available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Arsenic inhibits neurofilament transport and induces perikaryal accumulation of phosphorylated neurofilaments: Roles of JNK and GSK-3β

*Jason DeFuria, Thomas B. Shea**

Department of Biological Sciences and Biochemistry, Center Cell Neurobiology and Neurodegeneration Research, University of Massachusetts – Lowell, Lowell MA 01854, USA

ARTICLE INFO

Article history:
Accepted 7 April 2007
Available online 12 April 2007

Keywords:
Arsenic
Stress-activated protein kinase
JNK
Glycogen synthase kinase
Phosphorylation
Neurofilaments
Axonal transport
Environmental neurotoxins

ABSTRACT

The environmental neurotoxin arsenic has recently been associated with altered neurofilament (NF) content in sciatic nerve. We examined herein the impact of sodium arsenite (the inorganic form of arsenic) on NF dynamics. Treatment of differentiated NB2/d1 cells and cultured dorsal root ganglion neurons decreased NF transport into axonal neurites and increased perikaryal phospho-NF immunoreactivity. Both of these effects were prevented by a pharmacological inhibitor (SP600125) of c-*jun* terminal kinase and by expression of a dominant-negative form of this kinase. Arsenic-induced inhibition of NF transport was prevented by treatment with lithium, a selective inhibitor of glycogen synthase kinase-3beta. Pharmacological inhibitors of cyclin-dependent kinase 5 and p38 mitogen-activated protein kinase did not attenuate the effects of arsenic on NF dynamics. These latter findings suggest that this environmental neurotoxin could contribute to peripheral neuropathy by perturbing NF dynamics.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is characterized by a progressive loss of motor neurons, with eventual degeneration of muscles, resulting in paralysis and death. The full range of causative factors remains elusive despite decades of study. Studies from multiple laboratories implicate oxidative stress, mitochondrial dysfunction, aberrant calcium accumulation, axonal cytoskeleton perturbation, kinase hyperactivation, and/or exposure to environmental neurotoxins, at various stages in the disease progression (Bruijn et al., 2004; Strong, 2003). One hallmark of affected motor neurons in ALS is the accumulation of filamentous "spheroids" within proximal axons. These spheroids are comprised of disorganized neurofilaments (NFs). NFs, abundant within motor neurons, are 10-nm filaments composed of 3 subunits, termed NF-H, NF-M and NF-L (for high, medium and low with respect to molecular mass). Spheroid formation may result from inappropriate NF subunit stoichiometry and/or hyperphosphorylation (reviewed in Bruijn et al., 2004; Julien, 1999; Julien and Mushynski, 1998; Miller et al., 2002; Pant and Veeranna, 1995; Rao and Nixon, 2003). NF subunits are among the most highly phosphorylated proteins in the nervous system (for review, see Pant and Veeranna, 1995). The C-terminal regions, or "side-arms," of the subunits differ from each other. Extensive phosphorylation of NF-H and NF-M C-terminal "side-arms" (which extend away from the filament backbone) initiates within cell bodies and continues during axonal transport,

* *Corresponding author*. Fax: +1 978 934 3044.
E-mail address: Thomas_Shea@uml.edu (T.B. Shea).

0006-8993/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.brainres.2007.04.019

resulting in segregation of extensively phosphorylated NFs within axons, while hypophosphorylated NFs are largely confined to perikarya (Pant and Veeranna, 1995). Determination of factors causing spheroid formation is key to understanding their contribution to ALS neuropathology.

Several kinases have been reported to mediate C-terminal NF phosphorylation, and in some cases to interfere with NF axonal transport, including cyclin-dependent kinase 5 (cdk5; Bajaj and Miller, 1997; Bajaj et al., 1999; Guidato et al., 1996; Moran et al., 2005; Sharma et al., 1999; Shea et al., 2004; Sun et al., 1996), p 38 MAP kinase (Ackerley et al., 2004; Sasaki et al., 2006), GSK-3β (Ackerley et al., 2004; Bajaj and Miller, 1997; Chen et al., 2005; Guan et al., 1991; Guidato et al., 1996; Sasaki et al., 2006) and C-jun terminal kinase (JNK; Brownlees et al., 2000; Giasson and Mushynski, 1996; Liu et al., 1996; O'Ferrall et al., 2000). Activities of these kinases have been implicated as agents contributing to mislocalization of phospho-NF within perikarya and/or proximal axons, and in doing so, may contribute to the progression of neurodegenerative diseases including ALS (Ackerley et al., 2004; Harper and LoGrasso, 2001; Moran et al., 2005; Strong et al., 2001). Notably, the pattern of NF-H phosphorylation in spinal tissue from ALS patients was identical to that of normal individuals, suggesting that the accumulations of perikaryal/proximal axonal phospho-NFs consist of mislocated, but otherwise normally phosphorylated NFs. This supports the notion that precocious activation of the kinase(s) normally responsible for axonal NF bundling could promote aberrant accumulation. It is unclear whether NF accumulation represents a causative factor in neurodegeneration or is instead a downstream consequence observed in neurons that have already undergone extensive neurodegeneration as a result of already of antecedent trauma such as oxidative stress. However, removal of the C-terminal tail domain lessens ALS neuropathology (Lobsiger et al., 2005), suggesting a contributory role. Notably, NF spheroids accumulate kinesin and dynein (Toyoshima et al., 1998a,b), both of which participate in NF axonal transport (Shea and Flanagan, 2001); their concentration within spheroids may represent an apparent futile attempt of these motors to translocate NFs that are "trapped" within the spheroids. Motor entrapment may impair overall axonal transport. By compromising overall transport, and/or by destabilizing the axonal cytoskeleton, accumulation of NF spheroids may therefore represent the final insult that leads to degeneration of neurons already compromised by oxidative stress (e.g., Crosby, 2003; LaMonte et al., 2002). In addition, perikaryal NF inclusions have also been demonstrated to sequester SOD-1 and nitric oxide synthase (NOS; Sanelli et al., 2004), which could contribute to oxidative damage and disregulation of cytosolic calcium.

