Group II Metabotropic Glutamate Receptor Activation Attenuates Traumatic Neuronal Injury and Improves Neurological Recovery after Traumatic Brain Injury

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ABSTRACT

We examined the effects of modulating group II metabotropic glutamate receptors (mGluRs) on traumatic neuronal injury using both in vitro and in vivo models. Treatment with various selective group II mGluR agonists significantly decreased lactate dehydrogenase release, a marker of cell death, after traumatic injury to rat neuronal-glial cultures; injury-induced increases in cyclic AMP and glutamate levels were also significantly reduced by a group II agonist. The neuroprotective effects of group II agonists were markedly attenuated by co-administration of a group II antagonist or a membrane-permeable cyclic AMP analog and were additive to those provided by an N-methyl-D-aspartate receptor antagonist or a selective group I mGluR antagonist. Administration of a group II mGluR agonist 30 min after lateral fluid percussion-induced brain injury in rats significantly improved subsequent behavioral recovery as compared with vehicle-treated controls. Together these studies indicate that group II mGluR agonists protect against traumatic neuronal injury by attenuating glutamate release and cAMP levels and suggest a potential role for these agents in the treatment of clinical neurotrauma.

Activation of ionotropic glutamate receptors has been implicated as an important pathophysiological factor contributing to tissue damage after central nervous system trauma (Faden et al., 1989). Traumatic brain injury (TBI) induces marked elevations in extracellular glutamate levels (Faden et al., 1989), and treatment with N-methyl-D-aspartate receptor (NMDAR) antagonists limits post-traumatic tissue damage and improves neurological recovery following experimental TBI (Faden et al., 1989).

Released glutamate can also activate metabotropic glutamate receptors (mGluRs), which are coupled to second messenger cascades through G proteins (Pin and Duvoisin, 1990). Although the existence of mGluR was suggested as early as 1985 (Sladeczek et al., 1985), only the recent availability of relatively selective agonists and antagonists have made possible to address the potential physiological and pathophysiological roles of these receptors. mGluRs have been divided into three groups based on sequence homology and signal transduction mechanisms. Group I receptors (mGluR1, mGluR5) stimulate phospholipase C, leading to phosphoinositide hydrolysis and intracellular calcium mobilization; group II and group III receptors are negatively coupled to adenylyl cyclase activity but differ in their pharmacological properties (Pin and Duvoisin, 1990). Pharmacological studies have shown that activation of group I receptors leads to depolarization and excitation in a variety of model systems, whereas group II/III receptors seem to be involved in presynaptic depression (Sladeczek et al., 1985; Bolshakov and Siegelbaum, 1994).

Although a role for mGluR in modulating neuronal cell death has been suggested by a number of studies, early reports often produced inconsistent results due to the use of nonselective agents (Mukhin et al., 1996). With the recent development and use of selective agonists and antagonists, it seems that group I and group II/III metabotropic glutamate

ABBREVIATIONS: LDH, lactate dehydrogenase; mGluR, metabotropic glutamate receptor; TBI, traumatic brain injury; NMDAR, N-methyl-D-aspartate receptor; APDC, (S(2R,4R))-4-aminopyrrolidine-2,4-dicarboxylate; DIV, days in vitro; RT-PCR, reverse transcriptase-polymerase chain reaction; AIDA, (R,S)-1-aminooindan-1,5-dicarboxylic acid; DCG-I, (2S,2‘R,3‘R)-2-(2‘,3‘-dicarboxycyclopropyl)glycine; MCCG, (2S,3S,4S)-2-methyl-2-(carboxycyclopropyl)glycine; EGLU, (2S)-3-ethylglutamic acid; MK-801, (S(5R,10S)-(+-)5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine; LY354740, (1S,2S,5R,8S)-2aminobicyclo[3.1.0]hexane-2,6-dicarboxylate; 8-Br-cAMP, adenosine 3’5’-cyclic monophosphate, 8-bromo, sodium salt.
receptors may have opposite actions. Group I receptor activation exacerbates post-traumatic or posts ischemic neuronal loss, possibly by potentiating NMDA receptor activity (Fitzjohn et al., 1996; Mukhin et al., 1997a). In contrast, activation of group II or group III receptors may exert a protective effect due to their ability to inhibit presynaptic glutamate release (Nakanishi, 1994) or modulate adenylate cyclase activity (Buisson and Choi, 1995). We have recently shown that treatment with group III agonists significantly attenuates post-traumatic neuronal cell death in vitro (Faden et al., 1997).

