Molecular and Cellular Neuroscience 52 (2013) 140–151

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

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

The role of autophagy in Nmnat-mediated protection against hypoxia-induced dendrite degeneration

Yuhui Wen, R. Grace Zhai, Michael D. Kim *

Department of Molecular and Cellular Pharmacology, University of Miami, Miller School of Medicine, Miami, FL 33136, USA

ARTICLE INFO

Article history:
Received 26 June 2012
Revised 20 September 2012
Accepted 7 November 2012
Available online 15 November 2012

Keywords:
Nmnat
Autophagy
Hypoxia–ischemia
Dendrite
Neurodegeneration
Drosophila

ABSTRACT

The selective degeneration of dendrites precedes neuronal cell death in hypoxia–ischemia (HI) and is a neuropathological hallmark of stroke. While it is clear that a number of different molecular pathways likely contribute to neuronal cell death in HI, the mechanisms that govern HI-induced dendrite degeneration are largely unknown. Here, we show that the NAD synthase nicotinamide mononucleotide adenylyltransferase (Nmnat) functions endogenously to protect *Drosophila* class IV dendritic arborization (da) sensory neurons against hypoxia-induced dendritic damage. Whereas dendrites of wild-type class IV neurons are largely resistant to morphological changes during prolonged periods of hypoxia (<1.0% O_2), class IV neurons of *nmnat* heterozygous mutants exhibit significant dendrite loss and extensive fragmentation of the dendritic arbor under the same hypoxic conditions. Although basal levels of autophagy are required for neuronal survival, we demonstrate that autophagy is dispensable for maintaining the dendritic integrity of class IV neurons. However, we find that genetically blocking autophagy can suppress hypoxia-induced dendrite degeneration of *nmnat* heterozygous mutants in a cell-autonomous manner, suggestive of a self-destructive role for autophagy in this context. We further show that inducing autophagy by overexpression of the autophagy-specific kinase Atg1 is sufficient to cause dendrite degeneration of class IV neurons under hypoxia and that overexpression of Nmnat fails to protect class IV dendrites from the effects of Atg1 overexpression. Our studies reveal an essential neuroprotective role for endogenous Nmnat in hypoxia and demonstrate that Nmnat functions upstream of autophagy to mitigate the damage incurred by dendrites in neurons under hypoxic stress.

Introduction

Pathological alterations in dendritic structure are an early hallmark of brain injury in hypoxia–ischemia (HI). Transient ischemic episodes can induce rapid morphological changes in neurons, including extensive beading and swelling of dendrites, and the selective degeneration of dendrites during early ischemia likely serves as a precursor to neuronal cell death (Hori and Carpenter, 1994; Hsu and Buzsaki, 1993; Ikonomidou et al., 1989; Kitagawa et al., 1989; Matesic and Lin, 1994). Whereas much attention has focused on the mechanisms underlying neuronal death following HI (Lipton, 1999), relatively little is known of how HI can induce dendritic damage.

Macroautophagy (hereafter referred to as autophagy) is a highly conserved, ubiquitous process that is one of the major pathways responsible for the bulk degradation of proteins and organelles in response to starvation and other cellular stresses (Levine and Klionsky, 2004). Basal levels of autophagy are essential for neuronal survival (Hara et al., 2006; Komatsu et al., 2006), and accordingly, autophagic dysfunction has been linked to a growing number of neurodegenerative disorders (Levine and Kroemer, 2008). Neurons show increased autophagy following cerebral ischemia (Adhami et al., 2006; Carloni et al., 2008; Nitatori et al., 1995; Puyal et al., 2009; Rami et al., 2008; Wen et al., 2008; Zhu et al., 2005); however, the question of whether elevated levels of autophagy are neuroprotective or directly contribute to HI-induced neuronal cell death remains largely unresolved (Nakka et al., 2008; Uchiyama et al., 2008).

