Metabotropic Glutamate Receptors as Targets for Multipotential Treatment of Neurological Disorders

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Summary: Glutamate is a major excitatory neurotransmitter in the CNS that is involved in numerous cellular functions, including cell death and survival. Metabotropic glutamate receptors (mGluR) are G-protein coupled receptors that have been classified into three groups on the basis of signal transduction pathways and pharmacological profiles. Group I, II, and III mGluRs are found on cell types within and peripheral to the CNS, including neurons, microglia, astrocytes, oligodendrocytes, T- and B-cell lymphocytes, osteoblasts, hepatocytes, and endothelial cells, among others. These receptors have a number of effects on cells that can influence outcome after trauma, including reducing neuronal and oligodendroglial cell death, inflammation, and endothelial permeability. Thus, mGluRs are a promising multipotential therapeutic approach. Because the pathology of CNS trauma and neurodegeneration is multifactorial (including, for example, oxidative stress, mitochondrial breakdown, and inflammation), therapies that serve to modulate multiple pathophysiological pathways may prove more effective than those directed at a single target. This review examines the multipotential therapeutic utility of mGluR modulation in acute and chronic injury and neurodegeneration. **Key Words:** Astrocytes, inflammation, metabotropic glutamate receptors, microglia, neuron, neuroprotection.

INTRODUCTION

Glutamate is a major excitatory neurotransmitter in the CNS that regulates cellular and synaptic activity, plasticity, cell death and survival, learning and memory, pain perception, and motor activity.[1] Glutamate receptors are present in two forms: ionotropic and metabotropic. Ionotropic receptors, such as the NMDA and AMPA receptors, are ligand-gated ionic channels, whereas metabotropic glutamate receptors (mGluR) are G-protein coupled receptors. The latter have seven transmembrane domains and have been classified into three groups on the basis of signal transduction pathways and pharmacological profiles (Table 1).

Group I mGluRs comprise mGluR1 and mGluR5. They are localized in the postsynaptic density area at excitatory synaptic sites and function through $G\alpha_q$-proteins (FIG. 1). Group I agonists cause activation of phospholipase C (PLC), leading to release of calcium and activation of protein kinase C (PKC).[2] Downstream signaling pathways include mitogen-activated protein (MAP) kinases, ERK1 and ERK2, which can be inhibited by mGluR5 and mGluR1 antagonists such as MPEP and CPCCOEt.[2,3] Although less well studied, mGluR1 and 5 have been identified on other cell types including microglia, astrocytes, and oligodendrocytes.

Group II and III mGluRs are primarily localized presynaptically and are negatively coupled to adenylate cyclase (FIG. 1). Activation of these receptors results in feedback inhibition of glutamate release, through the inhibition of voltage-gated calcium entry into the cell. Similar to mGluR1 and mGluR5, group II and III mGluRs have also been identified on astrocytes and microglia.

After CNS trauma, the extracellular concentration of excitatory amino acids, including glutamate, is increased.[4,5] The expression of mGluRs are also altered by CNS trauma; group II mGluRs are reduced after spinal cord injury and traumatic brain injury,[6–8] whereas mGluR1 is increased rostral and caudal to the injury site after spinal cord injury, and mGluR5 remains unchanged.[7]

Multipotential treatments have become more attractive therapeutic strategies. Several recent review articles have emphasized the potential of multifunctional drug approaches.[9–11] Because the pathology of CNS trauma and neurodegeneration is multifactorial, therapies that serve

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Table 1. *Characteristics of Metabotropic Glutamate Receptor Groups 1–III*

mGluR Group	Subtype	Location	Transduction Mechanism	Agonists	Antagonists
Group I	mGluR1 mGluR5	Preferentially postsynaptic	↑ PLC ↑ PKC ↑ PLA2 ↑ Calcium release ↑ Adenylate cyclase	tADA CHPG DHPG 3HPG *S*-Sulfo-L-cysteine	LY367385 MCPG AIDA 4CPG CPCCOEt MPEP SIB-1893
Group II	mGluR2 mGluR3	Preferentially presynaptic	↓ Adenylate cyclase	DCG-IV LY354740 L-CCG-I APDC	LY341495 MCCG EGLU
Group III	mGluR4 mGluR6 mGluR7 mGluR8	Preferentially presynaptic	↓ Adenylate cyclase ↓ cGMP-PDE	L-AP4 L-SOP BzAPDC Homo-AMPA	CPPG MAP4 MSOP

3HPG = 2-amino-2-(3-hydroxyphenyl)acetic acid; 4CPG = (*S*)-4-carboxyphenylglycine; AIDA = 1-aminoindan-1,5-dicarboxylic acid; AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APDC = aminopyrrolidine-2,4-decarboxylate; APDC = aminopyrrolidine dicarboxylate; cGMP = cyclic guanosine monophosphate; CHPG = 2-chloro-5-hydroxyphenylglycine; CPCCOEt = 7-(hydroxyimino)cyclopropa(*b*)chromen-1α -carboxylate ethyl ester; CPPG = cyclopropyl-4-phosphonophenylglycine; DCG-IV = (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG = (*S*)-3,5-dihydroxyphenylglycine; EGLU = 2*S*-α-ethylglutamic acid; L-AP4 = l-(+)-2-amino-4-phosphonobutyrate; L-CCG-I = (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine; L-SOP = l-serine-*O*-phosphate; LY341495 = 2-amino-2-(2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid; LY354740 = 1*S*,2*S*,5*R*,6*S*-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate; LY367385 = (+)-2-methyl-4-carboxyphenylglycine; LY379268 = (−)-2-oxa-4-aminobicylco hexane-4,6-dicarboxylic acid; MAP4 = 2-amino-2-methyl-4-phosphonobutyrate; MCCG = 2-methyl-2-(2-carboxycyclopropyl)glycine; MCPG = α-methyl-4-carboxyphenylglycine; mGluR = metabotropic glutamate receptor; MPEP = 2-methyl-6-(phenylethynyl)-pyridine; MSOP = (R,S)-alpha-methyl-serine-O-phosphate; PDE = phosphodiesterase; PKC = protein kinase C; PLA2 = phospholipase A2; PLC = phospholipase C; SIB-1893 = (/E/)-2-methyl-6-(2-phenylethenyl)pyridine; tADA = *trans*-azetidine-2,4-dicarboxylic acid.

to modulate multiple pathophysiological pathways may prove more effective than those directed at a single target. Neurons, astrocytes, microglia, oligodendrocytes, endothelial cells, and circulating immune cells all play roles in response to acute and subacute injury, as well as in chronic neurodegeneration. Thus, treatments that target multiple cell types and associated pathways may prove most beneficial.

