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Unveiling the Functions of Presynaptic Metabotropic Glutamate Receptors in the Central Nervous System

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ABSTRACT

Metabotropic glutamate (mGlu) receptors, which include mGlu1–8 receptors, are a heterogeneous family of G-protein-coupled receptors which function to modulate brain excitability via presynaptic, postsynaptic and glial mechanisms. Certain members of this receptor family have been shown to function as presynaptic regulatory mechanisms to control release of neurotransmitters. In general, Gi-coupled mGlu receptor subtypes appear to negatively modulate excitatory (and possibly also inhibitory) neurotransmitter output when activated. Localization studies have shown that mGlu7 is restricted to the presynaptic grid at the site of vesicle fusion. These studies along with other evidence suggest that mGlu7 is the nerve terminal autoreceptor that regulates physiological release of glutamate. Other mGlu subtypes, in particular mGlu2, mGlu8,

and possibly mGlu4, are also localized presynaptically, but at perisynaptic sites outside the active zone of neurotransmitter release. Gi-coupled mGlu receptors also may exist on presynaptic elements of neighboring γ -aminobutyric acid (GABA) neurons where they play a role in heterosynaptic suppressions of GABA release. This suggests that these receptors may have evolved to monitor glutamate that has “spilled” out of the synapse. Thus, they may serve as the brain’s evolutionary mechanism to prevent pathological changes in neuronal excitability and thus maintain homeostasis. Recent progress on the molecular and pharmacological aspects of these presynaptic mGlu receptors is unveiling their functions and the therapeutic directions of agents designed for these novel glutamate receptor targets.

In the past decade there has been considerable progress in the field of metabotropic or G-protein-coupled glutamate (mGlu) receptors. For the most part, the cloning and identification of a novel heterogeneous family of mGlu receptors has driven this progress. There are currently eight known subtypes of mGlu receptors, which have been classified into three groups (see Table 1). Members of the mGlu receptor family are each G-protein-coupled receptors (GPCRs). Within each mGlu receptor group, there is ~70% sequence homology, whereas between the mGlu receptor subgroups there is lesser (~40%) homology. Group I mGlu receptors include mGlu1 and mGlu5, which when expressed are coupled via Gq to phospholipase C. Group II (mGlu2 and mGlu3) and group III (mGlu4,6,7,8) receptors are coupled to Gi and inhibit stimulated cAMP formation when expressed in cell lines. A number of gene splice variants for group I and III mGlu receptors are also

known, with most amino acid changes in the carboxyl-terminal regions that may be important in targeting receptors to regions of the cell (Boudin et al., 2000). These receptors each have unique but overlapping distributions in the central nervous system, and the functions of each subtype within these groups have recently been an active area of neuroscience research. In general, mGlu receptors appear to have evolved as modulatory mechanisms to control CNS excitability. Many disorders of the central nervous system, including psychiatric as well as neurological, have been linked to alterations in neuronal excitability via the glutamatergic system (Danysz et al., 1995). Thus, understanding ways to modulate CNS excitability by glutamate receptor mechanisms has broad therapeutic significance. In particular, certain members of the group II and group III mGlu receptors have been implicated in presynaptic negative modulation of excitatory glutamate and/or

ABBREVIATIONS: mGlu, metabotropic glutamate; GPCRs, G-protein-coupled receptors; CNS, central nervous system; GABA, γ -aminobutyric acid; EPSP, excitatory postsynaptic potential; MCPG, α -methyl-carboxyphenylglycine; 5HT, serotonin; L-AP4, L-2-amino-4-phosphonobutyric acid; 3,4-DCPG, (S)3,4-dicarboxyphenyl glycine; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; nRT, thalamic reticular nucleus.

