Mitigating Microglial-mediated Neuroinflammation: The Sur1-Trpm4 Channel Regulates Calcium-sensitive Induction of iNOS

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Introduction

Microglia, the resident immune cells of the central nervous system, play a critical role in health and disease. Following injury, microglia upregulate inducible nitric oxide synthase (iNOS), and can exert neurotoxic effects by releasing large quantities of nitric oxide (NO). Expression of iNOS, and other pro-inflammatory genes, is regulated in part by Ca2+-dependent transcription factors. The expression of the non-selective cation channel Sur1-Trpm4 may be one molecular mechanism by which microglia dynamically modulate Ca2+ influx. We hypothesized that microglial Sur1-Trpm4 plays a role in microglial-mediated neuroinflammation by regulating the calcium-sensitive induction of iNOS.

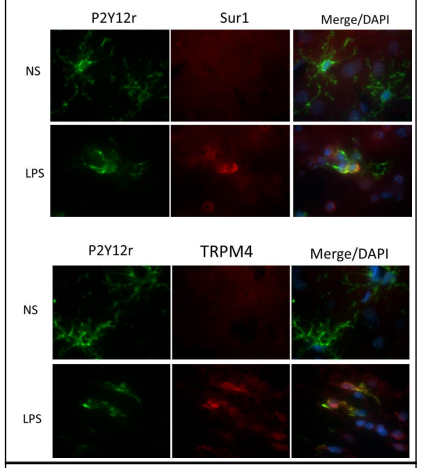
Methods

The TLR4 agonist lipopolysaccharide (LPS) was used as a stimulus to activate microglia. Standard techniques of molecular biology were employed to evaluate protein and gene expression. Functional Sur1-Trpm4 activity was evaluated electrophysiologically. Confocal microscopy and the calcium-sensitive fluorescent dye, Fluo-4, was used to measure intracellular Ca2+. iNOS activity was evaluated by measuring extracellular nitrite.

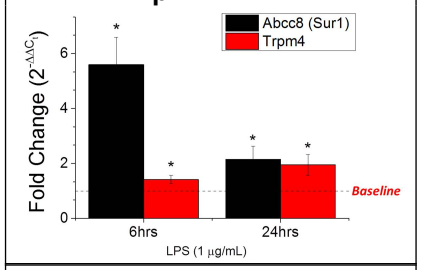
Results

LPS or normal saline (NS) was injected into the striatum of adult rats. Depicted are immunofluorescent labelings of coronal brain sections for P2Y12r (microglial marker), Sur1 and Trpm4.

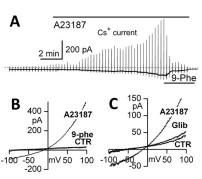
TLR4 ligation in vivo induces upregulation of Sur1 and Trpm4 in microglia



TLR4 ligation induces upregulation of Sur1 and Trpm4 mRNA

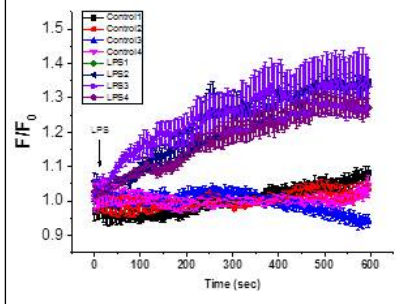


Functional Sur1-Trpm4 channels in microglia

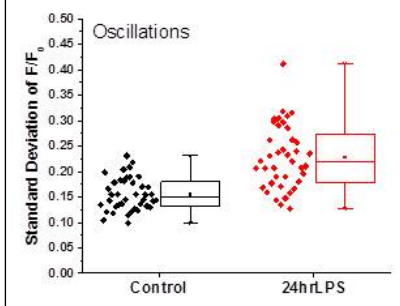


Confocal microscopy and the Ca2+-sensitive dye, Fluo-4 were used to measure intracellular Ca2+ in the N9 microglial cell line. Each trace represents an average of 10 cells.

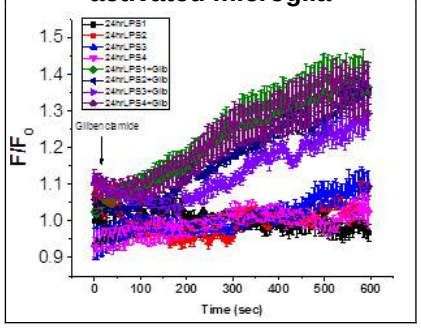
TLR4 ligation acutely induces a rise in intracellular Ca2+



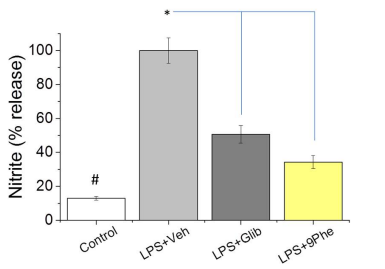
Chronic TLR4 ligation induces Ca2+ oscillations



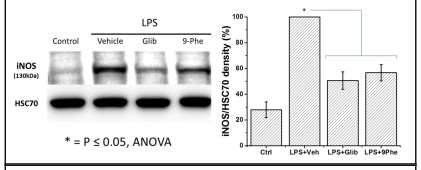
Blockade of Sur1-Trpm4 disrupts Ca2+ oscillations and increases intracellular Ca2+ in activated microglia



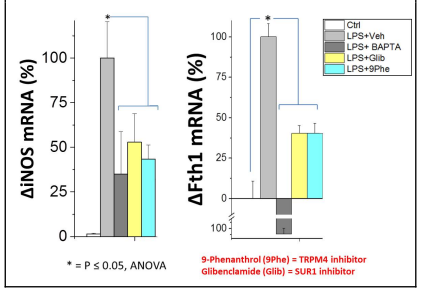
Inhibition of Sur1-Trpm4 reduces the production of nitrite



Inhibition of Sur1-Trpm4 reduces the induction of iNOS



Inhibition of Sur1-Trpm4 reduces the induction of iNOS by a Ca2+-sensitive mechanism



While enzymatic activity of iNOS is not Ca2+-dependent, mRNA expression is known to be Ca2+-sensitive. Ferritin H (Fth1, an inducible Ca2+-dependent iron binding protein) was evaluated as a positive control.

Conclusions

Our results strongly support our hypothesis that Sur1-Trpm4 regulates the calcium-sensitive induction of iNOS. These observations have impactful therapeutic implications. Inhibition of Sur1-Trpm4 using the well-tolerated sulfonylurea glibenclamide (a.k.a. glyburide) may be a promising approach to limit the deleterious effects of microglial-mediated neuroinflammation.

References

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