Peptides 32 (2011) 1244–1248

Contents lists available at ScienceDirect

Peptides

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

Neuregulin-1β regulates outgrowth of neurites and migration of neurofilament 200 neurons from dorsal root ganglial explants in vitro

Zhen Liu[a], Wei Gao[b], Yanping Wang[c], Weiwei Zhang[a], Huaxiang Liu[d,*], Zhenzhong Li[a,**]

[a] Department of Anatomy, Shandong University School of Medicine, 44 Wenhua Xi Road, Jinan, Shandong Province 250012, China
[b] Department of Pathology, Jinan Central Hospital, Jinan 250013, China
[c] Department of Internal Medicine, Shouguang Health School, Shouguang 262700, China
[d] Department of Rheumatology, Shandong University Qilu Hospital, 107 Wenhua Xi Road, Jinan, Shandong Province 250012, China

ARTICLE INFO

Article history:
Received 5 March 2011
Received in revised form 4 April 2011
Accepted 7 April 2011
Available online 15 April 2011

Keywords:
Neuregulin-1β
Neurofilament 200
Neuron
Dorsal root ganglion

ABSTRACT

Neuregulin-1β (NRG-1β) signaling has multiple functions in neurons. To assess NRG-1β on neurite outgrowth and neuronal migration in vitro, organotypic dorsal root ganglion (DRG) neuronal culture model was established. Neurite outgrowth and neuronal migration were evaluated using this culture model in the presence (5 nmol/L, 10 nmol/L, 20 nmol/L) or absence of NRG-1β. Neurofilament 200 (NF-200)-immunoreactive (IR) neurons were determined as the migrating neurons. The number of nerve fiber bundles extended from DRG explant increased significantly in the presence of NRG-1β (5 nmol/L, 23.0 ± 2.2, $P < 0.05$; 10 nmol/L, 27.0 ± 2.7, $P < 0.001$; 20 nmol/L, 30.8 ± 3.7, $P < 0.001$) as compared with that in the absence of NRG-1β (19.0 ± 2.2). The number of neurons migrating from DRG explants increased significantly in the presence of NRG-1β (5 nmol/L, 39.6 ± 5.0, $P < 0.05$; 10 nmol/L, 54.6 ± 6.7, $P < 0.001$; 20 nmol/L, 62.2 ± 5.7, $P < 0.001$) as compared with that in the absence of NRG-1β (31.6 ± 4.0). Moreover, the increase of the number of nerve fiber bundles and the number of migrating NF-200-IR neurons was dose-dependent for NRG-1β addition. The data in this study imply that NRG-1β promotes neurite outgrowth and neuronal migration from DRG explants in vitro.

1. Introduction

The neuregulins (NRGs) are a family of polypeptide factors which bind to the erbB family of tyrosine kinase receptors implicated in a wide range of roles in the development and function in different organs including the nervous system [7,19,21]. NRG-1/erbB signaling has multiple functions in neurons [16]. NRG-1/erbB signaling regulates neuronal development, migration, myelination, and synaptic maintenance [1,18,23,24]. Several recent reports demonstrated that NRG-1 is neuroprotective in vivo following ischemia [28–30] and in vitro ischemia model [3]. NRG-1 and its receptors, erbB receptors, are induced in neuronal cells following traumatic brain injury [4,25]. It has been suggested that multiple NRG-1 isoforms and NRG-1 receptor subunits erbB2, erbB3, and erbB4 are expressed in rat dorsal root ganglion (DRG) neurons [20]. Blockade of the endogenous NRG activity of nascent neurons in the DRG completely blocks the cholinergic neurotrophic factor (CNTF)-induced proliferation and reduces the neurotrophin-3 (NT-3)-mediated proliferation suggesting important role of NRG on the genesis and differentiation of neurons in the DRG [12]. 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 [9]. Whether NRG-1β signaling regulates neurite outgrowth and neuronal migration of DRG neurons remains unknown. In the present study, organotypic DRG neuronal culture model was established. Neurofilament 200 (NF-200) was used as a neuronal marker to distinguish the migrating DRG neurons from other cells [32]. Neurite outgrowth and neuronal migration were evaluated using this culture model in the presence or absence of NRG-1β.