TABLE 1
Classification, functions, and pharmacology of mGlu receptors

Receptor	Coupling	Localizations and Functions	Pharmacological Tools ^a
Group I mGlu1	Gq/↑ PLC	Primarily postsynaptic localization to neurons. Activation enhances post-synaptic glutamatergic excitability. Positive modulation of glutamate and GABA transmission suggested. Relative receptor expression noted in cerebellum. Involved in mediating LTD and LTP in certain synapses. Knockout animals exhibit motor learning and spatial learning deficits. Knockdown (antisense)-treated animals have reductions in excitatory responses to C-fiber (pain) inputs.	Prototypic group I agonists: 3,5-DHPG quisqualate Selective mGlu1 antagonist: CPCCOEt (noncompetitive)
mGlu5	Gq/↑ PLC	Primarily postsynaptic localization to neurons and glial cells. Positive modulation of glutamate and GABA neuronal transmission suggested. High expression through forebrain regions and in spinal cord. Involved in mediating NMDA-dependent LTP in certain synapses (CA1). Knockout animals exhibit deficits in acquisition and retention of spatial learning and contextual fear information. Pharmacological studies (MPEP) suggests a role in pain and anxiety states.	Selective mGlu5 antagonist: MPEP (noncompetitive)
Group II mGlu2	Gi, Go/↓ A.C.	Localizations pre- and postsynaptic in neurons. Negative modulation of glutamate and GABA neuronal transmission suggested. Relatively high expression levels noted in rat forebrain regions. Role in modulation of multiple forebrain limbic circuits (e.g. hippocampus) known. Loss of mGlu2/3 agonist suppressions of glutamate release and hippocampal LTD noted in mGlu2 knockout animals. No behavioral consequences in knockout animals yet reported. Spatial memory normal. Pharmacological data with mGlu2/3 agonists suggest possible antianxiety, antipsychotic, and neuroprotectant properties.	Prototypic group II agonists: DCG-IV 2R,4R-APDC LY354740 LY379268 Prototypic group II antagonists: EGLU LY341495 LY307452
mGlu3	Gi, Go/↓ A.C.	Primary localization postsynaptic to neurons and in glial cells. Role in modulation of GABA neuronal transmission noted. Phenotype of knockout animals not yet reported. Possible role in processing of noxious sensory stimuli within cortical thalamic circuits noted.	
Group III mGlu4	Gi, Go/↓ A.C.	Localizations presynaptic and postsynaptic in neurons. High receptor levels noted in rat cerebellum. Negative modulation of glutamate and GABA neuronal transmission suggested. Phenotype of knockout animals suggests role in motor function and seizure states in animals.	
mGlu6	Gi, Go/↓ A.C.	Highly localized to retinal tissues to dendrites of ON bipolar cells, with very low expression noted in brain tissue. Knockout animals suggest primary role is processing of visual sensory information.	Prototypic group III agonists: L-AP4 L-SOP PPG
mGlu7	Gi, Go/↓ A.C.	Localizations presynaptic and postsynaptic in neurons. Targeted presynaptically to the active zone of glutamate release. Negative modulation of glutamate and GABA neuronal transmission suggested. Phenotype of knockout animals suggests a role on amygdala-dependent learning and fear responses. Developmentally delayed seizures noted in knockout animals suggest a primary role in negative modulation of physiological glutamate release and possible the etiology of epilepsy.	Prototypic group III antagonists: CPPG MAP4
mGlu8	Gi, Go/↓ A.C.	Localizations pre- and postsynaptic in neurons. Negative modulation of glutamate (and possibly GABA neuronal transmission suggested). Likely presynaptic L-AP4 mGlu receptor in lateral perforant and neonatal spinal cord preparations. Phenotype of knockout animals not yet reported.	3,4-DCPG is a highly selective mGlu8 receptor agonist

A.C., adenylyl cyclase; LTD, long-term depression; LTP, long-term potentiation; 3,5-DHPG, 3,5-dihydroxyphenylglycine; CPCCOEt, 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester; MPEP, 2-methyl-6-(phenylethynyl)pyridine; DCG-IV (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine; 2R,4R-APDC, 2R,4R-4-aminopyrrolidine-2,4-dicarboxylic acid; EGLU (S)-α-ethylglutamic acid; L-SOP, S-serine-O-phosphate; PPG, (RS)-4-phosphonophenylglycine; MAP4, (S)-α-methyl-2-amino-4-phosphonobutanoic acid; CPPG, (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine; PLC, phospholipase C.

^a See Schoepp et al. (1999a) for a detailed review of pharmacological agents.

inhibitory GABA neuronal transmission, and that subject and its therapeutic implications are the focus of this article. For recent comprehensive reviews of other aspects of

mGlu receptors, see Anwyl (1999), Bockaert and Pin (1999), Bordi and Ugolini (1999), Pin et al. (1999), Schoepp et al. (1999a), and Cartmell and Schoepp (2000).

Evolution of mGlu Receptors May Implicate Them as an Important Mechanism for Modulation of Neuronal Excitability

Because of their general structure and related sequence homologies, mGlu receptors are classified as family 3 GPCRs, which also include GABA_B (or “metabotropic” GABA receptors), Ca²⁺-sensing receptors, and certain pheromone receptors (see Bockaert and Pin, 1999). All of these receptors have in common a large extracellular ligand recognition domain, seven transmembrane-spanning regions connected by three intracellular loops and three extracellular loops, and a number of conserved cysteine residues that may be involved in receptor conformation by the formation of possible intra- or intermolecular disulfide linkages. Recent data suggest that functional family 3 GPCRs exist in situ as either hetero- (GABA_B) or homodimers (Ca²⁺-sensing and mGlu receptors). Family 3 GPCRs, particularly mGlu receptors, GABA_B receptors, and Ca²⁺-sensing proteins, are each highly expressed in the nervous system of many species (as well as in peripheral tissues to various degrees) of mammals, *Drosophila*, and fish (for reviews see Marshall et al., 1999; Bockaert and Pin, 1999; Riccardi, 1999; Schoepp et al., 1999a; Couve et al., 2000). This receptor class has structural features (a bi-lobed structure with an open configuration in absence and closed configuration in the presence of ligand) reminiscent of bacterial periplasmic binding proteins that function to sense nutrients (including ions and amino acids) for cellular uptake. Thus, from an evolutionary perspective, family 3 GPCRs in general may have evolved from a common primordial function (possibly Ca²⁺ sensing, see Riccardi, 1999). During evolution, the structure and functions of different family 3 GPCR have obviously diverged. Nevertheless, as discussed below, in the case of GABA_B and mGlu receptors, certain related roles in nervous system function, namely presynaptic modulation of brain excitability, have been apparently retained.