This review examines the multipotential therapeutic utility of mGluR modulation in acute and chronic injury and neurodegeneration. Targeting mGluRs represents a multifunctional drug approach, in that they are expressed in a number of different cell types widely distributed throughout the CNS.[12]

METABOTROPIC GLUTAMATE RECEPTOR–MEDIATED EFFECTS

Neurons

Neurons express all three groups of mGluRs (Tables 2–4), depending on the location within the CNS. For example, neurons within the cortex and caudate–putamen express mGluR3 and mGluR5, but only mGluR3 is expressed in the septum.[13] mGluR1 is predominantly expressed in the CA3 region of the hippocampus.[14] After injury, the expression of mGluRs is altered. After kainate-induced seizures, for example, expression of mGluR3 and mGluR5 in $NeuN^+$ neurons increases,[13] and the expression of mGluR1 and mGluR2/3 are also chronically increased in neurons after spinal cord injury.[6]

Stimulation of group I mGluRs increases neuronal excitability[15] and has been linked to epileptic events.[14] Antagonists for mGluR1 and mGluR5 and agonists for mGluR group II and III have anticonvulsant activities. In normal tissue, activation of mGluR1a in the hippocampus potentiates signaling, facilitating induction of long-term potentiation.[14] Within neurons, group I mGluR receptors potentiate glutamatergic signaling. Addition of group I agonists, such as (*S*)-3,5-dihydroxyphenylglycine (DHPG), to neurons enhances the potency of NMDA-induced neuronal cell death.[16–18] In addition, DHPG enhances the release of arachidonic acid in response to NMDA administration to neuronal cultures, increasing neuronal cell death[18] via the production of superoxide and subsequent lipid peroxidation and DNA damage.[19]

Not surprisingly, antagonists of group I mGluRs are neuroprotective. For example, the mGluR1 antagonists AIDA, CPCCOEt, and LY367385 reduce neuronal cell death after mechanical trauma *in vitro*.[20] Furthermore, MCPG, AIDA, and 4CPG are neuroprotective when neurons are subjected to oxygen and glucose deprivation *in vitro*.[17] mGluR1 antagonists also limit cell death in response to the administration of NMDA[21] or staurospor-

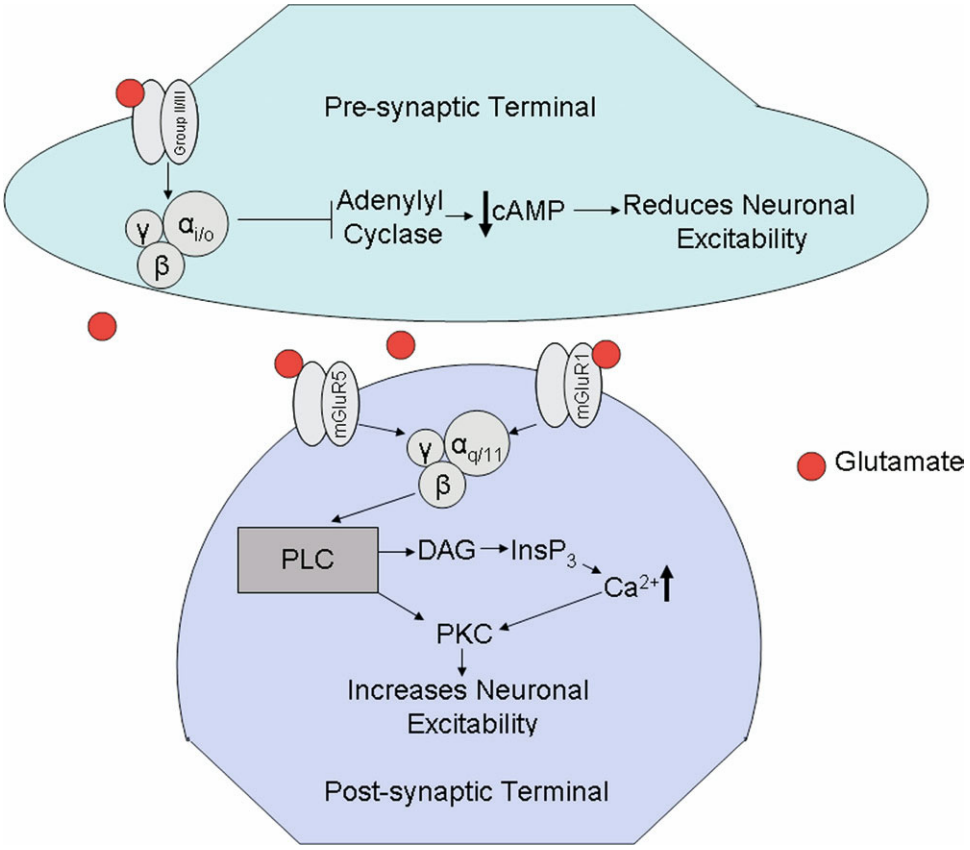

FIG. 1. Schematic of metabotropic glutamate receptor (mGluR) neuronal signaling. DAG = diacylglycerol; $InsP_3$ = inositol 1,4,5-triphosphate; PKC = protein kinase C; PLC = phospholipase C.

ine.[17] The mGluR5 antagonist MPEP also significantly reduced neuronal cell death in response to NMDA administration[22]; however, these effects were found when MPEP was applied in a concentration that was effective in blocking NMDA channel openings. Thus, neuroprotective effects may not be a result of direct mGluR5 activity, but rather from noncompetitive NMDA antagonistic properties. Further work has shown that neuroprotective properties of mGluR5 antagonists are mediated by direct actions on NMDA receptors, and similar protective effects are observed in mGluR5 knockout mice.[23]

Administration of β-amyloid (Aβ) to cortical neuronal cultures causes neuronal apoptosis, which is exacerbated by application of the mGluR1 antagonist AIDA.[24] In contrast, agonists of mGluR5 demonstrate neuroprotective properties. For example, the nonselective mGluR group I agonist DHPG or the selective mGluR5 agonist CHPG are neuroprotective when neurons are challenged

Table 2. *Cells Expressing Group I mGluR*

Cell Type	Receptor Subtype	Function
Neuron	mGluR1; mGluR5	Postsynaptic; operates through PLC activation and potentiation
Astrocyte	mGluR1[36]; mGluR5[6,36]	Elevates intracellular calcium[94] May contribute to neuronal death by increasing glutamate release
T lymphocyte	mGluR1; mGluR5[72]	mGluR5 reduces activation in a cAMP dependent fashion[72]
B lymphocyte	mGluR1; mGluR5[95]	Unknown
Microglia	mGluR5[12,36]	Attenuates activation (proliferation, production of inflammatory mediators)[38]
Hepatocytes	mGluR5[96]	May contribute to oxidative induced cell death
Pinealocytes	mGluR5[93]	Unknown
Melanocytes	mGluR1[97]; mGluR5[98]	Induces Proliferation
Oligodendrocytes	mGluR5[94]	Reduces oxidative stress[69] May limit cell death[99]
Endothelial cells	mGluR1; mGluR5[100]	Increase permeability[100]