2. Materials and methods

2.1. Organotypic DRG culture preparations

The organotypic DRG culture preparations utilized embryonic rats taken from the breeding colony of Wistar rats maintained in the Experimental Animal Center at Shandong University of China.

Abbreviations: CNTF, cholinergic neurotrophic factor; DRG, dorsal root ganglion; DRGs, dorsal root ganglia; DMEM/F-12, Dulbecco's Modified Eagle Medium with F-12 supplement; E15, embryonic day 15; Egr-3, early growth response 3; NF, neurofilament; NF-200, neurofilament 200; NF-H, neurofilament heavy chain; NF-L, neurofilament light chain; NF-M, neurofilament middle chain; NRG, neuregulin; NRGs, neuregulins; NT-3, neurotrophin-3; PBS, phosphate buffer saline; PI3K, phosphatidylinositol 3-kinase; PNS, peripheral nervous system.

* Corresponding author. Tel.: +86 531 8216 9654; fax: +86 531 8603 3058.
** Corresponding author. Tel.: +86 158 6379 3602.
E-mail addresses: huaxiangliu@yahoo.com.cn (H. Liu), zli@sdu.edu.cn, zhenzhongli@gmail.com (Z. Li).

0196-9781/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2011.04.005

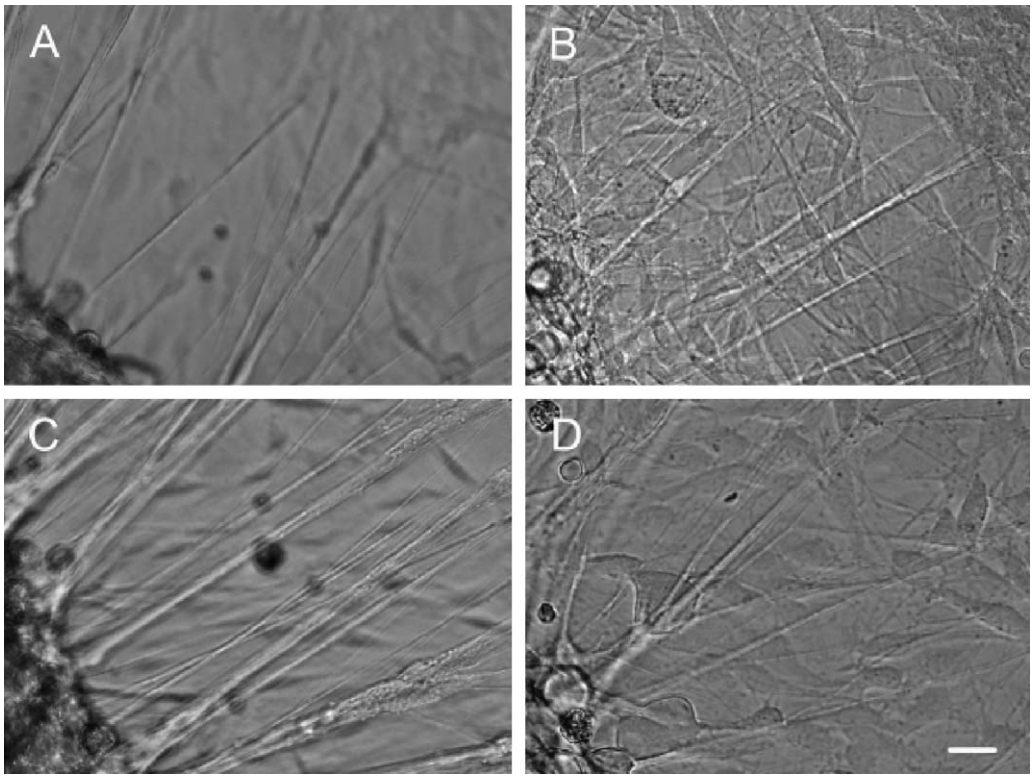

Fig. 1. Radial nerve fiber bundles extended from DRG explant 3 days after plating to the coverslip in the presence or absence of NRG-1β. Panel A: NRG-1β 5 nmol/L, Panel B: NRG-1β 10 nmol/L, Panel C: NRG-1β 20 nmol/L, Panel D: control. Scale bar = 25 μm.

Embryonic rats at embryonic day 15 (E15) were used for DRG organotypic DRG culture. Under aseptic conditions, the bilateral dorsal root ganglia (DRGs) were removed from each rat embryo and placed in culture media in the half of petri dishes. Each DRG explant was plated at the bottom of each well of 24-well clusters (Costar, Corning, NY, USA) and cultured in Dulbecco's Modified Eagle Medium with F-12 supplement (DMEM/F-12) (Invitrogen) media with 10% heat inactivated fetal bovine serum (Invitrogen), 20 μl/ml 1× B-27 (Invitrogen), 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C with 5% CO_2 with media change the second day.

2.2. Exposure of NRG-1β on DRG cultures

The organotypic DRG explants cultures were treated with 5 nmol/L NRG-1β (Peprotech), 10 nmol/L NRG-1β, and 20 nmol/L NRG-1β, respectively. The DRG explants were continuously cultured in growth media as control. All above cultures were incubated at 37 °C in a humidified 5% CO_2-air atmosphere for 3 days.

