

The role of metabotropic glutamate receptors in Alzheimer's disease

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Review

Abstract. While glutamatergic transmission is severely altered by early degeneration of cortico-cortical connections and hippocampal projections in Alzheimer's disease (AD), the role of glutamate receptors in the pathogenesis of AD is not yet defined clearly. Nonetheless, as reviewed here, the topographical distribution of different types of receptors likely contributes to the regional selective nature of neuronal degeneration. In particular, metabotropic glutamate receptors (mGluR) may contribute the pathogenesis of many neurological conditions and also regulate neuronal vulnerability against cytotoxic stress. Thus, we here discuss the possible role of mGluR in the pathogenesis of AD based on the results from other neurodegenerative diseases that may give us clues to solve the mysterious selective neurodegeneration evident in AD.

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GLUTAMATE RECEPTORS: ANTAGONISTIC PLEIOTROPY?

Glutamate receptors mediate most of the excitatory neurotransmission in the mammalian central nervous system and also participate in plastic changes in the efficacy of synaptic transmission underlying memory and learning, and the formation of neural networks during development (Mayer and Westbrook 1987, Monaghan et al. 1989). However, rather ironically, glutamate can also be excitotoxic to neurons in circumstances which there is excessive activation of glutamate receptors. In fact, excitotoxicity is thought of as a contributor of neuronal cell death during stress to the brain and in acute neurological disorders such as trauma and ischemia (Choi and Rothman 1990, Meldrum and Garthwaite 1990). Glutamate receptors have also been widely implicated in the mechanism of neuronal cell death in other chronic neurodegenerative diseases such as Parkinson's disease and Huntington's disease (Calabresi et al. 1999, Lynch and Guttman 2002, Pogocki 2003). Therefore, the regulation of glutamate receptor expression and their specific localization may affect intracellular signaling in each neuron and eventually determine not only the fate of those cells but the function of the central nervous system in specific pathological and physiological conditions.

Selective degeneration of populations of vulnerable neurons in the hippocampus and other cortical brain regions is one of the prominent features of Alzheimer's disease (AD). While the actual mechanism(s) responsible for the highly selective nature of such neuronal changes is incompletely understood (reviewed in Smith 1998), such selective neuronal vulnerability may arise through the differential expression of receptors, activation of which would lead to signal transduction alterations (McShea et al. 1999, Perry et al. 1999, Zhu et al. 2001c). While it is still not clear which receptor or receptor classes are involved in these signal transduction alterations in AD, the glutamate receptor family is likely to play a pivotal role based on the role proposed for glutamate in neuronal cell death. Supporting this, glutamatergic transmission is severely altered by the early degeneration of cortico-cortical connections and hippocampal projections in AD (Francis et al. 1993). While it is known that excitotoxicity may be a key player in many neurological diseases, the exact role played by excitotoxicity in AD has been hotly debated. There is limited evidence that directly supports

excitotoxicity in AD, but it is notable that the activation of glutamate receptors can increase the expression of tau protein and its phosphorylation (Couratier et al. 1996, Esclaire et al. 1997). Given that the increase of tau protein expression and phosphorylation is one of the hallmark pathologies in AD, a signal transduction pathway which is controlled *via* activation of glutamate receptors may play a role in the pathogenesis of AD. Thus, below we discuss the expression pattern of each glutamate receptor and its functional significance in the pathogenesis of AD.

IONOTROPIC GLUTAMATE RECEPTORS IN ALZHEIMER'S DISEASE

Glutamate receptors consist of two major classes which are ionotropic (iGluR) and metabotropic glutamate receptor (mGluR). The iGluRs are cation-specific ion channels and, are subdivided into three groups by their specific agonists, namely N-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid (KA). mGluRs are a family of G-protein-coupled and can be divided to three groups, I, II and III, according to their signal transduction pathways, pharmacology and sequence homology.