Table 3. *Cells Expressing Group II mGluR*

Cell Type	Receptor Subtype	Function
Neuron	mGluR2; mGluR3[91]	Reduces adenylate cyclase activity, reducing cAMP and neuronal excitability. mGluR2 may be less neuroprotective than mGluR3[91]
Astrocyte	mGluR3[12]	Increases growth factor production[92]
Microglia	mGluR2; mGluR3[41]	mGluR2 stimulates a neurotoxic phenotype, while mGluR3 is neuroprotective[40]
Pinealocytes	mGluR3[93]	Negatively regulate melatonin secretion
Oligodendrocytes	mGluR3[59,94,99]	May play a role in cell survival[99]

with nitric oxide (NO),[25] Aβ,[26] or platelet activating factor.[27] More specifically, mGluR5 agonists block apoptotic neuronal cell death,[17] and these effects are reversed by the mGluR5 antagonist MPEP.[26]

Group II and III mGluRs are presynaptic receptors on neurons that act to reduce glutamatergic signaling. As such, excitotoxicity may be reduced after trauma. A number of studies have shown neuroprotective activities of group II and group III mGluR agonists. For example, activation of these receptors with APDC, L-CCG-I, LY379268, or L-AP4 increases neuronal survival in response to several different challenges, including activation of endonucleases with NO donors,[25,28,29] platelet activating factor,[27] or NMDA challenge.[30] Electrophysiological function is also improved with group III agonist treatment after oxygen and glucose deprivation.[31] In neuronal and astrocyte mixed cultures, administration of DCG-IV, APDC, or L-CCG-I, group II mGluR agonists, or the group III mGluR agonists L-SOP or L-AP4 reduced Aβ-induced neuronal apoptosis.[32]

The group II mGluR agonists LY379268, DCG-IV, APDC, and LY354740 reduced neuronal release of lactate dehydrogenase after mechanical trauma *in vitro*.[33,34] These effects may have been specific to mGluR2, because administration of the mGluR2/3 antagonist EGLU reduced the observed neuroprotection but the mGluR3 antagonist β-acetyl-aspartyl-glutamate (β-NAAG) did not.[33] Furthermore, the mGluR3-specific agonist α-NAAG did not have the neuroprotective effects observed by LY379268, the nonspecific group II agonist.

Two specific agonists of group III mGluRs, L-SOP and L-AP4, also significantly reduced neuronal cell death after mechanical injury *in vitro*.[35] These neuroprotective effects were mediated by a reduction in cAMP, and antagonists of group III mGluRs exacerbated neuronal death.

Microglia

Metabotropic glutamate receptors are expressed in glial cells, where their activation exerts numerous effects that are crucial for glial cell function and glial–neuronal interaction under physiologic and pathologic conditions. mGluR5 mRNA has been detected in cultured microglia,[36] but has not been found in resident microglia of intact brain by *in situ* hybridization.[13] Although mGluR1 mRNA has not been detected in cultured microglia,[36] in humans mGluR1α immunoreactivity is colocalized with a subset of cells of microglia–macrophage lineage in multiple sclerosis lesions.[37] Recently, however, using immunocytochemistry and Western blotting, we found that mGluR5 protein is expressed in microglia cultured from rat brain,[38] whereas mGluR1α is negligibly expressed (FIG. 2). Double-labeling with the microglial markers OX42 and ED1 showed that microglial cells do express mGluR5 (FIG. 3C).

Preliminary experiments in which the group I mGluR agonist DHPG is applied to microglia stimulated with lipopolysaccharide demonstrate that DHPG has an inhibitory effect on microglial activation. Measures of microglial activation including NO production (FIG. 3A) and proliferation (FIG. 3B) were significantly reduced by the pretreatment of purified cortical microglia (96% pure)

Table 4. *Cells Expressing Group III mGluR*

Cell Type	Receptor Subtype	Function
Neuron	mGluR4; mGluR6; mGluR7; mGluR8	Reduces Adenylate cyclase activity, reducing cAMP and neuronal excitability
Astrocyte	mGluR4[42]; mGluR6; mGluR7; mGluR8[36]	Increases glutamate uptake, potentially providing neuroprotection[101]
Microglia	mGluR4; mGluR6; mGluR8[12,41]	Stimulates a neuroprotective phenotype [40,41]
Osteoblasts	mGluR4; mGluR8[102]	May play a role in proliferation[102]
Bone marrow stromal cells	mGluR4; mGluR8[103]	Reduces nitric oxide synthase activity[103]
Endothelial cells	mGluR4[100]	Increase permeability[100]

