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Research Report

Mg^{2+} ions reduce microglial and THP-1 cell neurotoxicity by inhibiting Ca^{2+} entry through purinergic channels

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ABSTRACT

Mg^{2+} is a known antagonist of some Ca^{2+} ion channels. It may therefore be able to counteract the toxic consequences of excessive Ca^{2+} entry into immune-type cells. Here we examined the effects of Mg^{2+} on inflammation induced by Ca^{2+} influx into microglia and THP-1 cells following activation of purinergic receptors. Using tissue culture, an inflammatory response was induced by treatment with either the P2X7 purinergic receptor agonist 2′,3′-[benzoyl-4-benzoyl]-ATP (BzATP) or the P2Y2,4 receptor agonist uridine 5′-triphosphate (UTP). Both microglia and THP-1 cells expressed the mRNAs for these receptors. Treatment produced a rapid rise in intracellular Ca^{2+} which was significantly reduced by Mg^{2+} or the calcium chelator BAPTA-AM. Purinergic receptor stimulation activated the intracellular inflammatory pathway P38 MAP kinase and NFκB. This caused release of TNFα, IL-6, nitrite ions and other materials that are neurotoxic to SH-SY5Y cells. These effects were all ameliorated by Mg^{2+}. They were also partly ameliorated by the P2X7R antagonists, oxATP and KN-62, the P2YR antagonist MRS2179, and the store operated Ca^{2+} channel blocker, SK96365. These results indicate that elevated Mg^{2+} is a broad spectrum inhibitor of Ca^{2+} entry into microglia or THP-1 cells. Mg^{2+} administration may be a strategy for reducing the damaging consequences Ca^{2+} induced neuroinflammation in degenerative neurological disorders such as Alzheimer disease and Parkinson disease.

1. Introduction

Magnesium ion (Mg^{2+}) is one of the most abundant divalent cation in cells. Mg^{2+} is known to be essential for enzymatic activity, structural stability of anions such as nucleotides, and maintaining body physiology (Romani and Scarpa, 2000). Although the abundance of Mg^{2+} may suggest that it could be involved in pathophysiological conditions, there are few studies concerning its effects in brain. One function may be to inhibit activities associated with stimulation of ATP-gated

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Abbreviations: oxATP, oxidized ATP; TNFα, tumor necrosis factor-alpha; BzATP, 2′,3′-[benzoyl-4-benzoyl]-ATP; UTP, uridine 5′-triphosphate; KN-62, 1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine]; MRS2179, N^6-methyl-2'-deoxyadenosine-3',5'-bisphosphate;1-[2-(4-methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl-1H-imidazolehydrochloride; SKF96365, 1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl-1H-imidazole hydrochloride; LPS, lipopolysaccharide

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P2X7 purinergic receptors (P2X7Rs), since they are known to be associated with neuroinflammation in brain diseases (Virginio et al., 1997; Chessell et al., 1998; Witting et al., 2004).

The purinergic P2X family of receptors are ionotropic channels concerned with influx and outflux of cationic current. The P2X7R subtype, unlike other P2XR members, can form oligomers to create a high conductance pore which allows considerable amounts of Ca^{2+} to enter cells (North, 2002). Elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$) is then coupled to a diversity of pathways modulating cellular responses (James and Butt, 2002; Takenouchi et al., 2007). Mg^{2+} may competitively inhibit Ca^{2+}-entry through these or other Ca^{2+} channels, suggesting that Mg^{2+} could play a role in Ca^{2+}-dependent cellular processes involved in the pathophysiology of neurological disease.

Expression of P2X7R is particularly robust in brain microglia, with evidence suggesting that activation of this receptor contributes to cell inflammatory responses mediated by a diversity of factors including TNFα (Hide et al., 2000), IL-1β (Ferrari et al., 1996; Chakfe et al., 2002) and IL-6 (Shigemoto-Mogami et al., 2001). Upregulation and activation of P2X7R has been demonstrated in AD animal models (Parvathenani et al., 2003) and in AD brain (McLarnon et al., 2006). Importantly, pharmacological inhibition of P2X7R with compounds such as oxidized ATP (oxATP) diminishes inflammatory reactivity in inflamed brain (Choi et al., 2007).

