Metabotropic glutamate receptors inhibit microglial glutamate release

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ABSTRACT

Pro-inflammatory stimuli evoke an export of glutamate from microglia that is sufficient to contribute to excitotoxicity in neighbouring neurons. Since microglia also express various glutamate receptors themselves, we were interested in the potential feedback of glutamate on this system. Several agonists of mGluRs (metabotropic glutamate receptors) were applied to primary rat microglia, and the export of glutamate into their culture medium was evoked by LPS (lipopolysaccharide). Agonists of group-II and -III mGluR ACPD [(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid] and L-AP4 [L-(+)-2-amino-4-phosphonobutyric acid] were both capable of completely blocking the glutamate export without interfering with the production of NO (nitric oxide); the group-I agonist tADA (trans-azetidine-2,4-dicarboxylic acid) was ineffective. Consistent with the possibility of feedback, inhibition of mGluR by MSPG [(R,S)-α-2-methyl-4sulfonophenylglycine] potentiated glutamate export. As the group-II and -III mGluR are coupled to $G\alpha_i$-containing G-proteins and the inhibition of adenylate cyclase, we explored the role of cAMP in this effect. Inhibition of cAMP-dependent protein kinase [also known as protein kinase A (PKA)] by H89 mimicked the effect of ACPD, and the mGluR agonist had its actions reversed by artificially sustaining cAMP through the PDE (phosphodiesterase) inhibitor IBMX (isobutylmethylxanthine) or the cAMP mimetic dbcAMP (dibutyryl cAMP). These data indicate that mGluR activation attenuates a potentially neurotoxic export of glutamate from activated microglia and implicate cAMP as a contributor to this aspect of microglial action.

Key words: cAMP, G-protein, lipopolysaccharide, microglia, protein kinase A, Xc⁻ transport.

INTRODUCTION

Neuroinflammation, mediated largely by microglia, has been implicated in several different neurological disorders from acute injuries such as stroke to chronic neurodegenerative conditions such as AD (Alzheimer's disease). When exhibiting their full repertoire of responses, microglia produce ROS (reactive oxygen species), proteases, cytokines, prostanoids and NO (nitric oxide). However, there is another mechanism of cytotoxicity in the CNS (central nervous system) that may explain its greater vulnerability to inflammation-related events: excitotoxicity. Evidence suggests that production of EAAs (excitatory amino acids) may be one of the critical determinants of microglial malactivation. Microglia can produce several defined agonists of EAA receptors, including glutamate, quinolinate and D-serine (Barger, 2004). While astrocytes could be expected to clear most of the glutamate exported by microglia under normal conditions, this function is compromised by ROS (Harris et al., 1996; Trotti et al., 1998) or pro-inflammatory cytokines (Kim et al., 2003; Korn et al., 2005). In addition, many microglia can be found intimately abutting neuronal somata and arbors (Wekerle, 2005), and glutamate receptors at such extrasynaptic positions preferentially contribute to excitotoxicity (Sattler et al., 2000). A role for EAAs in neurodegeneration during inflammation is evinced by the protective actions of glutamate receptor antagonists in many models of neuroinflammation (Espey et al., 1998; Mascarucci et al., 1998; Willard et al., 2000; Groom et al., 2003; Bossuet et al., 2004; Rosi et al., 2009). Beyond these experimental models, natural disease correlations also imply a link between neuroinflammation and excitotoxicity; AD, HIV encephalitis, multiple sclerosis, cerebral ischaemia, traumatic brain injury and ALS (amyotrophic lateral sclerosis) all have inflammation–excitotoxicity intersections.

We previously demonstrated that the vast majority of glutamate exported from activated microglia could be attributed to the Xc$^-$ exchange mechanism (Barger and Basile, 2001; Barger et al., 2007), consistent with Piani and Fontana (1994). The Xc$^-$ exchange relies on a glutamate/cystine antiporter that resides in the plasmalemma of most cells. This mechanism becomes extremely active in microglia because it is the primary route of internalizing cystine for the production of GSH. As activated microglia produce abundant ROS, they place themselves under severe oxidative stress. Although much of the superoxide produced by NADPHox (NADPH oxidase) is released from the cell, intracellular generation occurs as well (Kobayashi et al., 2001). Thus, the microglial oxidative burst creates a GSH shortage that is alleviated by cystine influx through the Xc$^-$ antiporter, extruding glutamate in the balance (Barger et al., 2007).