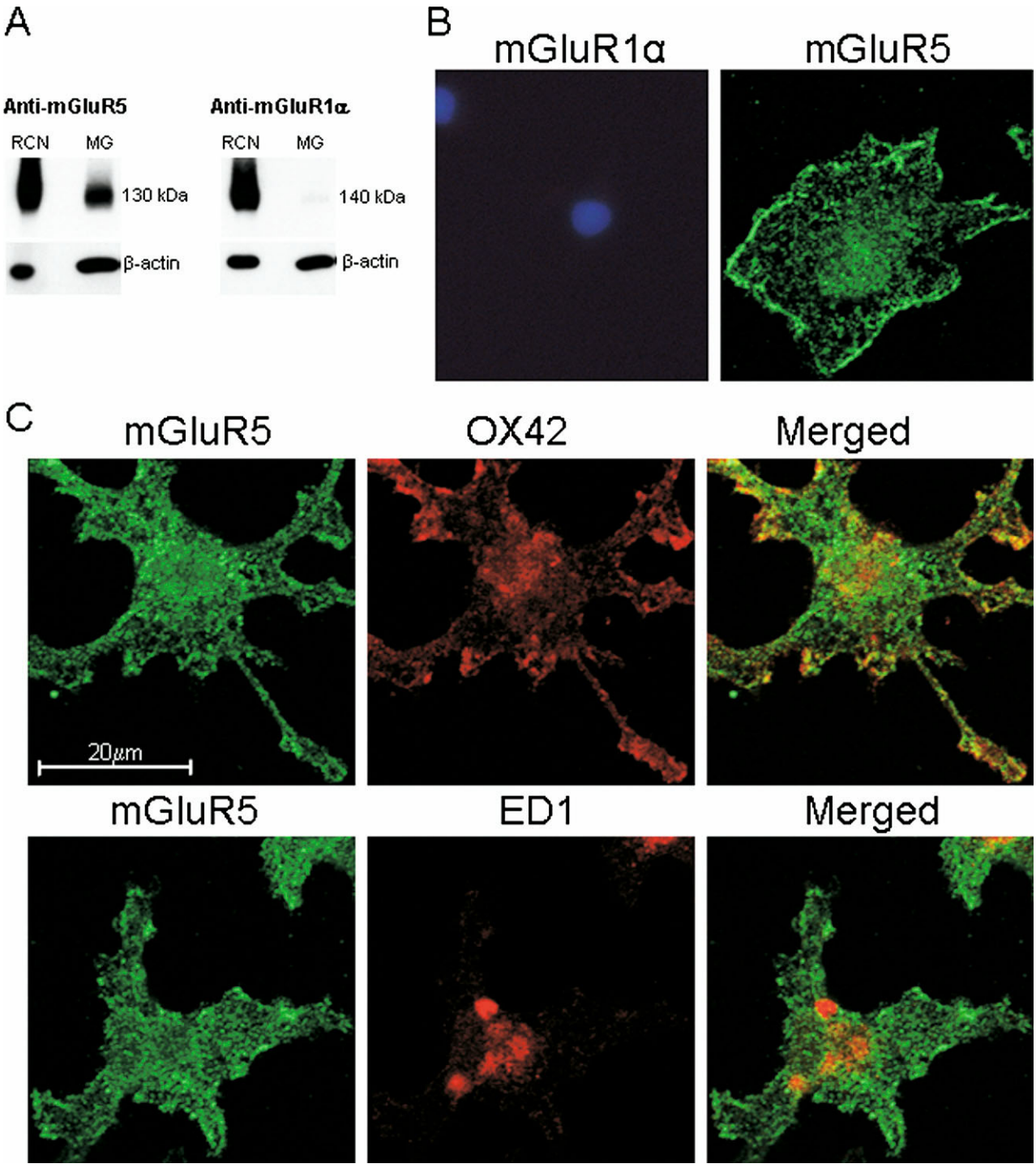

FIG. 2. Microglia express the group I mGluR receptors: mGluR5 and mGluR1α, as demonstrated by Western blot (A) and immunocytochemistry (B, C). Rat cortical neuron (RCN) samples were run alongside microglia (MG) as positive controls for the antibodies. To confirm microglial expression of mGluR5, cells were double-labeled with common markers for microglia, including OX42 and ED1 (C). Cell nuclei are stained with 4′,6-diamidino-2-phenylindole DAPI (blue). Reproduced with permission from Byrnes et al.[38]

with DHPG and the mGluR1 antagonist CPCCOEt prior to lipopolysaccharide stimulation. Furthermore, the mGluR5-specific agonist CHPG showed similar actions, suppressing NO, reactive oxygen species production, proliferation, and neurotoxicity.[38] These effects were not observed in microglial cultures from mGluR5 knockout mice.[38] Suppression of microglial activation by mGluR5 agonists is mediated by the $G\alpha_q$ signal transduction pathway, and requires activation of PLC, PKC, and calcium release.[38]

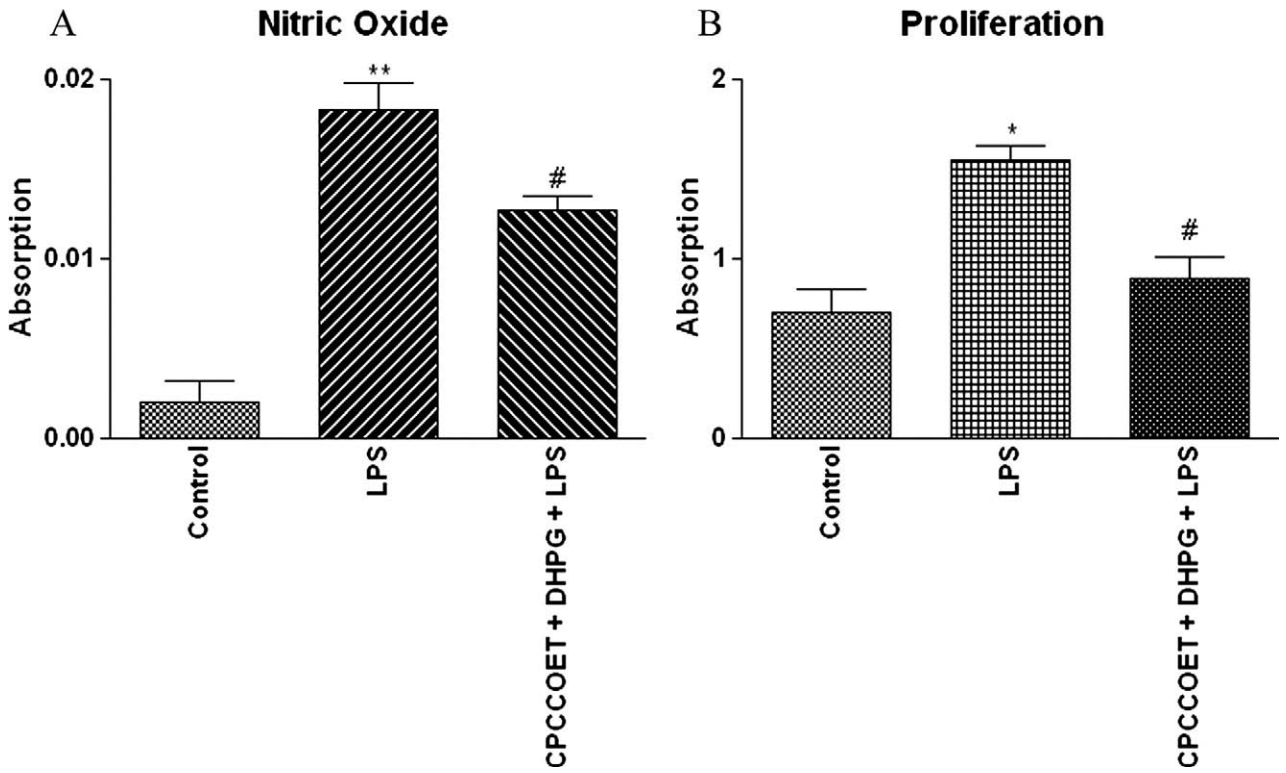

FIG. 3. Stimulation of mGluR5 with DHPG (mGluR1 is inhibited by the addition of CPCCOET) reduces lipopolysaccharide (LPS)-induced activation of microglia. Microglial activation was measured by NO production (a) and proliferation (b) at 24 h after stimulation. Both measurements were significantly inhibited by pretreatment with DHPG + CPCCOET. Error bars indicate standard error of the mean (± SEM). $^{**}p < 0.01$ vs. control; $\#p < 0.05$ vs. LPS. For abbreviations, see abbreviation list.