In the present studies, we investigated the role of group II mGlurR in post-traumatic neuronal injury, using well established in vitro and in vivo trauma models, and examined potential mechanisms of action.

Materials and Methods

Mixed Neuronal-Glial Cultures. Mixed rat neuronal-glial cultures were prepared as detailed previously (Mukhin et al., 1996, 1997a,b; Faden et al., 1997). Briefly, before injury neuronal-glial cultures were transferred to an incubation media containing minimal essential media with Earle’s salts (Mediatech, Herndon, VA) supplemented with 10 mM HEPES (pH 7.4; Biofluids, Rockville, MD), 1 mM glucose (Biofluids), 1% antibiotic/antimycotic (Biofluids). Cultures were incubated at 37°C for 30 min in the presence or absence of selective agonists and/or antagonists. Trauma was delivered using a device that produces 28 parallel 1.2-mm cuts uniformly distributed throughout the cell layer at 0.5-mm intervals. After injury, cells were incubated at 37°C for an additional 30 min, washed 5 times with incubation media, and incubated in the presence or absence of the same agonists and/or antagonists in 5% CO2 for 16 to 18 h at 37°C before assessment of injury.

Evaluation of Cyclic AMP (cAMP) Accumulation. Basal cAMP levels and changes in cAMP accumulation after injury were evaluated in 18 DIV neuronal-glial cultures. Media were completely aspirated, and 50 mM of acetonitrile was added to each well. Cells were harvested (3–4 samples per treatment, three wells per sample), and cAMP was extracted by ion-exchange chromatography using Amprep SAX minicolumns (100 mg; Amersham Corp., Arlington Heights, IL). cAMP was quantified using a scintillation proximity assay system (RPA 538; Amersham). Protein content in samples was evaluated by Bradford’s assay (Bio-Rad, Hercules, CA) with a bovine serum albumin standard (Pierce, Rockford, IL). Cultures contained 109 ± 0.6 fmol/sample of cAMP by this method.

Evaluation of mRNA Expression of Group II mGlurRs. The expression of mRNA encoding mGlurR2 and mGlurR3 was analyzed using a reverse transcriptase-polymerase chain reaction (RT-PCR) approach as previously described (Mukhin et al., 1998). In brief, total cellular RNA was isolated from neuronal-glial (18 DIV) and glial (28 DIV) cultures, and adult rat cortex using acidic phenol extraction and RNA was reverse transcribed with Moloney murine leukemia virus-RT (GIBCO, Grand Island, NY). The resulting cDNA was amplified by PCR for 35 cycles: denaturation for 30 s at 94°C, annealing for 15 s at 55°C, and primer extension for 45 s at 72°C. Amplified cDNA was analyzed in 2% agarose electrophoretic gels and stained with ethidium bromide. UV light gel images were captured and analyzed using the Image 1.59 program. The following primer sequences were used for cDNA amplification: mGlurR2, 5'-CTCCTGCG- CCTGACATGGCTGCTCCC-3'; mGlurR3, 5'-ATCACAGCCGCTTCACTGTAGT-3'; mGlurR3, 5'-AACAAACAGCCGAGGAGACAA-3'. The specificity of primers was confirmed by sequencing of the RT-PCR generated products as detailed elsewhere (Faden et al., 1997).

