Published in final edited form as:
Glia. 2009 April 1; 57(5): 550–560. doi:10.1002/glia.20783.

MGLUR5 ACTIVATION INHIBITS MICROGLIAL ASSOCIATED INFLAMMATION AND NEUROTOXICITY

Kimberly R. Byrnes[1], **Bogdan Stoica**[1], **David J. Loane**[1], **Angela Riccio**[1], **Margaret I. Davis**[2], and **Alan I. Faden**[1]

[1]Department of Neuroscience, Georgetown University Medical Center, 3970 Reservoir Rd, N.W., Washington, DC 20057;

[2]National Institute on Alcohol Abuse and Alcoholism, NIH, 5625 Fishers Lane, Rockville, MD 20852

Abstract

The group I metabotropic glutamate receptor 5 (mGluR5) can modulate addiction, pain and neuronal cell death. Expression of some mGluRs, such as group II and III mGluRs, has been reported in microglia and may affect their activation. However, the expression and role of mGluR5 in microglia is unclear. Using immunocytochemistry and Western blot, we demonstrate that mGluR5 protein is expressed in primary microglial cultures. Activation of mGluR5 using the selective agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) significantly reduces microglial activation in response to lipopolysaccharide (LPS), as indicated by a reduction in nitric oxide, reactive oxygen species and TNFα production. Microglial-induced neurotoxicity is also markedly reduced by CHPG treatment. The anti-inflammatory effects of CHPG are not observed in microglial cultures from mGluR5 knockout mice and are blocked by selective mGluR5 antagonists, suggesting these actions are mediated by the mGluR5 receptor. Anti-inflammatory actions of mGluR5 activation are attenuated by PLC and PKC inhibitors, as well as by calcium chelators, suggesting that mGluR5 activation in microglia involves the $G_{\alpha q}$-protein signal transduction pathway. These data indicate that microglial mGluR5 may represent a novel target for modulating neuroinflammation, an important component of both acute and chronic neurodegenerative disorders.

Keywords

metabotropic glutamate receptor; neurotoxic; nitric oxide; ROS; signal transduction

INTRODUCTION

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that modulate glutamatergic activity. Within the central nervous system, these receptors have been studied largely in neurons, although they are also found in glia. Classified into three groups based upon their signal transduction pathways and pharmacological profiles, activation of several mGluR subtypes affect neuronal viability both *in vitro* and after central nervous system injury. Within group I receptors, mGluR1 activation exacerbates neuronal death, particularly necrotic cell death (Allen et al. 2000). In contrast, activation of mGluR5 can inhibit caspase-dependent neuronal apoptosis in cell culture models (Movsesyan et al. 2004). Although

Address correspondence to: Kimberly R. Byrnes Room EP12A, New Research Building Department of Neuroscience 3970 Reservoir Rd., NW Washington, DC 20057 Phone: 202-687-5391. Fax: 202-687-0617. E-mail: krb27@georgetown.edu..

mGluR5 antagonists have also been reported to be neuroprotective, such effects appear to be unrelated to actions at the mGluR5 receptor (Lea et al. 2005).

In neurons and astrocytes, mGluR5 receptors are coupled to $G_{\alpha q}$-proteins and activation induces phospholipase C (PLC), leading to an increase in inositol triphosphate (IP3) and intracellular calcium, as well as activation of protein kinase C (PKC). Alternatively, mGluR5 is coupled to a $G_{\alpha s}$-protein in T-lympocytes; mGluR5 activation in these cells induces cAMP and reduces lymphocyte proliferation (Pacheco et al. 2004).

mGluRs have been identified on microglia, but their roles in neuroinflammation have received limited attention. Taylor and colleagues have reported modulatory effects by group II and III receptors in opposite directions *in vitro* (Taylor et al. 2002; Taylor et al. 2003), but such data are hard to reconcile with *in vivo* studies showing virtually identical neuroprotective profiles after activation of these receptors (Allen et al. 1999; Bond et al. 1999; Faden et al. 1997; Folbergrova et al. 2005; Movsesyan and Faden 2006; Vernon et al. 2007). Although the presence of mGluR5 mRNA has been observed in microglia (Biber et al. 1999), neither protein expression nor its role in microglial activation and neuroinflammation has been evaluated.

We show that mGluR5, but not mGluR1, is substantially expressed in microglia and negatively regulates the release of microglial associated inflammatory factors and related neurotoxicity. These effects are mediated in part by G-protein signal transduction mechanisms, including activation of the PLC - PKC signal transduction system.

MATERIALS AND METHODS

Microglial Cultures

Microglial cells were obtained from postnatal day 2 Sprague Dawley rat pups, day 2 mGluR5 knockout and wild-type mouse pups, or day 2 C57Bl6 mouse pups and cultured as described (Byrnes et al. 2006). Briefly, the whole brain was carefully dissected and homogenized in L15 media (Gibco, Carlsbad, CA). Mixed glial cultures were incubated for 8 – 10 days at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Media (Gibco) with 10% Fetal Calf Serum (Hyclone, Logan, UT), 1% L-glutamine (Gibco), 1% Sodium Pyruvate (Gibco), and 1% Pen/Strep (Fisher, Pittsburgh, PA). After the initial incubation, the cells were shaken for 1 hour at 100 rpm and 37°C. Detached microglia were collected and replated as purified cultures with greater than 96% purity.

Treatments

CHPG ((RS)-2-chloro-5-hydroxyphenylglycine; Tocris, Ellisville, MO) was applied to cells for 1 hr prior to lipopolysaccharide (LPS; 100ng/ml) stimulation. The mGluR5 antagonist MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine; 100μM) was a gift from Merck Research Laboratories (Rahway, NJ), and was administered 30 min prior to CHPG administration. Forskolin (50μM, Tocris) and dibutyryl cAMP (200μM, Biomol, Plymouth Meeting, PA) were administered 1 hr prior to LPS stimulation. SQ22356, RpcAMPs (Calbiochem, San Diego, CA), R0318220, U-73122 (10μM; BioMol, Plymouth Meeting, PA), and BAPTA-AM (1.5μM; Molecular Probes, Carlsbad, CA) were administered 30 minutes prior to CHPG administration. All chemicals were prepared and stored according to the manufacturer's guidelines.

Immunolabeling of Microglia

At 24 hours post-purification, microglia in 24-well plates on glass cover-slips were stimulated with LPS (100 ng/ml) or were untreated (control) for an additional 24 hours.

Cells were then fixed in 4% paraformaldehyde and subjected to standard immunohistochemistry, performed as described (Byrnes et al. 2006) using antibodies against mGluR1α and mGluR5 (Chemicon, Billerica, MA), OX42 (Serotec, Raleigh, NC), ED1 (Serotec), and Galectin-3 (Abcam, Cambridge, MA). Confocal fluorescence microscopy imaging was performed using Zeiss LSM 510 Meta confocal laser scanning microscope.

Microglial Proliferation and Viability

At various time points after application of mGluR agonists/antagonists, proliferation of microglia in 96-well plates was assessed using the MTS assay (MTS tetrazolium compound; Cell Titer 96® Aqueous One Solution, Promega, Madison, WI); cell death was assessed with the lactate dehydrogenase (LDH) release assay (CytoTox 96® nonradioactive cytotoxicity assay kit, Promega, Madison, WI). Both were performed according to the manufacturer's protocols.

Nitric Oxide Production

Nitric oxide (NO) production was assayed using the Griess Reagent Assay (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions.

TNFα Detection

TNFα protein secreted into the media was assessed using the TNFα ELISA kit according to the manufacturer's protocol (Endogen, Rockford, IL).