Microglia have been reported to have several types of glutamate receptor, including those of mGluR (metabotropic glutamate receptor) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid) classes (D'Antoni et al., 2008). Metabotropic groups I, II and III are represented on microglia through expression of individual subunits of these types (Noda et al., 2000; Byrnes et al., 2009a). Activation of mGluR from group I or III seem to inhibit microglial activation; group II agonists generally appear to evoke malactivation, although an agonist of mGluR3 – a member of group-II – inhibited the toxicity exhibited by microglia towards oligodendrocytes (Pinteaux-Jones et al., 2008). The individual mGluR groups are coupled to various G-proteins, activating PLC (phospholipase C; group I) or inhibiting adenylate cyclase (groups II and III). At least two antagonists of group-I mGluR have been reported to inhibit the activity of the Xc$^-$ system (Bridges et al., 2012), but inactivity of other group-I antagonists (Melendez et al., 2005) suggests that the effect on Xc$^-$ is not mediated by group-I mGluR. We have initiated a survey of the potential for glutamate to exert, via glutamate receptors, feedback on its own export from microglia.

MATERIALS AND METHODS

Materials
AMPA; DNQX (6,7-dinitroquinoxaline-2,3-dione); ACPD [(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid]; L-AP4 [L-(+)-2-amino-4-phosphonobutyric acid]; tADA (*trans*-azetidine-2,4-dicarboxylic acid); and MSPG [(R,S)-α-2-methyl-4sulfonophenylglycine] were from Tocris.

Primary microglia
Mixed glial cultures were established from neonatal (P0–P1) Sprague–Dawley rats (Harlan) as described previously (Barger and Basile, 2001). This use of animals was reviewed and approved by the Institutional Animal Care and Use Committee. Cultures were maintained in MEM (minimal essential medium; Invitrogen), Earle's salts supplemented to 10% with FBS (foetal bovine serum; Atlanta Biologicals) and 10 μg/ml gentamycin (Sigma). After approx. 16 days, the cultures were subjected to vigorous lavage to remove the microglia from the confluent astrocyte monolayer. The cell suspension was subjected to centrifugation at 110 g, and the pellet was resuspended in the complete culture medium described above and plated at a density of 375000 cells/cm^2 in 96-well plates (glutamate, nitrite and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assays) or 35-mm plates [qRT–PCR (quantitative reverse transcriptase–PCR)]. After 20 min, the medium was exchanged to remove most of the contaminating astrocytes. Experimental treatments were initiated within 24 h of plating, after changing the medium to serum-free MEM.

Glutamate assays
Accumulation of glutamate in the culture medium was assayed by monitoring the reaction catalysed by glutamate dehydrogenase, using iodonitrotetrazolium chloride as a colorimetric indicator, all supplied as a kit (R-Biopharm). Conversion of the iodonitrotetrazolium chloride into formazan was assessed as absorbance at 490 nm using a spectrophotometric plate reader (Bio-Rad). Standard curves were generated from spectrophotometer readings of reactions with known concentrations of glutamate in MEM. All other readings obtained were interpolated in the standard curve to achieve the corresponding glutamate concentration.

Nitrite assays
The accumulation of nitrite was used as an index of NO production and inflammatory activation in general. Medium samples from cell cultures were incubated with an equal volume of Griess reagent and assessed for absorbance at 540 nm as described previously (Barger et al., 2000).

MTT assay
After removal of the culture medium for glutamate and nitrite assays, cell numbers were assessed by their ability to effect chemical reduction of MTT (Sigma) to formazan. Briefly, MTT was added to all wells at 125 μg/ml in MEM/FBS and incubated (37℃, 5.5% CO$_2$) for 30 min. Then, all medium was removed from each well, and the formazan crystals were dissolved in 100 μl of DMSO. The plate was then read at 540 nm in a spectrophotometer.

Real-time (q) RT–PCR
Total RNA was prepared from microglia cultures with the RNeasy kit (Qiagen); RNA quality and quantity were assessed on an Agilent Bioanalyzer (Agilent). Two-step, real-time RT–PCR was performed on the unique subunit (xT) of the Xc

transporter. The RT reaction utilized 500 ng of RNA and TaqMan reverse transcription reagents (including random hexamers for priming). PCR was performed with the Power SYBR-Green PCR Master Mix (Applied Biosystems) in an ABI 7900HT Fast Real-time PCR System (Applied Biosystems). The xCT signal was quantified relative to that for 18S rRNA. Equal amounts of RT–PCR from each sample were pooled for use in standard curve reactions with each primer set. A melting curve was generated for both xCT and 18S rRNA to ensure that a single peak of the predicted T_m was produced and no primer–dimer complexes were present. The relative standard curve method was used to calculate the amplification difference between the samples for each primer set. This is performed by correcting for signal concentration with the concentration of 18S signal for each sample (signal conc./18S conc.). The xCT primers used were as follows; forward: 5′-CCC AGA TAT GCA TCG TCC TT; reverse: 5′-ACA ACC ATG AAG AGG CAG GT. Primer sequences for 18S were forward: 5′-TTC GAA CGT CTG CCC TAT CAA-3′; reverse: 5′-ATG GTA GGC ACG GCG ACT A-3′.