Cultured microglia express mRNA and protein for the group II mGluRs, mGluR2 and mGluR3.[39] Activation of mGluR2 and mGluR3 by selective agonists DCG-IV and L-CCG-I promotes a neurotoxic microglial phenotype. In addition, chromogranin A (CGA)-induced and Aβ (Aβ_{25-35})-induced microglial activation is modulated by group II mGluRs, because inhibition by the antagonist MCCG reduced toxin-induced microglial reactivity and related neurotoxicity.[39] More recently, activation of microglial mGluR2 was shown to exacerbate cell death, whereas activation of mGluR3 was protective in a model of myelin-induced microglial neurotoxicity.[40] Although mGluR3 mRNA has not been found in microglia from intact rat brain,[13] mGluR2/3 immunoreactivity has been observed in microglia and macrophage-like cells in autopsy brain samples from patients with multiple sclerosis.[37]

Microglia also express mRNA and protein for the group III mGluRs mGluR4, mGluR6, and mGluR8—but not mGluR7.[41] Activation of these receptors with the specific group III agonists L-AP4 or RS-PPG inhibited forskolin-induced cAMP production, linking them to the negative inhibition of adenylate cyclase. Agonists of group III mGluRs reduced microglial activation when stimulated with lipopolysaccharide, CGA, or Aβ_{25-35}, and agonist treatment reduced their neurotoxicity after microglial stimulation with lipopolysaccharide or CGA; thus, activation of group III mGluRs can protect neurons against microglial-mediated neurotoxicity.[41]

The protective effects of group III mGluR activation has been confirmed in a model of myelin-induced microglial neurotoxicity,[40] findings that may have important implications for the treatment of multiple sclerosis. Notably, analysis of human tissue samples revealed that mGluR8, but not mGluR4, was expressed in multiple sclerosis lesions, in particular in cells of the microglia–macrophage lineage with an amoeboid morphology.[42] With respect to lesion stage, it was found that the mGluR8 expression was strongly colocalized to actively demyelinating lesions.

Astrocytes

mGluR5 receptor mRNA has been detected in astrocytes isolated from young rats[43] and from adult rats.[44] The expression profile of mGluR5 in astrocytes appears to decrease during development,[44] but in the intact brain immunohistochemistry reveals mGluR5 protein expression.[45] The other group I mGluR, however, mGluR1, shows limited astrocytic expression. mGluR1a receptor mRNA or protein has not been detected in cultured cortical astrocytes grown in conventional medium or astrocyte-defined media.[46–48] In a small proportion (10%) of cultured astrocytes prepared from the spinal cord, how-

ever, mGluR1a receptors have been detected by immunohistochemistry.[49] These data are consistent with observations of human spinal cord from patients with amyotrophic lateral sclerosis.[50]

The expression of mGluR5 receptors is modulated by extracellular signals. Cultured astrocytes grown in conventional serum-containing medium show low expression, whereas expression is upregulated when cells are cultured in medium containing growth factors such as basic fibroblast growth factor, epidermal growth factor, or transforming growth factor-α (TGF-α).[46,48] It has been suggested that the expression profile induced by the growth medium mimics the activation of astrocytes during reactive gliosis. Accordingly, immunohistochemical analysis revealed mGluR5 expression in reactive astrocytes surrounding a lesion site or induced by epileptic seizures to be higher than in nonactivated astrocytes.[6,51–53] In humans, mGluR5 receptors have been found to be diffusely upregulated in reactive astrocytes under pathological conditions, such as multiple sclerosis[37] and amyotrophic lateral sclerosis.[50]

Activation of mGluR5 in astrocytes stimulates polyphosphoinositide (PI) hydrolysis[48] and generates oscillatory increases in intracellular calcium.[36,54] This results in the release of transmitters such as glutamate, which in turn modulates neuronal excitability and promotes synchronized activation of groups of neurons.[55] Activation of group I receptors by DHPG stimulates MAP kinase pathways[56] and selective activation of mGluR5 stimulates phospholipase D signaling in cultured cortical and hippocampal astrocytes.[57]

Among group II mGluRs, astrocytes express mGluR3 receptors *in vitro* and *in vivo,* whereas mGluR2 receptors are not expressed.[13,58,59] mGluR3 mRNA is expressed in cultured astrocytes,[36] but detection of mGluR3 protein has been unsuccessful with the current battery of mGluR3-specific antibodies.[60,61] Similar to mGluR5 expression, mGluR3 expression is upregulated in media containing growth factors in cultured astrocytes.[60]

mGluR2 and mGluR3 receptors are negatively coupled to adenylate cyclase, and activation of group II mGluRs by the selective agonist LY379268 reduces forskolin-stimulated cAMP formation in the absence of extracellular calcium but enhances cAMP formation in the presence of calcium.[62] This dual regulation of cAMP formation is unique to cultured astrocytes. In addition, activation of group II mGluRs amplifies the stimulation of cAMP formation mediated by β2-adrenergic receptors, leading to adenosine release in cultured astrocytes.[63] Activation of mGluR2/3 receptors also stimulates the MAP–ERK kinase and PI-3-kinase signaling pathways.[60,64] Stimulation of MAP kinase and PI-3-kinase pathways increases formation of TGF-β, which is neuroprotective,[65] and also protects against astrocytic damage caused by oxygen and glucose deprivation in culture.[66]

The expression of mGluR4 in astrocytes is controversial; some studies have detected the receptor in primary cultures of rat and mouse cortical astrocytes by RT-PCR and Western immunoblotting,[67] whereas others have not.[36,61] Neither mGluR6 nor mGluR7 expression has been detected in cultured astrocytes to date.[61] Although mGluR8 mRNA is not expressed in cortical astrocytes grown in conventional medium, it is upregulated in astrocytes grown in astrocyte-defined media. In humans, mGluR4 receptors are not found in resting astrocytes, but are detectable in reactive astrocytes of multiple sclerosis lesions.[42]

Other cell types

Studies have shown that mGluRs are expressed on a number of other CNS and peripheral cell types, including oligodendrocytes, lymphocytes, meningeal cells, pinealocytes, hepatocytes, osteoblasts, bone marrow cells, and pancreatic islet cells (for review, see Ferraguti and Shigemoto[12] and Maiese et al.[68]). Cultured oligodendrocytes prepared from neonatal rats express mGluR1α, mGluR2/3, mGluR4, and mGluR5 receptors. Expression of these receptor subtypes is developmentally regulated and is high in early and late oligodendrocyte precursors, and low or absent in immature and mature oligodendrocytes.[69] Rat O4- and O1-positive precursors and A2B5-positive early precursors from adult human brain express both mGlu3 and mGlu5 receptor proteins.[70,71] In oligodendrocytes, group I mGluR agonists reduce oxidative stress and excitotoxic cell death in a PKCα-mediated manner.[69] This effect is reversed by the mGluR5 antagonist MPEP, suggesting that mGluR5 mediates the protective effect.

Both T and B lymphocytes express group I, II, and III mGluRs.[72,73] In T cells, mGluR5 activation results in cAMP upregulation and an inhibition of T-cell activity.[72] Activation of mGluR1 in T cells resulted in increases in ERK1/2 phosphorylation and cell proliferation.[72] Activation of group III mGluRs with L-AP4 has been shown to increase levels of reactive oxygen species in lymphocytes and contribute to neuronal toxicity, which is contrary to its effects *in vivo.*[73]

THERAPEUTIC EFFECTS

mGluR1 antagonists

As has been discussed here, mGluR1 receptors are expressed on a number of cells within the CNS, including neurons, meningeal cells, microglia, astrocytes, T cells, and B cells (Table 2). Through interactions with the Homer proteins, group I mGluRs may also have effects on NMDA signaling,[74] and they potentiate NMDA-mediated neurotoxicity and increase arachidonic

acid release.[18] Furthermore, mGluR1 agonists increase T-cell proliferation and activation of the MAP kinase signal transduction cascade, increasing inflammation.[72] Therefore, antagonists of mGluR1 may have multimodal therapeutic effects after CNS trauma.