Western Blot Analysis. Immunoblot analysis for mGlurR2 and mGlurR3 protein was performed following a previously described protocol (Mukhin et al., 1998). Briefly, neuronal-glial (18 DIV) cultures were harvested from 96-well plates in 10 mM Tris-HCl buffer (pH 7.4) containing 100 mM phenylmethylsulfonyl fluoride (Sigma). Cells were lysed with a polytron, and crude membrane preparations were obtained by centrifugation at 33,000g for 20 min at 4°C. Pellets were washed, and protein concentration was determined by Bradford’s assay (Bio-Rad). Samples were stored at −80°C. Five micrograms of protein were separated by SDS-polyacrylamide gel electrophoresis in 7.5% acrylamide (Sigma) and transferred onto nitrocellulose (Bio-Rad). Primary affinity-purified rabbit anti-mGlurR23 monoclonal antibody directed against the carboxyl terminus (dilution 2.5 μg/ml; Chemicon, Temecula, CA) was used for immunostaining. This antibody does not distinguish between...
mGluR2 and mGluR3. Peroxidase-conjugated purified anti-rabbit IgG (dilution 2.5 μg/ml; Vector Laboratories, Burlingame, CA) and enhanced chemiluminescence Western blotting detection system (Amersham) were used to visualize primary antibody staining.

In Vivo Traumatic Brain Injury. Male Sprague-Dawley rats (Harlan, Frederick, MD) weighing 375 to 425 g were anesthetized with sodium pentobarbital (70 mg/kg i.p.; Abbott Laboratories, North Chicago, IL). Lateral fluid percussion-induced TBI was performed as described previously (Faden et al., 1997). In this model, a fluid wave is delivered to the extradural space through a craniotomy over left parietal cortex transiently deforming the underlying brain. Injury severity is related to the pressure induced; in the present studies, a moderate level of injury was chosen (2.4 atmospheres). Animals were randomly assembled to treatment with (1S,2S,5R,6S)-diaminocyclohexane-2,6-dicarboxylate (LY354740; 5 mg/kg, n = 12) or equal-volume normal saline (1 ml/kg, n = 13), administered through a femoral vein catheter 30 min after trauma. Behavioral tests were performed at 24 h and 1 and 2 weeks after TBI by an individual unaware of the treatment group. Neuroscores reflect a series of behavioral tests including ability to maintain position on an incline plane (left, right, vertical), forelimb flexion (left, right), and lateral pulsion (left, right). Each test was evaluated using an ordinal scale ranging from 0 (no function) to 5 (normal function). Total neuroscore thereafter could range from 0 to 35.

These tests show high interrater reliability and can discriminate drug treatment effect (Faden et al., 1989; Mukhin et al., 1996).

Drugs. Selective agonists and antagonists for metabotropic glutamate receptors (R,S)-1-aminoimidane-1,5-dicarboxylic acid (AIDA), (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV), (2S,3'S,4'S)-2-methyl-2-(carboxycyclopropyl)glycine (CCG), (2S)-ethylglycine butyramide acid (EGLU), as well as noncompetitive NMDA receptor antagonist (S(+),10S(+)-S-methyl-11,11-dihydro-5H-dibenzo[a,g]cyclohepten-5,10-imine (MK-801) were obtained from Toronto Cookson (St. Louis, MO). LY354740 was received from Lilly Research Laboratories (Eli Lilly and Company, Indianapolis, IN). (2R,4R)-4-Aminopyrrolidine-2,4-dicarboxylate (APDC) was synthesized in Drug Discovery Laboratory (Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington, DC). Adenosine 3',5'-cyclic monophosphate, 8-bromo, sodium salt (8-Br-cAMP), a cell-permeable cAMP analog (Hei et al., 1991), was obtained from Calbiochem (La Jolla, CA).

