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Research Paper

The anti-parkinsonian drug zonisamide reduces neuroinflammation: Role of microglial Na_v 1.6

Muhammad M. Hossain[a,b], Blair Weig[c], Kenneth Reuhl[c], Marla Gearing[d], Long-Jun Wu[e], Jason R. Richardson[a,b,f,*]

[a] Department of Pharmaceutical Sciences and Center for Neurodegenerative Disease and Aging, Northeast Ohio Medical University, Rootstown, OH, USA
[b] Department of Environmental and Occupational Medicine and Environmental and Occupational Health Sciences Institute, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ, USA
[c] Department of Pharmacology and Toxicology, Rutgers Ernest Mario School of Pharmacy Rutgers University, Piscataway, NJ, USA
[d] Department of Pathology and Laboratory Medicine, School of Medicine, Emory University, Atlanta, GA, USA
[e] Department of Neurology, Mayo Clinic, Rochester, MN, USA
[f] Department of Environmental Health Sciences, Robert Stemple School of Public Health and Social Work, Florida International University, Miami, FL, USA

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ABSTRACT

Parkinson's disease (PD), the second most common age-related progressive neurodegenerative disorder, is characterized by dopamine depletion and the loss of dopaminergic (DA) neurons with accompanying neuroinflammation. Zonisamide is an-anti-convulsant drug that has recently been shown to improve clinical symptoms of PD through its inhibition of monoamine oxidase B (MAO-B). However, zonisamide has additional targets, including voltage-gated sodium channels (Na_v), which may contribute to its reported neuroprotective role in preclinical models of PD. Here, we report that Na_v1.6 is highly expressed in microglia of post-mortem PD brain and of mice treated with the parkinsonism-inducing neurotoxin MPTP. Administration of zonisamide (20 mg/kg, i.p. every 4 h × 3) following a single injection of MPTP (12.5 mg/kg, s.c.) reduced microglial Na_v 1.6 and microglial activation in the striatum, as indicated by Iba-1 staining and mRNA expression of F4/80. MPTP increased the levels of the pro-inflammatory cytokine TNF-α and $gp91^{phox}$, and this was significantly reduced by zonisamide. Together, these findings suggest that zonisamide may reduce neuroinflammation through the down-regulation of microglial Na_v 1.6. Thus, in addition to its effects on parkinsonian symptoms through inhibition of MAO-B, zonisamide may have disease modifying potential through the inhibition of Na_v 1.6 and neuroinflammation.

1. Introduction

Neuroinflammation and microglial activation contributes to the pathogenesis of a variety of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD) (Bronzuoli et al., 2016; Heneka et al., 2015; Tansey and Goldberg, 2010; Wang et al., 2015). Microglia play an active role in neuroprotection and repair of neurons in the central nervous system following toxic insult and neuronal injury. However, persistent activation of microglia can mediate neuronal death and neurodegeneration by increasing the secretion of inflammatory molecules and cytokines, including tumor necrosis factor alpha (TNF-α) and reactive oxygen species (ROS) (Harrigan et al., 2008; Liu et al., 2010). Given the association between microglial activation and neurodegeneration, a significant amount of effort has been focused on identifying interventions that can dampen the neuroinflammatory response and slow the initiation or progression of neurodegeneration. Unfortunately, these interventions have not yet been successful in clinical trials.

Microglia express a number of ion channels, including Na^+ channels that regulate various aspects of inflammatory process, providing a potential target for intervention (Black et al., 2009; Hossain et al., 2017; Pappalardo et al., 2016; Richardson and Hossain, 2013). Although generation of action potentials is the primary function of voltage-gated sodium channels (VGSC), several recent studies demonstrated that VGSC can regulate a number of cellular functions such as morphological transformation, migration, and phagocytosis of microglia when stimulated with lipopolysaccharide (LPS) (Black et al., 2009; Stevens et al., 2013), suggesting potential immunomodulatory properties of

* Corresponding author at: Department of Environmental Health Sciences, Robert Stempel School of Public Health and Social Work, Florida International University, Miami, FL, USA.
E-mail address: jarichar@fiu.edu (J.R. Richardson).