Inhibition of mGluR1 receptors has beneficial effects after CNS damage. After spinal cord contusion injury, injections of the mGluR group I antagonist AIDA into the lesion site improved early locomotor recovery, as measured by standardized Basso–Beattie–Bresnahan (BBB) scores.[75] However, differences between animals receiving AIDA or vehicle disappeared by 28 days post injury, possibly because only single-dose administration was delivered. Beneficial effects were mediated by the mGluR1α receptor, in that the effects were also found with the mGluR1α-specific antagonist LY367385, but not the mGluR5-specific antagonist MPEP. Treatment with the mGluR1α antagonist also produced white and gray matter sparing; however, the cellular target of this effect was not investigated.

mGluR1 antagonists have also proved protective in traumatic brain injury models (FIG. 4). AIDA administration after lateral fluid percussion in rats significantly improved neuroscores and MRI-based lesion volume,[20] as well as reducing overall neuronal cell death.[76] Furthermore, the mGluR1 antagonist YM-202074 is neuroprotective after cerebral ischemia.[77] After middle cerebral artery occlusion in rats, YM-202074 administration within 2 h of the onset of ischemia significantly reduced infarct volumes in the brain and improved neurological scores.

mGluR5 agonists

mGluR5 can operate through either release of calcium from intracellular stores, similar to mGluR1, or it can activate the Src family of tyrosine kinases, producing intracellular signaling through the ERK/MAPK cascade.[78] As already discussed, expression of mGluR5 is found in a number of CNS and peripheral cells (Table 2),[12] and its actions in different cells have suggested strong possibilities for therapeutic potential. For example, mGluR5 agonists have shown antiapoptotic properties in neuronal cultures,[17,25–27] and have strong anti-inflammatory effects in microglial cultures.[38] In addition, group I mGluRs can activate PKC (FIG. 1), which can cause upregulation of inward rectifier potassium channels and reduce microglial activation.[79] mGluR5 activation also reduces excitotoxic death in oligodendrocyte cultures.[69] Cocultures of neurons and astrocytes suggested the requirement of astrocytes for a CHPG-mediated excitotoxicity.[80] In this coculture system, administration of the mGluR5 agonist CHPG significantly reduced NMDA-mediated currents after a stretch-injury; without astrocytes, CHPG did not modulate the NMDA responses. However, this study did not directly address the effects of CHPG on astrocytic cultures.

Although a number of reports indicate that treatment with the specific mGluR5 agonist CHPG is neuroprotective *in vitro* and *in vivo*, treatment with the mGluR5 antagonist MPEP may also provide neuroprotection. The fact that the group I receptors mGluR1 and mGluR5 have similar signaling pathways and intracellular effects led to the theory that inhibition of mGluR5, similar to mGluR1, would be neuroprotective. Indeed, administration of MPEP significantly reduced neuronal death after glutamate or NMDA exposure.[22]

Nonetheless, the confusing actions of mGluR5 agonism–antagonism is underscored in a 2001 study by Bao et al.,[81] in which CHPG or MPEP was administered after middle cerebral artery occlusion–induced focal ischemia. Both the agonist CHPG and antagonist MPEP reduced infarct volume when applied at 250 nmol concentrations. Application of MPEP after injection of 6-hydroxydopamine into the substantia nigra also attenuated neuronal loss.[82]

Preliminary studies in our laboratory have shown promising beneficial effects of mGluR5 agonists in a rat spinal cord contusion model and in a mouse traumatic brain injury model. Intrathecal administration of CHPG for 7 days after a moderately severe spinal cord contusion at T9 resulted in a significant improvement in function, as measured by the BBB score at 28 days post injury (FIG. 5).

This apparent confusion regarding mGluR5 agonists and antagonists was resolved by work of Lea et al.,[23] which definitively showed that neuroprotective actions of the mGluR5 antagonists MPEP or MTEP do not reflect actions at the mGluR5 receptor. Instead, MPEP acts to directly inhibit NMDA receptor signaling, and application of the antagonists in cultures lacking the mGluR5 receptors (mGluR5 knockouts) yields the same neuroprotective effects as in cultures from wild-type animals.

Group II and III mGluR agonists

Group II and III mGluRs are presynaptic receptors on neurons,[30] but are also expressed on microglia, astrocytes, and a number of other cell types (Tables 3 and 4). Activation of these receptors reduces glutamate release and GABAergic transmission in neurons,[83] thus potentially reducing excitotoxic cell death. Furthermore, group III mGluR activation induces neuroprotective phenotypes in microglia,[40,41] although nonspecific activation of the group II mGluR2/3 has shown neurotoxic effects[39], whereas the group II agonist DCG-IV induces brain-derived neurotrophic factor (BDNF) expression in microglia.[84,85] In astrocytes, activation of mGluR2/3 induces the release of neuroprotective TGF-β.[65] These findings suggest that group II and III mGluR agonists may also have multipotential therapeutic actions.

