Neurobiology of Aging 36 (2015) 1140–1150

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

"Preconditioning" with latrepirdine, an adenosine 5′-monophosphate-activated protein kinase activator, delays amyotrophic lateral sclerosis progression in SOD1^{G93A} mice

Karen S. Coughlan, Mollie R. Mitchem, Marion C. Hogg, Jochen H.M. Prehn*

Department of Physiology and Medical Physics, Centre for the Study of Neurological Disorders, Royal College of Surgeons in Ireland, Dublin, Ireland

ARTICLE INFO

Article history:
Received 20 March 2014
Received in revised form 28 August 2014
Accepted 18 September 2014
Available online 26 September 2014

Keywords:
Amyotrophic lateral sclerosis
AMPK
Bioenergetics
Pre-conditioning
Spinal cord
Motoneuron degeneration
SOD1

ABSTRACT

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is a master regulator of energy balance. As energy imbalance is documented as a key pathologic feature of amyotrophic lateral sclerosis (ALS), we investigated AMPK as a pharmacologic target in SOD1^{G93A} mice. We noted a strong activation of AMPK in lumbar spinal cords of SOD1^{G93A} mice. Pharmacologic activation of AMPK has shown protective effects in neuronal "preconditioning" models. We tested the hypothesis that "preconditioning" with a small molecule activator of AMPK, latrepirdine, exerts beneficial effects on disease progression. SOD1^{G93A} mice ($n = 24$ animals per group; sex and litter matched) were treated with latrepirdine (1 μg/kg, intraperitoneal) or vehicle from postnatal day 70 to 120. Treatment with latrepirdine increased AMPK activity in primary mouse motor neuron cultures and in SOD1^{G93A} lumbar spinal cords. Mice "preconditioned" with latrepirdine showed a delayed symptom onset and a significant increase in life span ($p < 0.01$). Our study suggests that "preconditioning" with latrepirdine may represent a possible therapeutic strategy for individuals harboring ALS-associated gene mutations who are at risk for developing ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that leads to the weakening of motor neurons, paralysis, and eventual death. There is no known cure, and limited treatment options are currently available. Of the pathologic hallmarks of ALS, mitochondrial defects and alterations in energy metabolism routinely correlate with disease progression in patients and in animal models of ALS. In SOD1^{G93A} mice, a mouse model of familial ALS, mutant SOD1 protein accumulates in mitochondria early during disease (Deng et al., 2006; Higgins et al., 2002; Luo et al., 2013). This build up of mutant protein is suggested to impair mitochondrial function (Israelson et al., 2010; Pasinelli et al., 2004; Song et al., 2013). In line with these findings, mitochondria had a metabolic switch from oxidative phosphorylation to glycolysis in transgenic SOD1^{G93A} mice, NSC34 cells (motor neuron-like cell line) and fibroblasts transfected with mutant SOD1 compared with controls (Allen et al., 2013; Mattiazzi et al., 2002; Richardson et al., 2013). Glucose utilization and adenosine triphosphate (ATP) levels have been shown to be significantly reduced in corticospinal and bulbospinal motor tracts and the motor cortex of SOD1^{G93A} before pathologic changes (Browne et al., 2006). Late-stage SOD1^{G93A} mice also display decreased glucose uptake into skeletal muscle (Smittkamp et al., 2014). Furthermore, SOD1^{G93A} and SOD1^{G86R} mice show a decrease in body weight and fat mass during disease progression, reduced levels of circulating triglycerides, and evidence of increased lipolysis (Dodge et al., 2013; Dupuis et al., 2004; Fergani et al., 2007; Kim et al., 2011a). ALS patients also exhibit higher resting levels of energy expenditure, weight loss, a hypermetabolic phenotype, and increased lipolysis (Bouteloup et al., 2009; Desport et al., 2005; Dodge et al., 2013; Dupuis et al., 2008; Funalot et al., 2009; Kasarskis et al., 1996). Hence, there is growing evidence for decreased glucose utilization and metabolic reprogramming in ALS.

