Neuroscience Letters 511 (2012) 52–57

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

The effects of neuregulin-1β on neuronal phenotypes of primary cultured dorsal root ganglion neurons by activation of PI3K/Akt

Zhen Liu[a], Hao Jiang[b], Hao Li[b], Huaxiang Liu[c], Xiaobo Xu[d], Zhenzhong Li[a,*]

[a] Department of Anatomy, Shandong University School of Medicine, Jinan 250012, China
[b] Department of Orthopaedics, Shandong University Qilu hospital, Jinan 250012, China
[c] Department of Rheumatology, Shandong University Qilu Hospital, Jinan 250012, China
[d] Neuroscience Program, University of Southern California, Los Angeles, California, 90089-2520, USA

ARTICLE INFO

Article history:
Received 30 August 2011
Received in revised form 16 January 2012
Accepted 18 January 2012

Keywords:
Neuregulin-1β
Calcitonin gene-related peptide
Neurofilament-200
Neuron
Dorsal root ganglion

ABSTRACT

Neuregulin-1β (NRG-1β) signaling has multiple functions in neurons. NRG-1 signaling regulates neuronal development, migration, myelination, and synaptic maintenance. The neuropeptide- and neurofilament (NF)-immunoreactive (IR) neurons are two major phenotypical classes in dorsal root ganglion (DRG). Whether NRG-1β influences DRG neuronal phenotypes remains unknown. To assess the effects of NRG-1β on DRG neuronal phenotypes, dissociated embryonic rat DRG neuronal culture model was established. Primary cultured DRG neurons were exposed to NRG-1β (5 nmol/L), NRG-1β (10 nmol/L), NRG-1β (20 nmol/L), NRG-1β (20 nmol/L) plus LY294002 (10 μmol/L) for 3 days, respectively. The DRG neurons were continuously exposed to growth media as control. After that, all above cultured DRG neurons were processed for double fluorescent labeling of calcitonin gene-related peptide (CGRP) or neurofilament-200 (NF-200) and microtubule associated protein 2 (MAP2). The percentage of CGRP-IR neurons and NF-200-IR neurons was counted. The expression of CGRP mRNA and NF-200 mRNA was analyzed by real time-PCR analysis. The percentage of CGRP-IR neurons but not NF-200-IR neurons increased significantly in the presence of NRG-1β as compared with that in the absence of NRG-1β. The levels of CGRP mRNA but not NF-200 mRNA increased significantly in the presence of NRG-1β as compared with that in the absence of NRG-1β. PI3K inhibitor LY294002 blocked the effects of NRG-1β. These results support an important role for exogenous NRG-1β in induction of the distinct neuronal phenotype response by activation of PI3K/Akt in sensory neurons.

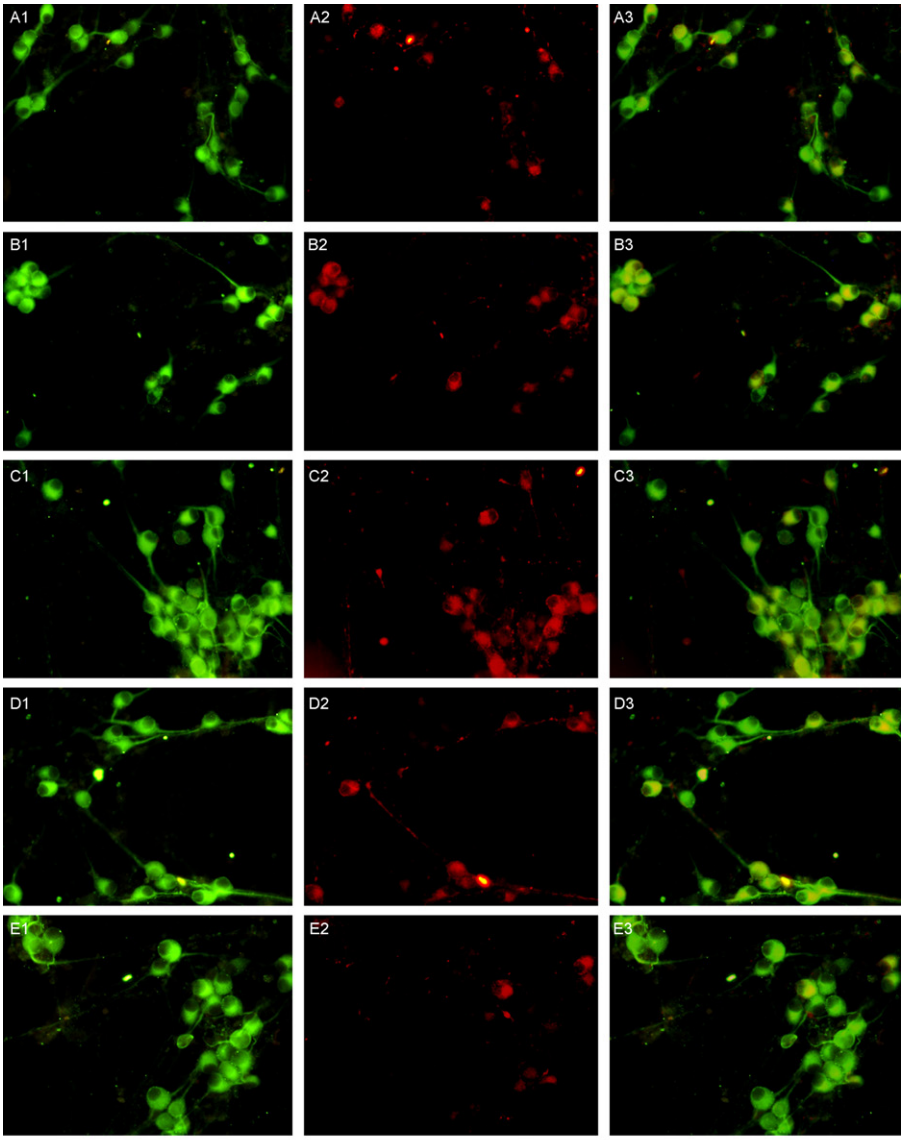
Neuregulin-1β (NRG-1β), an excitomotor of tyrosine kinase receptor (erbB) family, has multiple functions in the development of nervous system, adjusting the proliferation, migration, differentiation, myelination, and synaptic maintenance [3]. NRG-1 and erbB receptor expression was observed in dorsal root ganglion (DRG) of rat pups between embryonic day (E) 13 and postnatal day 15 [13]. Blockade of the endogenous NRG activity of nascent neurons in the DRG completely blocks the cholinergic neurotrophic factor-induced proliferation and reduces the neurotrophin-3-mediated proliferation suggesting important role of NRG on the genesis and differentiation of neurons in the DRG [8]. It has been shown that NRG-1β increases the outgrowth of primary neurites, neuronal area, total neurite length, and neuritic branching in E18 hippocampal neurons [6].