Data Analysis. Injury-induced LDH release was calculated by subtraction of background levels of LDH estimated for control (uninjured) neuronal-glial cultures from values obtained for injured cultures. Results are expressed as a percentage of injury-induced LDH release observed in injured cultures in absence of treatment. Student’s t test or analysis of variance (one-way ANOVA) followed by Student-Newman-Keuls test were used to compare differences across groups. Concentration-dependent changes in cell death (reflected by injury-induced LDH release) were analyzed using Spearman’s Rank Correlation test. Neuroscore data obtained from in vivo TBI experiments were analyzed using the Mann-Whitney U test after Kruskal-Wallis nonparametric ANOVA. A p value < .05 was considered statistically significant.

Results

Expression of Group II mGluR Receptors in Neuronal-Glial Cultures. Expression of both mGluR2 and mGluR3 was detected by qualitative RT-PCR in 18 DIV neuronal-glial cultures (Fig. 1a). The presence of group II mGluR protein in neuronal-glial cultures (18 DIV) was confirmed by Western blot analysis with antibody directed against mGluR2/mGluR3 (Fig. 1b).

Effects of Group II mGluR Agonists and Antagonists on In Vitro Injury-Induced Neuronal Cell Death. To evaluate the role of group II mGluRs in neuronal injury, we used three relatively selective agonists of group II mGluRs: DCG-IV (Ishida et al., 1993), APDC (Schoepf et al., 1995), and LY354740 (Monn et al., 1997). All three agonists demonstrated dose-dependent neuroprotection with similar maximal effects of approximately 26 to 30% (Fig. 2a–c). EC50 values for DCG-IV, APDC, and LY354740 were approximately 73 nM, 9 μM, and 34 nM, respectively. Treatment of injured cultures with DCG-IV at concentrations higher than 2.5 μM led to exacerbation of neuronal cell death (data not shown). None of the group II mGluR agonists had any effects in uninjured control sister cultures at the concentrations tested.

The group II antagonists MCCG (Jane et al., 1994; Knopfel et al., 1995) and EGLU (Jane et al., 1996) were used to further address the potential role of group II mGluR in secondary neuronal cell death. Treatment with EGLU (200–1000 μM) had no effect on injury-induced LDH release (Fig. 2d). MCCG had no effect at concentrations of 200 to 300 μM, but at concentrations above 500 μM MCCG attenuated injury-induced neurotoxicity (Fig. 2d). Neither EGLU nor MCCG had any effect on uninjured control sister neuronal-glial cultures. However, addition of EGLU (500 μM) reversed the neuroprotective effect of APDC (50 μM) (Fig. 2e).

Modulation of Glutamate Release. Basal levels of glutamate present in media removed from uninjured control cultures and those treated with LY354740 (100 nM) were approximately 0.27 nM. Significant increases in extracellular glutamate concentrations occurred by 2 h following injury and remained elevated at 4 h (Fig. 3a). Pretreatment with 100 nM LY354740 significantly attenuated injury-induced increases in glutamate levels at 2 h (Fig. 3a). However, extracellular glutamate levels were not statistically different in media removed from treated and untreated cultures at 4 h (Fig. 3a).

Modulation of cAMP Levels. To evaluate the possible role of adenylyl cyclase modulation in the neuroprotection provided by group II activation, we examined cAMP levels after injury in the presence or absence of group II mGluR agonists. Significant increases in cAMP levels were detectable 2 h after injury to neuronal-glial cultures and continued to rise at 4 h postinjury (Fig. 3b). Treatment with LY354740 (100 nM) significantly decreased injury-induced cAMP accumulation (Fig. 3b). Basal cAMP levels in neuronal-glial cul-
tures treated for 30 min with 200 μM APDC (93 ± 2, n = 6), 0.5 μM DCG-IV (86 ± 2, n = 6), or 100 nM LY354740 (89 ± 1, n = 6) were lower than basal cAMP levels in control untreated cultures (100 ± 1, n = 8, p < .05, ANOVA followed by Student-Newman-Keuls’ test).