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is a major sensor and regulator of energy homeostasis (Hardie et al., 2012). AMPK is a heterotrimeric protein composed of 1 catalytic subunit (α) and 2 regulatory subunits (β and γ), and it is expressed in most tissues including the central nervous system (CNS) (Culmsee et al., 2001). AMPK is activated by phosphorylation of the α subunit at the threonine 172 site when there is a high AMP:ATP ratio (Hawley et al., 1996). AMPK has also been shown to be activated in mutant SOD1-expressing primary mixed spinal cord cultures and in spinal cords of SOD1^{G93A} mice at postnatal day (PND) 90 (Lim et al., 2012; Perera et al., 2014). When activated in cells,

* Corresponding author at: Department of Physiology and Medical Physics, Centre for the Study of Neurological Disorders, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland. Tel.: + 353 1 402 2255; fax: + 353 1 402 2447.
E-mail address: JPrehn@rcsi.ie (J.H.M. Prehn).

0197-4580/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.09.022

AMPK decreases energy expenditure and increases glucose uptake and utilization, hence maintaining a bioenergetic homeostasis (Culmsee et al., 2001; Weisova et al., 2009). AMPK also stimulates fatty acid oxidation and inhibits lipogenesis and triglyceride synthesis (Hardie, 2007). Pretreatment of neurons with AMPK mimetics has been shown to increase the resistance of neurons to subsequent toxic challenges such as Ca^{2+} overloading (Anilkumar et al., 2013; Culmsee et al., 2001). We have recently also shown that AMPK mediates the preconditioning effects of mild mitochondrial uncoupling and complex inhibition in neurons (Weisova et al., 2012). However, prolonged AMPK activation during severe energetic stress can also lead to proapoptotic responses in neurons and can worsen neuronal injury (McCullough et al., 2005). Our laboratory has shown that this effect is mediated via the activation of proapoptotic Bcl-2 family proteins (Concannon et al., 2010; Davila et al., 2012; Kilbride et al., 2010). AMPK may therefore act as a "master switch" for neuronal survival decisions (Weisova et al., 2012). However, the role of AMPK in regulating metabolism in ALS and as a new therapeutic target warrants further investigation. Continual AMPK activation may be detrimental to severely stressed neurons (Concannon et al., 2010; McCullough et al., 2005); therefore, neuronal "preconditioning" rather than continuous treatment with an AMPK activator may represent a novel therapeutic approach for the treatment of subjects at risk of developing ALS. Preconditioning is the concept of applying a subthreshold stress to a tissue which induces tolerance in the case of a more robust stress later (Dirnagl et al., 2003). We have recently identified latrepirdine as a potent, small molecule activator of AMPK (Weisova et al., 2013). Latrepirdine crosses the blood-brain barrier and is active in the CNS (Wang et al., 2011). It activates AMPK when used in the nanomolar range and has been safely prescribed as a travel sickness remedy and anti-histaminergic agent since the early 1980s (Doody et al., 2008; Kieburtz et al., 2010; Matveeva et al., 1983; Wang et al., 2011; Weisova et al., 2013). Therefore, here we tested the hypothesis that "preconditioning" with latrepirdine exerts beneficial effects on disease progression in transgenic $SOD1^{G93A}$ mice.

2. Methods

2.1. Animals

$SOD1^{G93A}$ mice C57B6.Cg-Tg (SOD1G93 A) 1Gur/J were purchased from Jackson Laboratory (Bar Harbor, ME, USA). These mice are congenic on the C57Bl6 background and carry a high-copy number of the G93A mutant SOD1 transgene. Mice were housed in cages of between 3 and 5 mice, and all mice for this study were age, gender, and litter matched according to specific ALS preclinical trial guidelines (Ludolph et al., 2010). Mice were housed at constant temperature (22 °C) on a 12-hour light/dark cycle (7 AM on, 7 PM off), with *ad libitum* food and water available. All experiments were carried out under license (no. B100/4414) from the Department of Health and Children, Ireland, with ethical approval from the Royal College of Surgeons in Ireland Research Ethics Committee (REC625 b). (Supplementary Table 1—a record of all animals used in the study, Supplementary Table 2— a record of any animals excluded from the study).