Statistics

All data are representative of at least three replicated experiments. Concentration–response curves were analysed by ANOVA and Scheffe post-hoc test. Pairwise comparisons were made by Student's t-test. Values of P less than or equal to 0.05 were taken to be significant.

RESULTS

As previously reported, LPS (lipopolysaccharide) treatment of microglia caused an increased accumulation of glutamate in the culture medium. We were interested in exploring the potential consequences of feedback stimulation of glutamate receptors on microglia. While AMPA receptors desensitize rapidly and thus produce only small currents in the absence of cyclothiazide (Noda et al., 2000), we have observed large increases in intracellular calcium concentrations via AMPA receptors in a subset of microglia (data not shown). Eun et al. (2004) observed gene expression changes in microglia resulting from AMPA treatments at 500 μM. However, co-application of 30–300 μM AMPA had no appreciable effect on the export of glutamate triggered by LPS (Figure 1A). Indeed, there appeared to be a trend towards inhibition when AMPA receptors were antagonized by DNQX; this may have resulted from toxicity of the latter, however, as indicated by MTT assays of cellular metabolism (Figure 1B). ACPD, an agonist of group-I and-II mGluR, inhibited the LPS-triggered glutamate export substantially and dosimetrically (Figure 1A) without toxicity. To assess whether this effect was specific to glutamate or indicated a generalized anti-inflammatory effect, we assayed nitrite accumulation as a surrogate marker of NO production. ACPD had no apparent effect on microglial
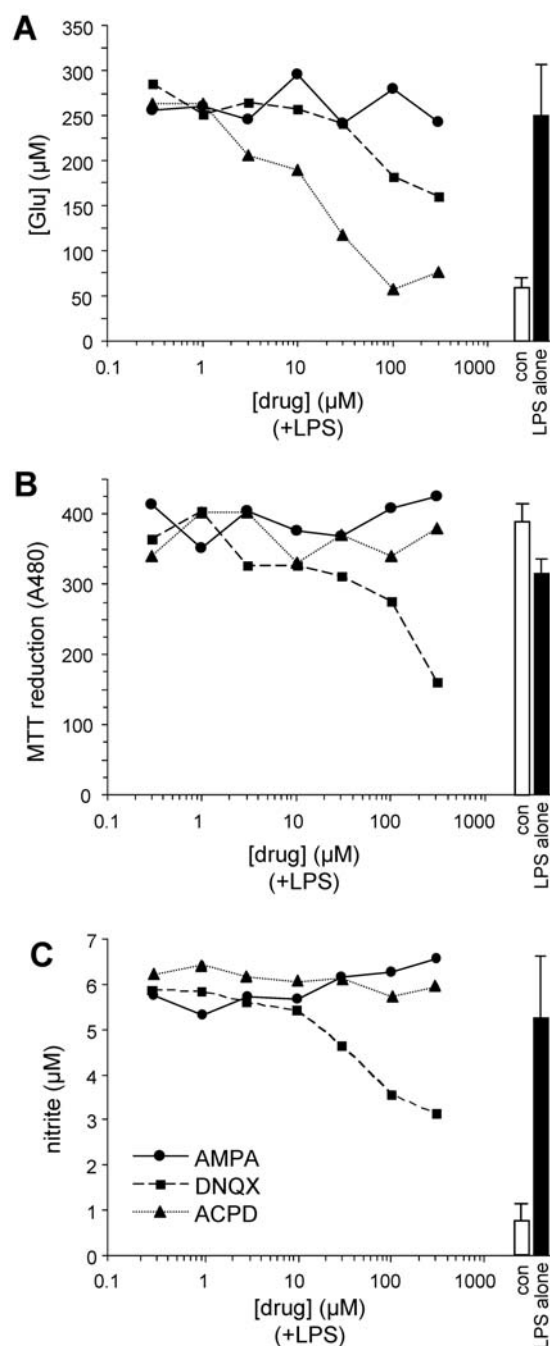

Figure 1 Specific inhibition of the export of glutamate from microglia by an mGluR agonist
Rat primary microglia were treated with the indicated concentrations of AMPA (●), DNQX (■) or ACPD (▲). LPS was then applied at 30 ng/ml. After 20 h, the culture medium was assayed for glutamate (**A**) and nitrite (**C**), and the cells were assayed for MTT reduction (**B**). The values obtained in untreated cultures are shown by the white bars ('con'), and the values obtained in cultures treated with LPS alone are shown by the black bars ('LPS alone'). Means of quadruplicate determinations are shown (+S.E.M. for 'con' and 'LPS alone'). Differences were statistically significant ($P\leq0.05$) for ACPD in (**A**) and for DNQX in (**B**) and (**C**).

production of NO (Figure 1C), suggesting a specific effect on some aspect of the chain of events leading to glutamate release. These findings indicate that glutamate exported from activated microglia may exert negative feedback on this event through the activation of mGluR.

