Roles of group I metabotropic glutamate receptors under physiological conditions and in neurodegeneration

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Since their cloning in 1991, metabotropic glutamate receptors (mGluRs) have been the subject of numerous studies, as it rapidly became apparent that this class of G-protein coupled receptors (GPCRs) participated in numerous functions in the central nervous system. Group I mGluR receptors function chiefly postsynaptically, as modulators of the ionotropic AMPA and NMDA glutamate receptors, and triggers of intracellular signaling pathways that could lead to long-term modifications of synaptic efficacy and to neurodegeneration. It is now clear that the activation of group I mGluR receptors has only minimum effects on synaptic transmission and regulation at low level of presynaptic activity; in contrast, they become engaged and exert potent effects on intracellular cascades at high frequency of stimulation. Moreover, when postsynaptic calcium reaches levels sufficient to activate the calcium-dependent protease calpain, calpain truncates the C-terminal domain of mGluR1α and changes its signaling properties to make it exclusively neurodegenerative. A new method using the transmembrane transport properties of the tat-peptide prevents neuronal degeneration following excessive activation of the NMDA receptors, which could occur in ischemia and various forms of excitotoxicity. Caution should be observed, however, regarding the numerous phenomena observed following the prolonged activation of group I mGluRs by exogenous agonists. © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

INTRODUCTION

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS) of mammals, and mediates its actions by interacting with two classes of receptors. In addition to ionotropic receptors responsible for fast excitatory neurotransmission in the CNS, glutamate also activates a number of metabotropic receptors, which belong to the G-protein coupled receptor (GPCR) family of receptors. GPCRs are composed of a single polypeptidic chain with seven transmembrane domains,1 and interact with a variety of trimeric G-proteins made up of an α subunit and a βγ complex. The four subclasses of Ga subunits determine the features of the transduction cascade activated by a particular GPCR, although the Gβγ complex may also interact with and activate several effectors. Thus, Gas and Gai are linked to stimulation (s) or inhibition (i) of adenylate cyclases; Gαq is linked to the activation of phospholipase C (PLC)-β, which cleaves phosphoinositide, PI(4,5)P2, into

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in the hippocampus, where mGluR1 is predominantly localized at the periphery and often in the GABAergic interneurons, whereas mGluR1α receptors are expressed almost exclusively in GABAergic interneurons, as revealed by immunohistochemical and in situ hybridization studies. However, electrophysiological studies have suggested their presence in CA1 pyramidal cells. mGluR5 receptors are diffusively expressed in hippocampus and are present in dendrites of both pyramidal cells and certain interneurons. Although this topic is controversial, it appears that mGluR1 is also localized in astrocytes, and their glial expression is increased under certain pathological conditions.

Within the postsynaptic density, group I mGluRs are mostly localized postsynaptically at glutamatergic synapses, although their exact cellular distribution varies depending on the isoforms. This distribution has been studied in great detail in the hippocampus, where mGluR1α receptors are expressed almost exclusively in GABAergic interneurons, as revealed by immunohistochemical and in situ hybridization studies. However, electrophysiological studies have suggested their presence in CA1 pyramidal cells. mGluR5 receptors are diffusively expressed in hippocampus and are present in dendrites of both pyramidal cells and certain interneurons. Although this topic is controversial, it appears that mGluR1 is also localized in astrocytes, and their glial expression is increased under certain pathological conditions.

Within the postsynaptic density, group I mGluRs are generally localized at the periphery and often in the perisynaptic region. As we discuss later, this perisynaptic localization has profound implications for the function of these receptors, as the concentration of glutamate they are exposed to is critically dependent on the distance from the release sites and the activity of glutamate transporters. It has also been repeatedly shown that group I mGluRs are linked to Shank and to NMDA receptors through the binding of their C-terminal domain to Homer. The association of NMDA receptors and group I mGluRs can be modified by pathological conditions and represents an important component of the regulation of glutamatergic transmission by intracellular signaling pathways.

