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MINIREVIEW

Location-Dependent Signaling of the Group 1 Metabotropic Glutamate Receptor mGlu5

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ABSTRACT

Although G protein–coupled receptors are primarily known for converting extracellular signals into intracellular responses, some receptors, such as the group 1 metabotropic glutamate receptor, mGlu5, are also localized on intracellular membranes where they can mediate both overlapping and unique signaling effects. Thus, besides "ligand bias," whereby a receptor's signaling modality can shift from G protein dependence to independence, canonical mGlu5 receptor signaling can also be influenced by "location bias" (i.e., the particular membrane and/or cell type from which it signals). Because mGlu5 receptors play important roles in both normal development and in disorders such as Fragile X syndrome, autism, epilepsy, addiction, anxiety, schizophrenia, pain, dyskinesias, and melanoma, a large number of drugs are being developed to allosterically target this receptor. Therefore, it is critical to understand how such drugs might be affecting mGlu5 receptor function on different membranes and in different brain regions. Further elucidation of the site(s) of action of these drugs may determine which signal pathways mediate therapeutic efficacy.

Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system signaling through both ionotropic and metabotropic glutamate receptors (mGlus) (Hermans and Challiss, 2001). mGlus are members of the class C G protein–coupled receptor (GPCR) superfamily characterized by large extracellular domains containing the endogenous agonist binding site (orthosteric) as well as sequences responsible for receptor dimerization. Eight different mGlu receptors have been described, which are further classified into three different groups based on amino-acid sequence homology, signal-transduction properties, and pharmacologic criteria (Niswender and Conn, 2010; Yin and Niswender, 2014). Group 1 mGlu receptors include $mGlu_1$ and $mGlu_5$, which couple to $G_{q/11}$, activate phospholipase C (PLC) β1, and subsequently lead to inositol 1,4,5-trisphosphate (IP_3) formation and calcium (Ca^{2+}) release from intracellular stores (Iacovelli et al., 2013). Given the abundant expression of mGlu5 receptors in areas of the brain involved in learning and memory, motivation, and emotion, as well as their known role in disorders such as Fragile X syndrome (FXS), autism spectrum disorder (ASD), Parkinson disease, addiction, schizophrenia, and pain, mGlu5 has been the focus of many studies exploring its structure, protein interactions, signaling properties, and therapeutic possibilities (Gasparini et al., 2013; Matosin and Newell, 2013; Nickols and Conn, 2014; Pop et al., 2014; Yin and Niswender, 2014). This review focuses on a lesser known mGlu5 receptor characteristic, its life as a receptor inside the cell. As such, mGlu5 receptors serve as prototypes for the growing number of GPCRs that regulate important cellular functions from intracellular membranes within the cell.

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ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AQ056, methyl(3a*R*,4*S*,7a*R*)-4-hydroxy-4-[(3-methylphenyl) ethynyl]octahydro-1*H*-indole-1-carboxylate; ASD, autism spectrum disorder; CaM, calmodulin; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CPCCOEt, 7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxylate ethyl ester; CREB, cAMP-responsive element binding protein; CTEP, 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine; DHPG, (*S*)-3,5-dihydroxyphenylglycine; Elk-1, ETS-domain transcription factor 1; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FITM, 4-fluoro-*N*-(4-(6-(isopropylamino)pyrimidin-4-yl) thiazol-2-yl)-*N*-methylbenzamide; FMRP, Fragile X Mental Retardation protein; FXS, Fragile X syndrome; GPCR, G protein–coupled receptor; GRK, G protein–coupled receptor kinase; IP_3, inositol 1,4,5-trisphosphate; LY393053, (±)-2-amino-2-(3-*cis* and *trans*-carboxycyclobutyl-3-(9-thioxanthyl) propionic acid; MAPK, mitogen-activated protein kinase; mGlu, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; mTOR, mammalian target of rapamycin; NAM, negative allosteric modulator; PI3K, phosphoinositide 3 kinase; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; RO4917523, 2-chloro-4-{[1-(4-fluorophenyl)-2,5-dimethyl-1*H*-imidazol-4-yl]ethynyl}pyridine.

General Features of mGlu5

The extracellular domain of the mGlu5 receptor consists of a so-called Venus flytrap motif, which contains the orthosteric binding site as well as a cysteine-rich domain (Cao et al., 2009). The latter mediates communication between the extracellular domain and the seven transmembrane domains (Doré et al., 2014). Stable, covalent mGlu5 receptor dimerization via the extracellular domain was first suggested by coimmunoprecipitation experiments (Romano et al., 1996) and was subsequently supported by crystallography data (Kunishima et al., 2000). More recent studies have shown that mGlu5 receptors heterodimerize with mGlu1 receptors (Beqollari and Kammermeier, 2010; Fuxe et al., 2012) as well as unrelated GPCRs, such as adensine A2A and dopamine D2 receptors (Ferré et al., 2002; Fuxe et al., 2003; Cabello et al., 2009), but not with group 2 or 3 receptors (Doumazane et al., 2011).

Pharmacologic and mutagenesis studies have revealed that there is at least one alternative (allosteric) site within the seven-transmembrane domain. Drugs binding at the allosteric site can either enhance or decrease activity at the orthosteric site or can be neutral in some cases (Niswender and Conn, 2010). The crystal structure of the seven-transmembrane region of the human mGlu5 receptor bound to the negative allosteric modulator (NAM) mavoglurant (AQ056 [methyl (3*aR*,4*S*,7*aR*)-4-hydroxy-4-[(3-methylphenyl)ethynyl]octahydro-1*H*-indole-1-carboxylate]; Novartis, Basel, Switzerland) was recently resolved (Doré et al., 2014). Together with a similar study that resolved the structure of mGlu1 receptors with the NAM, FITM [4-fluoro-*N*-(4-(6-(isopropylamino)pyrimidin-4-yl)thiazol-2-yl)-*N*-methylbenzamide] (Wu et al., 2014), important advances were made in the ability to design selective mGlu receptor modulators targeting the allosteric binding sites (Nickols and Conn, 2014).