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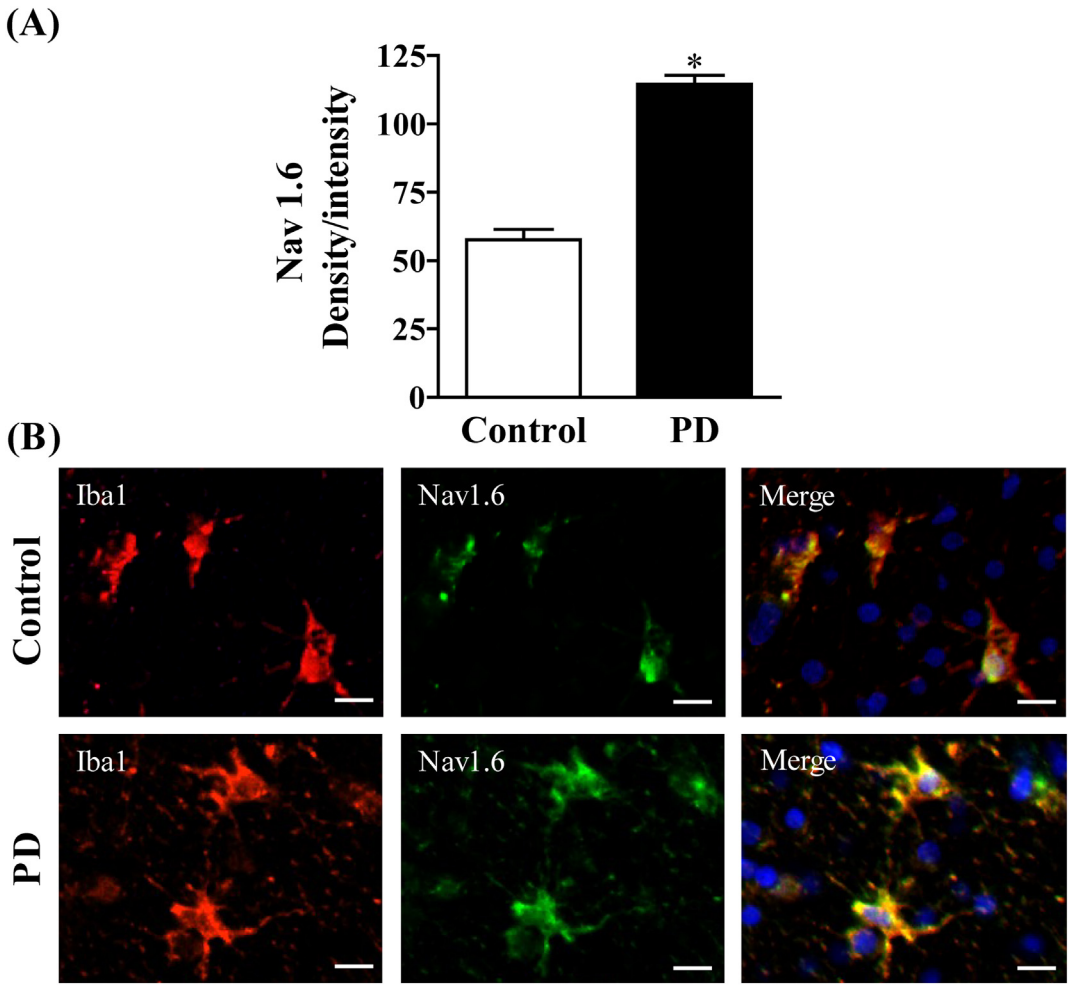

Fig. 1. (A) Semi-quantification of Nav1.6 staining.(B) Expression of $Na_v1.6$ in microglia of postmortem brain. Age matched control (Top panel) and PD (Bottom). Microglia were labeled with anti-Iba1 antibody (red) and expression of $Na_v1.6$ was visualized by immunolabeling with anti-$Na_v1.6$ antibody (green). Scale bar = 20 μM. Images were captured on a Zeiss Observer D1 microscope (Zeiss Inc., Thornwood, NY) with an X-Cite series 120Q fluorescent illuminator and a Jenoptik camera with ProgRes CapturePro 2.8 software (Jenoptik, Easthampton, MA). Optical density per intensity of fluorescence against Nav1.6 stain was semi-quantified in individual cells using ImageJ software (NIH). The values represent mean density/intensity ± SEM from 20 to 25 cells/section/brain. * indicates significant difference from control ($p < 0.05$) by Student's *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

VGSC. We recently reported that Na^+ influx through VGSC initiates activation of microglia and subsequently triggers an inflammatory pathway by accumulation of intracellular sodium [$(Na^+)i$] after exposure to LPS (Hossain et al., 2013) and pyrethroid insecticides (Hossain et al., 2017), which appears to involve Na_v 1.6. The Na_v 1.6 isoform is one of most abundantly expressed isoforms within peripheral and central nervous system of the adult (Goldin, 2001; Krzemien et al., 2000; Tzoumaka et al., 2000) and is also found to be a predominant isoform in microglia (Black et al., 2009; Black and Waxman, 2012; Hossain et al., 2017; Hossain et al., 2013). Additional studies from Waxman's group demonstrated that mice exhibit a significant upregulation of Na_v 1.6 in activated microglia in an experimental inflammatory/demyelinating model of multiple sclerosis (Craner et al., 2005). Furthermore, they reported that the expression of Na_v 1.6 expression increases with morphological transformation of microglia to an amoeboid like appearance. Together, these data suggest that microglial Na_v 1.6 plays an important role in regulation of microglial inflammation and could serve as a potential therapeutic target in neurodegeneration.

Zonisamide is an anti-convulsant drug approved by the FDA for the treatment of epilepsy (Sonsalla et al., 2010). The primary mechanism by which zonisamide is thought to exert its anti-epileptic effect is through inhibition of the voltage-gated sodium and T-type calcium channels (Biton, 2007; Kito et al., 1996; Matar et al., 2009; Okada et al., 2002). Recently, zonisamide has been reported to improve symptoms in PD patients when used in combination with other anti-parkinsonian drugs, likely through its ability to inhibit monoamine oxidase B (MAO-B) (Murata et al., 2007). In preclinical models, zonisamide has been shown to provide neuroprotection against seizure (Mares, 2010; Ueda et al., 2005) and ischemia (Minato et al., 1997; Owen et al., 1997). Pretreatment of mice with zonisamide attenuated the MPTP-induced reduction in striatal DA, DOPAC, and tyrosine hydroxylase (TH), which was likely the result of MAO-B inhibition and decreased MPP^+ formation (Sonsalla et al., 2010). More recent studies demonstrate that zonisamide can be protective when given after MPTP administration (Choudhury et al., 2011). However, the mechanisms responsible for this effect have not been fully elucidated.

In the present study, we investigated the expression of microglial $Na_v1.6$ in PD brain and MPTP mice. We further assessed the ability of zonisamide to prevent neuroinflammation in an acute mouse model of neuroinflammation produced by a single injection of MPTP (12.5 mg/kg, s.c.) that results in striatal injury and neuroinflammation within 12 h after MPTP administration (O'Callaghan et al., 1990). Our findings demonstrate that $Na_v1.6$ expression is increased in microglia in PD