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short report

Metabotropic glutamate receptor 4 positive allosteric modulators attenuate LPS-induced inflammation in microglial cells

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Abstract

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that contain a binding site for glutamate. mGluR4 is localized to presynaptic terminals in the substantia nigra pars compacta (SNpc). Previous studies have shown that mGluR4 plays a functional neuroprotective role in neurodegenerative disorders such as Parkinson's Disease (PD), but the role of mGluR4 in inflammatory response is currently unknown. In this study, we examined whether treatment of microglial cells with the mGluR4 positive allosteric modulators (PAMs) ADX and VU813 afforded protection against lipopolysaccharide (LPS)-induced inflammatory response. We found that pre-treatment of microglial cells with 1 nM, 10 nM, and 100 nM ADX and 1 nM, 100 nM, and 1000 nM VU813 attenuated LPS-induced tumor necrosis factor (TNF) release, suggesting that administration of the PAMs attenuates the pro-inflammatory response. These findings indicate that mGluR4 PAMs are not only protective against motor dysfunction in PD, but also have anti-inflammatory properties, suggesting that they have potential as novel therapeutic agents for the treatment of PD.

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative movement disorder in the United States. The substantia nigra pars compacta (SNpc) and striatum are the brain areas most affected in PD,[1] and the areas in which dopaminergic neurons and axonal projections are highly concentrated. PD causes progressive loss of dopaminergic neurons, resulting in motor deficits, as well as an increased accumulation of aggregated forms of the protein α-synuclein (α-syn) in neurons in the SNpc and in other parts of the brain. Such accumulation leads to microglial activation, production of inflammatory cytokines and chemokines, T-cell infiltration, and neurodegeneration.[2]

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors found on microglia that contain a binding site for glutamate. Upon the binding of glutamate to mGluRs, biochemical signaling cascades are activated that lead to changes in synaptic excitability and modulation of neuronal postsynaptic responses of neurons.[2]

mGluR4 has been found to be localized on presynaptic terminals in the SNpc[3] and has been shown to play a functional neuroprotective role in neurodegenerative disorders, but the exact relationship between mGluR4 localization and this role is unknown.[4] mGluR4 agonists, such as L-AP4, may therefore exert neuroprotective effects by increasing mGluR4 expression. Previously, selective activation of mGluR4 in rat models of PD was found to be functionally neuroprotective and also preserve motor function, suggesting that mGluR4 can be selectively targeted for PD therapies.[5, 6]

Inflammation in the brain is believed to play an important role in the neuronal cell death pathway in PD. Inflammation in the CNS is initiated by microglia, the resident immune cells of the central nervous system, which are responsible for responding to neuronal damage and removing damaged cells.[2] Inflammation and mGluR4 are both involved in PD progression, but the relationship between the two remains unknown. Although it has been shown that activation of mGluR4 is protective in PD,[4] possible anti-inflammatory properties have not yet been elucidated.

Allosteric binding sites, a target in drug therapies, are topographically different from endogenous binding sites, allowing for co-occupation of a single receptor by the ligand and an allosteric modulator. Positive allosteric modulators (PAMs) enhance the affinity of an agonist to bind to a binding site and have been shown to be protective in PD models.

Lipopolysaccharide (LPS) is a large molecule made up of a lipid and polysaccharide found in the outer membrane of Gram-negative, pathogenic bacteria. Upon binding to Toll-like receptor 4 (TLR4) expressed on immune cells such as microglia, LPS induces a strong pro-inflammatory cytokine response. Tumor necrosis factor (TNF, also referred to as TNFα) is a cytokine produced by LPS-activated immune cells and promotes an inflammatory response by activating surrounding cells (Figure 2).[7] In this study, we aimed to determine whether treatment of primary microglia cells with the synthetic mGluR4 PAMs ADX and VU813 attenuated lipopolysaccharide (LPS)-induced inflammation.

Materials and Methods

Microglia extraction

Primary microglia were isolated from wild type (WT) postnatal day 0 to 2 mouse pups as described by previously published protocols.[2] The brains were isolated, the meninges were removed, and the cells were allowed to dissociate. Mixed glial populations were plated and grown for 11 days or until they reached confluency. Upon confluency, microglia were isolated from the astrocyte bed by a mechanical shaking method previously described.[2] Prior to treatment, microglia were plated and allowed to adhere for 1 hour in serum-free and glutamate-free Sigma DMEM nutrient mixture.

Microglia treatment

After adhesion to the chamber slides, microglia were pre-treated with 1 nM, 10 nM, and 100 nM ADX and 1 nM, 100 nM, and 1000 nM VU813. As a positive control, microglial cells were also pre-treated with an mGluR4 agonist, L-AP4. Fifteen minutes after the addition of ADX, VU813, or L-AP4, 100 ng·mL^{-1} LPS was added to the cultures for 24 hours. At the end of the 24 hour treatment period, media was collected and analyzed by enzyme-linked immunosorbent assay (ELISA). The collected media was run according to the guidelines of the R&D Systems mouse TNF-a DuoSet ELISA assay.