Nmnat catalyzes a key step of NAD synthesis and the neuroprotective effects of Nmnat overexpression in response to injury and neurotoxic insults have been well documented (Coleman and Freeman, 2010). Further studies have provided evidence of an evolutionarily conserved role for endogenous Nmnat in axon and dendrite maintenance (Gilley and Coleman, 2010; Wen et al., 2011; Zhai et al., 2006). The recent finding that endogenous Nmnat is upregulated in the brains of adult flies exposed to hypoxia suggests that increasing Nmnat levels may serve as an adaptive response to hypoxic stress (Ali et al., 2011). We previously reported that Nmnat is required for maintaining dendritic coverage of *Drosophila* dendritic arborization (da) sensory neurons (Wen et al., 2011). In the present study, we set out to determine whether Nmnat functions endogenously to maintain

Abbreviations: CNS, central nervous system; da, dendritic arborization; HI, hypoxia–ischemia; NAD, nicotinamide adenine dinucleotide; PNS, peripheral nervous system; ROS, reactive oxygen species.

* Corresponding author at: Department of Molecular and Cellular Pharmacology, University of Miami, Miller School of Medicine, 1600 NW 10th Avenue, Miami, FL 33136, USA. Fax: +1 305 243 4555.
E-mail address: mkim2@med.miami.edu (M.D. Kim).

1044-7431/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2012.11.008

the dendritic integrity of da neurons under hypoxic stress. We show that, while class IV neurons of wild-type larvae exposed to hypoxia (<1.0% O_2) are largely resistant to morphological changes, class IV neurons of *nmnat* heterozygous mutants exhibit regression and fragmentation of dendrites under the same hypoxic conditions. This hypoxia-induced dendrite degeneration in *nmnat* heterozygous mutants is unrelated to apoptosis as expression of the caspase inhibitor p35 fails to rescue dendrite phenotypes associated with hypoxia. Surprisingly, we find that reducing *Atg1* function suppresses the dendrite degeneration observed in class IV neurons of hypoxia-exposed *nmnat* mutants and that cell-specific knockdown of autophagy-related genes rescues dendrite phenotypes associated with reduced *nmnat* function. We further demonstrate that overactivation of autophagy causes dendrite degeneration of class IV neurons under hypoxia and that overexpression of Nmnat fails to rescue autophagy-induced dendritic phenotypes. Our study supports a self-destructive role for autophagy in hypoxia and provides genetic evidence that *nmnat* functions upstream of autophagy to protect dendrites from damage induced by hypoxic stress.

Results

Dendrites of class IV da neurons are largely resistant to hypoxic stress

We investigated the in vivo effects of prolonged hypoxia on dendrite morphology by examining class IV da sensory neurons of *Drosophila* larvae exposed to varying degrees of hypoxia. Class IV neurons are multi-dendritic sensory neurons of the peripheral nervous system (PNS) that elaborate highly branched dendritic arbors that effectively cover the entire larval body wall (Grueber et al., 2002). Class IV neurons are ideal for these studies because their dendrite arborization patterns are highly stereotyped and their dendritic arbors are largely confined within a two-dimensional space between the epidermis and body wall muscles (Han et al., 2012; Kim et al., 2012), allowing for immediate and direct live visualization of dendrite morphology without any artifacts associated with fixation. We used the class IV neuron-specific *ppk-Gal4* reporter driving expression of a green fluorescent protein (GFP)-tagged membrane marker (mCD8::GFP) to examine class IV neurons in wild-type third instar larvae [96–100 h after egg laying (AEL)] that were subjected to varying times (4, 8, and 14 h) of hypoxia (<1.0% O_2). Despite prolonged hypoxia, GFP-expressing larvae remained viable under these conditions. Surprisingly, the dendritic arborization patterns of class IV neurons were largely unchanged in these larvae when compared with age-matched normoxia (21% O_2) controls (data not shown), and a slight, though statistically significant, reduction in the total number of terminal dendritic branches was only observed after 14 h of hypoxia exposure (Figs. 1A, D, and G). However, the total dendrite length of class IV neurons in hypoxia-exposed larvae at 14 h did not differ statistically from normoxia controls (Fig. 1H). Furthermore, despite prolonged hypoxia exposure, class IV dendritic arbors remained intact and did not exhibit any apparent degeneration phenotypes (Fig. 1I), suggesting that these neurons possess an endogenous protection mechanism against hypoxic stress.