GABA and glutamate, respectively, are the major inhibitory and excitatory neurotransmitter substances in the mammalian nervous systems. The balance of excitation/inhibition within neuronal circuits is highly dependent upon postsynaptic activation of ionotropic receptors for these ligands (GABA_A or AMPA/kainate/NMDA receptors). The related proteins, GABA_B and mGlu receptors, function in nervous tissues to recognize (“sense”) these respective ligands, but they serve a more modulatory role in the control of excitation/inhibition. It is interesting to consider that GABA_B and mGlu receptors are more related in functional and structural terms to each other than they are to their respective ionotropic receptor proteins, whose functions they apparently evolved to modulate. Recent data suggest that certain GABA_B and mGlu receptors are expressed presynaptically on both GABA and glutamate neurons where they monitor neuronal “spill-over” of their respective ligands, and play a role in heterosynaptic depression of either glutamate or GABA release (see Isaacson, 2000). The differential expression of these two metabotropic receptor systems may be important in determining the relative contribution of excitation versus inhibition in physiological and pathological states involving many circuits of the CNS. In other words, metabotropic amino acid receptors may have evolved as a primary mechanism to modulate neuronal excitability (see Fig. 1). In the case of mGlu

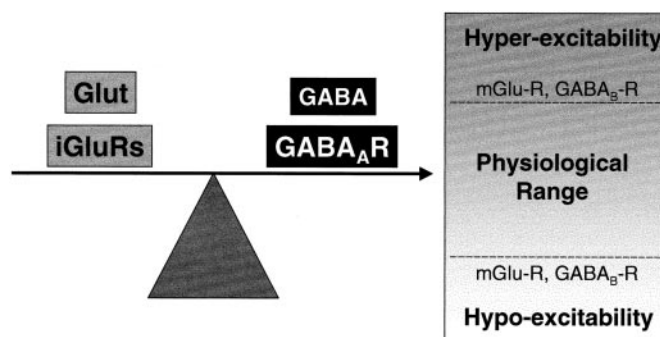


Fig. 1. Metabotropic glutamate and GABA_B receptors may have evolved to regulate neuronal excitability in the mammalian central nervous system. The amino acids L-glutamic acid (Glut) and GABA, respectively, are the primary excitatory and inhibitory neurotransmitter in the mammalian CNS. These substances mediate fast-synaptic transmission by activation of ionotropic glutamate receptors (iGluRs) and GABA_A receptors on postsynaptic neurons to control CNS excitability. In this manner, many physiological processes of the nervous system (motor, sensory, higher functions) are carried out. The modulatory roles of mGlu and GABA_B receptors (R) to control glutamate and GABA release have evolved as feedback monitors of glutamate and GABA levels within and outside the synaptic cleft. In this manner, these metabotropic receptors may play a role preventing pathological conditions of hyperexcitability (too much glutamate or not enough GABA) or hypoexcitability (not enough glutamate or too much GABA) within specific synapses where the receptors are expressed.

receptors, new information on localization and pharmacology of mGlu subtypes is beginning to reveal interesting details and possible therapeutic implications of these modulatory functions.

There is biochemical and electrophysiological evidence for a role of groups I, II, and III in the modulation of glutamate release (Anwyl, 1999; Cartmell and Schoepp, 2000). For the most part, group I mGlu receptors are considered to be primarily postsynaptic in localization, where they function to enhance cellular excitability via interactions with other postsynaptic processes (e.g., ionotropic receptors, ion channels) (see Bordi and Ugolini, 1999). Immunocytochemical studies have not yet confirmed a presynaptic localization of a group I mGlu receptor, although some biochemical evidence exists for a presynaptic role (see Cartmell and Schoepp, 2000). Nevertheless, a variety of approaches indicates that certain group II and group III mGlu receptor subtypes predominate on presynaptic elements where they function to regulate the release of glutamate in functionally diverse ways. Because a comprehensive review of the therapeutic aspects of mGlu modulation is beyond the scope of this article, select examples of therapeutic insights that have been gained from recent work are presented here.

Modulation of Glutamatergic Functions by Group II mGlu Receptor Subtypes

Of the group II metabotropic receptor subtypes, most of the data supports the conclusion that mGlu2 receptors are localized to preterminal axons of glutamate neurons where they function as a negative feedback mechanism to suppress further release of glutamate. mGlu3 receptors are primarily present postsynaptically on neurons and expressed in glia, where their functional role is less clear. Many studies use available antibodies and pharmacological agents that are group-II-selective (target both mGlu2 and mGlu3), so in many cases a clear distinction between the relative contribu-

tions of each subtype is difficult. For example, studies have clearly established that agonists for mGlu2/3 receptors reduce postsynaptic glutamate excitatory postsynaptic potentials (EPSPs) via a presynaptic mechanism in many synapses (reviewed by Anwyl, 1999). However, the presynaptic localization of mGlu2 to glutamate preterminal axons has been most clearly defined within the hippocampal formation. Here mGlu2 immunoreactivity illustrates a primary localization to subfields corresponding to the medial perforant and mossy fiber pathways (Shigemoto et al., 1997). Radioligand binding of the mGlu2/3-selective ligands, including either [³H]LY354740 (agonist) or [³H]LY341495 (antagonist), also supports this localization of mGlu2 (Schauflhauser et al., 1998; Wright et al., 2001). Unilateral lesions of entorhinal cortex, which contains cell bodies of the perforant path neurons, lead to ipsilateral loss of mGlu2/3 immunoreactivity (Shigemoto et al., 1997) and [³H]LY354740 binding (Richards et al., 2000) within perforant path terminal fields of the hippocampus that is associated with mGlu2 receptor labeling. Experiments with mGlu2 knockout mice most clearly indicate that mGlu2 is a presynaptic modulatory receptor in the hippocampus. Unlike wild-type animals, knockout mice deficient in mGlu2 receptors showed no immunostaining of mGlu2 within stratum lucidum of CA3 (mossy fiber terminal field) or stratum lacunosum moleculare of CA1 (medial perforant path terminal field) of hippocampus (Yokoi et al., 1996). In addition, in mGlu2-deficient mice, long-term depression induced by low-frequency stimulation of the mossy fiber CA3 synapses was abolished. Thus, presynaptic mGlu2 receptors appear to be essential for inducing long-term depression at this synapse, illustrating an important role in the modulation of neuronal excitability. However, that said, mGlu2 knockout animals appear to be functionally normal, with no alteration in basal synaptic transmission. This illustrates that mGlu2 receptors do not play a prominent role in acute regulation of excitatory synaptic transmission.