Although there is some evidence that "promiscuous" coupling to other G proteins occurs (Hermans and Challiss, 2001) the mGlu5 receptor predominately acts through $G_{q/11}$ to initiate the PLC/IP_3/Ca^{2+} cascade (Niswender and Conn, 2010). mGlu5 receptor–generated Ca^{2+} responses can vary depending upon the cell type in which the receptor is expressed. For instance, mGlu5 receptors exhibit oscillatory responses in heterologous cells, hippocampal neurons, and spinal cord neurons, whereas mGlu5 receptors induce a fast transient peak followed by a sustained increase in striatal neurons (Flint et al., 1999; Romano et al., 2001; Kettunen et al., 2002; Jong et al., 2005). This Ca^{2+} increase is regulated by channels such as the IP_3 receptor and the ryanodine receptor, which control Ca^{2+} release from internal stores (Rose and Konnerth, 2001; Bootman et al., 2002). The diversity of Ca^{2+} responses suggests that the cell type and environment are crucial factors in directing the spatiotemporal features of intracellular Ca^{2+} elevations.

mGlu5 Signaling from the Cell Surface. mGlu5 receptor–mediated Ca^{2+} induction activates a plethora of downstream signaling pathways, including the Ca^{2+}-sensing protein, calmodulin (CaM), which in turn interacts with CaM-dependent kinases (CaMKs), such as CaMKII and CaMKIV, leading to changes in gene transcription and translation (Wang and Zhuo, 2012). For example, cAMP-responsive element-binding protein (CREB), serum response factor, and histone deacetylase have all been shown to be upregulated by CaMK activation (Swulius and Waxham, 2008). Eukaryotic elongation factor 2 kinase is also upregulated, leading to phosphorylation of eukaryotic elongation factor 2, which inhibits general protein synthesis while upregulating local translation of specific synaptic proteins (Park et al., 2008).

Other mGlu5 receptor signaling effectors include the mitogen-activated protein kinase (MAPK) pathways such as the extracellular signal-regulated kinase (ERK1/2) pathway, the p38 MAPK pathway, and the c-Jun N-terminal kinase/stress–activated protein kinase pathway (Wang et al., 2007). MAPK signaling can also lead to activation of transcription factors such as ETS-domain transcription factor (Elk-1), CREB, activator protein 1, activating transcription factor 2, c-Jun, c-Rel, and nuclear factor κ-light-chain-enhancer of activated B cells (Yang and Sharrocks, 2006; Wang et al., 2007; Gladding et al., 2009). The ERK1/2 pathway has also been linked to regulation of protein synthesis by contributing to the formation of the eukaryotic translation initiation factor 4E complex, which is required for initiating translation (Banko et al., 2006).

mGlu5 receptors also regulate translation via connections to phosphoinositide 3 kinase enhancer-long, which activates the enzyme phosphoinositide 3 kinase (PI3K) (Rong et al., 2003; Ronesi and Huber, 2008). In turn, PI3K phosphorylates phosphoinositides to form lipids that activate downstream kinases, like protein kinase B (PKB) (Akt/PKB) (Franke et al., 1997; Chan et al., 1999) and phosphoinositide-dependent kinase 1 (Vanhaesebroeck and Alessi, 2000). The mammalian target of rapamycin (mTOR) is an important target of these kinases; activation of mTOR initiates protein translation (Hou and Klann, 2004). Thus, mGlu5 receptors regulate cellular functions not only by changing the transcriptional profile of the cell, but also by increasing local translation of dendritic mRNAs.

Both protein kinase C (PKC) and G protein–coupled receptor kinases (GRKs) contribute to the desensitization of many GPCRs including mGlu1/mGlu5 receptors. Although GRK phosphorylation of a given receptor facilitates the binding of β-arrestins that then act as adaptors leading to receptor internalization (Dhami and Ferguson, 2006), GRK2 appears to desensitize mGlu5 receptors in a phosphorylation and β-arrestin–independent manner (Ribeiro et al., 2009). Other proteins involved in mGlu5 receptor attenuation of signaling include CaMKII (Mundell et al., 2002), optineurin (Anborgh et al., 2005), calcineurin inhibitor protein (Ferreira et al., 2009), and proline-rich tyrosine kinase 2 (Nicodemo et al., 2010). At least in the latter case, attenuation involves a mechanism whereby the protein displaces $G_{q/11}$ from the receptor. Although mGlu1 receptors are internalized in a β-arrestin–dependent manner (Dale et al., 2001; Mundell et al., 2001; Iacovelli et al., 2003), there is little data showing this is the case for mGlu5 receptors. However, internalization of mGlu5 receptors can occur via clathrin-dependent (Bhattacharya et al., 2004) and clathrin-independent pathways (Fourgeaud et al., 2003). The former might be mediated by phospholipase D2, which recruits adaptor complexes of clathrin-coated pits. Thus, mGlu5 receptor G protein–dependent signaling, desensitization, and endocytosis involves many different pathways and partners, all of which can be regulated in a cell type–specific and/or receptor subtype–specific manner, adding to the diversity of responses.

mGlu5 Cell Receptor Surface Protein Interactions. mGlu5 receptor binding proteins assemble the receptor into

functional complexes at cellular targets including the synapse (Fagni et al., 2004). These protein interactions also serve as platforms for signaling, albeit ones not necessarily dependent upon G proteins. However, the term "G protein–independent" signaling is usually applied to β-arrestin–mediated signaling, which has contributed to the concept of ligand bias or biased agonism (Rajagopal et al., 2010; Kenakin, 2011, 2014; Reiter et al., 2012; Wisler et al., 2014). Biased agonism occurs when a ligand stabilizes a unique receptor conformation leading to the selective activation of either G protein–dependent pathways or G protein–independent pathways. For example, mGlu1 receptors reportedly display biased agonism in that agonists such as (*S*)-3,5-dihydroxyphenylglycine (DHPG) and quisqualate trigger mGlu1 receptor G protein signaling resulting in the transient activation of ERK, whereas succinic and glutaric acid trigger β-arrestin–mediated signaling, receptor internalization, sustained ERK activation, and cytoprotective signaling (Emery et al., 2010, 2012; Kammermeier, 2012). So-called "balanced" agonists, such as glutamate, aspartate, and cysteate, activate both pathways (Emery et al., 2012). These biased ligand effects were not observed for cells expressing mGlu5 receptors in which both glutamate and quisqualate produce transient ERK phosphorylation (Emery et al., 2010). Thus, ligand bias does not seem to play a role in mGlu5 receptor signaling at least in these contexts.

Other protein interactors that affect signaling by altering receptor activity, location, or protein/protein interactions include the scaffolding proteins Homer (Tu et al., 1998), tamalin (Kitano et al., 2002), Na^+/H^+ exchanger regulatory factor 2 (Paquet et al., 2006), cystic fibrosis transmembrane conductance regulator-associated ligand (Cheng et al., 2010), and preso1 (Hu et al., 2012); signaling proteins such as CaM (Minakami et al., 1997; Lee et al., 2008), seven in absentia homolog 1A (Siah-1A) (Ishikawa et al., 1999), protein phosphatase 2B/calcineurin (Alagarsamy et al., 2005), optineurin (Anborgh et al., 2005), protein phosphatase 1γ1/2 (Croci et al., 2003), protein phosphatase 2A (Mao et al., 2005), neuronal Ca^{2+}-binding protein 2 (Canela et al., 2009), calcineurin inhibitor protein (Ferreira et al., 2009), neurite-outgrowth–related rat brain protein (Norbin) (Wang et al., 2009), proline-rich tyrosine kinase 2 (Nicodemo et al., 2010), tyrosine kinase Fyn (Um et al., 2013), and CaMKIIα (Jin et al., 2013); and cytoskeletal proteins such as filamin-A (Enz, 2002) and actin-binding protein, α-actinin-1 (Cabello et al., 2007). Aside from protein-binding sites, there are also important phosphorylation sites on the mGlu5 receptor intracellular domain that controls receptor signaling and desensitization. As described, the phosphorylation status of mGlu5 receptors is regulated by kinases such as PKC, GRKs, CaMKII, and tyrosine kinases, along with protein phosphatases (Mao et al., 2008). Table 1 summarizes mGlu5 receptor interactors that regulate receptor signaling and trafficking.