The neuropeptide-immunoreactive (IR) and neurofilament (NF)-IR neurons are two major phenotypical classes in DRG. Neuropeptide-IR neurons are considered to be with unmyelinated or thinly myelinated nociceptive afferents which are considered to innervate skin and viscera. NF-IR neurons typically have myelinated axons which are considered to innervate muscle spindle [7]. Cultured neurons expressed neuropeptides with a time course and in proportions similar to those in vivo and NF-IR neurons are also present in DRG cell cultures [7]. Calcitonin gene-related peptide (CGRP) is a sensory neuron-associated neuropeptide [5]. CGRP-IR neurons represent neuropeptide-IR neurons in DRG [7]. NF-200-IR neurons represent NF-IR neurons in DRG [15]. Whether NRG-1β influences DRG neuronal phenotypes remains unknown. It has been suggested that the neuroprotective effects of NRG-1β on ischemia-induced neuronal death were prevented by inhibition of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in an in vitro rat ischemia model [4]. Whether PI3K/Akt signaling

Abbreviations: CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; E, embryonic day; IR, immunoreactive; MAP2, microtubule associated protein 2; NF, neurofilament; NRG, Neuregulin; PI3K, phosphatidylinositol 3-kinase.

* Corresponding author at: Department of Anatomy, Shandong University School of Medicine, 44 Wenhua Xi Road, Jinan, Shandong Province 250012, China. Tel.: +86 158 6379 3602.
E-mail address: zli@sdu.edu.cn (Z. Li).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2012.01.041



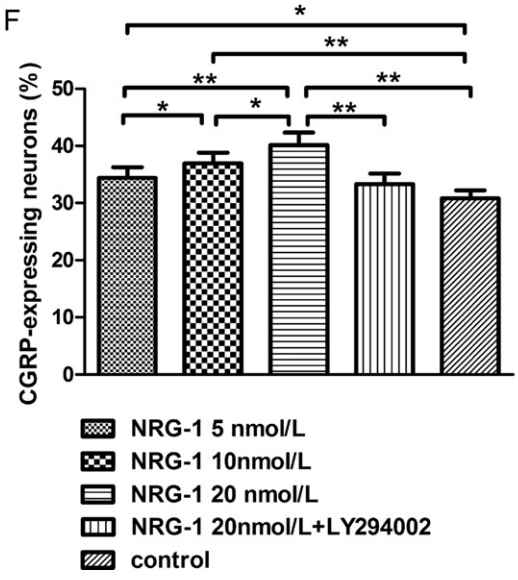


Fig. 1. Double fluorescent labeling of MAP2 and CGRP of cultured DRG neurons. (A) NRG-1β 5 nmol/L (A1: MAP2; A2: CGRP; A3: overlay of A1 and A2). (B) NRG-1β 10 nmol/L (B1: MAP2; B2: CGRP; B3: overlay of B1 and B2). (C) NRG-1β 20 nmol/L (C1: MAP2; C2: CGRP; C3: overlay of C1 and C2). (D) NRG-1β 20 nmol/L plus LY294002 (D1: MAP2; D2: CGRP; D3: overlay of D1 and D2). (E) Control (E1: MAP2; E2: CGRP; E3: overlay of E1 and E2). Scale bar = 50 μm. (F) The percentage of CGRP-IR neurons in DRG neuronal cultures ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.001$.