem 265:14176–14186
153. Okamoto T, Katada T, Murayama Y, Ui M, Ogata E, Nishimoto I (1990) A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. Cell 62:709–717

154. Okamoto T, Nishimoto I (1991) Analysis of stimulation-G protein subunit coupling by using active insulin-like growth factor II receptor peptide. Proc Natl Acad Sci USA 88:8020–8023
155. Okamoto T, Ohkuni Y, Ogata E, Nishimoto I (1991) Distinct mode of G protein activation due to single residue substitution of active IGF-II receptor peptide Arg2410-Lys2423: evidence for stimulation acceptor region other than C-terminus of Gi alpha. Biochem Biophys Res Commun 179:10–16
156. Nishimoto I (1993) The IGF-II receptor system: a G protein-linked mechanism. Mol Reprod Dev 35:398–406
157. Ikezu T, Okamoto T, Giambarella U, Yokota T, Nishimoto I (1995) In vivo coupling of insulin-like growth factor II/mannose 6-phosphate receptor to heteromeric G proteins. Distinct roles of cytoplasmic domains and signal sequestration by the receptor. J Biol Chem 270:29224–29228
158. Korner C, Nurnberg B, Uhde M, Braulke T (1995) Mannose 6-phosphate/insulin-like growth factor II receptor fails to interact with G-proteins, Analysis of mutant cytoplasmic receptor domains. J Biol Chem 270:287–295
159. Pfeifer A, Nurnberg B, Kamm S, Uhde M, Schultz G, Ruth P, Hofmann F (1995) Cyclic GMP-dependent protein kinase blocks pertussis toxin-sensitive hormone receptor signaling pathways in Chinese hamster ovary cells. J Biol Chem 270:9052–9059
160. Poiraudeau S, Lieberherr M, Kergosie N, Corvol MT (1997) Different mechanisms are involved in intracellular calcium increase by insulin-like growth factors 1 and 2 in articular chondrocytes: voltage-gated calcium channels, and/or phospholipase C coupled to a pertussis-sensitive G-protein. J Cell Biochem 64:414–422
161. Groskopf JC, Syu LJ, Saltiel AR, Linzer DI (1997) Proliferin induces endothelial cell chemotaxis through a G protein-coupled, mitogen-activated protein kinase-dependent pathway. Endocrinology 138:2835–2840
162. Walter HJ, Berry M, Hill DJ, Cwyfan-Hughes S, Holly JM, Logan A (1999) Distinct sites of insulin-like growth factor (IGF)-II expression and localization in lesioned rat brain: possible roles of IGF binding proteins (IGFBPs) in the mediation of IGF-II activity. Endocrinology 140:520–532
163. Araujo DM, Lapchak PA, Collier B, Chabot JG, Quirion R (1989) Insulin-like growth factor-1 (somatomedin-C) receptors in the rat brain: distribution and interaction with the hippocampal cholinergic system. Brain Res 484:130–138
164. Kar S, Seto D, Dore S, Hanisch U, Quirion R (1997) Insulin-like growth factors-I and -II differentially regulate endogenous acetylcholine release from the rat hippocampal formation. Proc Natl Acad Sci USA 94:14054–14059
165. Seto D, Zheng WH, McNicoll A, Collier B, Quirion R, Kar S (2002) Insulin-like growth factor-I inhibits endogenous acetylcholine release from the rat hippocampal formation: possible involvement of GABA in mediating the effects. Neuroscience 115:603–612
166. Rosenthal SM, Hsiao D, Silverman LA (1994) An insulin-like growth factor-II (IGF-II) analog with highly selective affinity for IGF-II receptors stimulates differentiation, but not IGF-I receptor down-regulation in muscle cells. Endocrinology 135:38–44
167. Forbes BE, Hartfield PJ, McNeil KA, Surinya KH, Milner SJ, Cosgrove LJ, Wallace JC (2002) Characteristics of binding of insulin-like growth factor (IGF)-I and IGF-II analogues to the type 1 IGF receptor determined by BIAcore analysis. Eur J Biochem 269:961–968
168. Rogers SA, Purchio AF, Hammerman MR (1990) Mannose 6-phosphate-containing peptides activate phospholipase C in proximal tubular basolateral membranes from canine kidney. J Biol Chem 265:9722–9727
169. Poiraudeau S, Lieberherr M, Kergosie N, Corvol MT (1997) Different mechanisms are involved in intracellular calcium increase by insulin-like growth factors 1 and 2 in articular chondrocytes: voltage-gated calcium channels, and/or phospholipase C coupled to a pertussis-sensitive G-protein. J Cell Biochem 64:414–422