Group 1 mGlu receptor interactions with Homer proteins are perhaps the best studied. As scaffolding proteins that interact with proline-rich sequences, Homers bring together mGlu1/mGlu5 receptors with IP_3 and ryanodine receptors, Shank, phosphoinositide 3 kinase enhancer-long, and Dynamin III (Bockaert et al., 2010). So-called long Homers have

TABLE 1
mGlu5 receptor interactors that regulate signaling and trafficking

Interactor	Effect on mGlu5 Receptors	References
α-Actin-1	Modulates receptor cell surface expression and function	Cabello et al. (2007)
β-Arrestin	Regulates receptor signaling and internalization	Emery et al. (2010, 2012), Rajagopal et al. (2010), Kenakin (2011, 2014), Reiter et al. (2012), and Wisler et al. (2014)
CAIN	Attenuates receptor endocytosis and signaling	Ferreira et al. (2009)
CAL	Modulates receptor expression	Cheng et al. (2010)
CaM	Regulates receptor signaling and localization	Minakami et al. (1997), Ishikawa et al. (1999), Lee et al. (2008), and Ko et al. (2012)
CaMKIIα	Regulates receptor signaling and desensitization	Mao et al. (2008) and Jin et al. (2013)
Filamin-A	Links mGlu5 to the actin cytoskeleton	Enz (2002)
Fyn	Regulates receptor signaling	Um et al. (2013)
GRK	Regulates receptor signaling and desensitization	Mao et al. (2008) and Kim et al. (2008)
Homer	Regulates receptor signaling and localization	Tu et al. (1998), Ango et al. (2001), Kammermeier (2008), and Bockaert et al. (2010)
NECAB2	Regulates receptor signaling	Canela et al. (2009)
NHERF-2	Regulates receptor activity and prolongs receptor-mediated calcium mobilization	Paquet et al. (2006)
Norbin	Increases receptor cell surface expression and modulates calcium signaling	Wang et al. (2009, 2010)
Optineurin	Regulates receptor desensitization	Anborgh et al. (2005)
PKC	Regulates protein–protein interactions, and mediates receptor signaling and desensitization	Catania et al. (1991), Aronica et al. (1993), Gereau and Heinemann (1998), Kammermeier and Ikeda (2002), Kim et al. (2005, 2008), and Ko et al. (2012)
PP1γ1/2	Regulates receptor desensitization	Croci et al. (2003)
PP2A	Regulates receptor signaling	Mao et al. (2005)
PP2B/CaN	Regulates receptor desensitization	Alagarsamy et al. (2005)
Preso 1	Enhances Homer binding and downregulates receptor signaling	Hu et al. (2012)
Pyk2	Regulates receptor signaling	Nicodemo et al. (2010)
Siah-1A	Decreases receptor surface expression and increases receptor endocytosis	Ishikawa et al. (1999) and Ko et al. (2012)
Tamalin	Promotes receptor intracellular trafficking and cell surface expression	Kitano et al. (2002)

CAIN, calcineurin inhibitor protein; CAL, cystic fibrosis transmembrane conductance regulator–associated ligand; CaN, calcineurin; NECAB2, neuronal Ca^{2+}-binding protein 2; NHERF-2, Na^+/H^+ exchanger regulatory factor 2; PP1γ1/2, protein phosphatase 1γ1/2; PP2A, protein phosphatase 2A; PP2B, protein phosphatase 2B; Pyk2, proline-rich tyrosine kinase 2.

a C-terminal coiled coil domain that allows them to form multiprotein complexes (Hayashi et al., 2006). Short Homers (e.g., Homer 1a) lack the coiled coil domain and thus act as dominant negative proteins (Xiao et al., 2000; Fagni et al., 2002). For example, expression of short Homer isoforms leads to agonist-independent/constitutive activation of mGlu1/mGlu5 receptors in human embryonic kidney cells (Ango et al., 2001). Disruption of mGlu5/Homer interactions can also block PI3K/AKT/mTOR signaling, which results in phenotypic behaviors similar to FXS (Ronesi et al., 2012). Thus, long and short Homers play critical roles in mGlu5 receptor signaling (Kammermeier, 2008).

Other key mGlu5 receptor interacting proteins, such as PKC, CaM, Siah-1A, and Norbin, also play central roles in integrating synaptic signals to direct mGlu5 receptor signaling and trafficking (Kim et al., 2005; Ko et al., 2012). For example, mGlu1/mGlu5-activated PKC feeds back to phosphorylate and desensitize these receptors (Catania et al., 1991; Aronica et al., 1993; Gereau and Heinemann, 1998; Kammermeier and Ikeda, 2002) and PKC-mediated phosphorylation of serine 839 is involved in the regulation of mGlu5 receptor–mediated Ca^{2+} oscillations (Kim et al., 2005, 2008). CaM binds to two sites in the C-terminal tail of the mGlu5 receptor, which blocks PKC phosphorylation; reciprocally, PKC phosphorylation blocks CaM binding (Minakami et al., 1997; Lee et al., 2008).

Besides PKC and CaM, Siah-1A also interacts with mGlu5 receptors competing with CaM for mGlu5 receptor binding (Ishikawa et al., 1999; Ko et al., 2012). In hippocampal neurons, activation of mGlu5 receptors leads to PKC phosphorylation, which decreases CaM binding. Subsequently, Siah-1A binds and ubiquitinates mGlu5 receptors, resulting in endocytosis and downregulation (Kim et al., 2005; Ko et al., 2012). Finally, Norbin also interacts with mGlu5 receptors via binding sites overlapping with those of CaM and Siah-1A (Wang et al., 2009). Norbin appears to modulate mGlu5 receptor oscillatory Ca^{2+} responses and increase mGlu5 receptor surface expression. Consistent with this, Norbin knockout mice display fewer surface mGlu5 receptors, have decreased mGlu5 receptor–driven synaptic changes, and show behavioral similarities to mGlu5 receptor knockout mice (Wang et al., 2009). Since Norbin interacts with membrane phospholipids, Norbin may facilitate mGlu5 receptor trafficking between the plasma membrane and intracellular compartments (Wang et al., 2010). Taken together, all of these studies indicate that mGlu1/mGlu5 receptor signaling is multifaceted, utilizing a wide variety of interacting proteins and pathways to fine-tune diverse integral responses.