We also examined the effects of cell-permeable cAMP analog 8-Br-cAMP on injury-induced neuronal cell death. Application of 8-Br-cAMP at concentrations of 250 and 500 μM did not affect injury-induced LDH release (data not shown), whereas 1000 μM 8-Br-cAMP significantly exacerbated injury (126 ± 5.9 versus 100 ± 4.7; n = 12–23, Student’s t test, p < .01). Moreover, the neuroprotective effects of both APDC (200 μM) and LY354740 (100 nM) were completely reversed by 8-Br-cAMP (500 μM) administration (Fig. 4).

**Combined Treatment with Group II mGluR Agonists and Postsynaptic Receptor Antagonists.** Based on our previous results, 10 μM MK-801 provides maximal inhibition of NMDA receptors (Mukhin et al., 1997a, 1998). At this concentration, MK-801 attenuated injury-induced neuronal death by 35 to 50% (Fig. 5a). Coapplication of MK-801 (10 μM) and the group II mGluR agonist APDC (200 μM) elicited significantly more neuroprotection than that afforded by treatment with MK-801 alone (Fig. 5a).

The selective group I mGluR-selective antagonist AIDA

![Fig. 2. Treatment with group II mGluR agonists attenuated injury-induced LDH release in neuronal-glial cultures (18–20 DIV). Activation of group II mGluR by LY354740 (a), APDC (b), and DCG-IV (c) exhibited dose-dependent protection. d, inhibition of group II mGluR by EGLU or MCCG did not exacerbate injury-induced LDH release. e, coadministration of EGLU with APDC completely reversed the neuroprotective effect of APDC. All treatments were applied 30 min before injury and for 16 to 18 h postinjury. Data are expressed as a percentage of injury-induced LDH release in the absence of treatment. Values represent mean ± S.E.M., n = 32 to 64 cultures per condition. *, p < .05 versus control; #, p < .05 versus APDC; ANOVA followed by Student-Newman-Keuls’ test.]
Pellicciari et al., 1995) was used to evaluate potential additive neuroprotective effects of group I and group II mGluR modulation. Treatment with 100 μM AIDA inhibited injury-induced cell death by approximately 25% (Fig. 5b). Significantly greater neuroprotection was produced by administration of APDC (200 μM) in combination with AIDA (100 μM) than that induced by either agent alone (Fig. 5b).

**Effects of Group II mGluR Activation on TBI In Vivo.**
To assess the potential role of group II mGluR in TBI-induced deficits in vivo, we administered LY354740 to rats subjected to fluid percussion TBI, a well characterized model of head trauma. Systemic administration of LY354740 (5 mg/kg) 30 min following TBI significantly improved neurologic outcome at 14 days as compared with animals receiving vehicle alone.
Animals treated with LY354740 exhibited significantly better scores at 14 days on each of the three tests used to assess functional recovery: ability to maintain position on an inclined plane, forced lateral pulsion, and forelimb contralateral during tail suspension (Fig. 6).

**Discussion**

In the present study, we demonstrated the dose-dependent neuroprotective effects of the selective group II mGluR agonists APDC and LY354740 and the relatively selective agonist DCG-IV against trauma-induced neuronal cell death in vitro. These results support the hypothesis that activation of group II mGluR protects against in vitro injury and are consistent with the findings reported by other investigators using different in vitro models and less selective agonists. For example, group II agonists were found to protect against injury in vitro neuronal cell death induced by ischemia (Opitz et al., 1994; Buisson and Choi, 1995) and by NMDA administration (Bruno et al., 1994; Buisson and Choi, 1995).