Group I mGluRs are coupled with numerous signaling pathways. One signaling pathway consists in the activation of PLC-β through the Gαq/11 family of G-proteins. Group I mGluRs can also activate some membrane channels, such as expand to transient receptor potential channel1 (TRPC1) and G protein-coupled inwardly-rectifying potassium channels (GIRK). Furthermore, group I mGluR1s activate phosphoinositide 3-kinase, MAP kinases, and mTOR and increase cAMP levels. In addition to the multiple signaling pathways coupled to group I mGluRs, the receptors themselves are also subjected to complex regulations: for instance, their activity can be directly regulated by the level of intracellular calcium, and they can also desensitize or remain constitutively activated.

A number of pharmacologic agents acting at various sites of group I mGluRs have been identified. It is now customary to designate ligands that bind to the same site as the endogenous ligand as orthosteric agents, while those binding at a different site are referred to as allosteric ligands. DHPG is generally considered to be an agonist for group I mGluRs, whereas LY367385 and AIDA are competitive antagonists of mGluR1. On the other hand, Ro-677476 is an allosteric agonist and CPCCOET an allosteric antagonist of mGluR1. The widely used compound MPEP is viewed as an allosteric antagonist of mGluR5.

We recently developed a computational model of a glutamatergic synapse incorporating a presynaptic element, a synaptic cleft, and a postsynaptic component with ionotropic and metabotropic glutamate receptors. This model permits the analysis of the respective contributions of different glutamate receptors to changes in intracellular calcium and IP3 concentrations in dendritic spines as a function of time following various patterns of synaptic stimulation. This model also provides quantitative information regarding the effect of group I mGluR localization and glutamate transporters on the postsynaptic dynamics of calcium and IP3 concentration profiles. In agreement with a previous report, our results clearly demonstrated that the location of group I mGluRs has a large impact on its activation, as the presence of glutamate transporters significantly reduces the availability of glutamate at locations distant from the release site (Figure 1). Our results also underscored the frequency dependency of group I mGluR...
activation and clearly showed that group I mGluR-mediated changes in IP3 and the resulting increase in intracellular calcium concentration are minimal at low frequency of synaptic activation and become significant only at high frequency of stimulation, and in particular with tetanic stimulation. Interestingly, both the role of the localization of group I mGluRs and its frequency dependency are accounted for by the presence of glutamate transporters, as they significantly reduce extracellular glutamate concentration at low frequency of presynaptic release, and even more so at high frequency of release by preventing
glutamate accumulation. These results are consistent with experimental evidence indicating that pharmacological inhibition of glutamate transporters greatly enhanced group I mGluR-mediated responses.20

GROUP I mGLURs AND SYNAPTIC PLASTICITY

The potential contribution of group I mGluRs in long-term potentiation (LTP) induction in various brain regions has been the subject of numerous reports, although the exact role of these receptors in various forms of LTP remains unclear.21 A number of studies suggest that group I mGluR activation facilitates the induction of NMDA receptor-dependent LTP. Activation of group I mGluRs by prolonged exogenous application of agonists results in significant changes in postsynaptic calcium concentration, which could lead to changes in synaptic efficacy, as has been repeatedly reported.22,23 In addition, it is important to stress that a recent report indicates that, in hippocampus, the endoplasmic reticulum is present mostly in large dendritic spines, suggesting that group I mGluR activation is able to modify synaptic function through increasing postsynaptic calcium levels only in a subset of hippocampal synapses.24 Finally, recent results implicate group I mGluRs in LTP in hippocampal interneurons,25 although the mechanisms underlying LTP at these synapses have been much less studied than at the synapses on principal neurons.

Similarly, the role of group I mGluR activation in long-term depression (LTD) induction, while extensively discussed, remains incompletely elucidated. Several mechanisms have been proposed to account for group I mGluR-dependent LTD. One such mechanism is clearly calcium-independent and appears to depend on interactions between the C-terminal domain of group I mGluRs and intracellular proteins such as Homer and downstream cascades.26–28 In particular, it appears that group I mGluR activation results in AMPA receptor endocytosis by a mechanism distinct from NMDA receptor-mediated endocytosis,29 possibly via the X-linked mental retardation protein OPHN1.30 However, in cerebellum, it appears that group I mGluR-dependent LTD requires calcium release from intracellular stores, resulting in internalization of AMPA receptors.31