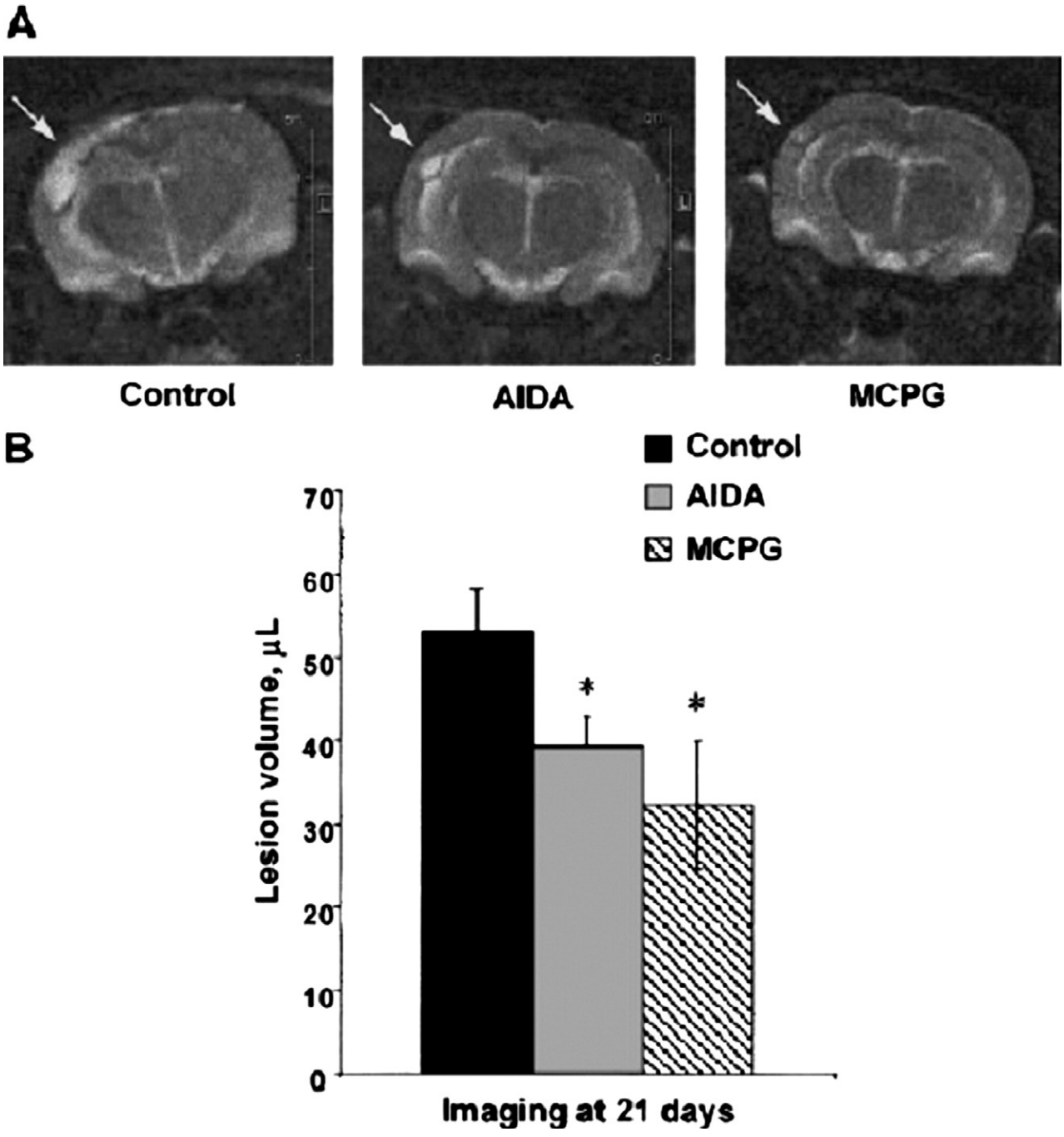

FIG. 4. AIDA- and MCPG-treated animals developed significantly smaller lesions after traumatic brain injury (TBI) than vehicle-treated control animals. (A) Representative T2-weighted magnetic resonance images of control and mGluR1 antagonist–treated rat brains at day 21 after TBI. (B) Summary of the effects of AIDA and MCPG treatments on lesion volume after TBI. The histograms represent average lesion volume (μL) at day 21 after injury (± SEM), as measured using T2-weighted MRI. $n = 9$ to 11 animals per treatment. $^*p < 0.05$ versus vehicle-treated controls using one-tailed t test. Reproduced with permission from Faden et al.[20] For abbreviations, see abbreviation list.

In vivo, the group II agonist LY379268 reduced neuronal loss in the hippocampus after global ischemia in a gerbil model and application of LY379268 up to 2 h after occlusion was neuroprotective in a rat model of focal ischemia.[30] These effects may reflect not only reduced neuronal death directly, but also the role of stimulating astrocytes in producing neuroprotective factors[65] and reducing glutamate release.[30]

In other models of CNS damage, such as spinal cord injury, traumatic brain injury, or excitotoxic injections, group II and III mGluRs provide neuroprotection. For example, after spinal cord injury, group II and III mGluR

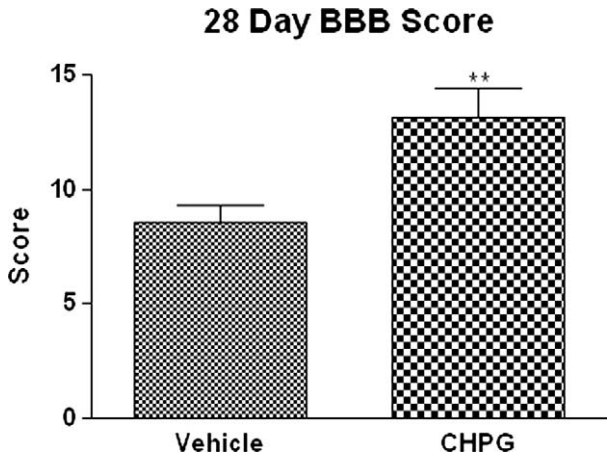

FIG. 5. Hindlimb locomotor function was assessed at day 28 after spinal cord injury in rats. CHPG treatment resulted in a significant improvement in Basso–Beattie–Bresnahan (BBB) score. Error bars indicate ± SEM; $^{**}p < 0.01$. For abbreviations, see abbreviation list.

agonists improve some measures of recovery, such as reduced allodynia.[86] Additionally, administration of the mGluR4 agonist (*R*,*S*)-phosphonophenylglycine (RS-PPG) reduces NMDA-induced neuronal death.[87] That these results are due to actions at the mGluR4 receptor is indicated by control experiments in mGluR4 knockout mice, which showed no neuroprotection with the addition of RS-PPG after NMDA administration. Administration of LY379268 30 minutes after controlled cortical impact injury in mice resulted in significant improvements in both motor and cognitive function (FIG. 6).[33] Similarly, treatment with LY354740 significantly improved neurological scores at 2 weeks post injury after lateral fluid percussion injury in rats.[34] Furthermore, blocking the breakdown of the endogenous mGluR2/3 neurotransmitter NAAG with ZJ-43 resulted in significant reductions in neuronal death and excitotoxicity.[88]

FUTURE WORK

To date, neurons have been the assumed target of mGluR agonists and antagonists *in vivo*. A number of other cells express functional mGluRs, however, which may play pathophysiological or protective roles after CNS injury. Therefore, more research remains to be done on the function of mGluRs in the various cell types and their specific roles *in vivo*. Conditional knockout models may help to discriminate specific cell contributions to neuroprotection and recovery.