The mGluR receptors stimulated by ACPD are groups I and II. Group-I receptors are coupled to activation of PLC and a resultant activation of PKC (protein kinase C) and release of intracellular calcium stores. If activation of PKC mediated the effect of ACPD, it might be expected that glutamate export would be similarly inhibited by direct activation of PKC or that the effects of ACPD would be sensitive to inhibitors of PKC. However, the PKC activator phorbol myristic acid was not able to mimic the effect of ACPD, and the PKC inhibitor bisindolylmaleimide I did not suppress the effect of ACPD (data not shown). Moreover, ACPD had no acute influence on intracellular calcium concentrations (data not shown). These findings are not consistent with activation of PLC. Therefore, we tested whether the effect of ACPD was mediated by group-II receptors, which are coupled with inhibition of adenylate cyclase via G-proteins containing the α_i subunit. Utilization of this signal-transduction pathway should thus require reduction in the levels of cAMP. This possibility was tested by examining the ability of ACPD to inhibit LPS-evoked glutamate export in the presence of IBMX (isobutylmethyl-xanthine), an inhibitor of the PDEs (phosphodiesterases) responsible for degrading cAMP. The raw glutamate-export data from these experiments produced an unusual concentration–response curve over concentrations of IBMX (Figure 2A). MTT assays suggested substantial toxicity at the two highest concentrations of this agent. To examine the glutamate being exported from live, healthy cells, we reanalysed the glutamate value of each culture well relative to the cellular metabolism (MTT value) for that well. This analysis removed the effect of presumed IBMX toxicity, revealing a concentration-dependent reversal of the ACPD effect by IBMX (Figure 2B). The dependency of ACPD's inhibition on cAMP modulation was further tested by the application of dbcAMP (dibutyryl cAMP). This agent had an effect similar to that of IBMX without evidence of toxicity (Figure 2C). Most effects of cAMP are mediated through PKA; therefore we tested the effects of PKA inhibitor H89 on glutamate release. H89 concentration-dependently mimicked the effect of ACPD, reducing the glutamate export triggered by LPS (Figure 3). Neither IBMX, dbcAMP nor H89 had any effect on glutamate export on its own; dbcAMP and H89 had no effect on MTT assessments of viability (data not shown).