The rank order of potency of group II agonists for inhibiting trauma-induced neuronal cell death in vitro was APDC (9 μM) > DCG-IV (73 nM) > MK-801 (34 nM). DCG-IV, which has been used as a group II agonist in some of the earlier studies (Ishida et al., 1993), showed biphasic effects in our in vitro system. At concentrations below 2.5 μM, this compound induced neuroprotection; however, at higher concentrations DCG-IV significantly exacerbated injury. This biphasic effect is consistent with the pharmacological profile of DCG-IV. At nanomolar concentrations, DCG-IV is an agonist at group II mGluR, whereas at micromolar concentrations it exhibits agonist activity at NMDA receptors (Ishida et al., 1993; Wilsch et al., 1994). APDC, DCG-IV, and LY354740 produced a similar maximal level of neuroprotection against in vitro trauma of approximately 25 to 30%. This degree of protection is similar to that provided by group I mGluR antagonists in this model (Mukhin et al., 1996, 1997a) and somewhat higher than effects of group III mGluR agonists (Faden et al., 1997). In comparison, NMDA antagonists elicit approximately 50% neuroprotection in this model (Mukhin et al., 1997a,b, 1998).

Perhaps surprisingly, the group II mGluR antagonists EGLU and MCCG did not exacerbate injury-induced LDH release. This finding is in contrast with our previous work using group III antagonists, which clearly exacerbate trauma-induced neuronal death in vitro (Faden et al., 1997). This suggests that group II receptors are not activated sufficiently in response to trauma to provide endogenous neuroprotection. Administration of MCCG at doses greater than 500 μM provided protection against trauma; however, this compound exhibits partial agonist activity at higher concentrations (Bushell et al., 1996). Although it may be argued that EGLU and MCCG may not be pure antagonists at the concentrations used (Kemp, 1994; Sekiyama et al., 1996), EGLU did completely reverse the neuroprotective effects of the selective group II agonist APDC.

Several potential mechanisms of neuroprotection by group II mGluR agonists against NMDA- or ischemia-induced neuronal cell death have been proposed. One possibility is that decreased neuronal cell death may be mediated by adenylate cyclase inhibition (Buisson and Choi, 1995). Increased levels of cAMP are found in the hippocampus following transient ischemia (Suyama, 1995) and in hippocampal slices after oxygen-glucose deprivation (Whittingham et al., 1984). Moreover, NMDAR activation causes significant increases in cAMP accumulation in rat brain cortical slices (Kemp, 1994) and in neuronal cultures (Buisson and Choi, 1995). Increases in cAMP levels activate protein kinase A with subsequent phosphorylation of ion channels and other target proteins, including NMDA receptors (Raman et al., 1996). cAMP is also capable of modulating ion channels in a protein kinase...
A-independent manner (Pedarzani and Storm, 1995), and increased levels of cAMP may alter free intracellular magnesium concentrations (Romani et al., 1991) and cellular bioenergetics. A reduced cellular bioenergetic state may contribute to NMDA receptor-mediated neurotoxicity (Novelli et al., 1988), and decreased free intracellular magnesium concentrations are correlated with poor neurological recovery after TBI (Vink et al., 1988).

A second possibility is that the neuroprotective effects of group II agonists result from inhibition of glutamate release (Nakanishi, 1994). Glutamate release inhibitors have been shown to produce neuroprotection in both in vivo trauma (Sun and Faden, 1995) and ischemia (Graham et al., 1993) models. In addition, Bruno and colleagues have suggested that neuroprotection by group II agonists against NMDA-induced excitotoxic neuronal cell death in vitro requires new protein synthesis and involves an interaction between neurons and astrocytes (Bruno et al., 1997).

In the present studies, treatment with group II mGluR agonists did reduce both cAMP levels and post-traumatic glutamate release, suggesting that one or both mechanisms may contribute to the neuroprotective responses observed. Attenuating glutamate release would be expected to limit both NMDA receptor and group I mGluR activation after trauma, each of which has been shown to contribute to post-traumatic neuronal cell death in this model (Mukhin et al., 1996, 1997b). A role for cAMP modulation is more speculative, although the ability of 8-Br-cAMP to exacerbate post-traumatic neuronal loss and correlations of cAMP levels to neuronal death are consistent with this interpretation.