ROS Detection

Intracellular reactive oxygen species (ROS) production was assessed at 24 hours after stimulation by measuring the oxidation of 5 (and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate-acetyl ester (CM-H2DCFDA; Molecular Probes, Eugene, OR). Media from microglia plated into 96-well plates was aspirated and replaced with warmed 1X PBS. CM-H2DCFDA (10 μM) was added to microglia and incubated for 45 minutes. Fluorescence was measured using excitation and emission wavelengths of 490 and 535 nm, respectively.

Neurotoxicity

Rat primary cortical neuronal cultures were derived from E18 rat cortices (Taconic, Germantown, NY) as previously described (Mukhin et al. 1998). At 24 hours after stimulation, microglia in transwell inserts were washed in media and inserted into 24-well plates containing neurons (neurons were at day 5 *in vitro*). Twenty-four hours later, microglia were removed and the LDH release assay was used to assess neuronal cell death.

^{3}H-PI Hydrolysis Asssay

Primary microglia were cultured in 96-well plates and incubated overnight with 0.625 μCi/well myo-[^{3}H]inositol (NEN, Boston, MA) to label the cell membrane phosphoinositides and perform the assay, as described previously (Surin et al. 2007). Briefly, cells were washed in Locke's buffer and incubated with or without 100μM CHPG for 1 hr at 37 °C in Locke's buffer containing 20 mM LiCl to block inositol phosphate degradation. Inositol phosphates were then extracted with 0.1 M HCl for 10 min. The separation of [^{3}H]inositol phosphates was performed by ion-exchange chromatography on AG 1-X8 resin (200–400 mesh; Bio-Rad Laboratories, Hercules, CA). Total [^{3}H]inositol phosphates were eluted from the columns with 0.5 ml of 0.1 M formic acid/1 M ammonium formate. The collected samples were mixed with Safety-Solve cocktail (RPI, Mount Prospect, IL) and measured by scintillation counting.

Statistical Analysis

Quantitative data are presented as mean +/- SEM. Data were analyzed using Student's t-test or one-way ANOVA, where appropriate. All statistical tests were performed using the GraphPad Prism Program, Version 3.02 for Windows (GraphPad Software, Inc. San Diego, CA). A p value < 0.05 was considered statistically significant.

RESULTS

Microglia express mGluR5

Western blot analysis demonstrated that mGluR5 protein is highly expressed in rat whole brain microglial cultures whereas expression of the other group I mGluR, mGluR1, is barely detectable in these cells (Fig. 1A). Rat cortical neuron (RCN) samples, which constitutively express all mGluR receptors, were run alongside as positive controls for the antibodies.

Immunocytochemistry was also used to confirm mGluR5 expression in microglial cultures (Fig. 1B). Immunolabeling for mGluR1α revealed no receptor expression on microglia (Fig. 1B). Strong mGluR5 immunolabeling was detected on the surface of primary microglia. mGluR5 labeling is found particularly on the cell periphery, consistent with membrane localization. Further, to confirm microglial expression, double-labeling was performed with common markers of microglia and mGluR5. mGluR5 was expressed on cells that were also positive for OX42 and ED1 (Fig. 1C). Primary antibodies were excluded from the incubation mix for immunocytochemistry to serve as negative controls and showed no labelling (data not shown).

mGluR5 stimulation reduces microglial activation

In order to determine if microglia respond to mGluR5 stimulation, microglia were cultured in 96-well plates and mGluR5 agonists/antagonists were added alone or in combination to determine their effect on microglial activation. Pre-treatment with CHPG was found to reduce expression of several independent markers of microglial activation, including ROS production, NO production, proliferation and TNFα production.

CHPG's effects were determined to be dose dependent, with maximal effects at 100μM, as measured by both ROS and NO production ($p<0.05$; Fig. 2A, B). CHPG addition without LPS stimulation had no effect on microglial ROS or NO production (Fig. 2A, B).

Addition of LPS (100ng/ml) to microglia resulted in an increase in proliferation over 24 hours, which was blocked by 1 hour pre-treatment with CHPG (100μM; $p<0.05$) (Fig. 3A). NO production was also significantly increased by 15 and 24 hours after LPS stimulation (Fig. 3B). Pre-treatment with CHPG significantly attenuated the LPS induced increase in NO production by 24 hours ($p<0.05$; Fig. 3B). TNFα production was up-regulated by LPS stimulation within 30 minutes and remained at increased levels through 24 hours ($p<0.001$; Fig. 3C). CHPG pre-treatment significantly attenuated TNFα production from 30 minutes through 8 hours, delaying the increase in TNFα observed after LPS stimulation.

Galectin-3, a marker of microglial activation (Byrnes et al. 2006), was also expressed when microglia were stimulated with LPS (Fig. 4). Pre-treatment with CHPG resulted in a loss of Galectin-3 staining, similar to control cultures (Fig. 4).

mGluR5 stimulation reduces neurotoxicity

Addition of activated microglia to neuronal cultures is known to induce neuronal cell death. In order to determine if mGluR5 plays a role in microglial-induced neurotoxicity, agonists/antagonists were added to microglia prior to co-culture with neurons. Stimulation of

microglia with LPS prior to co-culture significantly decreased neuron viability, as measured by neuronal LDH release ($p<0.05$; Fig. 5).

Pre-treatment of microglia with CHPG prior to LPS addition resulted in a significant increase in neuronal viability ($p<0.05$; Fig. 5). In order to eliminate the possibility of a direct effect of CHPG on neurons in this study, microglia were washed prior to addition to neurons. Neither LPS nor CHPG had any direct effect on neurons when added without microglia (data not shown). Addition of the selective mGluR5 antagonist MTEP (100 μM) prior to CHPG pre-treatment reversed the protective effect of CHPG, reducing neuronal viability ($p<0.05$; Fig. 5), suggesting that CHPG acts through the mGluR5 receptor.

The mGluR5 receptor is necessary for the action of CHPG

To confirm that CHPG is acting through the mGluR5 receptor, microglia from mGluR5 knockout (-/-) and wild-type (+/+) mice were obtained and stimulated with LPS. LPS stimulation increased microglial proliferation, NO production and ROS production in both knockout and wild-type cells (Fig. 6). However, one hour pre-treatment of microglia with CHPG attenuated the effects of LPS in wild-type microglia (Fig. 6A, C, E) but not those from mGluR5 knockouts (Fig. 6B, D, F), thus demonstrating that CHPG acts through mGluR5 receptor activation.

mGluR5 activation results in cAMP production

In T-lymphocytes, activation of mGluR5 receptors induces activation of adenylyl cyclase, cAMP production and PKA induction. To determine if this pathway is utilized in microglia, cAMP was measured in microglia following CHPG application. CHPG significantly increased cAMP production by 24 hours (Fig. 7A). To further investigate the potential role of cAMP in microglial activation, cAMP inducers, forskolin (50μM) and dibutyryl cAMP (dbcAMP; 200μM), were applied to microglia prior to LPS stimulation; each caused suppression of microglial activation as measured by NO production, similar to that of CHPG ($p<0.05$; Fig. 7B).

In order to determine if this pathway was required for CHPG's activities in microglia, cells were pre-treated with the adenylyl cyclase inhibitor SQ22356 (0.1 – 500 μM) or the PKA inhibitor RpcAMPs (10 – 500 μM) prior to addition of CHPG. Pre-treatment with either inhibitor failed to induce any significant reduction in CHPG's effects on microglial activation, as measured by ROS production (Fig. 7C, D), suggesting that cAMP production is not necessary for CHPG's activity in microglial cells.