It is thought that following the release of glutamate, the levels in the immediate area of the synapse are likely in the millimolar range. Nevertheless, the binding affinity of the cloned mGlu2 receptor for glutamate is low micromolar (Schoepp et al., 1999a). This being the case, any mGlu2 receptors in the immediate vicinity of synapse might be partially occupied or even saturated with glutamate ligand. Although pharmacological data of glutamate release suppression by mGlu2/3 agonists support a presynaptic location of mGlu2 receptors, immunocytochemical studies do not support the presence of mGlu2 in the glutamate synapse per se. Antibodies selective for mGlu2 or mGlu2/3 receptors generally show immunolabeling to membrane compartments distant from active release sites and postsynaptic specializations (see Fig. 2). Although mGlu2/3 agonists have been shown to suppress glutamate release and postsynaptic excitations in a number of excitatory synapses (see Anwyl, 1999), it has also been observed that concentrations of antagonists per se that block this agonist effect have little effect on evoked excitatory synaptic transmission. Thus, presynaptic mGlu2 receptors that mediate agonist-induced negative feedback do not appear to be activated by endogenous glutamate to exert negative feedback under "normal" conditions of excitatory synaptic transmission. This raises questions about the role of these mGlu2 receptors in excitatory synaptic events.

Electrophysiology experiments support the concept that

synaptic spillover of glutamate is necessary for synaptic activation of mGlu2 receptor-mediated negative feedback on glutamate release. Scanziani et al. (1997) showed that the occupancy of presynaptic inhibitory (presumably mGlu2) receptors, in the rat mossy fiber pathway, was frequency-dependent. Enhanced excitatory synaptic responses were noted in the presence of the competitive mGlu2 antagonist α -methyl-carboxyphenylglycine (MCPG) under conditions of high (1-Hz) but not low (0.05-Hz) frequency stimulation. Moreover, glutamate uptake blockade with *L-trans*-pyrrolidine-2,4-dicarboxylic acid also produced decreases in field EPSPs, but only under conditions high-frequency stimulation. These data suggest that mGlu2 receptors may have evolved as a neuronal mechanism to keep glutamate transmission within the physiological range and thus prevent hyperexcitability from interfering with normal brain functions. Certain pharmacological studies with mGlu2/3 receptor agonists further support this hypothesis, as systemically active mGlu2/3 agonist compounds such as LY354740 and LY379268 are active in animal models of anxiety, global ischemia, and psychosis, at doses that have minimal or no effects on the animal's normal functions (Schoepp et al., 1999a,b). However, as discussed below, other factors may contribute to these observations.

In the rat prefrontal cortex, Marek et al. (2000) have demonstrated that mGlu2/3 agonists such as LY354740 and LY379268 suppress both electrically evoked and serotonin (5HT)-evoked EPSPs by a presynaptic mechanism. In this system, 5HT-evoked EPSPs, in contrast to electrically evoked EPSPs, appear to involve presynaptic, impulse-flow-independent release of glutamate that is mediated by 5HT_{2A} receptors. These actions appear to be presynaptic, as selective lesions of cell bodies in thalamic nuclei that project glutamatergic axons to medial prefrontal cortex lead to loss of 5HT_{2A} receptor-induced EPSPs (Marek et al., 2001). Here, the mGlu2 receptors that mediate these inhibitory effects appear to be tonically activated, as enhanced 5HT or electrically evoked excitatory synaptic responses were produced by the presence of an mGlu2/3 receptor antagonist (LY341495) per se. This indicates that conditions for the occupancy of presynaptic inhibitory mGlu2 receptors by endogenous glutamate may depend on the synapses involved. Also, in certain synapses such as the prefrontal cortex, mGlu2 receptor-mediated negative feedback appears to play a role in nonimpulse-flow (e.g., 5HT_{2A} receptor)-dependent regulation of glutamate release. Activation and antagonism of 5HT_{2A} receptors in the prefrontal cortex are important in mediating the actions of certain hallucinogens and antipsychotic drugs, respectively. The ability of mGlu2/3 (and group III) receptor agonists to act as a "functional" 5HT_{2A} antagonists (see Marek and Aghajanian, 1998) may have important therapeutic implications. Prefrontal cortex 5HT_{2A} receptor antagonism has been associated with the efficacious effects of atypical antipsychotic drugs, and within this area of the brain mGlu2/3 receptor agonists share the pharmacology in this functional sense.

Modulation of Glutamatergic Functions by Group III mGlu Receptor Subtypes

Koerner and Cotman (1981) initially described that the diacidic amino acid analog *L*-2-amino-4-phosphonobutyric acid (*L*-AP4) selectively suppressed glutamate excitations by

a presynaptic mechanism in the lateral perforant pathway of the hippocampus. This inhibitory activity of L-AP4 on glutamate excitations was also observed in other preparations, including the mossy fiber synapse, lateral olfactory tract, and spinal cord (see Thomsen, 1997). Until the 1990s, presynaptic inhibition induced by L-AP4 was ascribed to a relatively nebulous "L-AP4" receptor. However, with the cloning of the group III mGlu receptors, which Nakanishi (1992) defined by their sensitivity to L-AP4, it was recognized that certain group III mGlu subtypes might be responsible for L-AP4-induced suppression of glutamate release. Current data suggest a role for mGlu7, mGlu8, and possibly mGlu4 as candidates for these presynaptic effects of L-AP4 in the brain (see Thomsen, 1997).