170. Singer WD, Brown HA, Sternweis PC (1997) Regulation of eurkaryotic phosphatidylionositol-specific phospholipase C and phospholipase D. Annu Rev Biochem 66:475–509
171. Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. Annu Rev Biochem 70:281–312
172. Vaughan PFT, Walker JH, Peers C (1999) The regulation of neurotransmitter secretion by protein kinase C. Mol Neurobiol 18:125–155
173. Johnson RM, Connelly PA, Sisk RB, Pobiner BF, Hewlett EL, Garrison JC (1986) Pertussis toxin or phorbol 12-myristate 13-acetate can distinguish between epidermal growth factor- and angiotensin-stimulated signals in hepatocytes. Proc Natl Acad Sci USA 83:2026–2032
174. Liang MN, Garrison JC (1991) The epidermal growth factor receptor is coupled to a pertussis toxin-sensitive guanine nucleotide regulatory protein in rat hepatocytes. J Biol Chem 266:13342–13349
175. Berven LA, Hughes BP, Barritt GJ (1994) A slowly ADP-ribosylated pertussis-toxin-sensitive GTP-binding regulatory protein is required for vasopressin-stimulated Ca2+ inflow in hepatocytes. Biochem J 299:399–407
176. Nair BG, Rashed HM, Patel TB (1989) Epidermal growth factor stimulates rat cardiac adenylate cyclase through a GTP-binding regulatory protein. Biochem J 264:563–571
177. Sun H, Seyer JM, Patel TB (1995) A region in the cytosolic domain of the epidermal growth factor receptor antithetically regulates the stimulatory and inhibitory guanine nucleotide-binding regulatory proteins of adenylyl cyclase. Proc Natl Acad Sci USA 92:2229–2233
178. Rothenberg PL, Kahn CR (1988) Insulin inhibits pertussis toxin-catalyzed ADP-ribosylation of G-proteins. Evidence for a novel interaction between insulin receptors and G-proteins. J Biol Chem 263:15546–15552
179. Ciaraldi TP, Maisel A (1989) Role of guanine nucleotide regulatory proteins in insulin stimulation of glucose transport in rat adipocytes. Influence of bacterial toxins. Biochem J 264:389–396
180. Dalle S, Imamura T, Rose DW, Worrall DS, Ugi S, Hupfeld CJ, Olefsky JM (2002) Insulin induces heterologous desensitization of G-protein-coupled receptor and insulin-like growth factor I signaling by downregulating beta-arrestin-1. Mol Cell Biol 22:6272–6285
181. Moxham CM, Malbon CC (1996) Insulin action impaired by deficiency of the G-protein subunit Giα2. Nature 379:840–844
182. Tao J, Malbon CC, Wang HY (2001) Galpha(i2) enhances insulin signaling via suppression of protein-tyrosine phosphatase 1B. J Biol Chem 276:39705–39712
183. Konopatskaya O, Shore AC, Tooke JE, Whatmore JL (2005) A role for heterotrimeric GTP-binding proteins and ERK1/2 in insulin-mediated, nitric-oxide-dependent, cyclic GMP production in human umbilical vein endothelial cells. Diabetologia 48:595–604
184. Nishimoto I, Ogata E, Kojima I (1989) Pertussis toxin inhibits the action of insulin-like growth factor-I. Biochem Biophys Res Commun 148:403–411
185. Hallak H, Seiler AE, Green JS, Ross BN, Rubin R (2000) Association of heterotrimeric G(i) with the insulin-like growth factor-I receptor. Release of G(betagamma) subunits upon receptor activation. J Biol Chem 275:2255–2258

186. Kuemmerle JF, Murthy KS (2001) Coupling of the insulin-like growth factor-I receptor tyrosine kinase to Gi2 in human intestinal smooth muscle: Gbetagamma -dependent mitogen-activated protein kinase activation and growth. J Biol Chem 276:7187–7194
187. Cunha RA, Malva JO, Ribeiro JA (1999) Kainate receptors coupled to G(i)/G(o) proteins in the rat hippocampus. Mol Pharmacol 56:429–433
188. Wang Y, Small DL, Stanimirovic DB, Morley P, Durkin JP (1997) AMPA receptor-mediated regulation of a Gi-protein in cortical neurons. Nature 389:502–504
189. Kawai F, Sterling P (1999) AMPA receptor activates a G protein that suppresses a cGMP-gated current. J Neurosci 19:2954–2959