Inhibition of phospholipase C blocks the effects of CHPG

In neurons and astrocytes, mGluR5 stimulation results in $G_{\alpha q}$ activation, triggering PLC phosphorylation, hydrolysis of phosphatidyl inositol (PI), PKC activation and calcium release. In order to determine if these signal transduction pathways were involved in CHPG signalling in microglia, hydrolysis of PI was assessed. CHPG treatment resulted in a significant increase PI hydrolysis ($p<0.01$; Fig. 8A), suggesting the activation of $G_{\alpha q}$. To further assess the role of this pathway, cells were pre-treated with inhibitors of PLC (U-73122) and PKC (R0318220) prior to CHPG and LPS addition. Using microglial activation outcomes such as ROS production, NO production and proliferation, U-73122 and R0318220 were found to block the effects of CHPG, returning NO, ROS and proliferation to LPS-induced levels (Fig. 8B, C, D). The calcium chelator (BAPTA-AM) also reversed the effects of CHPG (Fig. 8C). These inhibitors did not affect the activity of LPS or of microglia alone when introduced without CHPG pre-treatment (data not shown), demonstrating that their activities were specific for the CHPG signal transduction pathway.

DISCUSSION

Previous studies have reported the presence of mGluRs on immune cells such as microglia, macrophages and lymphocytes (Geurts et al. 2003; Pacheco et al. 2004; Rezzani et al. 2003; Taylor et al. 2005). Biber *et al* (Biber et al. 1999) demonstrated mGluR5 mRNA in microglia, but did not examine the protein or functional implications. The current study shows that in a purified rat microglia culture system, microglia express mGluR5 constitutively (Fig. 1), similar to T lymphocytes (Pacheco et al. 2004), while the other group I mGluR, mGluR1, is barely expressed.

Although mGluR1 and mGluR5 receptors share certain common signal transduction mechanisms, they have remarkably different profiles in CNS injury. Agonists of mGluR1 exacerbate necrotic cell death (Allen et al. 2000), whereas selective antagonists are neuroprotective *in vitro* and *in vivo* (Faden et al. 2001; Fei et al. 2006; Szydlowska et al. 2007). In contrast, activation of mGluR5 inhibits caspase dependent neuronal apoptosis in cell culture models (Movsesyan et al. 2004). Although mGluR5 antagonists have been reported to be neuroprotective, it has been convincingly established using mGluR5 knockout technology that such effects are unrelated to actions at the mGluR5 receptor and instead are mediated by direct actions on the NMDA receptor (Lea et al. 2005).

In the current work, we show that mGluR5 stimulation inhibits microglial activation *in vitro*. Markers of microglial activation, including proliferation, NO, TNFα and ROS production and Galectin-3 expression, as well as microglial-induced neurotoxicity were significantly attenuated by pre-treatment with the mGluR5 agonist CHPG. To verify that these effects were mediated by the mGluR5 receptor, rather than a non-receptor related mechanism, we demonstrated that CHPG's effects were lost in microglia from mGluR5 knockout mice and markedly attenuated by a selective mGluR5 receptor antagonist.

NO, TNFα and ROS each have been shown to contribute to neuronal damage and death *in vitro* and *in vivo* (He et al. 2003; Iravani et al. 2002; Jeohn et al. 2000; Juurlink and Paterson 1998; Lewen et al. 2000; Munch et al. 2003; Taylor et al. 2005; Zou and Crews 2005). For example, ROS induces lipid peroxidation, which results in cell membrane damage and cell death, as well as glutamate release to contribute to further excitotoxic cell death (Barger et al. 2007). NO is also associated with neuronal death *in vitro* and *in vivo*, through the production of the toxic metabolite peroxynitrite or via direct action on lipid membranes of the cell or mitochondria (Gibbons and Dragunow 2006; Mander and Brown 2005). TNFα can cause cell death directly by binding to neuronal TNF receptors linked to death domains that activate caspase-dependent apoptosis (Zhao et al. 2001). This cytokine can also induce glutamate release and enhance excitotoxicity (Zou and Crews 2005). In addition, TNFα can induce additional release of ROS, by inducing NADPH oxidase activity (Li et al. 2005). In our hands, LPS-stimulated microglia induced neuronal cell death, which was attenuated by CHPG treatment; it is unclear which of these factors, if not all, are responsible for the observed neurotoxicity. Because early suppression of TNFα is protective, whereas knockout of the receptor exacerbates damage after central nervous system injury, it has been suggested that this cytokine contributes to early pathophysiological changes but may facilitate subsequent plasticity (Bethea et al. 1999; Cheng et al. 1994; Kim et al. 2001; Knoblach and Faden 1998; Knoblach et al. 1999; Nakajima and Kohsaka 2004). In this regard, it should be noted that CHPG treatment of microglial cells markedly suppressed early TNFα release yet had modest to no effect on later cytokine levels. Finally, Galectin-3, a marker of microglial activation, was also up-regulated after LPS stimulation but reduced by CHPG treatment. Galectin-3 is a carbohydrate binding protein that is up-regulated following injury and plays a role in phagocytosis and perpetuation of the inflammatory response (Byrnes et al. 2006).

In lymphocytes, mGluR5 is coupled to a $G_{\alpha s}$-protein, with activation resulting in cAMP production (Pacheco et al. 2004). Our data indicate that treatment of microglia with CHPG induces cAMP production, and that other cAMP inducers, forskolin and dbcAMP, have similar effects on microglial activity. However, blocking the production of cAMP does not mitigate the effects of CHPG, suggesting that cAMP is not a critical component in the CHPG signal transduction pathway in microglia.

In neurons and astrocytes, mGluR5-mediated signal transduction involves activation of PLC and PKC, increased inositol triphosphate (IP3), and the release of intracellular calcium. Inhibitors of PLC (U-73122) and PKC (R0318220), as well as calcium chelation (BAPTA-AM), blocked CHPG's effects, suggesting that CHPG is operating through the PLC/PKC/calcium pathway. In the PLC/PKC pathway, activation of PLC leads to DAG synthesis and IP3 mediated release of Ca^{2+} (Pin and Duvoisin 1995). Both DAG and Ca^{2+} are capable of activating PKC, which has a multitude of actions within the cell, including activation of transcription factors and alteration of potassium channel activity (Endoh 2004; Mannaioni et al. 2001), which may alter microglial responses. Precisely how PKC affects microglial activation in response to CHPG remains to be elucidated. It should be noted that although Ro318220 is a PKC inhibitor, it also inhibits the activity of mitogen-activated protein kinase phosphatase-1 (MKP-1) (Guo et al. 1998). The latter enzyme is induced by TNFα, and operates to inactivate mitogen-activated protein kinases and JNK (Keyse 1995). As these actions can serve to modulate inflammation, they may reflect another potential mechanism of CHPG action within microglia.

Previous studies have demonstrated that PKC is also involved in the regulation of COX2 in activated microglia (Akundi et al. 2005), and that inhibitors of $PKC_{\alpha/\beta II}$ reduce microglial activation (Nikodemova et al. 2007). In our study, the pan-PKC inhibitor Ro318220 at the concentrations used (1.5μM) did not have an effect on microglial production of NO or on microglial proliferation, but did reverse the effect of CHPG, suggesting that different PKC isoforms may be involved in these two systems. Work has suggested that mGluR5 involves phosphorylation of the PKC_{ε} isoform (Lee and Ro 2007; Olive et al. 2005). However, the PKC isoforms that are involved in microglial activation or in mGluR5 signalling have not been fully elucidated.

CHPG provides protection in a rat model of focal brain ischemia (Bao et al. 2001). However, the authors did not evaluate potential mechanism of the neuroprotection, nor examine effects on microglia or inflammation. Our present findings suggest that neuroprotective effects of CHPG may reflect, in part, an mGluR5 receptor mediated reduction in the post-injury inflammatory response. As such, the present observations suggest a novel strategy for reducing neuroinflammation, which may have therapeutic potential in a number of neurological disorders in addition to stroke. Preliminary observations from our lab suggest that CHPG can limit microglial activation after traumatic brain or spinal cord injury in rodents. Moreover, as mGluR5 stimulation also exerts anti-apoptotic effects on neurons (Movsesyan et al. 2004), such treatment may have multi-potential neuroprotective actions. As both neuroinflammation and caspase-dependent neuronal apoptosis have been implicated in many acute and chronic neurodegenerative disorders (Block and Hong 2005; Tansey et al. 2007; Yakovlev and Faden 2001), mGluR5 agonist therapy may have broad therapeutic relevance.