Intracellular GPCRs

GPCRs have always been found within the cell, including in the endoplasmic reticulum (ER) where they are synthesized, folded, modified, and assembled, as well as in post-Golgi sorting vesicles on their way to the cell surface, or on endosomes that have just come off the membrane. Traditionally, however, GPCRs in these locations were not thought to be functional since 1) their ligand binding domains were directed toward the lumen of any given intracellular membrane and thus seemingly out of reach of external ligands, and 2) endocytosed receptors were likely desensitized and/or on their way for lysosomal destruction. The discovery that arrestins were not just involved in the desensitization and internalization of GPCRs but also served as critical signaling platforms from either the cell surface or from the endosome (Pierce et al., 2002; Shenoy and Lefkowitz, 2003) helped open the door to the concept of intracellular GPCR signaling. Since G protein activation may not be necessary for β-arrestin signaling (Pierce et al., 2002), endocytosed, intracellular GPCR signaling may constitute a G protein–independent pathway.

Just as biased agonism led to a paradigm shift in GPCR research and drug development, emerging data documenting G protein–dependent signaling from intracellular GPCRs should result in a similar marked change. Like β-arrestin signaling, G protein signaling from intracellular GPCRs may perform unique functions, such as activating different signaling systems, displaying unique desensitization patterns, and/or exhibiting distinctive patterns of subcellular distribution (Calebiro et al., 2009, 2010; Ferrandon et al., 2009; Vaniotis et al., 2011; Irannejad et al., 2013). To date, GPCRs have been found on endosomal membranes (β_2-adrenergic receptor; Irannejad et al., 2013), ER membranes (glycoprotein gp130; Meads and Medveczky, 2004), lysosomes (cannabinoid receptor CB1; Rozenfeld and Devi, 2008; ocular albinism type 1 receptor OA1; Shen et al., 2001; Lopez et al., 2008; Burgoyne et al., 2013), and within the nucleoplasm (angiotensin, apelin receptors; Lee et al., 2004). CB1 cannabinoid receptors are also reported to be on mitochondrial membranes where they appear to regulate neuronal metabolism (Bénard et al., 2012).

Many GPCRs are also found on inner nuclear membranes including endothelin receptors (Boivin et al., 2003; Vaniotis et al., 2011, 2013), β_1-, β_2-, and β_3-adrenergic receptors (Boivin et al., 2006; Merlen et al., 2013), platelet-activating factor receptors (Marrache et al., 2002), lysophosphatidic acid receptors (Gobeil et al., 2003), prostaglandin receptor EP_1 (Gobeil et al., 2002), bradykinin B_2 receptors (Lee et al., 2004; Savard et al., 2008), α_{1A}- and α_{1B}-adrenergic receptors (García-Cazarín et al., 2008; Wright et al., 2012), and angiotensin AT_1 and AT_2 receptors (Tadevosyan et al., 2010, 2012). Collectively, GPCRs expressed on the nuclear membrane include receptors from all three classes, present on many different cell types. Moreover, nuclear GPCRs have been detected in different organisms ranging from vertebrates to *Caenorhabditis elegans* and even plants, suggesting that nuclear localization is a general feature for GPCRs and it is evolutionarily conserved (Boivin et al., 2008; Vaniotis et al., 2011; Tadevosyan et al., 2012).

Because intracellular GPCRs would be oriented such that their intracellular domains are still in the cytoplasm, access to canonical signaling machinery is unimpeded. For GPCRs present on the inner nuclear membrane, many of the same signaling proteins are also observed in the nucleus and/or on nuclear membranes including heterotrimeric G proteins (Dupré and Hébert, 2006; Dupré et al., 2009), adenylyl cyclase (Schulze and Buchwalow, 1998), phospholipase A2 (Schievella et al., 1995), PLCβ (Kim et al., 1996), phospholipase D (Freyberg et al., 2001), regulator of G protein signaling proteins (Burchett, 2003), β-arrestin1 (Scott et al., 2002), GRKs (Johnson et al., 2004), and protein kinase A (Sastri et al., 2005). In addition, enzymes that are functional in the phosphoinositide metabolism or peptide ligand generation are also observed in the nuclei of different cell types (Vaniotis et al., 2011). Thus, it is not

surprising that activation of nuclear membrane localized GPCRs triggers classic second messenger systems such as adenylyl cyclase and subsequent protein kinase A activation (Boivin et al., 2006), and phospholipase activation that generates IP_3 and diacyglycerol (Kumar et al., 2008). IP_3 and diacyglycerol lead to the release of intraluminal (ER or nuclear) Ca^{2+} and the activation of PKC (Boivin et al., 2003, 2005). Additional downstream pathways affected by nuclear membrane receptors include activation of the kinases Akt/PKB, ERK and p38 MAPK (Marrache et al., 2002; Gobeil et al., 2003, 2006). Generation of nucleoplasmic Ca^{2+} may also affect a broad range of cellular processes including the initiation of gene expression (Bhattacharya et al., 1998, 1999; Gobeil et al., 2002; Boivin et al., 2006). In fact, nuclear membrane localized GPCRs regulate many different physiologic events, such as cell proliferation, survival, inflammatory responses, tumorigenesis, DNA synthesis, and transcription (Boivin et al., 2008; Vaniotis et al., 2011; Tadevosyan et al., 2012; Purgert et al., 2014).

Activation of Intracellular GPCRs. One of the most important questions about GPCRs on intracellular membranes is how they are activated. Since orthosteric binding sites would be within the endosome or in the luminal region of the ER or nucleus, extracellular ligands would have to cross both the plasma membrane as well as the intracellular membrane to activate intracellular receptors (O'Malley et al., 2003). A highly permeable ligand might freely cross such membranes, whereas a less permeable, charged ligand might require an active transport process. In terms of the group 1 mGlu receptors, we have shown that at least two uptake systems exist that are responsible for transporting glutamate into a neuron to activate mGlu1/mGlu5 receptors: the sodium-dependent excitatory amino acid transporters and the cystine/glutamate exchanger (Jong et al., 2005, 2007). Conditions that block the transporters (i.e., chloride-free buffers and the compound L-cystine for the cystine/glutamate exchanger; sodium free buffers and the compound, threo-β-benzyloxyaspartate for sodium-dependent excitatory amino acid transporters) reduced agonist uptake in heterologous models and striatal or hippocampal neurons (Jong et al., 2005, 2007; Purgert et al., 2014). Moreover, uptake of radiolabeled quisqualate and glutamate was observed in isolated nuclei, which could also be blocked with chloride-free buffers or by applying the transporter blockers L-cystine or threo-β-benzyloxyaspartate. Thus, for intracellular mGlu1/mGlu5 receptors, 90–95% of all ligand-induced intracellular responses can be accounted for by these transporters (Jong et al., 2005, 2007; Purgert et al., 2014).