190. Satake S, Saitow F, Rusakov D, Konishi S (2004) AMPA receptor-mediated presynaptic inhibition at cerebellar GABAergic synapses: a characterization of molecular mechanisms. Eur J Neurosci 19:2464–2474
191. Takago H, Nakamura Y, Takahashi T (2005) G protein-dependent presynaptic inhibition mediated by AMPA receptors at the calyx of Held. Proc Natl Acad Sci USA 102:7368–7373
192. Conway AM, Rakhit S, Pyne S, Pyne NJ (1999) Platelet-derived-growth factor stimulation of the p42/p44 mitogen-activated protein kinase pathway in airway smooth muscle: role of pertussis-toxin-sensitive G-proteins, c-Src tyrosine kinases and phosphoinositide 3-kinase. Biochem J 337:(Pt 2)171–177
193. Alderton F, Rakhit S, Kong KC, Palmer T, Sambi B, Pyne S, Pyne NJ (2001) Tethering of the platelet-derived growth factor beta receptor to G-protein-coupled receptors. A novel platform for integrative signaling by these receptor classes in mammalian cells. J Biol Chem 276:28578–28585
194. Krieger-Brauer HI, Medda P, Kather H (2000) Basic fibroblast growth factor utilizes both types of component subunits of Gs for dual signaling in human adipocytes. Stimulation of adenylyl cyclase via Galph(s) and inhibition of NADPH oxidase by Gbeta gamma(s). J Biol Chem 275:35920–35925
195. Frazier WA, Gao AG, Dimitry J, Chung J, Brown EJ, Lindberg FP, Linder ME (1999) The thrombospondin receptor integrin-associated protein (CD47) functionally couples to heterotrimeric Gi. J Biol Chem 274:8554–8560
196. Wang XQ, Lindberg FP, Frazier WA (1999) Integrin-associated protein stimulates alpha2beta1-dependent chemotaxis via Gi-mediated inhibition of adenylate cyclase and extracellular-regulated kinases. J Cell Biol 147:389–400
197. Gong X, Dubois DH, Miller DJ, Shur BD (1995) Activation of a G protein complex by aggregation of beta-1,4-galactosyltransferase on the surface of sperm. Science 269:1718–1721
198. Shi X, Amindari S, Paruchuru K, Skalla D, Burkin H, Shur BD, Miller DJ (2001) Cell surface beta-1,4-galactosyltransferase-I activates G protein-dependent exocytotic signaling. Development 128:645–654
199. Anand-Srivastava MB, Sairam MR, Cantin M (1990) Ring-deleted analogs of atrial natriuretic factor inhibit adenylate cyclase/cAMP system. Possible coupling of clearance atrial natriuretic factor receptors to adenylate cyclase/cAMP signal transduction system. J Biol Chem 265:8566–8572
200. Levin ER, Frank HJ (1991) Natriuretic peptides inhibit rat astroglial proliferation: mediation by C receptor. Am J Physiol 261(Pt 2):R453–R457
201. Hu RM, Levin ER, Pedram A, Frank HJ (1992) Atrial natriuretic peptide inhibits the production and secretion of endothelin from cultured endothelial cells. Mediation through the C receptor. J Biol Chem 267:17384–17389
202. Kiemer AK, Lehner MD, Hartung T, Vollmar AM (2002) Inhibition of cyclooxygenase-2 by natriuretic peptides. Endocrinology 143:846–852
203. Cenciarelli C, Hohman RJ, Atkinson TP, Gusovsky F, Weissman AM (1992) Evidence for GTP-binding protein involvement in the tyrosine phosphorylation of the T cell receptor zeta chain. J Biol Chem 267:14527–14530
204. Peter ME, Hall C, Ruhlmann A, Sancho J, Terhorst C (1992) The T-cell receptor zeta chain contains a GTP/GDP binding site. EMBO J 11:933–941
205. Stanners J, Kabouridis PS, McGuire KL, Tsoukas CD (1995) Interaction between G proteins and tyrosine kinases upon T cell receptor. CD3-mediated signaling. J Biol Chem 270:30635–30642
206. Lippert E, Jacques Y, Hermouet S (2000) Positive regulation of human T cell activation by Gi2 proteins and interleukin-8. J Leukoc Biol 67:742–748
207. Murthy KS, Teng B-Q, Zhou H, Jin J-G, Grider JR, Makhlouf GM (2000) G(i-1)/G(i-2)-dependent signaling by single-transmembrane natriuretic peptide clearance receptor. Am J Physiol Gastrointest Liver Physiol 278:G974–G980
208. Heuss C, Gerber U (2000) G-protein-independent signaling by G-protein-coupled receptors. Trends Neurosci 23:469–475