Acknowledgments

The authors would like to thank Dr. Ahdeah Pajoohesh-Ganji for her technical assistance and Dr. Jarda Wroblewski for instruction and use of the PI hydrolysis assay. The work was supported by NIH NINDS R01 grant 5R01NS037313-08.

References

Akundi RS, Candelario-Jalil E, Hess S, Hull M, Lieb K, Gebicke-Haerter PJ, Fiebich BL. Signal transduction pathways regulating cyclooxygenase-2 in lipopolysaccharide-activated primary rat microglia. Glia. 2005

Allen JW, Ivanova SA, Fan L, Espey MG, Basile AS, Faden AI. Group II metabotropic glutamate receptor activation attenuates traumatic neuronal injury and improves neurological recovery after traumatic brain injury. J Pharmacol Exp Ther. 1999; 290(1):112–20. [PubMed: 10381766]

Allen JW, Knoblach SM, Faden AI. Activation of group I metabotropic glutamate receptors reduces neuronal apoptosis but increases necrotic cell death in vitro. Cell Death Differ. 2000; 7(5):470–6. [PubMed: 10800080]

Bao WL, Williams AJ, Faden AI, Tortella FC. Selective mGluR5 receptor antagonist or agonist provides neuroprotection in a rat model of focal cerebral ischemia. Brain Res. 2001; 922(2):173–9. [PubMed: 11743947]

Barger SW, Goodwin ME, Porter MM, Beggs ML. Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. J Neurochem. 2007

Bethea JR, Nagashima H, Acosta MC, Briceno C, Gomez F, Marcillo AE, Loor K, Green J, Dietrich WD. Systemically administered interleukin-10 reduces tumor necrosis factor-alpha production and significantly improves functional recovery following traumatic spinal cord injury in rats. J Neurotrauma. 1999; 16(10):851–63. [PubMed: 10547095]

Biber K, Laurie DJ, Berthele A, Sommer B, Tolle TR, Gebicke-Harter PJ, van Calker D, Boddeke HW. Expression and signaling of group I metabotropic glutamate receptors in astrocytes and microglia. J Neurochem. 1999; 72(4):1671–80. [PubMed: 10098876]

Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol. 2005; 76(2):77–98. [PubMed: 16081203]

Bond A, Ragumoorthy N, Monn JA, Hicks CA, Ward MA, Lodge D, O'Neill MJ. LY379268, a potent and selective Group II metabotropic glutamate receptor agonist, is neuroprotective in gerbil global, but not focal, cerebral ischaemia. Neurosci Lett. 1999; 273(3):191–4. [PubMed: 10515191]

Byrnes KR, Garay J, Di Giovanni S, De Biase A, Knoblach SM, Hoffman EP, Movsesyan V, Faden AI. Expression of two temporally distinct microglia-related gene clusters after spinal cord injury. Glia. 2006; 53(4):420–33. [PubMed: 16345062]

Cheng B, Christakos S, Mattson MP. Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. Neuron. 1994; 12(1):139–53. [PubMed: 7507336]

Endoh T. Characterization of modulatory effects of postsynaptic metabotropic glutamate receptors on calcium currents in rat nucleus tractus solitarius. Brain Res. 2004; 1024(1-2):212–24. [PubMed: 15451384]

Faden AI, Ivanova SA, Yakovlev AG, Mukhin AG. Neuroprotective effects of group III mGluR in traumatic neuronal injury. J Neurotrauma. 1997; 14(12):885–95. [PubMed: 9475370]

Faden AI, O'Leary DM, Fan L, Bao W, Mullins PG, Movsesyan VA. Selective blockade of the mGluR1 receptor reduces traumatic neuronal injury in vitro and improvesoOutcome after brain trauma. Exp Neurol. 2001; 167(2):435–44. [PubMed: 11161632]

Fei Z, Zhang X, Bai HM, Jiang XF, Wang XL. Metabotropic glutamate receptor antagonists and agonists: potential neuroprotectors in diffuse brain injury. J Clin Neurosci. 2006; 13(10):1023–7. [PubMed: 17113985]

Folbergrova J, Druga R, Otahal J, Haugvicova R, Mares P, Kubova H. Seizures induced in immature rats by homocysteic acid and the associated brain damage are prevented by group II metabotropic glutamate receptor agonist (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate. Exp Neurol. 2005; 192(2):420–36. [PubMed: 15755559]

Geurts JJ, Wolswijk G, Bo L, van der Valk P, Polman CH, Troost D, Aronica E. Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis. Brain. 2003; 126(Pt 8):1755–66. [PubMed: 12805104]

Gibbons HM, Dragunow M. Microglia induce neural cell death via a proximity-dependent mechanism involving nitric oxide. Brain Res. 2006

Guo YL, Kang B, Williamson JR. Inhibition of the expression of mitogen-activated protein phosphatase-1 potentiates apoptosis induced by tumor necrosis factor-alpha in rat mesangial cells. J Biol Chem. 1998; 273(17):10362–6. [PubMed: 9553092]

He Y, Imam SZ, Dong Z, Jankovic J, Ali SF, Appel SH, Le W. Role of nitric oxide in rotenone-induced nigro-striatal injury. J Neurochem. 2003; 86(6):1338–45. [PubMed: 12950443]

Iravani MM, Kashefi K, Mander P, Rose S, Jenner P. Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience. 2002; 110(1):49–58. [PubMed: 11882372]

Jeohn GH, Kim WG, Hong JS. Time dependency of the action of nitric oxide in lipopolysaccharide-interferon-gamma-induced neuronal cell death in murine primary neuron-glia co-cultures. Brain Res. 2000; 880(1-2):173–7. [PubMed: 11033002]

Juurlink BH, Paterson PG. Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and nutritional management strategies. J Spinal Cord Med. 1998; 21(4):309–34. [PubMed: 10096045]

Keyse SM. An emerging family of dual specificity MAP kinase phosphatases. Biochim Biophys Acta. 1995; 1265(2-3):152–60. [PubMed: 7696343]

Kim GM, Xu J, Song SK, Yan P, Ku G, Xu XM, Hsu CY. Tumor necrosis factor receptor deletion reduces nuclear factor-kappaB activation, cellular inhibitor of apoptosis protein 2 expression, and functional recovery after traumatic spinal cord injury. J Neurosci. 2001; 21(17):6617–25. [PubMed: 11517251]

Knoblach SM, Faden AI. Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury. Exp Neurol. 1998; 153(1):143–51. [PubMed: 9743576]

Knoblach SM, Fan L, Faden AI. Early neuronal expression of tumor necrosis factor-alpha after experimental brain injury contributes to neurological impairment. J Neuroimmunol. 1999; 95(1-2): 115–25. [PubMed: 10229121]

Lea, PMt; Movsesyan, VA.; Faden, AI. Neuroprotective activity of the mGluR5 antagonists MPEP and MTEP against acute excitotoxicity differs and does not reflect actions at mGluR5 receptors. Br J Pharmacol. 2005; 145(4):527–34. [PubMed: 15821750]

Lee JS, Ro JY. Peripheral metabotropic glutamate receptor 5 mediates mechanical hypersensitivity in craniofacial muscle via protein kinase C dependent mechanisms. Neuroscience. 2007; 146(1):375–83. [PubMed: 17306466]

Lewen A, Matz P, Chan PH. Free radical pathways in CNS injury. J Neurotrauma. 2000; 17(10):871–90. [PubMed: 11063054]