Alternatively, ligands might be made in situ via localized biosynthetic machinery. Evidence exists that nuclear-localized prostaglandin receptors can be activated in this manner (Boivin et al., 2008). On the other hand, known ligands for peptidergic receptors reside within large dense core vesicles, making activation of intracellular receptors problematic. Conceivably, vesicles could refuse with intracellular membranes leading to receptor activation (Boivin et al., 2008) or, as has been described for the apelin receptor, ligand activation at the cell surface leads to receptor internalization and transport into the nucleoplasm (Lee et al., 2004). Finally, it is also possible that activation of nuclear localized GPCRs may not need ligands. Many GPCRs exhibit constitutive ligand–independent activity that might allow nuclear receptors to function (Chidiac et al., 1994; Boivin et al., 2008).

The functionality of these intracellular GPCRs has been assessed using a range of readouts with clear evidence showing that as long as ligand is either made in situ or transported to the site of action, the receptor can be activated (Boivin et al., 2008; Vaniotis et al., 2011; Tadevosyan et al., 2012). Recent, elegant evidence using conformation-specific single-domain antibodies to directly assess activation of the β_2-adrenergic receptor confirmed bona fide GPCR signaling from early endosomes (Irannejad et al., 2013). Additional evidence comes from strategies using pharmacological isolation of endogenous intracellular mGlu5 receptors on either the ER or nuclear membrane of primary hippocampal or striatal neurons (Jong et al., 2009; Kumar et al., 2012; Purgert et al., 2014). Interestingly, a mutant version of the V_2 vasopressin receptor, which cannot be trafficked to the plasma membrane and instead localizes within intracellular compartments, responded to agonists, suggesting that intracellular receptors can be functional even when mistrafficked (Robben et al., 2009).

The physiologic function of most intracellular GPCRs is unknown. That there is a physiologic role is perhaps best exemplified by OA1 (GPR143), a pigment cell-specific GPCR that is mutated in patients with ocular albinism type 1 (Shen et al., 2001). OA1 is exclusively localized on endolysomal/melanosomal membranes, never getting to the cell surface (Schiaffino, 2010). By contrast, many peptide receptors, start on the cell surface and then get trafficked to intracellular domains including the nucleoplasm (Lee et al., 2004). As described, how intracellular GPCRs are activated is also largely unknown but can range from endogenous stimuli that activate the receptor at the cell surface followed by endocytosis to de novo ligands yet to be discovered. Although G protein–dependent signaling is seen in many cases (e.g., OA1 couples to $G\alpha_{i3}$; Young et al., 2011; mGlu5 receptors couple to $G\alpha_{q/11}$; Kumar et al., 2008), β-arrestin–dependent signaling may also occur. Receptor heterodimers potentially create additional ligand signaling opportunities and, as in the case of group 1 mGlu receptors, proteins like Homer1a can lead to agonist-independent receptor activation (Ango et al., 2001). Agonist-independent activation of the pituitary adenylate cyclase–activating polypeptide PACAP 1 receptor also occurs due to a close association with the insulin-like growth factor 1 receptor and subsequent transactivation by Src; this constitutive interaction plays the dominant role in the antiapoptotic activity of insulin-like growth factor 1 (Delcourt et al., 2007). Taken together, the present data argue strongly that irrespective of how they are activated, intracellular GPCRs play a dynamic role in generating and shaping intracellular signaling pathways.

Intracellular mGlu5 Receptors

Localization. The intracellular localization of mGlu5 receptors has been well documented. Electron microscopy studies revealed that the mGlu5 receptor not only localizes on postsynaptic membranes and extrasynaptic regions but that most of this receptor is intracellular (Hubert et al., 2001; López-Bendito et al., 2002; O'Malley et al., 2003; Kuwajima et al., 2004; Mitrano et al., 2008, 2010). For example, 50–90% of mGlu5 receptors localize to different intracellular membranes in many different brain regions (Hubert et al., 2001; Paquet and Smith, 2003; Kuwajima et al., 2004). Our own

studies revealed that mGlu5 receptor immunogold particles were found on inner nuclear, outer nuclear, and ER membranes as well as in more traditional synaptic locations of rat visual cortex (O'Malley et al., 2003). Using differential permeabilization along with antibodies directed to the mGlu5 receptor's N or C terminus revealed that the topology of the receptor is such that the N terminus is located in the lumen of the nuclear membrane (Fig. 1; O'Malley et al., 2003; Jong et al., 2005). Direct activation of nuclear mGlu5 receptors by transported agonists triggered Ca^{2+} responses in purified nuclei that could be blocked by mGlu5 receptor–specific antagonists, such as 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (O'Malley et al., 2003; Jong et al., 2005; Purgert et al., 2014). Therefore, isolated nuclei can respond to mGlu5 receptor ligands by generating the expected second messengers without cytoplasmic input.

Ultrastructure studies also revealed large numbers of mGlu5 receptor gold particles on ER membranes (O'Malley et al., 2003; Mitrano and Smith, 2007; Mitrano et al., 2008, 2010). To show functionality of ER-localized mGlu5 receptors, we 1) uncaged 4-methoxy-7-nitroindolinyl–glutamate using laser-induced photolysis onto dendrites in the presence of mGlu5 receptor impermeable antagonists as well as other ionotropic and metabotropic receptor antagonists (Purgert et al., 2014), and 2) we puffed quisqualate onto a dendrite in the presence of the same inhibitors (Y.J. Jong, unpublished data). Only the region of the dendrite juxtaposed to the uncaging spot (or the microspritzer) exhibited a change in fluorescence, whereas proximal regions did not (Purgert et al., 2014). Therefore, activation of dendritic, intracellular mGlu5 receptors also leads to in situ Ca^{2+} changes with neither input to nor output from the cell soma (Purgert et al., 2014).