While the expression level and localization of iGluR have been extensively studied in AD, the regulation of iGluR expression is still controversial (Lee et al. 2002a). Indeed, while some authors report that the NMDA glutamate receptor subunit NR1 is markedly increased in vulnerable neurons of AD (Ikonomovic et al. 1999), other reports indicate that there is a reduction of NMDA receptors in AD (Hynd et al. 2001, Sze et al. 2001, Ulas and Cotman 1997), or no difference between AD and age-matched controls (Bi and Sze 2002, Panegyres et al. 2002, Thorns et al. 1997, Wakabayashi et al. 1999). Despite these disparity, it is important to note that the distribution of NMDA receptors does, however, correlate with the predilection for neurofibrillary tangles and neuritic plaques in hippocampal subfields (Geddes and Cotman 1986). Although it is not clear whether NMDA expression is decreased in AD, it should be noted that elective decrease of NMDA receptors may effect the memory dysfunction in AD. For example, a recent study clearly showed NMDA receptors play a pivotal role in memory formation (Clayton et al. 2002, Nakazawa et al. 2002) and, therefore, it is plausible that alterations of NMDA receptors may be responsible for the decreased

memory function that is clinically evident in patient with AD. Indeed, memory impairments are evident when NMDA antagonists are injected into different brain structures in animal experiments (Castellano et al. 2001) and glutamate levels in cerebrospinal fluid (CSF) and tissue are decreased in AD (Hyman et al. 1987, Kuiper et al. 2000). Thus, it is likely that NMDA receptors may contribute significantly to the pathophysiology in AD *via* degeneration of synaptic activity rather than cell death *via* excitotoxicity.

Although the role of AMPA or kainate receptors in the pathogenesis of AD has not been fully elucidated, specific populations of neurons that degenerate preferentially in AD have been found to exhibit an unusually high susceptibility to AMPA- or kainate receptor-mediated injury (Page et al. 1991, Weiss et al. 1994) suggesting that AMPA or kainate receptors might contribute to the pathogenesis of AD. While there are some conflicting results, AMPA receptors appear to be reduced in the vulnerable regions and their reduction is correlated with neurofibrillary tangle formation (Armstrong et al. 1994, Ikonomic et al. 1995). AMPA binding is also markedly reduced in the subiculum and hippocampal CA1 area and the magnitude of the change correlates with neuronal loss within the subiculum. Notably, the changes in ligand binding are minimal in brain regions that are resistant to pathological lesions such as dentate gyrus and the CA3 area in AD (Dewar et al. 1991). Immunohistochemical and immunoblot studies also show a consistent decrease of every subunit in vulnerable regions of AD brain (Armstrong et al. 1994, Aronica et al. 1998, Ikonomic et al. 1995, 1997, Thorns et al. 1997, Wakabayashi et al. 1999, Yasuda et al. 1995). Indeed, data shows there were large decreases in GluR1 and GluR2/3 subunits in brain regions which are affected and associated with neurofibrillary changes in AD. In addition, the notion that a loss of GluR2/3 immunolabeling precedes the development of neurofibrillary changes in AD suggests that a decrease or loss of these receptors may be important in the development of neurofibrillary changes in AD (Ikonomic et al. 1997).

The expression and localization of kainate receptors in AD has not been studied in detail. Kainate binding is significantly reduced in the parahippocampal gyrus, while in a number of other hippocampal areas (e.g., dentate gyrus, CA3), the binding of ligand is minimally altered in AD (Dewar et al. 1991). In contrast to hippocampal regions, kainate receptor binding is signif-

icantly increased by approximately 70% in deep layers of AD frontal cortex compared with controls and there is a positive correlation between kainate binding and senile plaque number in deep cortical layers (Chalmers et al. 1990). However, in caudate nucleus, kainate binding is unaltered (Pearce and Bowen 1984). Immunohistochemistry result also show a decrease of GluR5/6/7 subunits in AD-vulnerable regions such as the CA1 hippocampal area (Aronica et al. 1998).

METABOTROPIC GLUTAMATE RECEPTORS

mGluRs have been cloned and classified into three groups and eight subtypes according to their second messenger association, sequence homology and agonist selectivity (Pin and Duvoisin 1995, Pin et al. 1999). Group I mGluRs (mGluR1 and mGluR5) are known to functionally connect with polyphosphoinositide (PI) hydrolysis and are negatively coupled with K^+ channels (Chuang et al. 2000, Schoepp et al. 1999). Group II and Group III mGluRs are negatively coupled to adenylyl cyclase and thought to act as presynaptic autoreceptors, regulating glutamate transmission (Shigemoto et al. 1997). Group II mGluRs, comprising mGluR2 and mGluR3, reduce cyclic adenosine monophosphate (cAMP) formation under activation by specific agonist, but also can activate mitogen-activated protein (MAP) kinase and PI-3-kinase pathways (Ferraguti et al. 1999, Phillips et al. 1998). In recent years, potent, selective and systemically active agonists ((1S,2S,5R,6S-2-amino-bicyclo[3.1.0]hexane-4,6-dicarboxylate or LY354740, (1R,4R,5S,6R-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-icarboxylate or LY379268) and antagonists ((2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid or LY341495) for Group II mGluRs have been discovered. Group II agonists have been reported to be neuroprotective *in vitro* (Kingston et al. 1999) and systemic administration of these molecules provides neuroprotection *in vivo* (Bond et al. 2000) and block phencyclidine-induced behavioral effects (Cartmell et al. 2000). Group III mGluRs, including mGluR4, mGluR6, mGluR7, and mGluR8, have similar signal transduction pathways to group II mGluRs (Bruno et al. 2001, Iacovelli et al. 2002).