These results suggest that the depression of glutamate export by ACPD requires a reduction in the levels of cAMP, most probably afforded by activation of Gα_i by group-II mGluR. ACPD is an agonist of group-I and -II mGluR. To further examine the role of Gα_i-coupled receptors in the attenuation of glutamate release, we tested agonists more specific to mGluR of groups I and III. Application of L-AP4, a type-III agonist, reproduced the effects of ACPD precisely (Figure 4A). However, the type-I agonist tADA had no effect (Figure 4B).
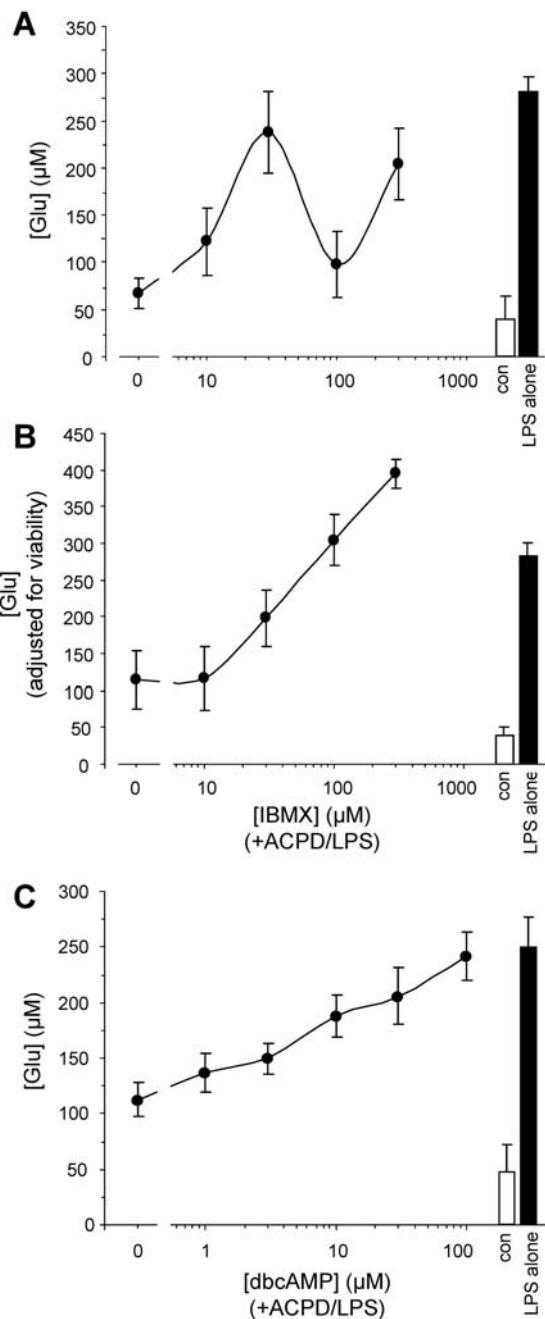
Figure 2 Inhibitory effect of ACPD reversed by agents sustaining cAMP
Rat primary microglia were treated with 100 μM ACPD and the indicated concentrations of IBMX (**A**, **B**) or dbcAMP (**C**). LPS was then applied at 30 ng/ml. After 20 h, the culture medium was assayed for glutamate, and the cells were assayed for MTT reduction. Raw values for glutamate are reported in (**A**) and (**C**). In (**B**), the glutamate values are shown after factoring by a denominator based on the MTT value and hence report a value related to the amount of glutamate exported per live cell. The values obtained in untreated cultures are shown by the white bars ('con'), and the values obtained in cultures treated with LPS alone are shown by the black bars ('LPS alone'). Values represent the means ± S.E.M. of quadruplicate determinations. Above 10 μM, both IBMX and dbcAMP produced differences that were statistically significant.
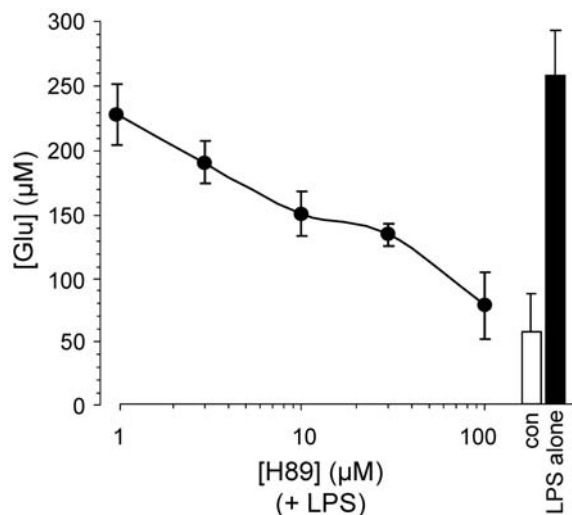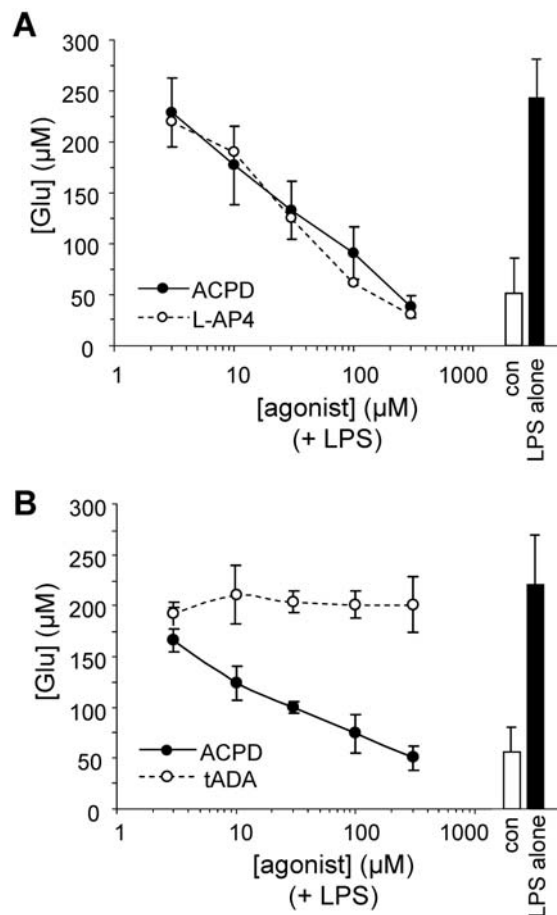
Figure 3 Inhibition of cAMP-dependent protein kinase mimics the effect of ACPD
Rat primary microglia were treated with the indicated concentrations of H89. LPS was then applied at 30 ng/ml. After 20 h, the culture medium was assayed for glutamate. The value obtained in untreated cultures is shown by the white bar ('con'), and the value obtained in cultures treated with LPS alone is shown by the black bar ('LPS alone'). Values represent the means $\pm$ S.E.M. of quadruplicate determinations. Above 3 µM, values were significantly different from LPS alone.