Nmnat functions endogenously to maintain dendritic integrity of class IV neurons under hypoxic stress

We previously found that Nmnat is cell-autonomously required for the proper maintenance of class IV dendrites and that it functions as a neuroprotective factor against progressive dendritic loss (Wen et al., 2011). The recent finding that Nmnat is upregulated in response to hypoxia and can mitigate the negative effects of oxidative stress on adult *Drosophila* lifespan (Ali et al., 2011) led us to investigate whether endogenous Nmnat serves a neuroprotective role in class IV neurons under hypoxia. Consistent with our previous results (Wen et al., 2011), we found that class IV neurons in larvae heterozygous for a loss-of-function mutation in *nmnat* ($nmnat^{\Delta 4792}$) exhibited significant reductions in both the total number of terminal dendritic branches (25.5%) and total dendrite length (13.7%) under normoxia when compared to wild-type controls (Figs. 1B, G, and H). However, when $nmnat^{\Delta 4792}$ heterozygous mutant larvae were placed under 14 h of hypoxia, the total number of terminal dendritic branches and the total dendrite length of class IV neurons were further reduced by 49.2% and 23.8%, respectively, when compared to class IV neurons of wild-type larvae under the same hypoxic conditions (Figs. 1E, G, and H). In addition to reduced dendritic branching, class IV neurons in hypoxia-exposed $nmnat^{\Delta 4792}$ heterozygotes exhibited extensive beading and fragmentation of the dendritic arbor (Fig. 1E). Both proximal and distal dendrites were affected as these neurons showed breaks at regular intervals along the entire lengths of the dendritic branches (Fig. 1E). We calculated the dendrite degeneration index (DI) for each genotype by summing the lengths of all breaks along the dendrites and dividing that sum by the total dendrite length. Whereas the DI for wild-type class IV neurons in hypoxia was 0.02, the DI for class IV neurons of hypoxia-exposed $nmnat^{\Delta 4792}$ heterozygous mutants was 0.16 (Fig. 1I), with 90% of these neurons having a DI greater than 0.10 as opposed to 0% for wild-type.

We then determined whether Nmnat functions cell-autonomously to protect class IV neurons under hypoxia by using *ppk-Gal4* to express a wild-type Nmnat transgene specifically in class IV neurons of both wild-type and $nmnat^{\Delta 4792}$ heterozygous mutant larvae. While overexpression of Nmnat did not significantly alter the dendritic properties of wild-type class IV neurons under normoxia or hypoxia (Figs. 1G, and H), overexpression of Nmnat fully rescued the dendritic phenotypes of class IV neurons in $nmnat^{\Delta 4792}$ heterozygotes under both normoxic and hypoxic conditions (Figs. 1C, F–I). Class IV-specific rescue restored total dendrite branching and dendrite lengths to that of wild-type controls and effectively prevented the degeneration of class IV dendrites under hypoxia (Figs. 1G–I). Collectively, these results demonstrate that endogenous Nmnat functions cell-autonomously to maintain the dendritic integrity of class IV neurons under hypoxic stress.

Overexpression of HSF and HIF-1α/Sima rescues hypoxia-induced dendrite degeneration in nmnat mutants

The *Drosophila* hypoxia-inducible factor 1α (HIF-1α) ortholog Similar (Sima) indirectly upregulates Nmnat expression in response to hypoxia through the induction of heat shock factor (HSF), which functions as a central regulator of *nmnat* transcription under stress conditions, and overexpression of either HSF or Sima has been shown to upregulate Nmnat in response to hypoxic stress (Ali et al., 2011). We therefore tested whether selective overexpression of HSF or Sima could rescue hypoxia-induced dendritic phenotypes of class IV neurons in $nmnat^{\Delta 4792}$ heterozygous mutants. Overexpression of HSF or Sima had no effect on the development or maintenance of class IV dendrites in wild-type larvae under both normoxic and hypoxic conditions (Supplementary Fig. S1I–N). Although overexpression of HSF or Sima partially suppressed the loss of terminal dendritic branches observed in $nmnat^{\Delta 4792}$ heterozygotes under normoxia, the total dendrite length remained unchanged in these mutants (Supplementary Fig. S1A–D, and I–N). This may be explained by moderate increases in Nmnat expression that are observed upon HSF or Sima overexpression in non-stress conditions (Ali et al., 2011). On the other hand, we found that overexpression of HSF or Sima fully rescued the dendrite regression and degeneration phenotypes of class IV neurons in hypoxia-exposed $nmnat^{\Delta 4792}$ heterozygotes (Supplementary Fig. S1E–N), consistent with the ability of either transcription factor to significantly upregulate Nmnat expression upon exposure to stress (Ali et al., 2011). These data provide further