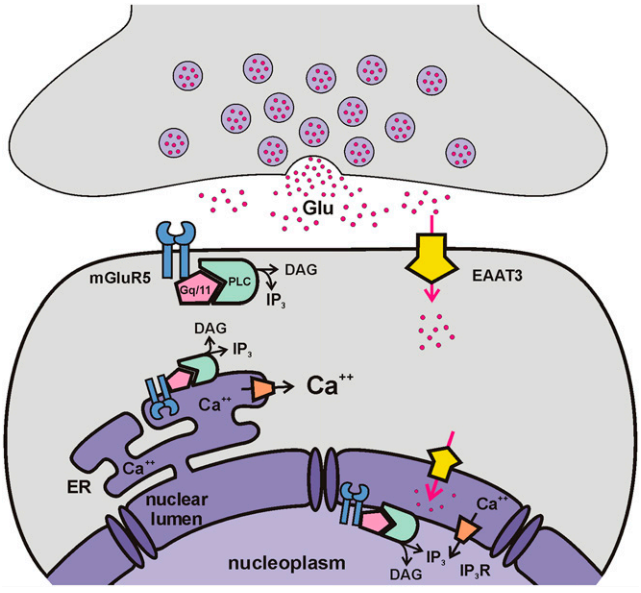

Fig. 1. Proposed model of cell surface and intracellular mGlu5 receptor activation by glutamate. Topology of the intracellular mGlu5 receptor is based on ultrastructural, genetic, immunologic, and pharmacologic evidence. Glutamate release from presynaptic vesicles can activate cell surface mGlu5 receptors as well as be taken up postsynaptically via transporters such as excitatory EAAT3. EAAT3 is also present on intracellular membranes including the ER and the outer nuclear membrane, which allows glutamate access to intracellular mGlu5 receptors. Ca^{2+} within the nuclear envelope is continuous with the ER pool. Like plasma membrane receptors, intracellular mGlu5 receptors couple to the $G_{q/11}$/PLC/IP_3 pathway. DAG, diacylglycerol; EAAT, sodium-dependent excitatory amino acid transporter; Glu, glutamate; IP_3R, IP_3 receptor.

Membrane Targeting of mGlu5 Receptors. Certain GPCRs such as the apelin, angiotensin AT_1 and bradykinin B_2 receptors use a canonical nuclear localization signal for nuclear import after receptor activation on the cell surface (Lee et al., 2004; Morinelli et al., 2007; Wright et al., 2012). Unlike these receptors, mGlu5 has no obvious nuclear localization signal, nor is it apparent that mGlu5 receptors are trafficked to nuclear membranes by endocytosis followed by reverse transport (Y.J. Jong and I. Sergin, unpublished data). Instead, it appears that there are sequences within the C terminus of the mGlu5 receptor that are responsible for targeting the receptor to at least the inner nuclear membrane (I. Sergin, unpublished data). Since there are still mGlu5 receptors present on the outer nuclear membrane, the ER, and the cell surface, membrane-selective targeting has yet to be achieved. Thus, the complexity of unequivocally targeting mGlu5 receptors to one membrane versus another precludes defining intracellular receptor function via genetic isolation at this time.

Pharmacologic Isolation of Intracellular mGlu5 Function. In theory, pharmacological isolation of intracellular GPCR function can be achieved using a combination of impermeable, nontransported drugs together with permeable or transported ones. Drug permeability can be gauged using lipophilicity values (logP) in which a logP > 2 is considered to be readily membrane permeable (Lester et al., 2012). For example, the mGlu5 receptor antagonist, MPEP, has a log P of 3.3, which is in agreement with its ability to block all mGlu5 receptor responses on and within the cell (e.g., Jong et al., 2005). By contrast, logP values for agonists such as glutamate (-2.7), DHPG (-2.4), and quisqualate (-3.9) are consistent with the notion that they are membrane impermeable. Thus, for any of these compounds to get into the cell, there must be an active transport/exchange process like the sodium or chloride-dependent processes described above (Jong et al., 2005, 2009; Kumar et al., 2008). Besides impermeable, nontransported agonists, impermeable, nontransported antagonists exist, such as the Lilly compound, LY393053 [(±)-2-amino-2-(3-*cis* and *trans*-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid] (Kingston et al., 2002), which can block cell surface–mediated mGlu5 receptor responses (Jong et al., 2005, 2009; Kumar et al., 2008, 2012; Purgert et al., 2014). By contrast, LY393053 did not block uptake of quisqualate or glutamate into the cells and thus did not block the functional Ca^{2+} responses generated by the intracellular mGlu5 receptor (Jong et al., 2005; Purgert et al., 2014). Thus, strategies exist by which the function of intracellular GPCRs can be deduced.

Signaling Pathways Activated by Intracellular mGlu5 Receptors. Using permeable and impermeable as well as intracellularly transported and nontransported agonists/antagonists, we have identified downstream pathways that are specifically activated by intracellular mGlu5 receptors (Fig. 2). For example, treatment of striatal neurons with the impermeable intracellularly transported mGlu5 receptor agonist quisqualate leads to the phosphorylation of ERK1/2, Elk-1, and CaMKII (Jong et al., 2009). These results were specific for quisqualate and were not observed when cells were treated with the impermeable, nontransported agonist DHPG or in mGlu5 receptor knockout cultures. Since quisqualate can also activate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

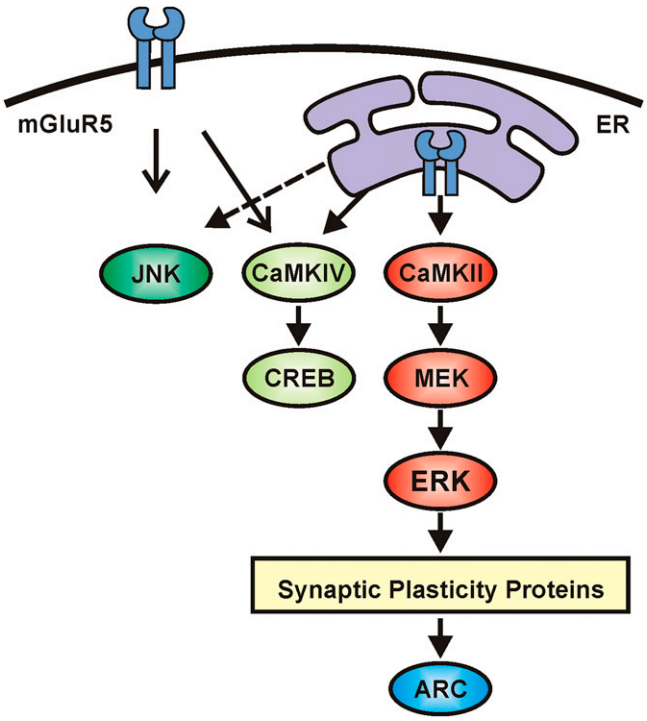

Fig. 2. Intracellular mGlu5 receptors activate signaling cascades distinct from cell surface counterparts in striatal neurons. Activation of either cell surface or intracellular mGlu5 receptors leads to JNK, CaMKIV, and CREB phosphorylation, whereas activation of intracellular mGlu5 receptors leads to CaMKII, MEK, and ERK1/2 phosphorylation and Arc upregulation. JNK, c-Jun N-terminal kinase; MEK, MAPK kinase.