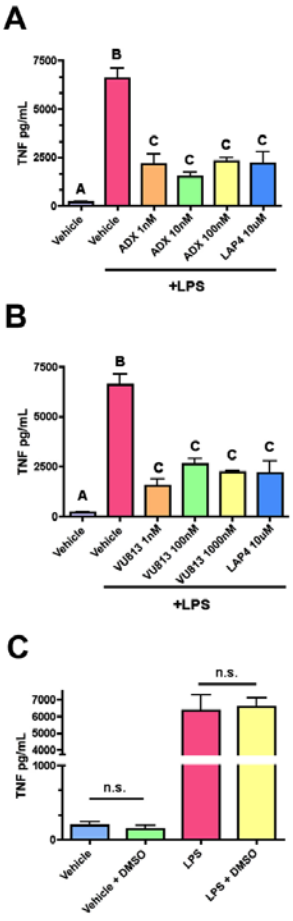

Figure 1. Administration of mGluR4 PAMs protects against LPS-induced TNF release from microglial cells. (A) Administration of ADX at the concentrations of 1 nM, 10 nM, and 100 nM 15 minutes prior to 100 ng·mL^{-1} LPS treatment attenuates TNF release as measured by ELISA. (B) Administration of VU813 at concentrations of 1 nM, 100 nM, and 1000 nM 15 minutes prior to 100 ng·mL^{-1} LPS stimulation attenuates TNF release as measured by ELISA. (C) Treatment of primary microglia with 0.1% DMSO did not induce significant TNF release over vehicle and LPS controls. Data was analyzed by one-way ANOVA with Tukey's multiple comparison post-hoc test. A-B $p<0.001$, B-C $p<0.001$, A-C $p<0.05$, n.s. not significant.

Results

In this study, a TNF ELISA was used to measure the amount of LPS-induced TNF released by the microglia. We found that pre-treatment with ADX and VU813 attenuated the LPS-induced TNF release by microglial cells (Figure 1A, 1B), indicating that mGluR4 PAMs and the mGluR4 agonist L-AP4 have anti-inflammatory properties.

In order to ensure the anti-inflammatory effect of ADX and VU813 was mediated by the PAMs themselves and not the DMSO vehicle, we prepared primary microglial cells as explained above and treated with 0.1% DMSO for 15 minutes prior to stimulation with LPS for 24 hours. We found that the concentrations of TNF in the serum-free media and DMSO vehicle samples were relatively similar. This effect was also observed with LPS treatment (Figure 1c).

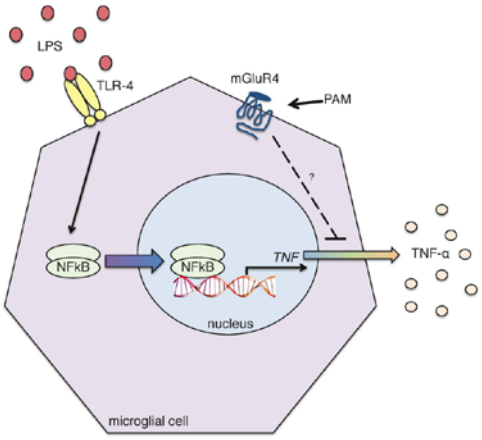

Figure 2. Schematic showing that LPS binds to TLR-4 on microglial cells, initiating a signaling cascade that results in TNF gene transcription and subsequent release from the cell. mGluR4 PAMs bind to mGluR4 receptors expressed on the surface of microglial cells, where downstream events result in the inhibition of TNF release from the cell.

Discussion

The decreased levels of TNF expression in the samples treated with ADX and VU813 support our initial hypothesis that ADX and VU813 attenuate LPS-induced inflammation in microglia.

As shown in Figure 1, the level of attenuation achieved by ADX at concentrations of 1 nM, 10 nM, and 100 nM and VU813 at concentrations of 1 nM, 100 nM, and 1000 nM were relatively similar, suggesting that the drug doses administered exceeded the dose response, but were still highly effective in blocking LPS-induced inflammation in microglial cells.

According to Figure 2, the PAMs used in this experiment bind to mGluR4 and block TNF release, which was initially activated by LPS. Although the pathway that blocks TNF release when

mGluR4 is activated remains unknown, future study of this pathway may reveal a role for PAMs in the modulation of PD symptoms beyond inflammation.

Lowering the administered concentrations of the PAMs could result in varied protectiveness against inflammation, including inflammation from stimuli other than LPS, such as α-syn. Further studies involving decreased PAM concentrations must be conducted to determine a dose response curve for PAM administration *in vitro*. To determine the viability of PAM administration as a treatment for PD, *in vivo* future studies will use neurotoxin and α-syn based animal models of PD. Studies using animal models will allow for the observation of the effect of PAMs on inflammatory responses *in vivo*, while also allowing for behavioral tests of motor function. Motor function loss is progressive in PD and may correlate with the level of inflammation found in PD brains. If a correlative relationship between inflammation and decreased motor function is found, the role of the PAMs may be extended to a possible treatment option for degenerative motor function in PD. Possible *in vivo* complications may include using higher than necessary concentrations of the PAMs in order to establish a drug dose response curve.

Conclusion

This study tested whether mGluR4 PAMs are protective against LPS-induced inflammation in microglia, which was quantitatively measured by TNF release. Compared to the LPS-induced TNF release in the positive control sample, the ADX and VU813 compounds showed a significant decrease in TNF expression, suggesting that administration of the PAMs attenuated LPS-induced inflammatory response. As mGluR4 activation has previously been shown to be protective in PD animal models through behavioral and motor tests,[6] future animal studies will show whether they can be targeted to protect against inflammation in PD. Various findings obtained by animal models of PD have suggested that neuroinflammation manifested by glial reactions is an important contributor to the pathogenesis of PD.[8] These results, coupled with future findings, will hopefully lead to the development of alternative therapeutic strategies for PD specifically targeting mGluR4.

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