There are many reports of neuroprotection with mGluRs, especially group II mGluR agonists and group I mGluR antagonists. For example, agonists for Group II mGluRs have been reported to protect against

apoptotic and excitotoxic stimuli *in vitro* (Allen et al. 1999, Copani et al. 1995, Kingston et al. 1999, Matarredona et al. 2001). These *in vitro* studies have been supported by reports that Group II mGluRs agonists are also neuroprotective *in vivo* (Bond et al. 2000, Chiamulera et al. 1992, Miyamoto et al. 1997). It is possible that direct activation of pre-synaptic mGluR2 receptors protects neurons against excitotoxic degeneration by the inhibition of glutamate release (Buisson and Choi 1995, Buisson et al. 1996). In support of this idea, the selective group II mGluR agonist, LY354740 reduces veratradine-evoked striatal amino acid release (Battaglia et al. 1997) and field excitatory postsynaptic potentials (fEPSP) in rat hippocampal slices (Kilbride et al. 1998).

Although the nature of group III receptors is less well understood than the other types of mGluRs, agonists of group III receptors are expected to play similar neuroprotective role in neurons because of the overlapping signal transduction pathways between group III and group II mGluRs. In agreement with this, recent studies have demonstrated that agonists of group III mGluRs protect cultured neurons against excitotoxicity (Bruno et al. 1995a, 2000, Gasparini et al. 1999) and mechanical injury (Faden et al. 1997). The regulation of NMDA receptors and the PI-3-kinase pathway might be the mechanisms of neuroprotection by agonists of group III mGluRs (Bruno et al. 2001).

THE ROLE OF METABOTROPIC GLUTAMATE RECEPTORS IN PATHOLOGICAL CONDITIONS

While there is limited information on the role of mGluRs in the pathogenesis of AD, it is likely that mGluRs do play a significant role. In this regard, a recent study showed that the activation of mGluRs by specific agonists can modulate the MAP kinase pathway (Ferraguti et al. 1999, Iacovelli et al. 2002, Otani et al. 1999) and it is from a plethora of studies that MAP kinase pathways are centrally involved in the pathogenesis of AD (Zhu et al. 2000, 2001a,b,c). Moreover, it has been recently suggested that the differential expression of mGluRs on specific neuronal populations might be responsible for selective neuronal degeneration and dysfunction in many different types of neurological diseases such as amyotrophic lateral sclerosis (ALS) (Laslo et al. 2001, Tomiyama et al. 2001) and Down's syndrome (DS) patients (Oka and Takashima 1999).

Down's syndrome

In comparison with controls, DS brains clearly show higher expression of mGluR5 in mostly large pyramidal neurons in cerebral cortex (Oka and Takashima 1999). Over the age of forty gestational weeks, neurons immunoreactive to mGluR5 were observed in both control and DS brains. However, in control cerebral cortex, reactive neurons were sparse, and definitely immunoreactive neurons were only occasionally observed. Although the mechanism of the up-regulation is unknown, the overexpression of mGluR5 may be related to the pathological state of amyloid precursor protein (APP) metabolism in DS. Because, similar to AD, amyloid- β plaque is found in DS brain and DS is caused by trisomy 21 and APP is also located in chromosome 21, it has been suggested that the pathogenesis of DS may be related with overexpression of APP and its product amyloid- β . Interestingly, group I mGluRs are known to regulate the metabolism of APP and accelerates its processing into non-amyloidogenic APP (Croucher et al. 2003, Lee et al. 1996). While amyloid- β production in glutamatergic neurons in the cortex and hippocampus may be enhanced by deficits in glutamatergic neurotransmission, it is also known that soluble APP has neuroprotective and neurotropic functions (Furukawa et al. 1996, Smith-Swintosky et al. 1994). The activation of group I mGluRs enhances the release of soluble APP in neuron and astrocytes. In addition, secretory processing of APP by group I mGluR is mimicked by phorbol esters and blocked by PKC inhibitors, suggesting that the activation of PKC by group I mGluR may mediate soluble APP. The relationship between APP processing and mGluR is also demonstrated by *in vivo* experiments. For example, the activation of mGluR by ACPD (trans-1-aminocyclopentane-1, 3-dicarboxylic acid), a potent agonist for both group I and II mGluRs, increases the release of soluble APP in rat retina (Croucher et al. 2003). Based on this result, together with the observation of a lack of deleterious effects of mGluR activation on retinal neurons, it is proposed that mGluR plays a physiological role in mediating the release of soluble APP, an action which may have important functional and therapeutic implications for AD. However, the injection of ACPD in hippocampus of guinea pig caused neurodegeneration of the CA1 hippocampal region (Stephenson and Clemens 1998). In the region of neurodegeneration, amyloid- β is localized in the cytosol as a form of punctuate intraneuronal