In general, when compared with mGlu7 or mGlu2/3 receptors, the expressions of mGlu4 and mGlu8 receptors are somewhat more restricted in distribution in the CNS. Also, mGlu6 receptors have been shown in the retina, but they are not prominently expressed in the CNS. mGlu4 receptors are most prominently expressed in the cerebellum, where they have been studied in most detail, but they are also found in other brain and spinal regions to some extent (see Thomsen, 1997). Immunocytochemical studies indicate that mGlu4 receptors are expressed on presynaptic terminals and are present postsynaptically on neurons (Bradley et al., 1999). mGlu4 receptors are also suggested to be the peripheral taste receptors responsible for "unami" taste sensation to monosodium glutamate (Kinnamon and Margolskee, 1996). Within the cerebellum, localization and electrophysiological studies suggest that mGlu4 receptors mediate presynaptic inhibitory effects of L-AP4 on parallel fiber synapses to Purkinje cell dendrites. A presynaptic localization of mGlu4a receptors along the membranes of cerebellar parallel fiber terminals, with interspaced clusters of receptors along parallel fibers at intervals of 40 to 80 nM, was reported by Mateos et al. (1999).

Consistent with a role in modulation of parallel fiber-Purkinje cell synaptic transmission, mGlu4 receptor knockout mice have a loss of L-AP4-induced presynaptic inhibition of Purkinje cell synapses (Pekhletski et al., 1996). When compared with wild-type, these animals were deficient on a motor-learning test (rotorod), suggesting that expression of mGlu4 at parallel fiber-Purkinje cell synapses are important for normal motor function.

When compared with mGlu4 receptors, less is known about mGlu8 receptor distribution and functions, as fewer studies with antibodies to mGlu8 receptors have been reported, and the phenotype of mGlu8 receptor knockouts has not yet been described. In the rat and mouse, mRNA for the mGlu8 receptor is highly expressed in olfactory bulb (Duvoisin et al., 1995; Saugstad et al., 1997), suggesting that mGlu8 may be the "L-AP4" receptor responsible for presynaptic inhibition in the lateral olfactory tract. A presynaptic localization of mGlu8 receptors in projection neurons of the olfactory bulb in rats is supported by a study (Kinoshita et al., 1996) showing that transection of the lateral olfactory tract leads to decreases in mGlu8a immunoreactivity in layer 1a of the pyriform cortex (which is the target area for these glutamatergic projection neurons). The precise localization of mGlu8 receptors at the subcellular level is not yet clear. Electron microscopy studies show localization in close proximity to, but not necessarily within, the presynaptic specialization of asymmetrical synapses (Kinoshita et al., 1996). Like with mGlu2 receptors, glutamate has low micromolar affinity at mGlu8 receptors (Wright et al., 2000), possibly indicating a perisynaptic localization and similar functional role in extrasynaptic glutamate modulation of glutamate release (Fig. 2). mGlu8 receptors are also expressed to a lesser degree in other brain areas including cerebral cortex and cerebellum, but little is yet known of mGlu8 functions in these regions (Duvoisin et al., 1995; Saugstad et al., 1997).