Li JM, Fan LM, Christie MR, Shah AM. Acute tumor necrosis factor alpha signaling via NADPH oxidase in microvascular endothelial cells: role of p47phox phosphorylation and binding to TRAF4. Mol Cell Biol. 2005; 25(6):2320–30. [PubMed: 15743827]

Mander P, Brown GC. Activation of microglial NADPH oxidase is synergistic with glial iNOS expression in inducing neuronal death: a dual-key mechanism of inflammatory neurodegeneration. J Neuroinflammation. 2005; 2:20. [PubMed: 16156895]

Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ. Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. J Neurosci. 2001; 21(16):5925–34. [PubMed: 11487615]

Movsesyan VA, Faden AI. Neuroprotective effects of selective group II mGluR activation in brain trauma and traumatic neuronal injury. J Neurotrauma. 2006; 23(2):117–27. [PubMed: 16503796]

Movsesyan VA, Stoica BA, Faden AI. MGLuR5 activation reduces beta-amyloid-induced cell death in primary neuronal cultures and attenuates translocation of cytochrome c and apoptosis-inducing factor. J Neurochem. 2004; 89(6):1528–36. [PubMed: 15189356]

Mukhin AG, Ivanova SA, Allen JW, Faden AI. Mechanical injury to neuronal/glial cultures in microplates: role of NMDA receptors and pH in secondary neuronal cell death. J Neurosci Res. 1998; 51(6):748–58. [PubMed: 9545088]

Munch G, Gasic-Milenkovic J, Dukic-Stefanovic S, Kuhla B, Heinrich K, Riederer P, Huttunen HJ, Founds H, Sajithlal G. Microglial activation induces cell death, inhibits neurite outgrowth and

causes neurite retraction of differentiated neuroblastoma cells. Exp Brain Res. 2003; 150(1):1–8. [PubMed: 12698210]

Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. Curr Drug Targets Cardiovasc Haematol Disord. 2004; 4(1):65–84. [PubMed: 15032653]

Nikodemova M, Watters JJ, Jackson SJ, Yang SK, Duncan ID. Minocycline down-regulates MHC II expression in microglia and macrophages through inhibition of IRF-1 and protein kinase C (PKC)alpha/betaII. J Biol Chem. 2007; 282(20):15208–16. [PubMed: 17395590]

Olive MF, McGeehan AJ, Kinder JR, McMahon T, Hodge CW, Janak PH, Messing RO. The mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine decreases ethanol consumption via a protein kinase C epsilon-dependent mechanism. Mol Pharmacol. 2005; 67(2):349–55. [PubMed: 15548766]

Pacheco R, Ciruela F, Casado V, Mallol J, Gallart T, Lluis C, Franco R. Group I metabotropic glutamate receptors mediate a dual role of glutamate in T cell activation. J Biol Chem. 2004; 279(32):33352–8. [PubMed: 15184389]

Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmacology. 1995; 34(1):1–26. [PubMed: 7623957]

Rezzani R, Corsetti G, Rodella L, Angoscini P, Lonati C, Bianchi R. Cyclosporine-A treatment inhibits the expression of metabotropic glutamate receptors in rat thymus. Acta Histochem. 2003; 105(1):81–7. [PubMed: 12666991]

Surin A, Pshenichkin S, Grajkowska E, Surina E, Wroblewski JT. Cyclothiazide selectively inhibits mGluR1 receptors interacting with a common allosteric site for non-competitive antagonists. Neuropharmacology. 2007; 52(3):744–54. [PubMed: 17095021]

Szydlowska K, Kaminska B, Baude A, Parsons CG, Danysz W. Neuroprotective activity of selective mGlu1 and mGlu5 antagonists in vitro and in vivo. Eur J Pharmacol. 2007; 554(1):18–29. [PubMed: 17109843]

Tansey MG, McCoy MK, Frank-Cannon TC. Neuroinflammatory mechanisms in Parkinson's disease: Potential environmental triggers, pathways, and targets for early therapeutic intervention. Exp Neurol. 2007; 208(1):1–25. [PubMed: 17720159]

Taylor DL, Diemel LT, Cuzner ML, Pocock JM. Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin A, a peptide up-regulated in Alzheimer's disease. J Neurochem. 2002; 82(5):1179–91. [PubMed: 12358765]

Taylor DL, Diemel LT, Pocock JM. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. J Neurosci. 2003; 23(6):2150–60. [PubMed: 12657674]

Taylor DL, Jones F, Kubota ES, Pocock JM. Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. J Neurosci. 2005; 25(11):2952–64. [PubMed: 15772355]

Vernon AC, Zbarsky V, Datla KP, Dexter DT, Croucher MJ. Selective activation of group III metabotropic glutamate receptors by L-(+)-2-amino-4-phosphonobutryic acid protects the nigrostriatal system against 6-hydroxydopamine toxicity in vivo. J Pharmacol Exp Ther. 2007; 320(1):397–409. [PubMed: 17012606]

Yakovlev AG, Faden AI. Caspase-dependent apoptotic pathways in CNS injury. Mol Neurobiol. 2001; 24(1-3):131–44. [PubMed: 11831549]

Zhao X, Bausano B, Pike BR, Newcomb-Fernandez JK, Wang KK, Shohami E, Ringger NC, DeFord SM, Anderson DK, Hayes RL. TNF-alpha stimulates caspase-3 activation and apoptotic cell death in primary septo-hippocampal cultures. J Neurosci Res. 2001; 64(2):121–31. [PubMed: 11288141]

Zou JY, Crews FT. TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. Brain Res. 2005; 1034(1-2):11–24. [PubMed: 15713255]

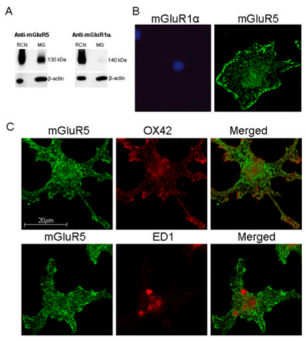

Figure 1.

Microglia express the group I mGluR receptors: mGluR5 and mGluR1α. Western blot of rat microglia cultures demonstrated high mGluR5 protein expression but low mGluR1α expression (A). Rat cortical neuron (RCN) samples were run alongside as positive controls for the antibodies. Immunocytochemistry of microglia (B) demonstrate labelling for mGluR5 (green), but not mGluR1α (green). Cell nuclei are indicated with DAPI (blue). To confirm microglial expression of mGluR5, cells were double-labeled with common markers for microglia, including OX42 and ED1 (C).

