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Restoring endoplasmic reticulum homeostasis improves functional recovery after spinal cord injury

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ABSTRACT

The endoplasmic reticulum (ER) stress response (ERSR) is activated to maintain protein homeostasis or induce apoptosis in the ER in response to distinct cellular insults including hypoxia, inflammation, and oxidative damage. Recently, we showed ERSR activation in a mouse model of a contusive spinal cord injury (SCI) and an improved hindlimb locomotor function following SCI when the pro-apoptotic arm of ERSR was genetically inhibited. The objective of the current study was to explore if the pharmacological enhancement of the homeostatic arm of the ERSR pathway can improve the functional outcome after SCI. Salubrinal enhances the homeostatic arm of the ERSR by increasing phosphorylation of eIF2α. Salubrinal significantly enhanced the levels of phosphorylated eIF2α protein and modulated the downstream ERSR effectors assessed at the lesion epicenter 6 h post-SCI. Hindlimb locomotion showed significant improvement in animals treated with salubrinal. Treadmill-based-gait assessment showed a significant increase in maximum speed of coordinated walking and a decrease in rear stance time and stride length in salubrinal-treated animals. This improved functional recovery corresponded with increased white matter sparing and decreased oligodendrocyte apoptosis. In addition, salubrinal protected cultured mouse oligodendrocyte progenitor cells against the ER stress-inducing toxin tunicamycin. These data suggest that boosting the homeostatic arm of the ERSR reduces oligodendrocyte loss after traumatic SCI and support the contention that pharmacological targeting of the ERSR after CNS trauma is a therapeutically viable approach.

Introduction

Despite numerous research endeavors and technological advances, effective clinical therapy after spinal cord injury (SCI) still remains obscure. This unavailability of treatment is due to the complex pathophysiology that ensues after SCI which includes inflammation, hypoxia, excitotoxicity, disruption of the blood brain barrier, ischemia and demyelination (Rowland et al., 2008). Therefore, studies are required to explore additional molecular mechanisms for identification of cellular targets for therapeutic intervention. Recent reports demonstrated activation of the endoplasmic reticulum (ER) stress response (ERSR) after a moderate contusive SCI in rats (Penas et al., 2007) and mice (Ohri et al., 2011) and improved functional recovery in hindlimb locomotion of mice deficient in the ERSR pro-apoptotic effector, C/EBP (CCAAT enhancer binding protein) homologous protein (CHOP) (Ohri et al., 2011).

The ERSR, mediated through three distinct pathways, is an evolutionary conserved cellular mechanism that is activated in response to insults that disrupt cellular homeostasis (Schroder, 2008; Tabas and Ron, 2011). The protein RNA (PKR)-like kinase (PERK) phosphorylates the α-subunit of elongation initiation factor 2α (eIF2α) and inhibits global protein synthesis. Activated inositol-requiring protein-1α (IRE-1α) splices X-box-binding protein 1 (XBP1) mRNA (Yoshida et al., 2001) which then activates ERSR specific transcription. The third pathway of the ERSR comprises the proteolytic processing of activating transcription factor-6 (ATF6) (Haze et al., 1999). The main objective of the ERSR is restoration of ER function and, in consequence, cytoprotection. However, the ERSR may also activate apoptosis if ER function cannot be timely restored (Tabas and Ron, 2011). CHOP is one of the major effectors of the apoptotic arm of the ERSR (Ron and Habener, 1992). Involvement of ERSR-activated cell death has been reported in ischemic stroke (Lange et al., 2008), multiple sclerosis (Lin et al., 2006), and Alzheimer's disease (Milhavet et al., 2002).

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Our previous study showing improved functional recovery post-SCI with genetic ablation of CHOP indicated a major role of ERSR-mediated apoptosis in the pathogenesis of SCI. That functional recovery correlated with an increase in white matter sparing and transcript levels of myelin basic protein (MBP) and claudin 11 (Ohri et al., 2011) suggesting oligodendrocyte protection. However, the role of the homeostatic component of ERSR in SCI pathogenesis has not been addressed. In addition, a possibility to improve the outcome of SCI by pharmacological modulation of the ERSR has also yet to be evaluated.

Salubrinal is a small molecule inhibitor of the protein phosphatase 1 (PP1) complex that reverses PERK-mediated phosphorylation of eIF2α. As a result, salubrinal enhances the PERK-eIF2α signaling during ERSR while protecting a wide range of cells against ER stress-induced cell death (Boyce et al., 2005). Salubrinal reduced kainic acid-induced ER stress and neuronal death in vivo and in vitro (Sokka et al., 2007) and ameliorated hypomyelination and oligodendrocyte loss in cultured hippocampal slices exposed to IFN-γ (Lin et al., 2008). Here, we demonstrate that salubrinal attenuates ER stress-induced apoptosis of oligodendrocyte precursor cells (OPCs) in vitro and oligodendrocytes in vivo and improves functional recovery of hindlimb locomotion in a mouse model of traumatic SCI.

Materials and methods

Animals

Procedures were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996) and the University of Louisville Institutional Animal Care and Use Committee. C57Bl/6 female mice (6–8 weeks) were obtained from Harlan (Indianapolis, IN). Growth arrest and DNA-damage protein 34 knockout (GADD34$^{-/-}$) mice on a 100% C57Bl/6 background were procured from MMRRC (Cat. No — 30266, Chapel Hill, NC) and bred in-house.