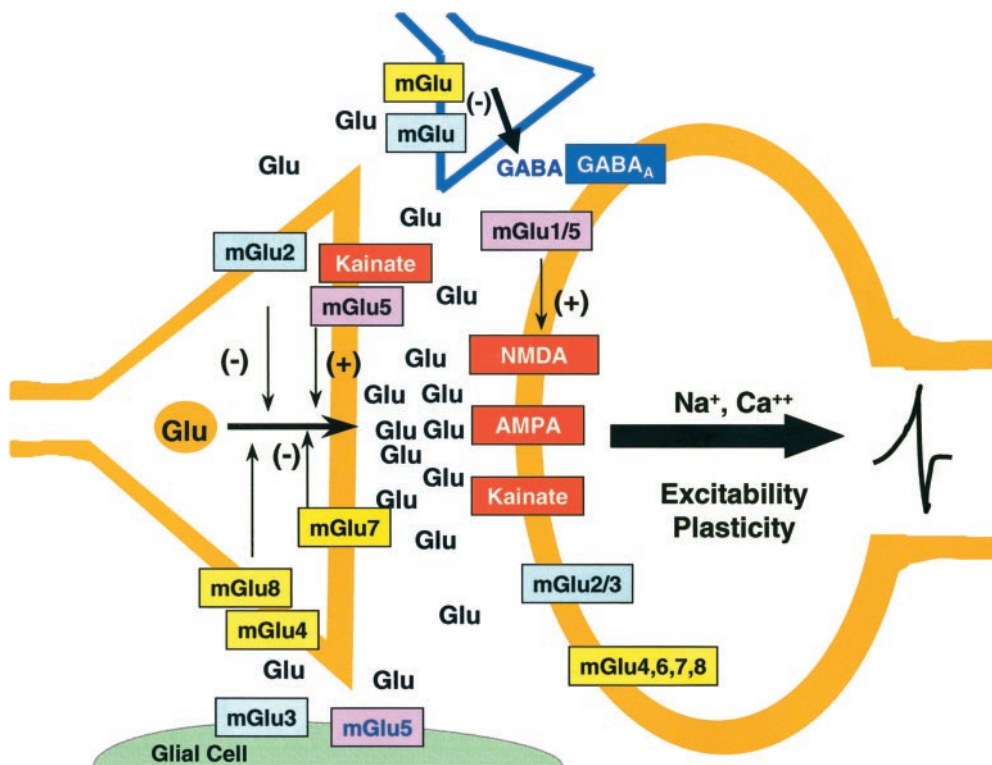


Fig. 2. General cellular localizations and cellular functions of mGlu receptor subtypes. In general, ionotropic glutamate receptors (AMPA, NMDA, and kainate subtypes) are postsynaptic where they function to mediate fast-excitations and synaptic plasticity associated with opening of sodium- and calcium-permeable ligand-gated ion channels. Metabotropic glutamate receptors are present at presynaptic, postsynaptic, glial, and heterosynaptic localizations where they function to monitor glutamate levels and provide positive feedback (group I mGlu receptors mGlu1/5) or negative feedback (group II mGlu receptors, mGlu2/3; and group III mGlu receptors, mGlu4/6/7/8) to decrease further release of neurotransmitters or change postsynaptic excitability to glutamate. The differential expression of these subtypes to certain synapses, cells, and cellular compartments allows for "finer" control of excitations throughout the CNS. Thus, drugs acting on metabotropic receptors may have therapeutic potential to treat a wide range of neurological and psychiatric conditions involving altered excitability of circuits in the brain and spinal cord.

Both mGlu4 and mGlu8 receptors have been shown to be expressed to a certain extent within certain subfields of the hippocampus, and their possible role in synaptic transmission at hippocampal pathways has been studied in some detail (Bradley et al., 1996; Bradley et al., 1999; Shigemoto et al., 1997). Among the group III mGlu subtypes, mGlu8 receptors are pharmacologically distinguished by sensitivity to the antagonist MCPG (Saugstad et al., 1997; Schoepp et al., 1999a). L-AP4 presynaptic inhibition of evoked excitations in the lateral perforant pathway of the hippocampus is also blocked by MCPG, suggesting a role for mGlu8 receptors in that glutamatergic pathway. Consistent with this conclusion, immunocytochemical studies have shown selective labeling of mGlu8 receptors to the terminal fields of the lateral perforant pathway (CA3 stratum lacunosum moleculare), and loss of this labeling following perforant path lesions (Shigemoto et al., 1997). Very recently, the compound (*S*)-3,4-dicarboxyphenylglycine (3,4-DCPG) has been described as a potent and highly selective mGlu8 receptor agonist, with no activity at cloned mGlu4, mGlu6, or mGlu7 receptors at concentrations that fully activate cloned mGlu8 receptors (Thomas et al., 2001). 3,4-DCPG appears to activate mGlu receptors on primary afferent glutamatergic terminals in the neonatal spinal cord to suppress evoked excitations, suggesting a role for mGlu8 in modulation of spinal sensory inputs (Thomas et al., 2001). This new mGlu8 agonist should be useful to further explore mGlu8 receptor function in the brain.

While mGlu8 receptors are relatively restricted to the terminal subfields of the dentate gyrus, the initial work of Bradley et al. (1996) showed mGlu4a staining was in cell bodies and dendrites of pyramidal neurons, granule cells, and scattered interneurons throughout the hippocampus. However, later work by this group using a more specific antibody (Bradley et al., 1999) suggested a more limited distribution of mGlu4a within the hippocampus. Here high expression was noted in the molecular layer of the dentate gyrus, stratum-moleculare of CA1 and stratum oriens of the CA3 area. Importantly, this immunoreactivity was not present in the mGlu4a knockout mouse. Within the basal ganglia, mGlu4a immunoreactivity was shown to be on presynaptic axonal elements of striatopallidal neurons, as quinolinic acid lesions of these neurons decreased mGlu4a receptor immunoreactivity in the globus pallidus. At the electron microscope level, mGlu4 receptors are found postsynaptically at asymmetrical (presumably glutamatergic) synapses, and presynaptic at both asymmetrical and symmetrical (presumably GABAergic) synapses (Bradley et al., 1999). Thus, mGlu4 receptors may have pre- and postsynaptic functions, and they may be involved in both homo- and heterosynaptic modulation in these brain regions. Experiments designed to examine hippocampal functions in mGlu4 receptor knockout mice suggest a role in the processing of spatial information. Although mGlu4 receptor mutants were not impaired in the water maze task, they exhibited enhanced performance in a spatial reversal learning task (Gérald et al., 1998). This phenotype may have been due to a decrease in the animal's memory retention of the original platform localization, producing a shorter escape latency to find a new platform location. In any case, this illustrates a possible role for mGlu4 receptors in hippocampal processing of spatial information.