granules and this amyloid beta level is correlated with the onset of neurodegeneration.

The activation of group I mGluRs produces excitatory effects in neurons and it has been suggested that these receptors facilitate the induction of excitotoxic neuronal death (Pin and Duvoisin 1995). Antagonists for group I mGluRs show protective effects *in vivo* and *in vitro* (Battaglia et al. 2001, Bruno et al. 1999). The modulation of NMDA receptors and enhancing of GABA release is suggested as a mechanism of neuroprotection (Bruno et al. 2001, Pizzi et al. 1999). In contrary to the results with antagonists, the results with agonists for group I mGluRs have yielded conflicting data. In some cases protective effects have been reported, while other investigators report cytotoxic effects (Bao et al. 2001, Bruno et al. 1995b, Pizzi et al. 1996, 1999). However, in many earlier studies the selectivity of the ligands was not ideal (some of the compounds activated NMDA receptors) and in general more consistent neuroprotective results have been obtained with selective mGluR1 ((S)-(+)- α -amino-4-carboxy-2-methylbenzeneacetic acid or LY367385) and mGluR5 (2-Methyl-6-([3,5-³H]-phenylethynyl)pyridine or MPEP) antagonists (Bruno et al. 1999, O'Neill 2001).

While the expression pattern of group I mGluR is totally unknown in AD, we suspect, and have evidence to support, that the further study of group I mGluR in AD may be helpful to understand the mechanism of selective neuronal degeneration and APP processing in AD.

Multiple sclerosis (MS)

The expression pattern of group I mGluRs in MS differs significantly from those in control tissue (Geurts et al. 2003). For example, in MS, strong mGluR1 immunoreactivity is observed in axons of the subcortical white matter, particularly in the center of actively demyelinating lesions and in the borders of chronic active lesions. The axonal localization of mGluR1 is also found in normal appearing MS white matter, but axons in control white matter are generally negative suggesting an early role. Additionally, mGluR1 axonal labeling is associated with the presence of non-phosphorylated neurofilaments, a sensitive marker for axonal injury. Finally, a diffuse increase in the expression of mGluR5 and mGluR2/3 is detected in reactive astrocytes in MS lesions. Nonetheless, despite these consistent findings, the physiological significance of the presence of mGluRs in astrocytes is still unclear.

Amyotrophic lateral sclerosis (ALS)

In ALS cases, the intensity of immunoreactivity of group I mGluRs (mGluR1 and mGluR5) and group II mGluR appear to be increased in cells with typical astroglial morphology in both gray and white matter of spinal cord (Aronica et al. 2001). Regional differences in immunoreactivity are apparent in ALS compared to control and, in particular, mGluR expression is increased in reactive glial cells in both gray and white matter of ALS spinal cord. Although the pathophysiological relevance of upregulation of mGluRs in reactive astrocytes is unclear, it may represent a critical mechanism for modulation of glial function and changes in glial-neuronal communication in the course of neurodegeneration of ALS. Indeed, it has been proposed the glial mGluR may participate in the communication between neurons and glial cells or may protect neurons from excitotoxic injury. Under pathological conditions, such as brain trauma and cerebral ischemia, astrocytes can limit brain damage by various means including the production of trophic factors (Kettenmann 1996) and glutamate release. Glutamate *via* astrocyte receptors, in particular group I mGluRs, can elicit a rise in intracellular calcium, which is one of the most important signaling systems in astrocytes, inducing characteristic morphological and functional changes in these cells. Previous studies have shown that mGluR5 activity, protein, and mRNA levels are up-regulated by growth factors such as FGF and EGF (Miller et al. 1995). It should be noted that the level of these growth factors are markedly regulated in the brain by trauma and cerebral ischemia which are also characterized by the conversion of resting astrocytes into reactive astrocytes. These conditions are also associated with increased release of glutamate, which may evoke a rise in the production of some of the factors up-regulating mGluR5 expression. These factors may themselves be produced and secreted by astrocytes in response to glutamate (Pechan et al. 1993). In addition, agonists for mGluR modulate glutamate transport and eventually reduce glutamate release from astrocytes (Ye and Sontheimer 1999). Indeed, the selective group I mGluR agonist produced a significant down-regulation of glutamate transporter proteins such as GLAST and GLT-1 in astrocytes (Aronica et al. 2003).