2.2. Drugs and "preconditioning" treatment of $SOD1^{G93A}$tg mice

Latrepirdine (MDV1100) (Medivation, San Fracisco, CA, USA) stock was dissolved in ultrapure H_2O. For in vitro use, latrepirdine was dissolved in culture media, and cells were treated at concentrations stated. For in vivo use, latrepirdine was diluted in 1X PBS (vehicle). $SOD1^{G93A}$ mice were given latrepirdine (1 μg/kg/d intraperitoneal injection) or PBS vehicle commencing at PND 70 until PND 120.

2.3. Assessment of life span and disease progression in vivo

For life span and motor function assessment, animals were age, gender, and litter matched in accordance with recent ALS guidelines for the generation of preclinical data (Ludolph et al., 2010). Tests were performed blind by a single observer, twice weekly with a day interval between testing. Mice were trained for use of motor function equipment at PND 80. Motor function tests included Rotarod (Stoelting, IL, USA), grip strength (Ugo, Basile, Italy), and stride length measurements (Gurney et al., 1994). End stage of ALS disease progression was determined by the extreme weakening of hind limbs and loss of righting reflex within 30 seconds of when mice were placed on their back (Ludolph et al., 2010).

2.4. Assessment of motor neuron survival in vivo

Cryoprotected lumbar spinal cord samples were sectioned (20 μM in thickness) from L1 to L5 and Nissl stained with cresyl violet (0.1%). Nissl positive motor neuron cells were counted (according to the predetermined inclusion criteria, cells must be between 30 μM and 80 μM in diameter, have a dark nucleolus, and be multipolar in structure) in every third section in the ventral horn region of lumbar spinal cords and motor neuron survival assessed.

2.5. Primary motor neuron cell culture

Primary mixed motor neuron cultures were prepared from E13 C57Bl6 mouse embryos (Sebastia et al., 2009). Briefly, donor animals were terminally anesthetized and embryos removed by hysterectomy. Spinal cords were dissected, the meninges removed, and the ventral tissue was incubated in 0.025% trypsin (Sigma, Wicklow, Ireland) for 10 minutes at 37 °C. The cells were then transferred into Neurobasal medium (supplemented with 2% horse serum [Biosciences, Dublin, Ireland], 2% B27 [Biosciences], 0.1% fungizone [Invitrogen, Dublin, Ireland], GDNF [Promega, Southampton, UK; 2 ng/mL], CNTF [R&D Systems, UK; 1 ng/mL], 100 U/mL penicillin, and 100 μg/mL streptomycin), with 0.1 mg/mL DNAse 1, (Sigma), and gently dissociated. The cell suspension was centrifuged and the pellet re-suspended in Neurobasal medium. Cells were seeded onto Poly-L-Ornithine/Laminin (Sigma) coated coverslips at a density of 5×10^4 cells/well in a 24-well plate and maintained in Neurobasal medium at 37 °C and 5% CO_2.

2.6. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide assay

Cells were plated at 1×10^3 cells/well in a 96-well plate. At 7 days in vitro (DIV), 7 cells were treated with varying concentrations of latrepirdine (0.01 nM, 0.1 nM, 1 nM, 10 nM, and 100 nM) for 24 hours. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (20 μL; Sigma) was added to each well and incubated for 4 hours at 37 °C. The media was carefully removed from each well so as not to remove any crystals. DMSO (200 μL) was added to each well, and the plate was placed on an orbital shaker for approximately 10 minutes to allow the crystals to dissolve. The absorbance was measured at 560 nm absorbance using the Tecan GENios plate reader.

2.7. Immunocytochemistry and confocal imaging

At DIV, 7 cells were treated with latrepirdine (0.1 nM), or rapamycin (200 nM) or vehicle for 24 hours and cathepsin inhibitors pepstatin A and E64D (Calbiochem, Merck Biosciences, Nottingham, UK) for 23 hours. Cells were fixed with 3% paraformaldehyde for 12 minutes at 37 °C. Coverslips were washed 3 times and permeabilised with 0.1% PBS-tritonX-100. Cells were blocked in 5% goat serum (Sigma), immunostained with primary antibodies; SMI-32