Isolation of mouse OPCs from cortex

Mouse cortices were dissected from whole brains of wild type and GADD34$^{-/-}$ postnatal day 5–7 pups (Dincman et al., 2012). Briefly, tissue was dissociated using the Neural Tissue Dissociation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. OPC-A media was prepared by adding 2.1 g/l $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO) to DMEM-F12 without HEPES powder (Invitrogen, Carlsbad, CA) and supplemented with N2 supplement (1%), B27 supplement (2%), penicillin/streptomycin (1%, all from Invitrogen), BSA (0.01%, Sigma), 40 ng/ml FGF2 (Millipore, Billerica, MA), and 20 ng/ml PDGFaa (Sigma). Average yield was 8–10 × 10^6 cells/brain with a viability of 85–95%. OPCs were enriched using magnetic cell sorting (MACS) with rat anti-mouse IgM magnetic beads (10% in MACS buffer). Between 9000 and 15,000 cells/cm^2 were plated on a PDL/laminin-coated 10 cm tissue culture dish, and incubated at 37 °C, 5% CO_2.

Spinal cord injury and injections

Mice were anesthetized by an intraperitoneal injection of 400 mg/kg body weight Avertin (2,2,2-tribromoethanol in 0.02 ml of 1.25% 2-methyl-2-butanol in saline, Sigma). A laminectomy was done at the T9 vertebrae and moderate contusion injuries (50 kdyn force/400–800 μm displacement) were performed using an IH impactor (Scheff et al., 2003; Infinite Horizons Inc., Lexington, KY) as described previously (Benton et al., 2008; Han et al., 2010; Ohri et al., 2011). Experimental controls included sham animals with the T9 laminectomy only. Salubrinal (1 or 5 mg/kg; Sigma) or vehicle was administered through the jugular vein immediately after surgery and then through the tail vein (1×/day) for three days. Animals were given 1 ml of sterile saline and 0.1 ml of gentamycin subcutaneously post-surgery and on the 3rd and 5th day post-surgery, and 0.1 ml of bupronorphine subcutaneously on the day of surgery and for the next 2 days.

RNA extraction, reverse transcriptase PCR

Total RNA was extracted from treated OPCs and spinal cord tissue of sham, vehicle- and salubrinal-contused wild type (n = 4/group)

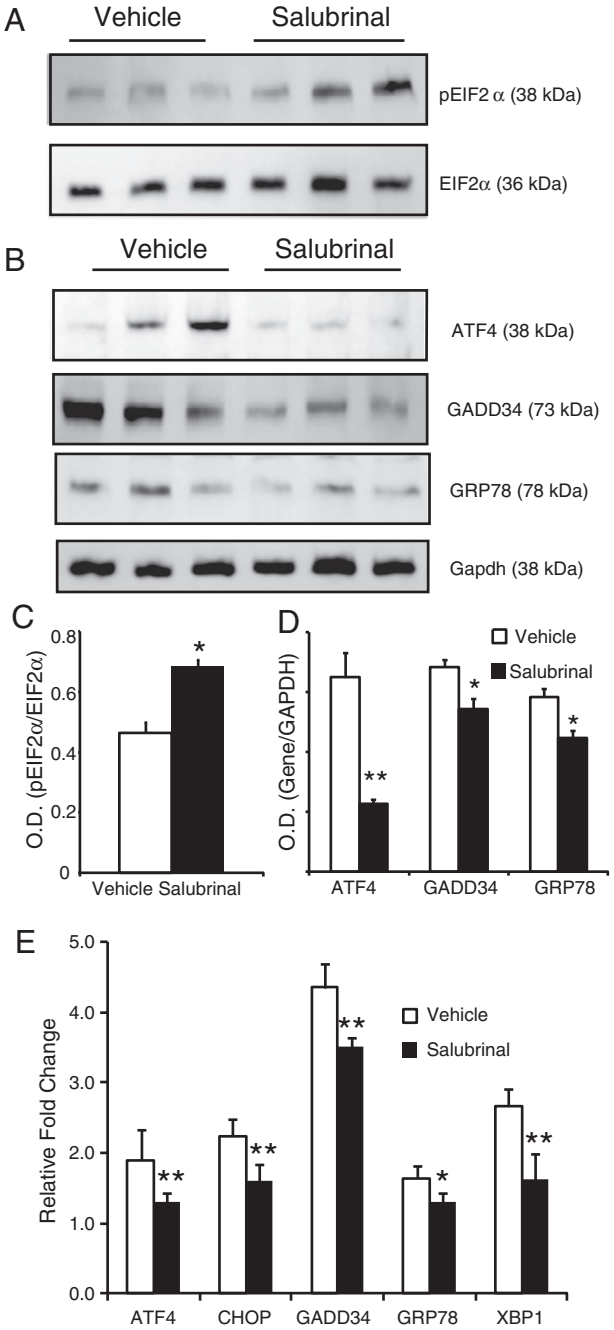

Fig. 1. Salubrinal treatment results in increased phosphorylation of eIF2α and modulates the key ERSR markers in vivo at 6 h post-injury. (A) Western blots show that salubrinal increases phosphorylated eIF2α levels at the injury epicenter of contused spinal cords. (B) Western blots show a decrease in the levels of ATF4, GADD34 and GRP78 at the injury epicenter in salubrinal-treated animals as compared to vehicle-treated group. (C,D) Quantification of the Western blots in (A,B) reveal significant differences in protein levels of ERSR markers as indicated. (E) Salubrinal leads to significant decreases in transcript levels of various ERSR markers analyzed by qRT-PCR. Transcript levels are expressed as fold changes compared with respective levels in sham controls. Data are the mean ± SD [n = 3 (A,B); n = 4 (E), *p < 0.05, **p < 0.01)].