Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Neurology 196 (2005) 184 – 194

Experimental Neurology

www.elsevier.com/locate/yexnr

The Janus role of c-Jun: Cell death versus survival and regeneration of neonatal sympathetic and sensory neurons

Charlotta Lindwall*, Martin Kanje

Lund University, Department of Cell and Organism Biology, Animal Physiology Building, Helgonavägen 3B, SE-223 62 Lund, Sweden

Received 30 May 2005; revised 19 July 2005; accepted 27 July 2005
Available online 29 August 2005

Abstract

We investigated the functional outcome of c-Jun activation in sympathetic and sensory neurons of neonatal rat superior cervical ganglion (SCG) and dorsal root ganglion (DRG), respectively. Distinctly different roles of c-Jun activation have been suggested for these two types of neurons. In dissociated sympathetic neurons, c-Jun has been demonstrated to promote apoptosis, whereas in sensory neurons it stimulates axonal outgrowth. In organ-cultured ganglia, we found that c-Jun was activated within 24 h of explantation in both types of neurons, and that the JNK inhibitor SP600125 could mitigate this response. In both types of neurons, c-Jun activation was also reduced by NGF treatment. Inhibition of c-Jun activation did not affect the viability of sympathetic neurons, whereas the number of apoptotic sensory neurons increased. Furthermore, inhibition of c-Jun reduced axonal outgrowth from both SCG and DRG. Thus, in organ culture, c-Jun activation may be required for axonal outgrowth and, at least in sensory neurons, it promotes survival. The role of ATF3, a neuronal marker of injury and a c-Jun dimerization partner, was also examined. We found an ATF3 induction in both SCG and DRG neurons, a response, which was reduced by JNK inhibition. The reduction of ATF3 upon JNK inhibition was much larger in DRG than in SCG, a result which might account for the higher number of apoptotic neurons in JNK inhibitor exposed DRG. Taken together, and contrary to our expectations, neonatal sympathetic and sensory neurons seem to respond to axonal injury similarly with respect to c-Jun activation, and in no case was this activation pro-apoptotic.

Keywords: c-Jun; DRG; SCG; NGF; SP600125 rat; Development

Introduction

Evidence is mounting regarding the dichotomous role of c-Jun N-terminal kinase (JNK) -mediated c-Jun activation (for review, see Herdegen et al., 1997b) in neuronal stress responses. Activation of c-Jun seems to be associated with cell death in neonatal sympathetic neurons deprived of nerve growth factor (NGF) (Bruckner et al., 2001; Eilers et al., 2001; Estus et al., 1994; Ham et al., 2000; Kanamoto et al., 2000; Maroney et al., 1999; Palmada et al., 2002; Whitfield et al., 2001), and in neonatal hippocampal neurons (Schlingensiepen et al., 1993). c-Jun activation may also be involved in neurodegeneration (Herdegen and Waetzig, 2001). In contrast, an increasing number of studies, including ours, indicate that JNK-mediated c-Jun activation occurs as a response to axonal injury and that this activation is required for axonal outgrowth (Broude et al., 1997; Kenney and Kocsis, 1998; Lindwall et al., 2004; Raivich et al., 2004; Robinson, 1995; Schaden et al., 1994; Waetzig and Herdegen, 2003) and not apoptosis.

The difference between the pro-apoptotic role of c-Jun in sympathetic neurons and c-Jun activation for survival and neurite outgrowth in sensory neurons is striking. Thus, we considered the possibility that the dichotomous role, or the

Abbreviations: ATF2/3, activating transcription factor 2/3; CDK, cyclin-dependent kinase; DRG, dorsal root ganglion/ganglia; Hsp27, heat shock protein 27; IR, immunoreactivity; JNK, c-Jun N-terminal kinase; L, lumbar; NGF, nerve growth factor; PBS, phosphate-buffered saline; SCG, superior cervical ganglion/ganglia.

* Corresponding author. Fax: +46 46 222 4539.
E-mail address: charlotta.lindwall@cob.lu.se (C. Lindwall).

0014-4886/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2005.07.022

Janus facet, of c-Jun activation in sympathetic versus sensory neurons could be due to the neuronal type or the different developmental stages of the neurons used for the experiments, i.e. neonatal and adult neurons. We also hypothesized that the differences could be accounted for by the experimental culture conditions. For instance, the pro-apoptotic activity of c-Jun has been demonstrated in dissociated cultures of neonatal sympathetic neurons deprived of support cells by DNA damaging agents such as fluorodeoxyuridine. These cultures were also maintained in serum-containing medium and were treated with NGF for several days prior to neurotrophin withdrawal. In contrast, the results from sensory neurons, where c-Jun activation was found to be a prerequisite for axonal outgrowth, were obtained in organ-cultured ganglia or dissociated cell cultures, which were neither subjected to NGF or serum treatment (Lindwall et al., 2004). These differences could be important with respect to c-Jun activation and its functional outcome. To investigate this possibility, we decided to compare the effects of c-Jun activation on neuronal survival and axonal outgrowth in organ-cultured neonatal SCG and DRG of the same age. Since c-Jun may also be involved in the induction of survival-associated factors like activating transcription factor 3 (ATF3) (Cai et al., 2000; Lindwall et al., 2004), we also investigated the induction of this transcription factor in both types of ganglia.