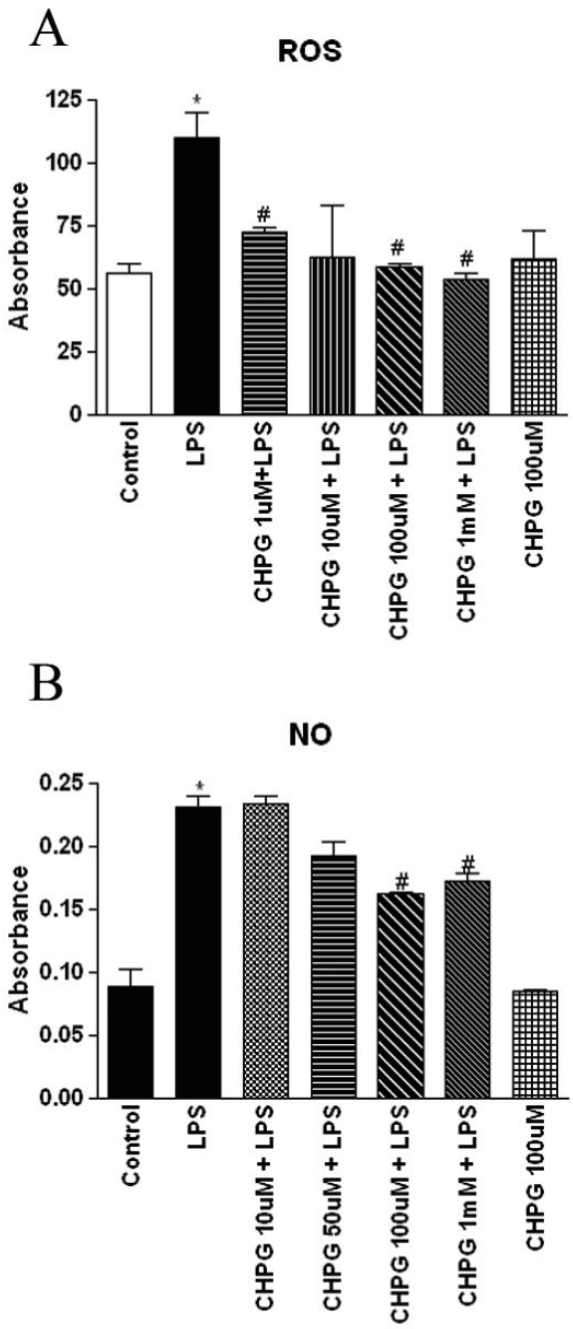

Figure 2.

CHPG inhibits LPS-induced activation in microglia in a dose-dependent manner. Microglial activation was measured by ROS production (A), and NO production (B) at 24 hours after stimulation. CHPG stimulation alone, without LPS, had no effect on ROS or NO production. Bars represent mean +/- SEM; *p<0.05 vs. control; **p<0.001 vs. control; #p<0.05 vs. LPS; ##p<0.001 vs. LPS.

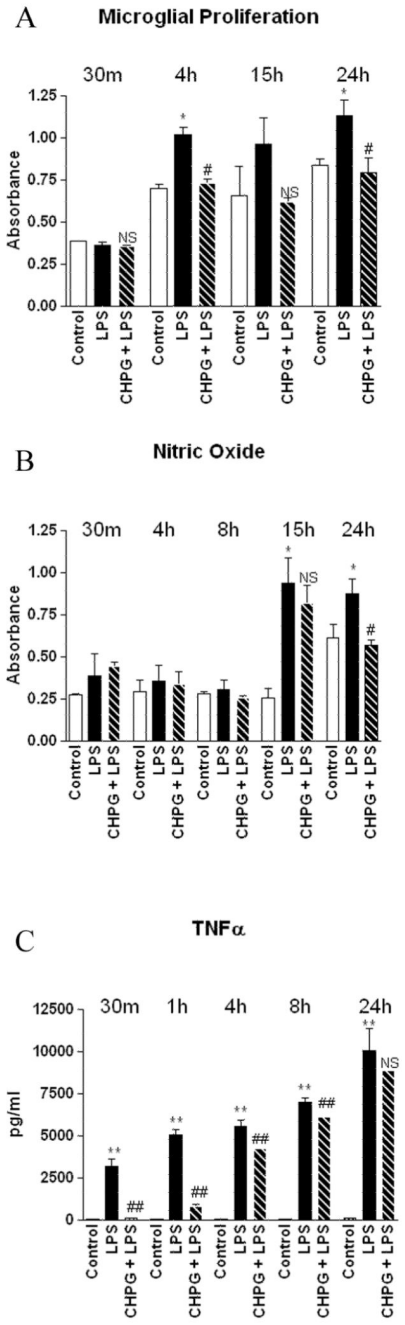

Figure 3.

CHPG inhibits LPS-induced activation in microglia over time. Microglial activation was measured by proliferation (A), NO production (B) and TNFα production (C). All aspects were significantly inhibited by pre-treatment with the mGluR5 agonist, CHPG (100 μM). All measurements were assessed from 30 minutes to 24 hours. Bars represent mean +/- SEM; *p<0.05 vs. control; **p<0.001 vs. control; #p<0.05 vs. LPS; ##p<0.001 vs. LPS.

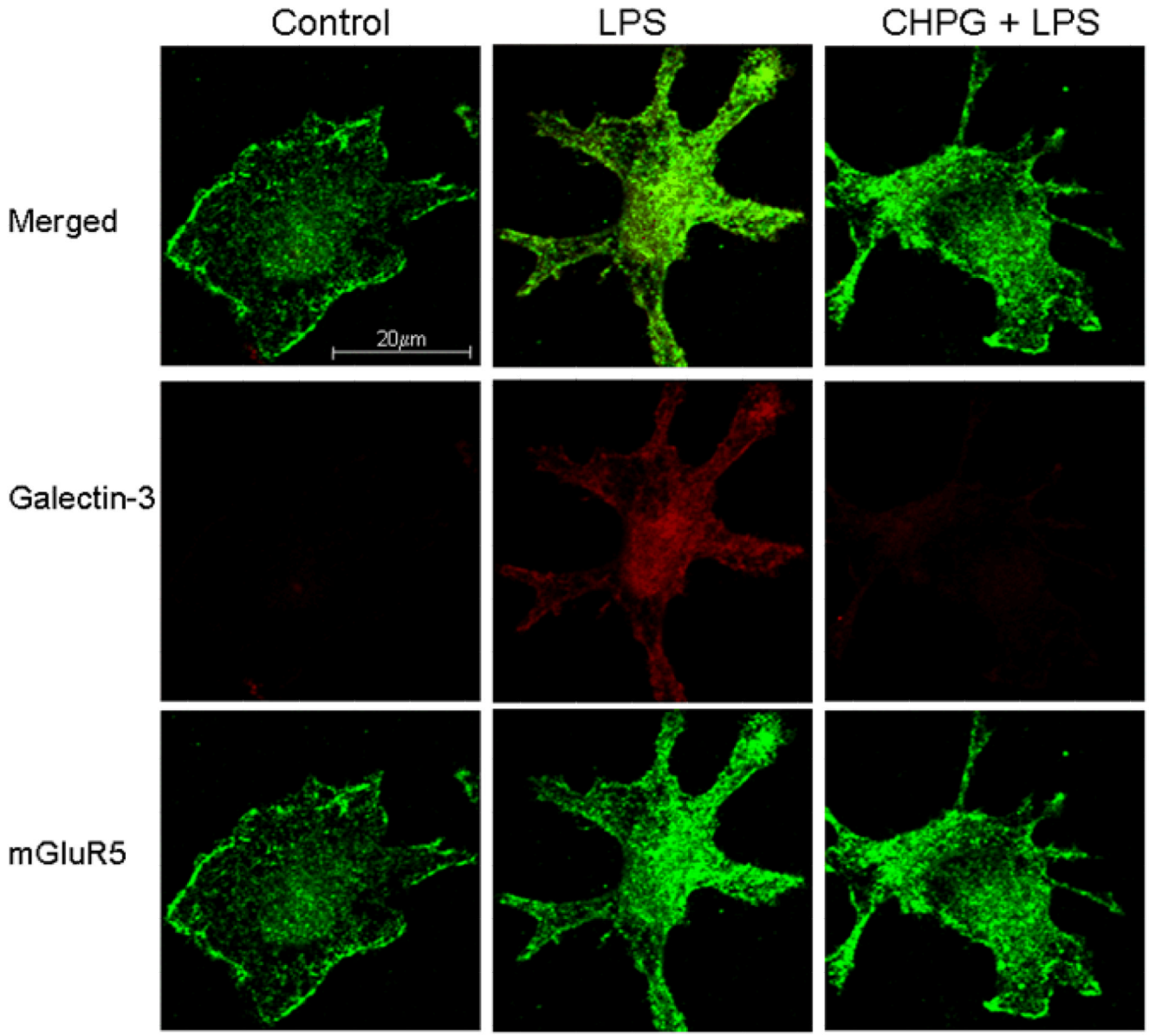

Figure 4.
CHPG inhibits expression of Galectin-3. Microglia, which are mGluR5 positive, do not express Galectin-3 under control conditions. Upon stimulation with LPS, microglia express Galectin-3. This expression is inhibited by CHPG treatment.