The conditions under which group I mGluRs are activated remain highly debated. In hippocampal CA1 pyramidal neurons, group I mGluR-dependent LTD is typically induced with prolonged low-frequency synaptic stimulation (1–3 Hz, 5–15 min) of the Schaffer collateral axons (or with long application of group I mGluR agonists). In contrast, 20 Hz was reported to be the minimum frequency below which synaptic activation fails to evoke group I mGluR-mediated EPSCs in rat cerebellar slices of rat.32 Given the perisynaptic localization of group I mGluRs, this frequency dependency of group I mGluR activation has generally been considered to reflect the accumulation of glutamate because of the saturation of glutamate transporter activity.33–35 In addition, even when glutamate transporters are blocked, one presynaptic cell releases insufficient glutamate to evoke a group I mGluR-mediated current in a CA1 cell, and consequently group I mGluR-LTD cannot be induced by a single presynaptic release.36 These apparently contradictory results could be explained by assuming that the discharge of multiple Schaffer collaterals onto a single postsynaptic cell is necessary for group I mGluR-LTD. The group I mGluR model discussed above and described in greater details by Greget et al. could thus be extremely useful to analyze group I mGluR activation under physiological conditions (nonsteady-state conditions) or pathological conditions.18

GROUP I mGLURs AND NEURODEGENERATION

The roles of metabotropic glutamate receptors, especially of group I mGluRs in neurodegeneration, remain controversial. Activation of group I mGluRs appears to be neuroprotective under various conditions. Application of the group I mGluR agonist, DHPG, prevented nitric oxide, hydrogen peroxide, or platelet-activating factor-induced neurotoxicity in neuronal cultures.37,38 Activation of group I mGluRs also protected neurons from oxidative stress.39 In organotypic hippocampal slice cultures, mGluR1 activation protected against NMDA-induced excitotoxicity.40 Recent studies indicated that the neuroprotective effects of group I mGluRs were mediated by the activation of PI3K-Akt signaling through the formation of an mGluR-Homer-PIKE-L signaling complex.9 Activation of Akt and neuroprotection by group I mGluRs were also reported in other studies.41,42

In contrast, numerous experiments demonstrate neurotoxic effects of group I mGluR activation. In models of cerebral ischemia, activation of group I mGluRs, especially of mGluR1, is neurotoxic while antagonists of mGluR1 are neuroprotective. The neurotoxic effects of mGluR1 activation in ischemia might be due to their effects on cytosolic free Ca2+ and their stimulation of glutamate release.43 Similarly, antagonists of group I mGluRs were shown to reduce kainic-acid-induced hippocampal dysfunction.

We recently discovered a new mechanism that links all of the elements discussed above, which
suggests the existence of a positive feedback loop that could play a critical role in ischemia-induced neuronal death and, potentially, in other forms of neurodegenerative diseases. In brief, we found that NMDA receptor activation results in calpain-mediated truncation of the C-terminal domain of mGluR1α, one of the isoforms of mGluR1 (Figure 2). As a result of this truncation, mGluR1α loses its neuroprotective signaling through the Homer-PI3K-Akt pathway, but maintains a normal calcium signaling function through the PLCβ activation and IP3 formation.

In other words, truncated mGluR1α becomes an exclusively ‘neurodegenerative receptor’. These results account for the previously discussed discrepancy in the literature regarding the potential role of mGluR1 in ischemia-induced damage. They also indicate that the development of classic agonists or antagonists of mGluR1 into therapeutic agents for the treatment of stroke or other neurodegenerative diseases could be extremely challenging.