2.3. Determination of neurite outgrowth from DRG explants

The number of nerve fiber bundles extended from DRG explants was counted at 3 days of culture age. Each nerve fiber bundle extended from the edge of each DRG explant as far as 200 μm was counted. The length of nerve fiber bundle which is less than 200 μm was not counted in this experiment. Nerve fiber bundles extended from a quarter of each DRG explant (as shown in Fig. 1) was counted. Because the whole DRG explant is too large to examine all the nerve fiber bundles around each explant in one visual field, so that only a quarter of each DRG explant with its nerve fiber bundles was counted in each sample.

2.4. Immunofluorescent labeling of NF-200

At 3 days of culture age, all NRG-1β treated and control DRG explants cultures were processed for immunofluorescent labeling of NF-200. The DRG cells on coverslips were rinsed quickly one time in 0.1 mol/L Sorenson's phosphate buffer to remove media. The DRG cells were then fixed in 4% paraformaldehyde, pH 7.4, for 20 min at 4 °C. After washing in 0.1 mol/L phosphate buffer saline (PBS) for 3 times, the DRG cells were blocked by 1% normal goat serum in 0.6% Triton PBS to block non-specific sites and permeabilize cells. After that, the samples were incubated with mouse monoclonal anti-NF-200 (1:400, Abcam) overnight at 4 °C. After washing in 0.1 mol/L PBS 3 times, the samples were incubated with goat anti-mouse conjugated to Cy2 (1:200, Abcam) for 60 min in dark. After washing in 0.1 mol/L PBS, the DRG cells were coverslipped immediately with Vectashield anti-fade mounting media (Vecto Laboratories, Inc.) and stored at 4 °C until observation by fluorescence microscopy.

2.5. Determination of neuron migration from DRG explants

The migrating neurons were determinated as NF-200-IR neurons. NF-200-IR neurons were observed under a fluorescence microscopy (Olympus) with 20× objective lens. NF-200-IR neurons in one visual field at the edge of DRG explant were counted as the migrating neurons in each sample.

2.6. Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was evaluated with SPSS software by one-way ANOVA followed by the Student–Newman–Keuls test for significance to compare the differences among various groups. Significance was determined as $P < 0.05$.