Since influx of Ca^{2+} is a critical component of P2X7R activation, we reasoned that any maneuvers which diminished entry of Ca^{2+} could reduce brain inflammation. One such strategy could involve increasing levels of external Mg^{2+} since this procedure is known to block Ca^{2+} influx through voltage-gated Ca^{2+} and NMDA channels (Ikonomidou and Turski, 1996; Kwak and Weiss, 2006; Deshpande et al., 2008). However, microglia do not express either of these types of channels (Färber and Kettenmann, 2005). There are previous studies indicating Mg^{2+} inhibition of Ca^{2+} entry through membrane pores (Alloisio et al., 2010; Acuña-Castillo et al., 2007; Mooren et al., 2001). Interestingly, a number of divalent ions including Mg^{2+} are reported as allosteric modulators which alter ATP binding to P2X7R (Virginio et al., 1997). Previously, calcium spectrofluorescence showed BzATP stimulation of microglial cells elicited a biphasic increase in $[Ca^{2+}]_i$; the initial phase results from activation of P2YR since it was unaffected by oxATP or SKF96365. The prolonged phase of the Ca^{2+} response is suggested to result from activation of P2X7R and SOC since this component was sensitive to oxATP and SKF96365 treatment. However, there has been no study demonstrating that Mg^{2+} inhibition of P2YR and SOC also attenuates Ca^{2+} entry and the accompanying microglia-mediated inflammation.

In this work, we induced an inflammatory response in human microglia and human monocyte THP-1 cells using the P2X7 receptor stimulator, 2′,3′-[benzoyl-4-benzoyl]-ATP (BzATP). We also induced an inflammatory response with the P2Y2,4 receptor stimulator, uridine 5′-triphosphate (UTP) (Burnstock, 2007). We then examined the effects of Mg^{2+} treatment as an inhibitor of these stimulations. Outcome measures of the Mg^{2+} effect were reductions in P38 MAP kinase and NFκB activation, plus reductions in the extracellular release of the pro-inflammatory cytokines TNFα and IL-6 and materials that are neurotoxic to SH-SY5Y neuroblastoma cells.

We established that these inflammatory responses were Ca^{2+}-dependent by showing that intracellular Ca^{2+} was dramatically increased by such stimulation and that the response was significantly reduced by treatment with the Ca^{2+} chelator BAPTA-AM. We reinforced these results by demonstrating that the inflammatory effects were attenuated by specific blockers of the receptors governing the calcium channels involved. These included the P2X7R antagonists, oxATP and KN-62, the P2Y antagonist MRS2179, and the antagonist for the TRP channel (also known as the store operated channel or SOC) and low voltage-activated LVA T-type calcium channel, SKF35395. In each case Mg^{2+} enhanced the blocking effect.

Finally, we showed that the inhibitory effects of Mg^{2+} applied when the cells were primed with an alternative and potent inflammatory stimulus, LPS plus IFNγ (Klegeris et al., 2005). We conclude that Mg^{2+} is a broad spectrum inhibitor of neuroinflammation which acts by blocking Ca^{2+} entry through cell surface channels.

2. Results

The results of the RT-PCR experiments are shown in Fig. 1A. Microglia and THP-1 cells expressed mRNAs for the ionotropic receptors P2X1, 4, and 7R and the metabotropic receptors P2Y1, 2, and 6R. THP-1 cells, but not microglia, expressed P2Y4R and, very weakly, P2X5R and P2Y12R. Bands were not detected for the ionotropic receptors P2X2, 3 or 6. SH-SY5Y cells expressed mRNAs for P2X1, 2, 3, 4, and 6R, and P2Y1, 4, 6 and 12R. Equivalent protein loading of lanes is demonstrated by equal intensities of the housekeeping GAPDH bands. These findings were duplicated in 3 independent experiments. It is important to note that SH-SY5Y cells expressed P2Y4R mRNA, but control experiments indicate that direct treatment of these cells with UTP (100 μM) did not change their viability (SFig. 7B).

Fig. 1B shows companion western blotting results illustrating protein expression of P2X7R by THP-1 cells and human microglia but not by SH-SY5Y cells. Equal loading was demonstrated by the bands of equal intensity for α-tubulin. This result is consistent with many previous studies showing that P2X7R is preferentially expressed by immune type cells, but not by neurons (North, 2002; Skaper et al., 2010).

We next explored whether stimulation of P2X7R in microglia with BzATP or LPS/IFNγ/BzATP would induce an intracellular inflammatory response and whether this would be attenuated by Mg^{2+}. Microglia were treated for 30 min at 37 °C with BzATP or LPS/IFNγ/BzATP with or without the addition of 10 mM Mg^{2+} (LPS/IFNγ, 2 h preincubation). The cells were separated from the supernatants, exposed to lysis buffer and the proteins separated by SDS-PAGE. The resulting Western blots are shown in Fig. 2. They demonstrate induction of phospho-NFκB (P65 NFκB) and phospho-P38 MAP kinase (P38 MAPK). BzATP treatment alone increased phospho-NFκB by 7 fold and phospho-P38 expression by 4.2 fold and this was potentiated 2- to 3-fold by prior priming for 2 h with LPS/IFNγ/BzATP (phospho-NFκB: 2-fold and phospho-P38 MAP kinase: 3 fold, $p<0.01$). Total P38 MAPK and NFκB proteins were used as the loading control. Mg^{2+} significantly attenuated this