The ability of group-II and -III agonists to inhibit glutamate export demonstrates sufficiency in impacting this phenomenon. If glutamate accumulating extracellularly actually has the potential to moderate its own export via activation of these receptors, antagonists would be expected relieve this squelching effect and cause an even larger accumulation of glutamate extracellularly. To assess the impact of mGluR activation by ambient glutamate, we measured glutamate accumulation in the presence of MSPG, a group-II and -III antagonist (Jane et al., 1995; cf. Chung et al., 1997). Indeed, MSPG inhibited the effect of ACPD, confirming that the latter exerted its effect via mGluR (Figure 5A). When MSPG was applied in the face of glutamate export elicited by a modest amount of LPS (2 ng/ml), there was a trend towards amplification of glutamate levels in the conditioned medium corresponding to MSPG concentration, culminating in a significant elevation at 300 µM MSPG (Figure 5B). While a trend towards greater glutamate release was observed in response to MSPG alone, the trend remained below the level of significance.

Together, the above data indicate reductions in cellular cAMP inhibit glutamate export from microglia activated by a pro-inflammatory stimulus. Such release was previously found to depend on the Xc$^-$ exchange system, which reciprocally transports cystine and glutamate (Barger et al., 2007). Expression of the unique subunit of this transporter, xCT, is dependent on a cAMP response element in the promoter of its gene. As an initial step, we analysed mRNA levels of xCT in response to manipulation of the cAMP system. Consistent with previous findings, dbcAMP and IBMX elevated the expression of xCT (Figure 6A). ACPD was able to suppress the effect of


Figure 4 Group-II and Group-III mGluR mediate inhibition of microglial glutamate export
Rat primary microglia were treated with the indicated concentrations of ACPD (●), L-AP4 (○, **A**) or tADA (○, **B**). LPS was then applied at 30 ng/ml. After 20 h, the culture medium was assayed for glutamate. The values obtained in untreated cultures are shown by the white bars ('con'), and the values obtained in cultures treated with LPS alone are shown by the black bars ('LPS alone'). Means of quadruplicate determinations are shown (+S.E.M. for 'con' and 'LPS alone'). Differences were statistically significant ($P \leq 0.05$) for ACPD in (**A**) and (**B**) and for L-AP4 in **A**.

IBMX, an effect that may owe to the low concentration of IBMX applied, necessary to avoid cytotoxicity. Next, we tested LPS and ACPD to determine if the effects of the latter might involve suppression of xCT transcription via reduction in cAMP levels. LPS stimulated a considerable increase in expression, as demonstrated previously (Barger et al., 2007), but ACPD was unable to suppress this response (Figure 6B).
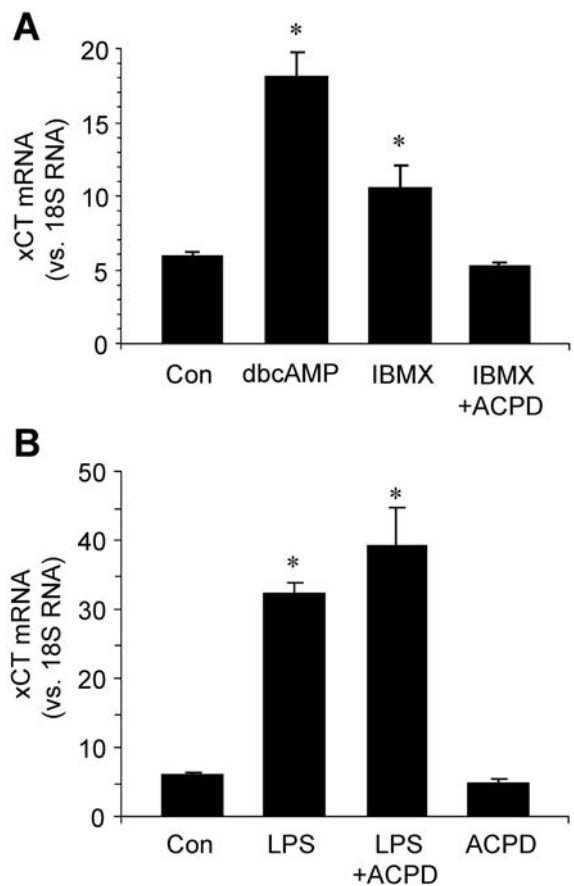
DISCUSSION