mGlu7 receptors are highly expressed throughout the fore-

brain, brainstem, and spinal cord regions of the CNS (Bradley et al., 1998). In particular, mGlu7 receptors may represent an autoreceptor in certain synapses that provide negative feedback to limit further release of glutamate under normal physiological conditions of excitatory synaptic transmission (see Fig. 2). In cells expressing recombinant human or rat mGlu7 receptors, multiple laboratories observed that almost millimolar concentrations of glutamate were required to functionally activate this receptor (e.g., as measured by suppression of stimulated cAMP formation) (see Schoepp et al., 1999a). This relative insensitivity to glutamate activation when expressed in cell lines called into question whether non-neuronal cells expressing mGlu7 receptors were coupled as effectively as they might be in their native environment. However, radioligand binding studies with [³H]LY341495 in human mGlu7 receptor-expressing cell membranes have shown that the affinity of mGlu7 for glutamate is also relatively low ($K_i = 869 \mu\text{M}$) (Wright et al., 2000). Shigemoto et al. (1996) showed that mGlu7a receptor immunoreactivity in the rat hippocampus was restricted to the presynaptic grid or site of vesicle fusion. The lower affinity of glutamate for mGlu7 receptors is consistent with its localization in the synaptic cleft and function as an autoreceptor. To exist as a dynamic regulator of physiological glutamate release, the mGlu7 receptor cannot be fully occupied under basal conditions. Conceptually, the attainment of millimolar concentrations of glutamate at the presynaptic grid upon release would then occupy mGlu7 sites and activate its regulatory functions to further limit glutamate exocytosis. In general, mGlu7 receptor protein and mRNA are relatively more widespread in distribution throughout the neuro-axis when compared with other presynaptic mGlu receptors (e.g., mGlu2, mGlu4, and mGlu8), possibly indicating a more prominent role in normal regulation of synaptic glutamate release. Nevertheless, like other mGlu receptors, mGlu7 expression is more concentrated in certain areas and appears to be specifically targeted to certain synapses. Thus, not all glutamatergic neurons appear to express (or need) an mGlu7 autoreceptor regulatory mechanism to maintain normal excitatory functions.

The expression of mGlu7 receptors in glutamatergic nerve terminals of the perforant path is supported by loss of mGlu7a immunoreactivity following lesions of entorhinal cortex (Shigemoto et al., 1997). Colchicine lesions of the dentate granule cells also produced loss of mGlu7 receptor immunoreactivity in the CA3 of the hippocampus, indicating a presynaptic role of mGlu7 in the mossy fiber pathway. Interestingly, terminals of pyramidal neurons, which were presynaptic to the population of interneurons expressing postsynaptic mGlu1 receptors, expressed ~10-fold higher levels of mGlu7 receptors when compared with terminals making synaptic contacts with other pyramidal neurons or interneurons. This suggests that mGlu7 receptors may regulate release of glutamate at certain synapses based on what other receptors are expressed postsynaptically. A recent study (Boudin et al., 2000) suggests that the targeting of the mGlu7a receptor to the presynaptic membrane is dependent upon binding to PICK1, a PDZ domain binding protein. The PDZ domain binding site for the mGlu7a receptor is within the extreme carboxyl terminus of the receptor, and this sequence appears to confer PICK1 binding and receptor targeting, as mGlu2, another presynaptic mGlu receptor, did not bind to PICK1 and an mGlu7a receptor mutant lacking crit-

ical amino acids led to lack of presynaptic receptor clustering in hippocampal neurons. A presynaptic localization of mGlu7a receptors has also been demonstrated on glutamatergic terminals of the corticostriatal pathway (Kosinski et al., 1999), and the mGlu7a receptor appears to be expressed postsynaptically on neurons within the striatum as well. These data suggest a prominent role for mGlu7 in the extra-pyramidal control of movement and possibly in the etiology of movement disorders.

Neuronal cell bodies of dorsal root ganglion neurons and their axon terminals within the dorsal horn of the spinal cord also express mGlu7 receptors (Ohishi et al., 1995). Here, loss of mGlu7 immunoreactivity following rhizotomy indicates a regulatory role of mGlu7 in control of excitatory sensory information at the level of the spinal cord. However, that said, mGlu7 receptor knockout animals did not exhibit any abnormalities in pain sensitivity (Masugi et al., 1999), and the possible role of mGlu7 receptors in sensory transmission of noxious and non-noxious stimuli remains to be determined.

The targeted disruption of mGlu7 receptor expression in mice does lend some additional support to the notion of an autoreceptor role for mGlu7 protein. mGlu7 receptor-deficient mice were reported to develop epileptic seizures at ~12 weeks of age (Masugi et al., 1999), possibly due to their inability to regulate synaptic levels of glutamate into adulthood. Interestingly, in young knockout mice (prior to developing seizures), there was noted a prominent loss of presynaptic mGlu7 receptors within the amygdala complex. Indeed, mGlu7 receptor-deficient animals exhibited deficits in fear responses (freezing behavior following foot shock) and conditioned taste aversion (avoidance of taste stimuli that was associated with a toxic effect) when compared with responses in wild-type animals. These data suggest a role of mGlu7 receptors in the animal's expression of amygdala-dependent aversion learning and the expression of amygdala-dependent fear responses.

Modulation of Inhibitory Neurotransmission by Group II/III mGlu Receptors

Extra-synaptic localization of both group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu7, mGlu8) mGlu receptors to nonglutamatergic neurons has been described and suggests a possible presynaptic heteroreceptor role for these receptors. Indeed, electrophysiological and biochemical studies have shown that mGlu2/3 receptor agonists and the group-III-selective agonist L-AP4 will suppress the release of GABA from neurons (see Anwyl, 1999; Cartmell and Schoepp, 2000). mGlu2/3 receptor agonists and L-AP4 will reversibly reduce the amplitude of GABA-mediated inhibitory postsynaptic potentials in a number of tissues including cerebral cortex, hippocampus, thalamus, and spinal cord (Anwyl, 1999). Thus, empirically mGlu receptor-mediated presynaptic modulation of GABA release may be a mechanism for enhancing cell excitability. However, the overall effects of this modulation would be dependent on the circuits these inhibitory interneurons are involved in controlling. For example, in CA1 of the hippocampus, Semyanov and Kullman (2000) demonstrated that the group III mGlu receptor agonist L-AP4 depresses GABAergic inhibitory postsynaptic currents in interneurons to a greater extent than GABAergic

inhibitory postsynaptic currents in pyramidal neurons. The selective depression of GABAergic transmission to interneurons was enhanced by glutamate uptake blockade and was prevented by α -methylserine-*O*-phosphate, a group III receptor antagonist. These data indicate that glutamate spillover from excitatory terminals may selectively disinhibit these inhibitory interneurons (via a decrease in GABA release on to other interneurons), and thus in fact lead to an overall suppression of excitatory synaptic transmission.