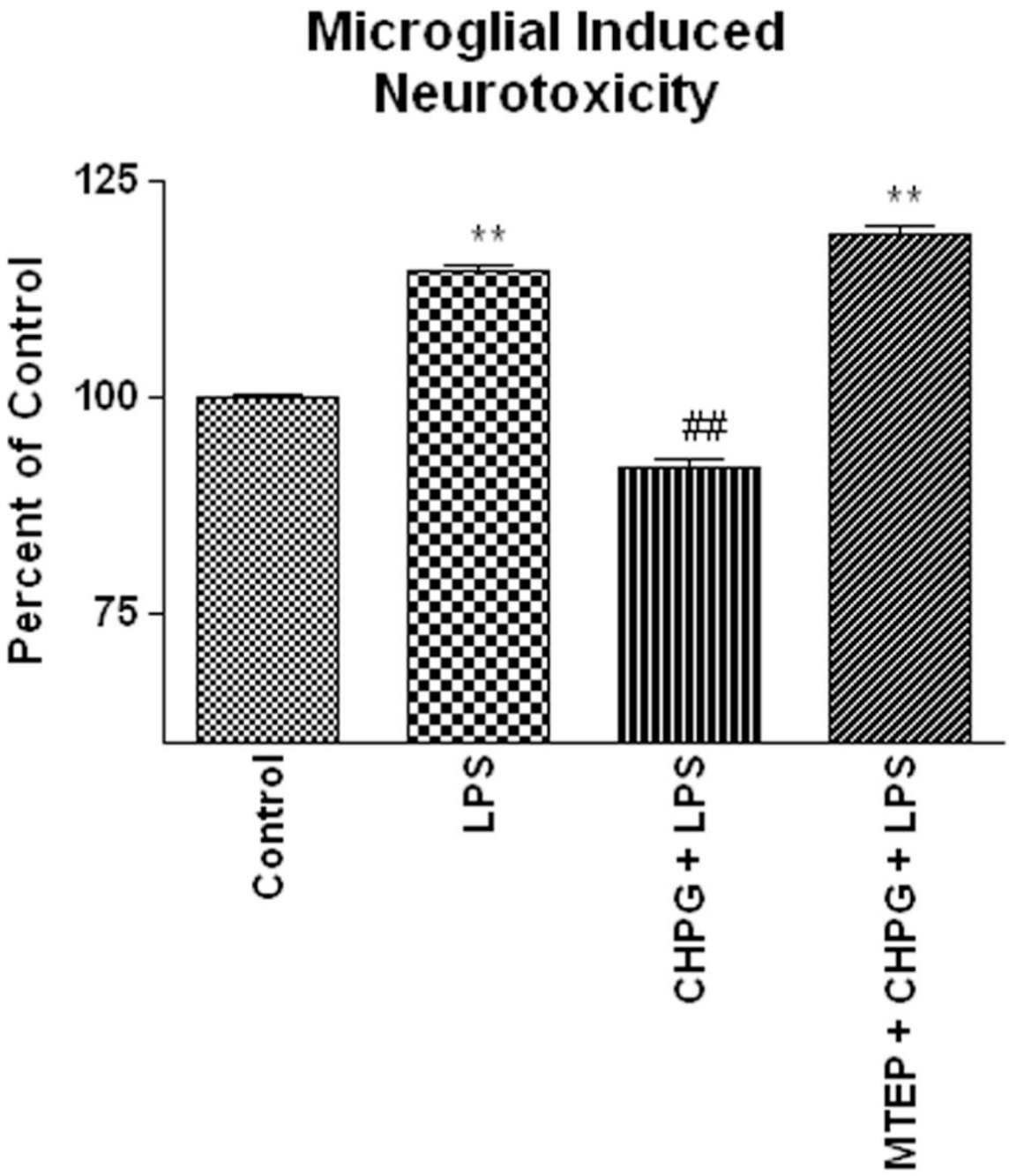

Figure 5.

CHPG inhibits microglial-induced neurotoxicity. LDH release, indicating cell death, was assayed from neuronal media at 24 hours after co-incubation with LPS-stimulated microglia. LDH release was increased by the co-incubation of LPS-stimulated microglia with neurons; this was reversed by the pre-treatment of microglia with CHPG. Addition of the mGluR5 antagonist, MTEP (100 μM), inhibited the effect of CHPG on microglial-induced neurotoxicity, demonstrating an mGluR5-mediated effect by CHPG. Bars represent mean +/- SEM; *p<0.05 vs. control; #p<0.05 vs. LPS; +p<0.05 vs. CHPG + LPS.

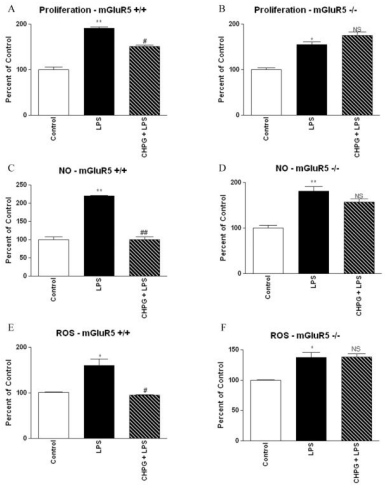

Figure 6.

The effects of CHPG are mediated by the mGluR5 receptor. Microglia cultured from mGluR5 wild-type (+/+, A, C, E) or knockout (-/-, B, D, F) were pre-treated with CHPG prior to stimulation with LPS. LPS resulted in a significant increase in microglial proliferation (A, B), NO release (C, D) and ROS production (E, F) that was attenuated by CHPG in wild-type microglia (A, C, E). However, proliferation and NO production were not attenuated in knockout microglia (B, D, F). Bars represent mean +/- SEM; *p<0.05 vs. control; **p<0.001 vs. control; #p<0.05 vs. LPS; NS: not significant vs. LPS.