We identified the site of calpain-mediated truncation and generated a small peptide consisting of...
the sequence of amino acids surrounding the cutting site and linked it to the human immunodeficiency virus (HIV) tat-peptide, which has recently been demonstrated to represent a good strategy to carry peptides and other cargoes across cell membranes.\textsuperscript{45} Remarkably, this tat-mGluR1 peptide prevented mGluR1\(\alpha\) truncation and was neuroprotective against glutamate toxicity in neuronal cultures. Systemic injection of this peptide in mice before kainic acid injection also prevented mGluR1\(\alpha\) truncation and neurotoxicity. These results clearly suggest that the prevention of mGluR1 truncation represents a major step in reducing excitotoxic damage. Our findings regarding NMDA-induced mGluR1\(\alpha\) truncation provide a possible explanation for the previously discussed contradictory experimental data: before NMDA application or onset of ischemia, mGluR1\(\alpha\) receptors are coupled to the PI3K-Akt signaling and their activation is neuroprotective. Although mGluR1\(\alpha\) activation leads to calcium release from internal stores, the extent of calcium release might be too low and transient to produce significant toxic effects. Following NMDA application or onset of ischemia, NMDA receptor activation would induce calpain-mediated truncation of mGluR1\(\alpha\). As a result, the neuroprotective effect of the mGluR1\(\alpha\)–Homer-PI3K-Akt signaling cascade would be disrupted. In addition, mGluR1\(\alpha\)-dependent calcium release from intracellular stores would further contribute to calcium overload because of calcium influx through NMDA receptors and thus enhance neurotoxicity. Thus, NMDA receptor activation followed by calpain-mediated truncation of mGluR1\(\alpha\) constitutes a positive feedback loop for excitotoxicity.

These results also indicate that novel approaches that target the functions of mGluR1\(\alpha\), such as the one using a tat-mGluR1 peptide, might be extremely promising to reduce neuronal damage resulting from the overstimulation of glutamate receptors. The target is downstream of NMDA receptors and, therefore, eliminates all the problems associated with widespread inhibition of NMDA receptors. Such an approach also avoids the nonspecific side effects of calpain inhibitors, as it potentially allows the selective blockade of calpain-mediated truncation of mGluR1 without inhibiting overall calpain activity. As the peptide itself should be devoid of physiological activity, it might even be possible to envisage a chronic treatment for slowly developing neurodegenerative diseases. Although there are still some questions regarding the mechanisms of entry of the cargoes attached to the tat-peptide, this peptide has proven to be a remarkable tool to facilitate cell entry of a variety of molecules, from peptides to protein to oligonucleotides (Box 1).\textsuperscript{45}

### Box 1

**HOW DOES THE tat-mGluR1 WORK?**

While the tat-mGluR1 peptide prevented calpain-mediated truncation of mGluR1\(\alpha\), the exact mechanism underlying this effect is not completely understood. It is possible that the peptide provides a competitive interaction with calpain for mGluR1; however, it does not prevent calpain-mediated truncation of another calpain substrate, \(\alpha\)-spectrin.\textsuperscript{1} Previous studies with other tat-coupled peptides have indicated that peripheral injection of these peptides is associated with brain penetration, and it is therefore likely that the tat-mGluR1 peptide we used did gain access to the brain.\textsuperscript{2,3,44,47,48}

Additional results further expended the neuroprotective effects of tat-mGluR1 peptide to hypoxia/ischemia-induced neuronal damage. In particular, tat-mGluR1 completely blocked both 30 and 45 min OGD-induced cell death in cultured hippocampal slices.\textsuperscript{46}

Compared to its complete neuroprotection in the *in vitro* OGD model, the tat-mGluR1 peptide exhibited a significant but incomplete protective effect in the *in vivo* hypoxia/ischemia model in neonate rats, as measured with Nissl staining. The effect was more complete when considering the hippocampus only, a result that might be related to a higher level of expression of the receptors in this brain structure as compared to the whole brain. Interestingly, tat-mGluR1 peptide still completely prevented H/I-induced mGluR1\(\alpha\) truncation, suggesting that other factors are also involved in H/I-induced neuronal death.\textsuperscript{46}

Moreover, several approaches are using a similar strategy (coupling of tat-peptide with various peptides or oligonucleotides) to provide protection against ischemia (peptide inhibitor of the Jun-C-terminal kinase (JNK)),\textsuperscript{49} increased neurotrophin signaling (peptide inhibitor of the protein tyrosine phosphatase (PTP)),\textsuperscript{50} and protection against radiation-induced apoptosis (BH4 peptide domain of the anti-apoptotic protein Bcl-xL).\textsuperscript{51} We believe that the current tat-mGluR1 peptide provides a useful tool to obtain the proof of concept that the mechanism we identified might be involved in a variety of disorders in which excitotoxicity has been implicated. Future studies will be directed at testing the effects of this peptide in animal models of stroke and at identifying modifications of the peptide sequence that could improve its potency and selectivity. While several groups are attempting to use cell penetrating peptides to treat a variety of...
diseases, several obstacles still need to be overcome to bring this approach to the clinic, and in particular the issue of immunogenicity of the peptides.