The work of Mitchell and Silver (2000) indicates that the spillover of glutamate from mossy fiber terminals can activate presynaptic mGlu receptors on GABAergic nerve terminals, and this leads to inhibition of GABA release onto principal excitatory neurons. This effect was mimicked by the nonselective mGlu agonist (\pm)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid, thus the mGlu receptor subtype responsible for this effect is not known. In any case, this represents a heterosynaptic mechanism by which inhibitory interneurons sense excitatory activity of neighboring excitatory synapses. In this manner, the efficacy of the active excitatory fibers onto CA3 pyramidal cells can be enhanced by locally reducing GABAergic inhibition.

As another example, most data suggest that activation of group III mGlu receptors are neuroprotectant in animal models *in vivo*, presumably due to the presynaptic suppression of glutamate release and reduced excitotoxicity mediated postsynaptically via ionotropic receptors (see Nicoletti et al., 1996). However, using cultured mouse striatal GABAergic neurons (which express mGlu7 receptors), Lafon-Cazal et al. (1999) showed that activation of presumable mGlu7 receptors with L-AP4 inhibited GABA release and ultimately enhanced neurotoxicity induced by NMDA. Thus, this expression of mGlu receptors in heterologous synapses to suppress GABAergic transmission needs to be considered when targeting mGlu receptors to suppress brain excitations in pathological states. This factor may explain why systemic administration of mGlu2/3 receptor agonists such as LY354740 and LY379268 *per se* to rats is not associated with profound suppressions on normal brain excitability (as measured by glucose utilization) (Lam et al., 1999). Possibly, coincident heterosynaptic inhibition of GABA release by these agonists contributes to counter any direct decreases in excitatory synaptic transmission on principal cells. Thus, overall, the actions of these types of drugs may be dependent on the relative roles of mGlu receptors to modulate presynaptic suppressions of glutamate versus GABA release in that functional circuit.

For example, thalamic relay neurons make excitatory synaptic contacts with GABAergic cells of the thalamic reticular nucleus (nRT), and they have been shown to express mGlu3 and mGlu4a receptors (Neto et al., 2000a). Interestingly, mGlu4 receptor knockout mice were found to be resistant to absence seizures induced by systemic administration of the GABA_A receptor antagonists such as bicuculline (Snead et al., 2000). The injection of a mGlu4 antagonist into the nRT of normal animals mimicked the resistance to bicuculline-induced seizures seen in the mGlu4a knockout animal. Conversely, nRT injection of an mGlu4 agonist to wild-type mice exacerbated bicuculline-induced seizures. These studies suggest a role for mGlu4 receptor-mediated modulation of thalamocortical GABAergic functions and a possible role for mGlu4a in pathological states such as absence seizures. It is

also suggested that modulation of GABAergic neurotransmission by mGlu4 receptor antagonist drugs may be useful to treat absence seizures in humans.

Furthermore, the relative roles of GABAergic inhibition and glutamatergic excitation within the brain can be altered in pathological states, and this may play a role in determining the ultimate actions of mGlu-selective compounds. For example, group II mGlu receptor agonists have been shown to produce hyperpolarization of GABAergic cells of nRT (Cox and Sherman, 1999), presumably due to activation of mGlu3 receptors (as mGlu2 receptor mRNA is not expressed in these cells) (Neto et al., 2000a). Interestingly, the induction of monoarthritis in rats by unilateral injection of complete Freund's adjuvant into the animal's tibiotarsal joint has been shown to produce a time-dependent and regionally specific up-regulation of mGlu3 receptor mRNA in nRT (Neto et al., 2000b). The direct injection of the mGlu2/3 antagonist (2S)- α -ethylglutamic acid in the nRT attenuated arthritic behavioral scores in these animals (Neto and Castro-Lopes, 2000). These studies suggest a possible role for mGlu3 modulation of nRT GABAergic functions in the central processing of certain noxious sensory stimuli, and a possible therapeutic avenue of mGlu3-selective antagonist compounds to treat certain forms for chronic pain. This is an interesting new concept, as group II and III receptor agonists have anti-pain effects in certain models, presumably due to reductions in pathologically enhanced neuronal hyperexcitability (Neugebauer et al., 2000). Ultimately, the effects of systemic mGlu antagonism (e.g., mGlu3) need to be further explored to investigate the optimal *in vivo* receptor profile for producing an mGlu receptor active anti-pain drug.

Conclusions

The identification of multiple mGlu receptor subtypes via molecular techniques, along with rapid advances in knowledge of their regional, cellular, and subcellular localizations, is providing new insights into how neuronal cell excitability is modulated in pathological as well as physiological states in animals and humans. Pharmacological studies and experiments with transgenic animals are filling in details of the roles of specific mGlu receptor subtypes in specific synapses, circuits, and brain regions. Ultimately, another benefit from this work may also be the development of highly novel, safe, and effective pharmacological agents to treat a range of neurological and psychiatric disorders in humans.

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