The environmental toxin arsenic induces neurotoxicity in adults, and profound developmental abnormalities of the nervous system (e.g., Ferm and Hanlon, 1986; Nagaraja and Desiraju, 1993; Rodriguez et al., 2003). Environmental exposure results primarily from consumption of drinking water contaminated with inorganic arsenic (Liu et al., 2004a,b). Since orally administered arsenic readily crosses the placenta and enters fetal tissue, maternal exposure can induce developmental abnormalities in utero (Chattopadhyay et al., 2002; Ferm and Hanlon, 1986) including permanent changes in genetic expression in fetuses (Liu et al., 2004a,b). Since environmental genotoxic agents may play a role in neurodegeneration (Eizirik et al., 1996), it remains possible that antecedent exposure, possibly including that in utero, to one or more environmental neurotoxins could represent one contributing factor to late-life neuropathological conditions such as ALS. The cytoskeleton may represent one target in arsenic toxicity (Li and Chou, 1992; van Bergen en Henegouwen and Linnemans, 1987). Decreased levels of NFs are observed in sciatic nerve following arsenic exposure (Vahidnia et al., 2006). In addition, activity of the NF kinases p38 MAP, ERK and JNK is increased by arsenic treatment (Cooper et al., 2006; Giafis et al., 2006; Kajiguchi et al., 2006; Kannan-Thulasiraman et al., 2006; Namgugn and Xia, 2001; Potin et al., 2007). These latter findings leave open the possibility that this environmental neurotoxin could contribute to neuropathological conditions by perturbing NF dynamics. To test this hypothesis, we examined the impact of arsenic treatment on NF transport and distribution in cultured neuroblastoma and neurons.

2. Results

Differentiated NB2a/d1 cells expressing GFP-M were incubated for 24 h with or without 0.15 or 1 μM sodium arsenite. The fluorescent intensity of perikarya and axonal neurites were then quantified for ≥10 transfected cells in each of 2 cultures under each condition (total≥20 cells/condition) and the mean % GFP-M that had translocated into axonal neurites was determined. Exposure to 0.15 and 1 μM sodium arsenite inhibited transport of GFP-M by 14±3% and 28±5%, respectively, and induced a 20–30% increase in perikaryal NF phospho-epitopes (Fig. 1). Perikarya of arsenic-treated cultures displayed thick "bundles" of phospho-NFs (e.g. Shea et al., 2004).

Since arsenic has been reported to stimulate kinase activity in a variety of cellular systems, the involvement of candidate NF kinases in the effects of arsenic on NF transport and levels of perikaryal NF phospho-epitopes was probed by co-treatment of these cultures with pharmacological inhibitors active against cdk5 (roscovitine), p38 MAP kinase (SB202190), GSK-3β (lithium) and JNK (SP600125). Cultures receiving 0.15 μM arsenic were treated for the last 2 h with these inhibitors (Fig. 2). As previously observed (Shea et al., 2004), treatment with roscovitine increased NF transport and decreased levels of RT97 within perikarya of NB2a/d1 cells. Co-treatment with roscovitine increased transport in arsenic-treated cells by the same degree that this inhibitor increased transport versus that observed in control cells. Similarly, co-treatment with roscovitine decreased perikaryal RT97 in arsenic-treated cells by the same degree that it decreased RT97 in control perikarya. Similar to effects of roscovitine, SB202190 increased transport in arsenic-treated cells to the same relative extent that this inhibitor increased transport in control cells. SB202190 did not alter the increase in perikaryal RT97 induced by arsenic. Lithium did not affect NF transport or perikaryal RT97 in isolation. However, co-treatment with lithium attenuated the extent of the arsenic-induced decrease in NF transport. Lithium did not alter the increase in perikaryal RT97 induced by arsenic. Treatment with SP600125 attenuated both NF