Finally, it is worth pointing out that the model of the glutamatergic synapse we have developed and that integrates all the different types of glutamate receptors will be extremely valuable in determining better ways to provide neuroprotection. In particular, it is clear that under conditions of excitotoxicity, which are associated with impaired glutamate uptake and massive release of glutamate, it is expected that group I mGluR will be activated for long periods of time, and that together with NMDA receptor activation, this will lead to massive increase in postsynaptic calcium concentration. Our model will be able to test any number of hypotheses that could be advanced to offer neuroprotection by targeting various elements of the complex signaling cascades present at glutamatergic synapses.

GROUP II AND III mGLURs AND NEUROPROTECTION

While this is not the main topic of this study, it is worth to mention the roles of groups II and III mGluRs in neuroprotection, as there is an abundant literature indicating that the activation of these two groups of mGluRs provides neuroprotection in a variety of models of neurodegeneration. While it was initially proposed that neuroprotection from groups II and III mGluRs was due to the presynaptic inhibition of glutamate release, more recent results point to a much more complex effect of these receptors on a number of neuroprotective pathways. In particular, the presence of groups II and III mGluRs on astrocyte membranes has provided a much broader spectrum of potential mechanisms by which mGluRs can exert neuroprotective effects through the release of neuroprotective cytokines from astrocytes. Another potential mechanism by which mGluR3 is neuroprotective would be through increased glutathione concentration and increased antioxidant defense mechanisms.

GROUP I mGLURs AND NEUROPSYCHIATRIC DISORDERS

In addition to the potential role of mGluRI receptors in ischemia and neurodegeneration, these receptors have been postulated to participate in many other types of CNS disorders. First, a number of evidence support a role for mGluRI in some of the symptoms of schizophrenia. It was initially reported that mGluRI antagonists can improve prepulse inhibition, which is often used as an animal model of sensory gating, a brain process that is altered in human schizophrenic patients. This suggested that mGluR1 antagonists could be useful compounds for treating schizophrenia. However, more recently, it was proposed that positive allosteric modulators of mGluR1 could be used to treat the positive and cognitive symptoms of schizophrenia. This hypothesis is consistent with the previously discussed role of mGluR1 in regulating LTP induction. An extensive literature also suggests that mGluRI receptors are involved in anxiety and depression disorders. In this case, it has been proposed that mGluRI antagonists could decrease the overactivation of NMDA receptors that has been hypothesized to underlie anxiety disorders. Similarly, antagonists of mGluRI were shown to have beneficial effects in various animal models of depression. Notably, the mGluR5 antagonist, MPEP, has been shown to correct several of the phenotypes present in the mouse model of the fragile X syndrome, including cognitive impairment, seizure activity, and morphological alterations. Finally, antagonists as well as negative allosteric modulators of mGluR5 have been shown to reduce the dyskinesia induced by chronic treatment with L-DOPA in various animal models of Parkinson’s disease.

CONCLUSION

It is clear that much has been learned regarding the properties of mGluRI since their initial cloning in the laboratory of Professor Nakanishi in 1991. Not only do we understand much better the signaling mechanisms used by these receptors to mediate their multiple functions in various parts of the brain, but also a large number of compounds acting as agonists, antagonists, and positive and negative allosteric modulators have been produced and tested in a variety of animal models. While the initial hope of using some of these molecules for the treatment of various human disorders has not yet been fulfilled, there are nevertheless numerous efforts to bring them to the final stages of clinical trials. Part of the difficulties to fulfill this goal is due to the fact that the conditions under which these receptors are physiologically activated as well as the modifications of their functioning in various human disorders are not yet well understood. In particular, as we discussed above, a critical property of these receptors is that they are likely to be rarely activated, or rather that their activation is only going to be meaningful under certain types of neuronal activity, i.e., high frequency of stimulation. This critical feature of the receptors raises some concerns regarding the extensive literature reporting effects of
prolonged activation of the receptors by exogenous agonists, as this clearly represents non-physiological conditions. There is therefore a clear need to better understand the mechanisms underlying the effects of the various modulators of these receptors under both physiological and pathological conditions.

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**FURTHER READING**