When activated by pro-inflammatory stimuli, microglia export substantial levels of glutamate. The consequences could range from interference with normal neurotransmission
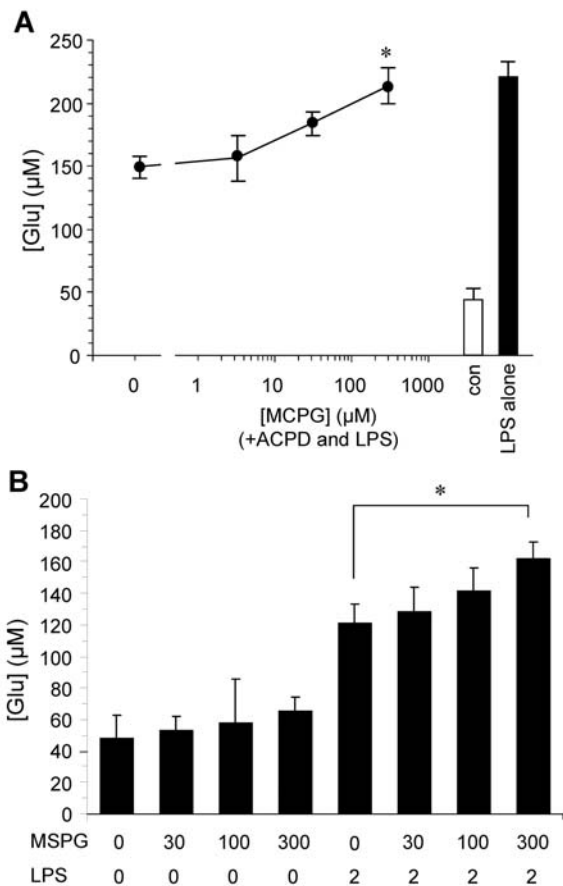
Figure 5 Blockade of mGluR accentuates glutamate export
Rat primary microglia were exposed to MSPG at the concentrations indicated (μM) and culture medium was assayed for glutamate after 20 h. (**A**) Cultures were treated with 30 μM ACPD and 30 ng/ml LPS, with or without MSPG (*$P<0.05$ against 0 MSPG). The values obtained in untreated cultures are shown by the white bar ('con'), and the values obtained in cultures treated with LPS alone are shown by the black bar ('LPS alone'). (**B**) Cultures were treated with MSPG alone or in combination with LPS (2 ng/ml) (*$P<0.05$).

Figure 6 ACPD does not interfere with induction of the xCT subunit of the Xc⁻ transporter
Rat primary microglia were treated for 20 h, then RNA was prepared and subjected to quantitative RT–PCR for xCT mRNA and 18S rRNA. Values are reflected as the ratio of the xCT signal to that of 18S rRNA and represent the means ± S.E.M. of triplicate determinations. (**A**) Cultures were treated with dbcAMP (100 μM), IBMX (30 μM) or IBMX+ACPD (the latter at 100 μM). *$P≤0.01$ against control or the combination of IBMX+ACPD. (**B**) Cultures were treated with LPS (30 ng/ml), ACPD (100 μM) or a combination of the two. (*$P≤0.01$ against control or ACPD alone)

to excitotoxicity for neurons in the vicinity. A greater understanding of the mechanisms controlling the glutamate export may lead to therapeutic alleviation of the neurode-generative effects of neuroinflammation in various conditions such as in multiple sclerosis, brain abscess, ischaemia, traumatic brain injury and AD. Here, we have documented the potential for a feedback mechanism by which glutamate exported from microglia acts through group-II and -III mGluR. In the presence of ACPD (a group-I and -II agonist) or L-AP3 (a group-III agonist), the amount of glutamate exported in response to LPS was suppressed, while accumulation of nitrite was unaffected; also unaffected was the induction of xCT expression in response to LPS. Antagonism of group-II and -III receptors by MSPG created conditions permissive for a super-induction of glutamate export in response to LPS. These data imply that glutamate, including that exported by activated microglia themselves, can act on mGluR to suppress some facet of the glutamate export

mechanism without having an impact on the fundamental pro-inflammatory activation of microglia. This phenomenon may influence the ultimate levels of glutamate that are exported from microglia.

