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Research paper

Effect of FGF-2 and sciatic nerve grafting on ChAT expression in dorsal root ganglia neurons of spinal cord transected rats

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HIGHLIGHTS

- Transected spinal cord as a model for study of DRG regeneration.
- Peripheral nerve grafts as a favorable environment to DRG neuroprotection.
- FGF-2 potentiates neuroprotective effect in DRG after spinal cord injury.
- FGF-2 plus sciatic nerve fragment improve DRG plasticity of rats submitted to complete transections of spinal cord.

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ABSTRACT

Neurotrophic factors and peripheral nerves are known to be good substrates for bridging CNS trauma. The involvement of fibroblast growth factor-2 (FGF-2) activation in the dorsal root ganglion (DRG) was examined following spinal cord injury in the rat. We evaluated whether FGF-2 increases the ability of a sciatic nerve graft to enhance neuronal plasticity, in a gap promoted by complete transection of the spinal cord. The rats were subjected to a 4 mm-long gap at low thoracic level and were repaired with saline (Saline or control group, $n = 10$), or fragment of the sciatic nerve (Nerve group, $n = 10$), or fragment of the sciatic nerve to which FGF-2 (Nerve + FGF-2 group, $n = 10$) had been added immediately after lesion. The effects of the FGF-2 and fragment of the sciatic nerve grafts on neuronal plasticity were investigated using choline acetyl transferase (ChAT)-immunoreactivity of neurons in the dorsal root ganglion after 8 weeks. Preservation of the area and diameter of neuronal cell bodies in dorsal root ganglion (DRG) was seen in animals treated with the sciatic nerve, an effect enhanced by the addition of FGF-2. Thus, the addition of exogenous FGF-2 to a sciatic nerve fragment grafted in a gap of the rat spinal cord submitted to complete transection was able to improve neuroprotection in the DRG. The results emphasized that the manipulation of the microenvironment in the wound might amplify the regenerative capacity of peripheral neurons.

1. Introduction

Trophic factors, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and glia-derived neurotrophic factor (GDNF) shows important and selective effects on survival and phenotypic expression of primary sensory neurons in the dorsal root ganglion (DRG) following nervous system injury and peripheral inflammation Trophic factors, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and glia-derived neurotrophic factor (GDNF) show important and selective effects on survival and phenotypic expression of primary sensory neurons in the dorsal root ganglion (DRG) following nervous system injury and peripheral inflammation [1–4].

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Heparin-binding growth factors include the family of fibroblast growth factors (FGFs) and may stimulate mitogenesis in a variety of cells with mesodermal or neuroectodermal origin. FGFs and its high affinity tyrosine kinase fibroblast growth factor receptors (FGFRs) are expressed in the peripheral nervous system and are known to regulate the development and modulation of DRG neurons [5–9].

Following peripheral nerve injury, FGF-2 increases in DRG neurons [10,11]. This increase may suggest functional roles of FGF-2 on neuronal survival or signal transduction through the FGFRs in the DRG. Indeed, FGF-2 exogenously applied shows a neuroprotective effect on adult sensory neurons after injury [12].

In the periphery, DRG has been shown to contain both FGF-1 protein and mRNA [13]. Whereas FGF-2 immunoreactivity in the embryonic DRG is present in almost all neurons [14], expression in postnatal and adult DRG becomes restricted to the somatostatin positive subpopulation of sensory neurons [6].

Evidence from several *in vitro* and *in vivo* studies has shown that FGF-2 is not only present in the nervous system but also mediates survival-promoting effects and stimulates the transmitter metabolism of several neurons during development and after injury [15,16]. Application of FGF-2 to the proximal stump of the transectioned sciatic nerve prevents the lesion-induced death of sensory neurons of DRG L4-L6 [12].

Animal studies have shown that if continuity is restored between the spinal cord and the ventral roots of nerves at the lumbar [17] or cervical [18–20] levels of the spinal cord, motor neuron axons can regrow into their respective peripheral nerves with concomitant recovery of motor functions. The return of motor functions after implantation of avulsed spinal nerve roots into the spinal cord has also been reported in one clinical case [21], as well as in some animal studies [22–24].

