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Research Report

Stress-induced heat shock protein 27 expression and its role in dorsal root ganglion neuronal survival

*M. Elaine Dodge, Jinguo Wang, Clifford Guy, Sherri Rankin, Masuma Rahimtula, Karen M. Mearow**

Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3V6

ARTICLE INFO

Article history:
Accepted 10 November 2005
Available online 27 December 2005

Theme:
Development and regeneration
Topic:
Neuronal death

Keywords:
Hsp27
DRG
NGF
Heat shock
siRNA

ABSTRACT

Heat shock protein 27 (Hsp27), a molecular chaperone ubiquitously expressed in many cell types, has been shown to play a role in protecting neurons from cellular stresses. Unlike adult DRG neurons in vitro, neonatal DRG neurons require NGF for survival; withdrawal of NGF results in apoptosis of a majority of neonatal neurons. We hypothesized that Hsp27 contributes to the neurotrophin-independent survival of adult DRG neurons. Constitutive Hsp27 expression is higher in adult DRG neurons compared to neonates, although both upregulate Hsp27 expression after heat shock (HS). We found that increasing endogenous Hsp27 by HS in neonatal neurons was able to inhibit NGF withdrawal-induced apoptosis. Heat shock of adult and neonatal neurons also resulted in Akt activation, which could be a mechanism for the increased survival. Hsp27 siRNA treatment of adult neurons effected a decreased expression of Hsp27, which correlated with increased apoptosis in these neurons. Downregulation of Hsp27 via siRNA also blocked the HS-induced rescue of neonatal neurons after NGF withdrawal. These results indicate that physiologically induced upregulation of Hsp27 is sufficient to provide some degree of neuronal protection. Further, this induction appears to be regulated by the transcriptional activation of HSF1 as shown by HSF1 nuclear translocation and by EMSA analyses of HSF1 binding to nuclear protein.

1. Introduction

Living cells possess a number of mechanisms to cope with various stresses such as radiation, oxidants, chemicals or heat, although one common response is the accumulation or activation of a set of highly conserved cellular proteins known as heat shock proteins (Hsps) (Hightower, 1991). Hsps can function as chaperones playing a key role in protein folding and transport, and also have important functions in the prevention of apoptosis and interactions with antiapoptotic signaling proteins (Arrigo, 2001; Beere, 2004; Morimoto et al., 1997; Nollen and Morimoto, 2002; Ohtsuka and Suzuki, 2000). A conditioning stress, such as a mild heat stress, is sufficient to induce the Hsp response and provide cells with a protective response against subsequent potentially lethal insults (Mailhos et al., 1993; Quigney et al., 2003).

There are different families of heat shock proteins that vary in their function and purpose within the cell. For example, Hsp70 and Hsp90 appear to be the major proteins induced by stress in the nervous system, providing protection against a variety of insults (Franklin et al., 2005; Ohtsuka and Suzuki, 2000; Richter-Landsberg and Goldbaum, 2003). In addition, a

* *Corresponding author*. Fax: +1 709 777 8281.
E-mail address: kmearow@mun.ca. (K.M. Mearow).

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doi:10.1016/j.brainres.2005.11.008

family of small heat shock proteins (sHsp), which includes Hsp25/27 and α-crystallin, has been shown to be upregulated by stress. Hsp27 has protective effects against heat shock, oxidative stress and other models of cellular injury in a variety of cell types, including neurons (e.g., Arrigo et al., 2005; Benn et al., 2002; Landry et al., 1989; Latchman, 2005; Lewis et al., 1999; Wagstaff et al., 1999; Zourlidou et al., 2004).

In response to cellular stress, the transcription factor heat shock factor-1 (HSF-1) has been reported to play a crucial role in cell protection (Ahn and Thiele, 2003; Christians et al., 2002; Pirkkala et al., 2001; Wu, 1995). HSF-1 is important for Hsp gene expression, and its activation leads to a conformational change from a monomeric form to an active, DNA-binding trimeric form. This trimeric form translocates to the cell nucleus where it can bind to the heat shock element (HSE) portion of the Hsp promoter and activate Hsp gene expression (Tonkiss and Calderwood, 2005; Wu, 1995). Neurons have relatively low levels of HSF compared to glial cells which has been suggested to result in a relatively compromised heat shock response (Tonkiss and Calderwood, 2005).