(AMPA) receptors albeit at higher concentrations, ionotropic glutamate receptor antagonists as well as an mGlu1 receptor antagonist were always present to further assure specificity (Jong et al., 2005). Moreover, phosphorylation of ERK1/2, Elk-1, and CaMKII was blocked by the permeable antagonist MPEP but not by LY393053, the impermeable, nontransported antagonist. By contrast, CaMKIV activation, c-Jun N-terminal kinase pathway activation and CREB phosphorylation were induced by both DHPG and quisqualate. Downstream targets of Elk-1 activation, such as Erg1 and cFos, were also activated by intracellular mGlu5 receptors as were Fos, Fos related antigen, Fosl1, Fosl2, and Fosb (Jong et al., 2009). Thus, intracellular mGlu5 receptors generate distinct Ca^{2+} responses as well as downstream signaling cascades separate from their cell surface counterparts.

Pharmacological isolation also allows the use of unbiased bioinformatics approaches to determine what other genes might be affected by plasma membrane or intracellular receptor activation. This approach showed that many of the transcripts upregulated by quisqualate alone were transcription factors involved in neuronal survival and growth (activating transcription factor 3, nuclear receptor subfamily 4 group A member 1, Tribbles homolog 1, cAMP responsive element modulator, JunB, and AT-rich interactive domain-containing protein 5A) as well as effector proteins, such as activity-regulated cytoskeletal-associated protein (Arc), which is involved in gene regulation and synaptic plasticity (Kumar et al., 2012). Because Arc is critical for long-term memory and synaptic plasticity, these studies suggest that intracellular mGlu5 receptors play a major role in the transcriptional regulation of genes associated with sustained synaptic transmission (Kumar et al., 2012). Taken together, at least in striatal neurons, intracellular mGlu5 receptors can activate different pathways than mGlu5 receptors on the cell surface.

To determine whether mGlu5 receptors signal from intracellular membranes of other cell types, such as excitatory pyramidal neurons in the hippocampus, we used dissociated rat CA1 hippocampal cultures and slice preparations to localize and characterize endogenous receptors. As in the striatum, mGlu5 receptors were highly expressed on CA1 neurons both on the cell surface and intracellular membranes. Interestingly, DHPG induced oscillatory Ca^{2+} responses in dissociated CA1 neurons, whereas only intracellular mGlu5 receptor activation (quisqualate plus LY393053) triggered sustained high-amplitude Ca^{2+} increases in dendrites. Using an ex vivo slice approach, an important role for intracellular mGlu5 receptors was also seen for electrically induced and chemically induced long-term depression, but not for long-term potentiation in acute hippocampal slices (Purgert et al., 2014). In addition, whereas striatal cultures require activation of intracellular mGlu5 receptors for activation of ERK and CaMKII (Jong et al., 2009), cell surface receptors are responsible for upregulation of these kinases in hippocampal slices (Gallagher et al., 2004). This could be explained by the following: 1) cell type–specific differences in scaffolding or signaling molecules associated with the receptor; 2) differences in cultured neurons versus acute slices, which keep physiologic connections intact; and/or 3) age-related differences (neonatal cultures versus P30 slices). This type of context-dependent signaling might also arise since CA1 pyramidal hippocampal neurons are glutamatergic, excitatory neurons, whereas the striatum is predominated by GABAergic, inhibitory medium spiny neurons.

mGlu5 receptors are also highly expressed in spinal cord dorsal horn lamina I-II (Alvarez et al., 2000; Pitcher et al., 2007) an important area of pain transmission. In rats with persistent pain, mGlu5 receptor agonists are known to be pronociceptive, whereas antagonists are antinociceptive (Montana and Gereau 2011). For example, the mGlu5 receptor antagonist, fenobam, is analgesic in rodents (Montana et al., 2011) and mGlu5 receptor knockout mice have reduced nociceptive behaviors (Montana and Gereau 2011). Cornea-Hébert et al. (2009) recently discovered that nerve injury leads to increased mGlu5 receptors on spinal cord nuclear membranes. These data suggest that intracellular mGlu5 receptors may play a critical role in pain meditation. If so, these data would represent a bona fide physiologic paradigm in which intracellular receptors play a dominant role. Thus, targeting drugs to intracellular mGlu5 receptors might also lead to new therapeutic tools for chronic inflammatory pain.

mGlu5 Receptors and Disease

mGlu5 receptors are known to play important roles in neuronal function and synaptic plasticity, and defects in mGlu5 receptor signaling are thought to cause a variety of disorders. Through contributions to synaptic plasticity, mGlu5 receptors have been implicated in neuronal processes such as learning and memory as well as disorders including FXS, tuberous sclerosis, autism, epilepsy, schizophrenia, anxiety, neuropathic pain, addiction, Alzheimer disease, Parkinson disease, L-DOPA–induced dyskinesias, and gastroesophageal reflux disease

(Catania et al., 2007; Cleva and Olive, 2011; Krueger and Bear, 2011; Blandini and Armentero, 2012; Gray et al., 2012). mGlu5 receptors may also play a role in disease processes outside the synapse. Because of their importance in regulating pathways related to cell growth, differentiation, and metabolism, mGlu5 receptors have been implicated in malignancies such as melanoma (Pollock et al., 2003; Choi et al., 2011) and glioma (Aronica et al., 2003). Indeed, various glutamate receptor antagonists have had some success in limiting growth of certain malignancies (Willard and Koochekpour, 2013). Thus, there is great interest in developing therapeutics targeting mGlu5 receptors to correct the underlying cellular defects present in a broad variety of disorders.

FXS. One disorder for which mGlu5 receptor antagonists are in clinical trials is FXS, the most common inherited form of autism caused by genetic inactivation of the Fragile X Mental Retardation protein (FMRP; Maurin et al., 2014). A prominent hypothesis of FXS/ASD is that symptoms arise due to exaggerated mGlu5 receptor signaling that normally opposes FMRP function (Pop et al., 2014). This notion comes from studies showing that FMRP acts as a translational repressor of subsets of neuronal mRNAs, including ones involved in synaptic plasticity, such as AMPA receptors, CaMKIIα, and Arc. Activation of mGlu5 receptors initiates signaling pathways that are normally kept in check by FMRP leading to enhanced protein synthesis, synaptic mRNA translation, and the loss of surface-expressed AMPA receptors (Garber et al., 2008). In the Fmr1 knockout, negative regulation is lost leading to enhanced mGlu5 receptor signaling (Bear et al., 2004). These studies led to the prediction that mGlu5 receptor antagonists should restore the normal synaptic balance and thus improve behavioral phenotypes (Bear et al., 2008).