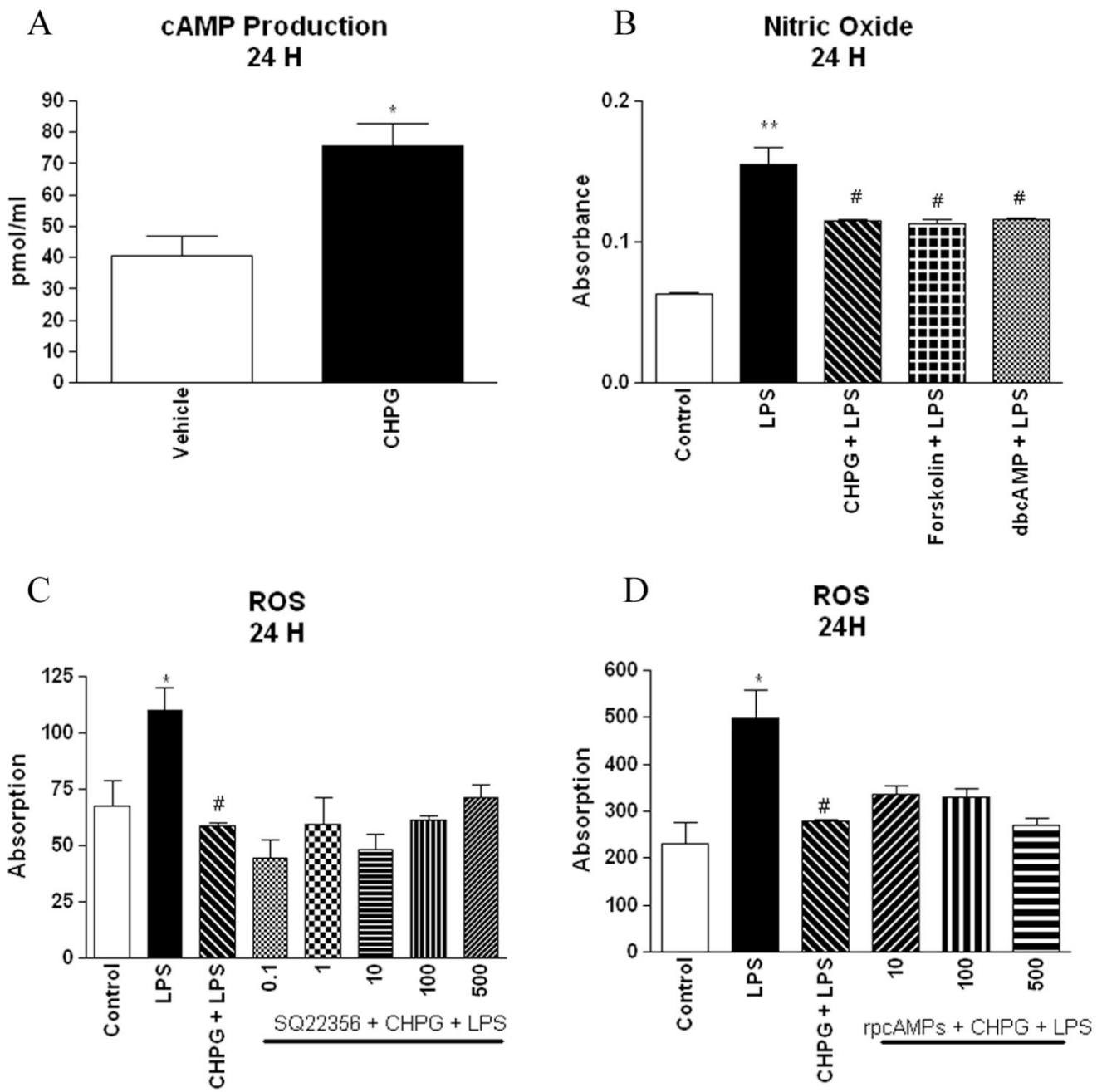

Figure 7.

CHPG induces the production of cAMP, but it is not necessary for CHPG-induced reduction in microglial activity. cAMP expression was increased by CHPG treatment, in comparison to control treatment (A). NO was used as an outcome measure to determine the impact of cAMP inducers forskolin (50μM) and dbcAMP (200 μM) on LPS-stimulated microglia (B). NO production was significantly reduced by CHPG, and similar inhibition was found with forskolin and dbcAMP. However, blocking cAMP production with the adenylyl cyclase inhibitor SQ22356 (0.1 - 500μM; C) or rpcAMPs (10 - 500 μM, D) did not have any significant effect on the reduction in ROS production induced by CHPG. Bars represent mean +/- SEM; *p<0.05 vs. control; **p<0.001 vs. control; #p<0.05 vs. LPS.

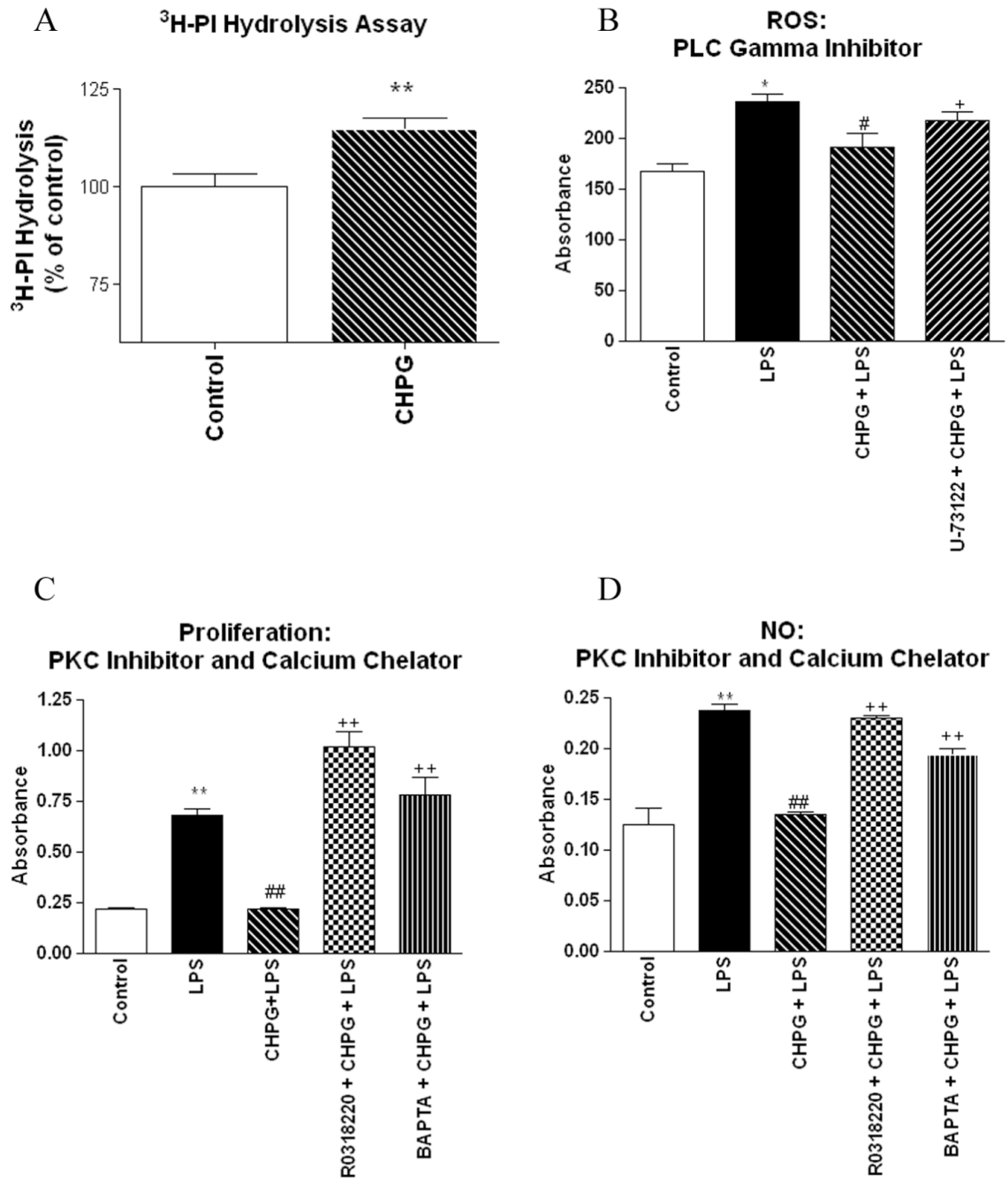

Figure 8.

Transduction pathways involved in CHPG signalling in microglia indicate the involvement of PLC, PKC and calcium. PI hydrolysis, as measured by incorporation of radioactive H into samples, was significantly increased CHPG treated cultures 24 hours after addition of CHPG (A). Pre-treatment of microglial cultures with the PLCβ inhibitor U-73122 blocked the ability of CHPG to reduce LPS-induced ROS production at 10μM (B). CHPG-induced reduction of proliferation was also blocked by the calcium chelator BAPTA-AM and the PKC inhibitor R0318220 (10μM and 1.5μM, respectively; C). R0318220 and BAPTA-AM also inhibited CHPG's effects on NO production (D). Bars represent mean +/- SEM;

*p<0.05 vs. control; **p<0.001 vs. control; #p<0.05 vs. LPS; +p<0.05 vs. CHPG + LPS; + +p<0.001 vs. CHPG + LPS.