The production and release of different growth factors is also regulated by glial group II mGluR (Bruno et al. 1998). It is proposed that transient activation of group II mGluR (presumably mGluR3) in astrocytes

leads to an increased formation and release of TGF β , which in turn protects neighboring neurons.

Alzheimer's disease

In regard to group II mGluR, we recently found mGluR2 is specifically increased in hippocampal neurons in AD (Lee et al. 2004). This aberrant expression is closely associated with hyperphosphorylated tau deposition and hence neurofibrillary changes. In contrast, the expression level of mGluR2 in the dentate gyrus granular neurons is unchanged and is specifically increased only in neurofibrillary tangle containing pyramidal neurons in CA3 regions in AD. Since dentate gyrus granular neurons and CA3 pyramidal neurons are more resistant to pathologic insults of AD (Braak and Braak 1991), the patterns of aberrant expression of mGluR2 correlate with the degree of severity of neurofibrillary pathology.

Activation of Group II mGluRs is reported to protect neurons against excitotoxic degeneration by the inhibition of glutamate release (Buisson and Choi 1995, Buisson et al. 1996). Further studies have reported that potent and selective Group II mGluR agonists protect against excitotoxicity *in vitro* (Kingston et al. 1999) and global ischaemia *in vivo* (Bond et al. 2000). In addition, amyloid-beta peptide (25-35)-induced apoptosis in cultured cortical neurons is also substantially attenuated by the group II and III specific agonists respectively (Copani et al. 1995). The protective effect of mGluR agonists is potentiated by mixed culture with glial cells and it suggests the activation of group II and III mGluRs in glial cells may play a key role in this protective mechanism (Bruno et al. 1998). Recently, Poli and colleagues reported that expression of group II mGluR provides a major defensive mechanism against brain damage in anoxia-tolerant species (Poli et al. 2003). Using different fish species, they found that expression of mGluR2/3 was substantially higher in the brain of anoxia-tolerant species than in the brain of species that are highly vulnerable to anoxic damage although expression of mGluR1 and mGluR5 was similar in the brain of all species examined. It suggests the possible mechanism which expression of specific type of mGluR may regulate vulnerability of each cell population and, in turn, the selective neurodegeneration shown in AD and other neurodegenerative diseases is possibly regulated by the expression of specific types of mGluR. Overall, the specific regulation of each mGluRs in neuron or glial cells may participate in selective neuronal dysfunction and

degeneration in AD and further study is required to elucidate the regulation of mGluR in AD and its consequence.

CONCLUSIONS

As hypothesized above, the selective degeneration observed in AD and other neurological diseases may be regulated by the topographical distribution of different types of receptor. Glutamate receptors have been suspected to be as the type of receptor as glutamatergic transmission is severely altered by early degeneration of cortico-cortical connections and hippocampal projections in AD. Thus, glutamate receptors represent a novel target for drug intervention and the differential regulation of glutamate receptors may provide important insights into the pathogenesis of AD. While the expression and possible role of iGluR in AD has been widely studied during the last decade, the study for mGluR has just started (Lee et al. 2002a). As shown in this review, the expression of mGluR is dynamically regulated in specific brain regions and cells under different types of pathological conditions. However, the nature of mGluR in AD is not well understood although many lines of evidence show the possible links between mGluR expression and AD such as regulation of APP and selective neuronal vulnerability against cytotoxic stress. Therefore, future investigations that address the expression pattern of each mGluR and its pathological significance will not only enhance the clinical therapeutic utility for AD, but also foster greater understanding of the pathogenesis of AD.

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