In support of this model, children with autism have increased mGlu5 receptor levels compared with age-matched controls (Fatemi and Folsom, 2011). Moreover, a de novo mutation in the mGlu5 receptor itself has been found in a child with ASD (Iossifov et al., 2012). In addition, genes downstream of the mGlu5 receptor form intricate signaling and scaffolding networks, many of whose members have also been implicated in ASD (e.g., neuronal Ca^{2+}-binding protein 2, phosphatase and tensin homolog, mTOR, tuberous sclerosis complex 1/2, Shanks, SAP90/PSD-95–associated proteins, and postsynaptic density-95) as well as neurexin–neuroligin complexes (Banerjee et al., 2014; D'Antoni et al., 2014). Thus, there may be a common synaptic mechanism for this complex disorder. Given that mGlu5 receptors serve as a high-level drug-able entry point into FXS/ASD, many pharmaceuticals have targeted this receptor for drug development (Pop et al., 2014).

Consistent with this hypothesis, MPEP and fenobam improve phenotypes associated with the disorder in various animal models (Pop et al., 2014). An even more selective, long-acting mGlu5 antagonist, CTEP [2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine], also corrects many features of FXS in Fmr1 knockout mice (Lindemann et al., 2011; Michalon et al., 2012). These findings prompted clinical trials with mGlu5 receptor NAMs that exhibited the best pharmacokinetic profiles such as the Novartis drug, mavoglurant (AFQ056) (Jacquemont et al., 2011; Gomez-Mancilla et al., 2014) or the Roche mGlu5 NAM, RO4917523 [2-chloro-4-{[1-(4-fluorophenyl)-2,5-dimethyl-1*H*-imidazol-4-yl] ethynyl}pyridine], a clinical derivative of CTEP (Lindemann et al., 2011). Despite early promise, both drug trials were recently discontinued due to negative outcome results (http://www.fraxa.org/fragile-x-clinical-trials-mglur-theory/).

Although outcomes are difficult to define as well as measure, one possibility for a lack of efficacy is the development of tolerance (http://www.fraxa.org/fragile-x-clinical-trials-mglur-theory/) and another possiblity is differential inhibition of mGlu5 receptors on cell surface or intracellular membranes. For example, most drug candidates are designed to be potent, bioavailable, and metabolically stable, yet every compound scaffold has unique chemical properties. Since populations of neurons can also have unique membrane constituents and lipophilic properties, ligand parameters worked out in heterologous cell types might not reflect what happens in a given neuron. Conceivably, differential membrane properties might contribute to receptor location bias and underlie differential efficacy. In theory, drug candidates with preferred pharmacokinetic outcomes might be targeted for further optimization of the preferred cell surface and/or intracellular response. Given that mGlu5 receptors appear to play a key role in FXS, understanding the signaling pathways associated with spatially restricted mGlu5 receptor signaling may aid in defining early intervention points.

Are There Other Intracellular mGlu Receptors?

Given the pivotal role played by group 1 mGlu receptors throughout development and in disease, we tested whether, like mGlu5 receptors, mGlu1 could also function as an intracellular receptor. As with mGlu5 receptors, ultrastructural studies reported approximately 40–60% of mGlu1 receptors on intracellular membranes depending upon the brain region examined (Hubert et al., 2001; Kuwajima et al., 2004, 2007; Mitrano et al., 2008). Moreover, we showed that mGlu1 receptors were present on nuclear membranes in the cortex, olfactory bulb, thalamus, and cerebellum (Jong et al., 2007). Real-time measurements confirmed that changes in nuclear Ca^{2+} levels resulted from direct activation of mGlu1 receptors on the nuclear membranes of cortical neurons that could be blocked by the mGlu1 receptor–specific antagonist CPCCOEt [7-(hydroxyimino)cyclopropa[*b*]chromen-1α-carboxylate ethyl ester]. Uptake studies suggested that like mGlu5 receptors, ligand transport across cortical nuclear membranes occurred via sodium-dependent and -independent processes. Finally, increasing levels of nuclear mGlu1 receptors were observed throughout postnatal development, pointing to an important role for cell surface and nuclear mGlu1 receptors in the control of brain development (Jong et al., 2007). Therefore, mGlu1 receptors also appear to serve as a critical intracellular regulator.

It is less clear that group 2/group 3 mGlu receptors serve in this role. There is some ultrastructural evidence suggesting that antibodies recognizing mGlu2/mGlu3 receptors were distributed intracellularly in rat globus pallidus (Poisik et al., 2005). Most studies, however, have shown group 2/group 3 receptors to be primarily at the plasma membrane whether at the axon terminal or postsynaptically. Thus, intracellular localization may be a function restricted to group 1 mGlu receptors.

Summary

It is increasingly clear that a variety of biased signaling modalities can affect GPCR signaling, such as ligand bias, receptor bias, location bias, and cell-type bias. In the case of

the group 1 mGlu receptors, different cellular locations of receptors (cell surface versus intracellular) mediate both overlapping and unique signaling effects. The functions of mGlu5 receptors are also subject to cell type bias in that signaling in the hippocampus differs from that in the striatum. Finally, other explanations that may contribute to complexities in mGlu5 receptor signaling include age-related alterations in downstream signaling effects, differences in signaling among mGlu5 receptor isoforms, and/or the possibility of heterodimerization or formation of multimeric complexes with other GPCRs.

As briefly described above and thoroughly reviewed elsewhere (Dhami and Ferguson, 2006; Esseltine and Ferguson, 2013), there are reports indicating that mGlu5 receptors undergo agonist-dependent and -independent endocytosis that may or may not involve β-arrestin (Fourgeaud et al., 2003; Dhami and Ferguson, 2006; Ko et al., 2012). These data suggest that there are multiple complex mechanisms associated with trafficking and regulating mGlu5 receptor location. It will be of great interest and potential clinical utility to discover how all of these various pathways come together to regulate intracellular mGlu5 receptors. Clearly, there is a need for modeling the complex dynamics of all of the different receptor populations in order to discern the effect that spatial segregation has on signaling regulation.

Given the breadth of the mGlu5 receptor target market, many drugs are being developed for this receptor (Blandini and Armentero, 2012; Duty, 2012). Our data suggest that whether a ligand gets across a given cellular membrane may change a receptor's functional response. Thus, drugs with a desirable pharmacokinetic outcome might be further optimized for a desirable cell surface and/or intracellular response. Given that NAMs are in clinical trials for FXS and other disorders, it is critical to understand whether they are differentially affecting receptor function in relevant areas of the brain. Further elucidation of the site of action of these drugs may determine those signal pathways mediating therapeutic efficacy.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Jong, Sergin, Purgert, O'Malley.

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