In addition, because acute and chronic CNS disorders share similar mechanisms of neuronal death, including inflammation and neuronal apoptosis, mGluRs may also provide therapeutic targets for chronic neurodegenerative disorders. For example, mGluR1 is strongly expressed in the substantia nigra,[12] and group I mGluRs facilitate inhibitory signaling in the striatal pathways.[74] Antagonists against mGluR1, such as LY367385, attenuate the loss of dopamine and tyrosine hydroxylase-positive neurons after injection of 6-hydroxydopamine into the substantia nigra.[82] mGluR1 is also expressed in the hippocampus,[12] and inhibition of mGluR1 signaling may be beneficial in Alzheimer's disease.[89] Group II and III agonists also reduce GABAergic and glutamatergic transmission in the basal ganglia, and improve motor activity in experimental models of Parkinson's disease (for review, see Maiese et al.,[68] Benarroch,[74] and Rouse et al.[90]). The group II agonist LY354740 reduces muscle rigidity and catalepsy in an animal model of Parkinson's disease.[91]

CONCLUSION

Although mGluRs are less frequently investigated than neuronal expression, it is clear that they are expressed on a number of different cell types. Group I, II, and III mGluRs are found on cell types within, and peripheral to, the CNS, including neurons, microglia, astrocytes, oligodendrocytes, T and B lymphocytes, osteoblasts, hepatocytes, and endothelial cells, among others (Tables 2–4). These receptors have a number of effects on cells that can influence outcome after trauma. It is now well known that activation of neuronal mGluR1 exacerbates necrosis, and mGluR1 activation has also been shown to increase permeability of endothelial cells after injury, which may exacerbate inflammation and injury. mGluR5 activation in neurons deters neuronal apoptosis, and provides protection in oligodendrocytes exposed to oxidative stress. Stimulation of the group II

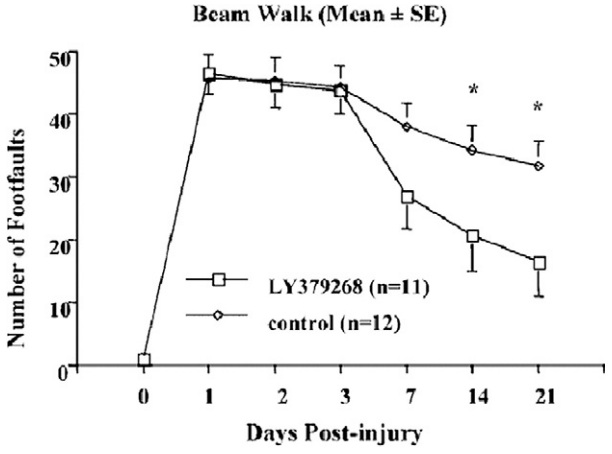

FIG. 6. Treatment with LY379268 improved motor recovery after brain injury in mice. A beam walking task was used to discriminate fine motor coordination differences between treated and control animals that had been subjected to controlled cortical impact injury. LY379268 or vehicle was administered intravenously at 30 min after trauma. A repeated-measures analysis of variance (ANOVA) yielded significant differences between these two groups ($p < 0.05$) at days 14 and 21. Reproduced with permission from Movsesyan and Faden.[33] For abbreviations, see abbreviation list.

and III mGluRs is neuroprotective, reducing neuronal death after a variety of mechanical or chemical injuries. In microglia, activation of mGluR5, mGluR3, and group III mGluRs has an anti-inflammatory effect, reducing neurotoxicity and production of inflammatory mediators. mGluR5 also exerts anti-inflammatory effects when activated in T lymphocytes. mGluR2 stimulation, on the other hand, promotes a neurotoxic microglial phenotype. Stimulation of mGluR3 and group III mGluRs in astrocytes may also have neuroprotective effects, stimulating growth factor expression and glutamate clearance.

Modulation of mGluRs represents an attractive multipotential therapeutic strategy for both acute and chronic neurodegenerative disorders. *In vivo*, the activation of mGluR5 and group II and III mGluRs has been met with improved recovery after both traumatic brain injury and spinal cord injury, and antagonism of mGluR1 has similar effects, reflecting the multiple actions of these receptors *in vitro*.

Potential therapeutic effects of mGluR modulation include reduction of inflammation, decreased excitotoxicity, and inhibition of both necrotic and apoptotic cell death. The treatment regimen must be optimized for each injury or neurodegenerative model, however, with a focus on time of application and dose to most appropriately respond to each situation. The response of individual cell types to injury or disease was briefly reviewed here, and should be considered when applying mGluR agonists or antagonists as multipotential treatment strategies. For example, the increase in proliferation of microglia occurs quickly after spinal cord injury, and is maintained for months,[92] suggesting a long therapeutic window for mGluR5 modification of microglial-mediated inflammation after spinal cord injury. Furthermore, mGluR2/3 is chronically increased in neurons after spinal cord injury,[6] suggesting the possibility of delayed treatments.

Much research remains to be done to fully characterize the potential targets and therapeutic approaches to optimize mGluRs as a multipotential intervention. Recent reviews extensively outline critical considerations for the development of multitarget-directed ligands for treatment of neurodegenerative disorders.[10,93]

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Abbreviations: 4CPG = (S)-4-carboxyphenylglycine, 6-OHDA = 6-hydroxydopamine, Aβ = β-amyloid, AIDA = 1-aminoindan-1, 5-dicarboxylic acid, ALS = amyotrophic lateral sclerosis, AMPA = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, APDC = aminopyrrolidine-2,4-decarboxylate, BBB = Basso-Beattie-Bresnahan scale, cAMP = cyclic adenosine monophosphate, CHPG = (RS)-2-Chloro-5-hydroxyphenylglycine, CGA = chromogranin A, CNS = central nervous system, CPCCOEt = 7-(hydroxyimino)cyclopropa[b]chromen-la-carboxylate ethyl ester, DCG-IV = (2S,2′R,3′R)-2-(2′,3′-Dicarboxycyclopropyl)glycine, DHPG = (S)-3,5-dihydroxyphenylglycine, EGLU = 2*S*-α-ethylglutamic acid, ERK = extracellular signal-regulated kinase, LAP4 = L-(+)-2-amino-4-phosphonobutyrate, L-CCG-I = (2S,1′S,2′S)-2-(carboxycyclopropyl)glycine, LDH = lactate dehydrogenase, LPS = lipopolysaccharide, L-SOP = L-serine-O-phosphate, LTP = long-term potentiation, LY354740 = 1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate, LY367385 = (+)-2-Methyl-4-carboxyphenylglycine, LY379268 = (–)-2-oxa-4-aminobicylco hexane-4,6-dicarboxylic acid, MCPG = α-methyl-4-carboxyphenylglycine, mGluR = metabotropic glutamate receptor, MAPK = mitogen-activated protein kinase, MPEP = 2-methyl-6-(phenylethynyl)-pyridine, MTEP = 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine, MS = multiple sclerosis, NAAG = N-Acetyl-aspartyl-glutamate, NO = nitric oxide, NMDA = N-methyl-D-aspartic acid, PD = Parkinson's disease, PLC = phospholipase C, PAF = platelet activating factor, PI = polyphosphoinositide, PSD = postsynaptic density, PKC = protein kinase C, ROS = reactive oxygen species, SCI = spinal cord injury, TGF-β = transforming growth factor-β, TBI = traumatic brain injury, TH = tyrosine hyrdoxylase, YM-202074 = N-cyclohexyl-6-{[(2-methoxyethyl)(methyl)amino]methyl}-N-methylthiazolo [3,2-a]benzimidazole-2-carboxamide.

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