Expression of group I and group III mGluRs as well as NMDAR was previously demonstrated in our neuronal-glial cultures, and this model was successfully used to study the roles of group I mGluR (Mukhin et al., 1996, 1997a) and group III mGluR (Faden et al., 1997) in injury-induced neuronal cell death. In the present studies, we demonstrated...
expression of group II mGluR in our neuronal-glial cultures as detected by RT-PCR and confirmed by Western blotting. Therefore, this model was used to study the possible additive neuroprotective effects of agonists at presynaptic group II mGluR and antagonists of postsynaptic glutamate receptors (i.e., NMDAR and group I mGluR). Treatment with MK-801 (10 μM) completely blocks NMDA-induced neurotoxicity in our neuronal-glial cultures and significantly reduces injury-induced LDH release (Mukhin et al., 1997a, 1998). However, coadministration of maximally effective concentrations of MK-801 and APDC provided significant additional neuroprotection above that produced by either agent alone. This added protection may result in part from decreased glutamate release, leading to a reduction in the neurotoxic effects mediated by group I mGluR activation (Mukhin et al., 1996, 1997b).

Injury-induced neuronal cell death in vitro was also further decreased by combined treatment with APDC and the group I mGluR antagonist AIDA, as compared with application of either drug alone. It has been shown previously that group I mGluR-mediated neurotoxicity may result, in part, from NMDA receptor potentiation (Fitzjohn et al., 1996; Mukhin et al., 1997a). However, this toxicity may also reflect increased arachidonic acid release (Aramori and Nakanishi, 1992; Dumuis et al., 1993) and/or a synergistic interaction between group I and group II mGluR. Simultaneous activation of group I and II mGluR leads to stimulation of phosphoinositide hydrolysis (Schoepp et al., 1996b) and stimulation of agonist-evoked increases in cAMP formation (Schoepp et al., 1996a). The observation that the neuroprotective actions of group II mGluR agonists are additive to those with NMDA or group I mGluR antagonists suggests that combination therapy may be useful in the treatment of clinically relevant traumatic brain injury.

To evaluate the possible role of group II mGluR in trauma-mediated neuronal death in vivo and to determine the possible therapeutic benefits of group II agonists against in vivo TBI, we administered LY354740 (5 mg/kg) i.v. 30 min after induction of fluid percussion TBI in rats. The dose of LY354740 used was based upon data from other in vivo studies using this compound and corresponds to a moderate to moderately high dose (Monn et al., 1997). This treatment significantly improved neurological recovery at 14 days postinjury. Surprisingly, the degree of protection provided by LY354740 was comparable with our previous experience with competitive and noncompetitive NMDAR antagonists in this model (Faden et al., 1989). However, in contrast to NMDAR antagonists, LY354740 exhibits few side effects and is generally well tolerated by experimental animals (Monn et al., 1997). Thus, group II mGluR modulation may be a useful treatment strategy for clinical head injury. Furthermore, our in vitro results suggest that combination therapy composed of a group II agonist and postsynaptic glutamate receptor antagonists may provide additional neuroprotection. This treatment paradigm may allow a reduction in the dose of NMDAR antagonists, thereby reducing unwanted side effects.

In summary, we have demonstrated the neuroprotective effects of group II mGluR activation against in vitro trauma-induced neuronal death using two selective agonists, APDC and LY354740, and the relatively selective agonist DCG-IV. A specific role for group II mGluR was further suggested by complete blockade of the effects of APDC by the group II mGluR antagonist EGLU. In addition, our results implicate both inhibition of glutamate release and decreased injury-induced cAMP levels as mechanisms of group II mGluR-mediated neuroprotection. Additional neuroprotection was also seen after coapplication of group II mGluR and a NMDAR antagonist or a group I mGluR antagonist. Finally, the marked neuroprotection produced by LY354740 against in vivo TBI indicates that group II mGluR agonists may prove to be beneficial in clinical head injury management.

References


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