Additional data suggest that the influence of mGluR activations in this system occurs through modulation of the levels of the intracellular second messenger cAMP. ACPD and L-AP4 both act on mGluR that activate Gα_i to inhibit adenylate cyclase. The effect of ACPD was reversed by IBMX and dbcAMP. IBMX inhibits PDEs and consequently stabilizes the levels of cAMP, even after inhibition of adenylate cyclase by Gα_i; dbcAMP functionally replaces cAMP lost through such inhibition. Finally, an inhibitor of cAMP-dependent protein kinase acted like ACPD and L-AP4 to suppress the glutamate release. We interpret our results as evidence that group-II and group-III mGluR inhibit glutamate export through a suppression of cAMP levels. Although ACPD can also activate group-I mGluR, these receptors do not appear to be involved

in the phenomenon observed, as glutamate export was unaffected by tADA or by manipulations of PLC cascades, the signal-transduction pathway activated by group-I mGluR. MSPG appeared to confirm an active feedback inhibition, as LPS exerted a greater effect in the presence of this group-II and -III antagonist; and while MSPG has often been characterized as a 'non-selective' mGluR antagonist, it fails to modulate inositol phospholipid metabolism stimulated by group-I agonists (Chung et al., 1997). The lack of involvement of group-I mGluR contrasts somewhat with other evidence that activation of these receptors attenuates the neurotoxicity exhibited by activated microglia (Byrnes et al., 2009b). On the other hand, group-III mGluR have been reported to exert a similar influence on microglial neurotoxicity (Taylor et al., 2003).

At this point, it is not clear what dictates the role of cAMP in microglial activation. Clearly, this second messenger contributes differentially in different paradigms. Elevations of cAMP have been associated with both stimulatory (Sowa and Przewlocki, 1994; Mullet et al., 1997; Park et al., 1997) and inhibitory (Peters-Golden, 2009) effects on macrophage activation (Okado-Matsumoto et al., 2000). In microglia, there is ambivalent data as well. Bradykinin inhibits microglial release of TNF (tumour necrosis factor) and IL-1 β (interleukin-1β) in a cAMP-dependent manner, and this effect is mimicked by dbcAMP (Noda et al., 2007). More general inhibitions of microglial activation by cAMP-dependent mechanisms are well documented (Colton and Chernyshev, 1996; Aloisi et al., 1999; Caggiano and Kraig, 1999; Zhang et al., 2002). However, agents that elevate or mimic cAMP have been shown to potentiate NO release stimulated by Aβ (amyloid β-peptide) in microglia (Pyo et al., 1999), and PKA or AC inhibitors reduced nitrite production in microglia stimulated with adrenaline (Dello Russo et al., 2004).

It bears reiterating that the cAMP-dependent inhibition of glutamate export documented here was not accompanied by inhibition of LPS's ability to elevate xCT mRNA. As predicted by the findings of Gochenauer and Robinson (2001), agents that elevate or mimic cAMP here boosted expression of xCT. ACPD suppressed the effect of a modest concentration of IBMX in this regard (its effects against dbcAMP were not tested, as dbcAMP essentially bypasses adenylate cyclase, the point of impact of mGluR agonism). However, ACPD did not suppress the gene induction stimulated by LPS. This may indicate that LPS and cAMP enhance transcription of xCT via distinct mechanisms, but it must also be considered that the strength of stimulus was simply too large to overcome in the case of LPS. Nevertheless, the data add to a growing body of evidence that Xc⁻ transport activity is not strictly correlated with xCT expression. For instance, we previously showed that inhibitors of macromolecular synthesis had little effect on the export of glutamate evoked by LPS (Barger et al., 2007). Of course, xCT gene transcription is not tantamount to protein synthesis and trafficking to its site of action in the plasmalemma. There may exist additional mechanisms of regulation by post-translational modification

of the transporter subunits, but we have also argued for consideration of simple mass-action stimulation, predicated on the depletion of GSH in activated microglia (Barger et al., 2007).

The mGluR- and cAMP-dependent modulations of glutamate export also failed to correlate with another response to pro-inflammatory activation: NO generation. NO has diverse effects in the CNS, but some evidence suggests that its production by NOS (nitric oxide synthase) – 2 largely in non-neuronal cells – has a neuroprotective effect, perhaps in contrast with the NO generated within neurons by NOS-1 (Barger and Basile, 2001; Colton et al., 2006 and references therein). Together, these findings suggest that a reduction in cAMP levels might have a modulatory effect on pro-inflammatory activation that is uniquely beneficial for the CNS, predicated on the unique impacts of glutamate (i.e., neurotransmission and excitotoxicity) in this compartment. Perhaps, cAMP signalling represents an inroad that could be exploited to shift the microglial response to one that retains beneficial aspects of activation while attenuating the threat of glutamatergic toxicity.

In conclusion, our results suggest that activation of Gα_i-coupled receptors, including group-II and group-III mGluRs, suppresses the export of potentially neurotoxic glutamate from activated microglia. As the effect may be limited to the glutamate release, the findings behove an exploration of strategies aimed at this modality in order to shift microglial responses to those that retain beneficial aspects (e.g., phagocytosis, production of neurotrophins) while attenuating the maladaptive bent towards excitotoxicity. Development of pharmacological strategies in this tack may benefit from mechanistic determination of the cAMP-dependent events in this effect.

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