Our results show that organ-cultured neonatal sensory and sympathetic neurons respond to axonal injury by JNK-mediated c-Jun activation and ATF3 induction. By inhibiting JNK in these ganglia, we also demonstrate that JNK signaling is important for axonal outgrowth in both types of neurons.

Materials and methods

Animals and cell culture

Sprague–Dawley postnatal day 1 rat pups (own breeding) were used. The experiments were approved by the local ethics committee at Lund University and carried out according to the European Communities Council Directive regarding care and use of animals for experimental procedures. A total of 22 rat pups were killed by heart puncture after hypothermia by placing the pups for 5 min on ice. The superior cervical ganglia (SCG) and the 4th and 5th lumbar (L4 and L5) dorsal root ganglia (DRG) were removed by dissection and either fixed directly (n = 2) or cultured in RPMI-1640 medium (Sigma-Aldrich Co LTD, U.K.), supplemented with antibiotics (100 U/ml penicillin, 100 U/ml streptomycin, and 250 ng/ml amphoretoricin B; Gibco, UK) and glutamine (Gibco, UK). The cultured ganglia were either whole-mounted (n = 8) in 35 mm plastic culture dishes with 5 μl Matrigel® (Collaborative research Inc., USA) or cultured free-floating in 4-well chambers (n = 12). All cultures were maintained for 24 h at 37°C in a humidified atmosphere of 93.5% O_2 and 6.5% CO_2 before fixation and further processing for immunohistochemistry. Axonal outgrowth from the Matrigel®-mounted ganglia was measured at 24 h after explantation with a phase contrast microscope equipped with a scaled ocular. The linear distance between the transection site and the front line of regenerative axons was regarded as the axonal outgrowth distance.

Culture treatments

NGF was used at 50 ng/ml (β-NGF; Peprotech, UK) and the JNK inhibitor SP600125 (1,9-pyrazoloanthrone, Calbiochem, Germany) was used at 10 μM. The stock solution of SP600125 was prepared in dimethyl sulfoxide (DMSO, Merck, Germany) at 20 mM, which was then diluted to the final concentration in RPMI-1640 medium. Control ganglia received DMSO at a concentration corresponding to the experimental ganglia, however not exceeding 1%.

Immunohistochemistry

The ganglia were fixed in Stefanini's fixative (2% formaldehyde, 0.1% picric acid in 0.1 M phosphate buffer, pH 7.2)

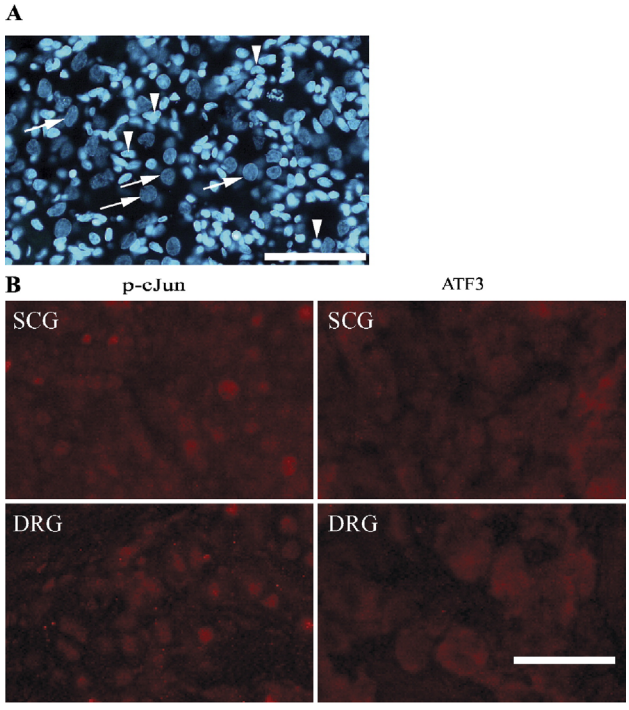

Fig. 1. Illustration of the criteria for neuron/glia discrimination using nuclear bisbenzimide staining on a neonatal DRG section (A) and p-c-Jun and ATF3 IR positive neuronal nuclei (B) in freshly dissected rat neonatal SCG and DRG sections. (A) Neurons were distinguished from glial cells by their larger nuclear size and their lower bisbenzimide staining intensity. Arrows point to neuronal nuclei, whereas arrowheads indicate glial cells. (B) Low levels of both p-c-Jun and ATF3 IR were found in the majority of SCG and DRG neuronal nuclei in the uninjured ganglia. The images are representative of 6 sections obtained from two animals. Scale bars = 50 μm.