Dorsal root ganglion (DRG) sensory neurons are known to be dependent upon neurotrophins for their embryonic development and differentiation. During this early embryonic period, their dependence is based upon neurotrophins such as NT3 and NGF; however, dependence is shifted primarily to NGF during late embryonic development (Memberg and Hall, 1995; Molliver and Snider, 1997; Ruit et al., 1992; Vogelbaum et al., 1998; White et al., 1996). It is well established that early neonatal DRG neurons are dependent on NGF for survival, and when NGF is withdrawn from culture, these neurons die via an apoptotic mode of cell death, likely by inhibition of pro-survival signaling (e.g., PI3K → Akt) and activation of pro-apoptotic genes c-Jun and bax (Datta et al., 1997; Deckwerth et al., 1996; Tong et al., 1996; Vogelbaum et al., 1998).

On the other hand, adult DRG neurons do not require NGF for survival and survive up to 2 weeks in culture in the absence of NGF (Dodge et al., 2002). The mechanism by which developing DRG neurons become less sensitive to harmful stimuli as they progress to adulthood is the subject of much study and a number of molecular candidates, including Hsp27, have been suggested (Benn et al., 2002; Fernyhough et al., 2005; Walsh et al., 2004). For example, it has been reported that the developmental regulation of Hsp27 may play a role in the decreased vulnerability of mature DRG neurons to injury or

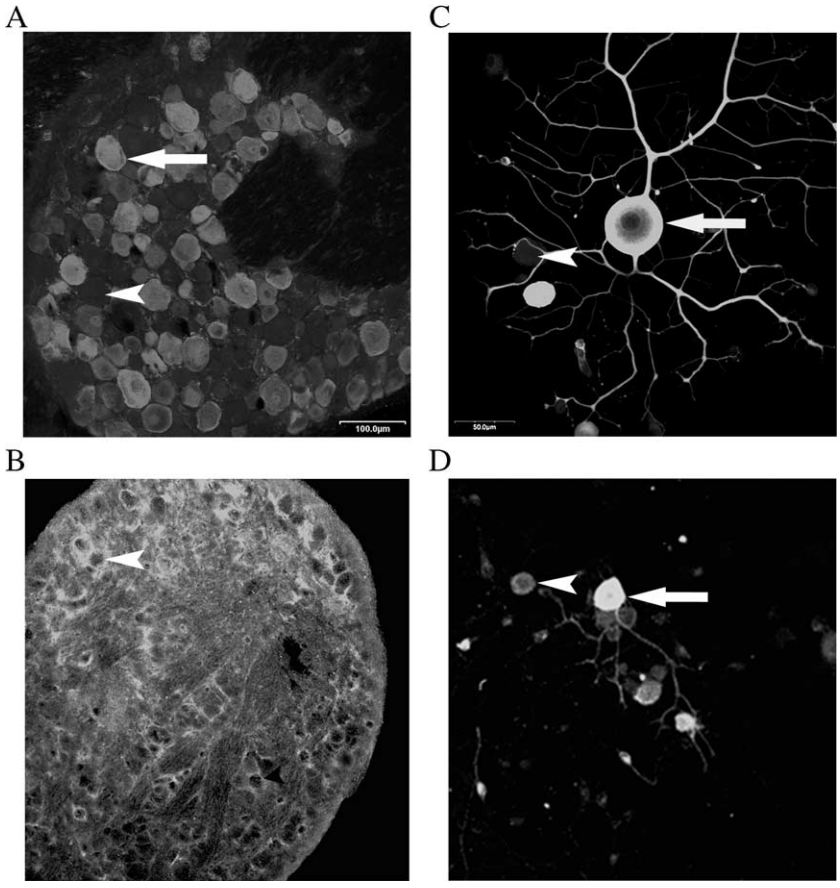

Fig. 1 – Expression of Hsp27 in adult and neonatal DRG cryosections and primary cultures. (A, B) DRGs were extracted from adult (A) and neonatal (B) rats, frozen in liquid nitrogen and sectioned into 8 μm (adult) and 16 μm (neonate) thick sections. Cell counts of sections from cervical, thoracic, lumbar and sacral regions together for expression levels of Hsp27 show that there are a significantly greater number of cells in adult DRGs expressing Hsp27. (C, D) Neuronal cultures were also prepared and immunostained with an antibody to Hsp27. (C) Adult neuronal cultures show robust, intense staining for Hsp27 in both the cell body and the surrounding neurites. (D) Neonatal neurons show faint or no staining for Hsp27. Arrowheads indicate cells lacking Hsp27 expression, whereas arrows indicate cells immunopositive for Hsp27. Scale bar, 50 μm.