FGF-1 and FGF-2 treated cultures can promote a significant increase in neurite outgrowth of ventral spinal cord neurons and stem cells, suggesting that both FGFs can influence neuronal development [25,26].

In order to determine functional roles of FGF-2 in the peripheral nervous system we analyzed the expression of choline acetyl transferase (ChAT) in spinal ganglia after spinal cord lesion treated with FGF-2.

2. Materials and methods

2.1. Animal treatment

Adult male Wistar rats ($n = 30$) from the Federal University of Pernambuco, Brazil (body weight [b.w.] 180–200 g), were used in the present study. The study was conducted according to protocols approved by the Animal Care and Use Ethic Committee at the Federal University of Rio Grande do Norte using the San Poiley outbreeding method [27].

2.2. Microneurosurgery

Rats were pre-anaesthetized with an intramuscular injection of ketamine chloridrate 10% (Agener União, Brazil, 0,1 ml/100 g b.w.) and xylazine 2% (Agener União, Brazil, 0,01 ml/100 g b.w.) and then anesthetized with isoflurane inhalation (Isoflorine®) (Cristália, Brazil). A small laminectomy at the tenth/eleventh thoracic levels was performed. Complete transections created a 4 mm-long gap at the eleventh/twelfth spinal cord levels. A gelfoam soaked in 10 μl of 0,9% saline was left at the bottom of the gap close to the vertebral body in 10 rats (Saline or Control group). Another group of 10 rats received gelfoam filled with sciatic nerve fragment (Nerve group). Additionally, a further 10 rats received sciatic nerve fragment and 10 μl of a FGF-2 (Santa Cruz Biotechnology, Inc. USA, 1 μg/10 μl/animal) solution (Nerve + FGF-2 group). Four mm-long sciatic nerve fragments were grafted close to the edges of the spinal cord stumps [28]. All animals had received sodium cephalexin (40 mg/kg/day, Eurofarma, Brasil) prior to surgery, in order to prevent secondary infection.

2.3. Tissue preparation, sectioning and samppling

After a 2 months-survival, animals were deeply anaesthetized with Isoflorine® (Cristália, Brasil) and euthanized by transcardiac perfusion with 100 ml 0,9% saline at room temperature followed by 500 ml of fixative solution (4 °C) [29,30]. The fixative consisted of 4% paraformaldehyde (w/v) in 0.1 M phosphate buffer, pH 7.4. The DRGs adjacent to the wounds were removed, kept in the fixative solution at 4 °C for 90 min, and then rinsed in 10% sucrose (Merck, Germany) dissolved in 0.1 M phosphate buffered saline (PBS), pH 7.4, for 48 h.

A group of 6 uninjured animals were also perfused and their DRGs from the same location in the thorax were taken out. A microtome (Leica, SM 2000R, Germany) was used to prepare 20 μm thicken transversal frozen sections from the DRG.

2.4. Immunohistochemical procedures for ChAT

Immunoreactivity was detected using the avidin–biotin peroxidase technique [31–33]. Series of sections were washed 2 × 10 min in 0.1 M PBS, pH 7.4 and incubated with 5% normal goat serum (NGS, Sigma) for 30 min at room temperature. Series were then incubated for 24 h at 4 °C with goat monoclonal antisera against ChAT (Millipore, diluted 1:100). The antibodies were diluted in PBS containing 0.3% Triton X-100 (Sigma) and 1% bovine serum albumin (Sigma). The series of sections were washed again in PBS (2 × 10 min) and incubated with biotinylated donkey anti-goat (ChAT) immunoglobulins diluted 1:1000 (Jackson, USA) for 1 hour. The sections were washed again in PBS and incubated with an avidin–biotin peroxidase complex (both diluted 1:100, Vectastain, Vector) for 90 min. Immunoreactivity was visualized using 3-3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) as a chromogen and H_2O_2 as a substrate.

2.5. Morphometric/microdensitometric image analysis

The ChAT immunoreactivity was measured in one section per rat in the ganglion central region. An optical microscope (Olympus BX41) with a 40× objective in bright field was used to obtain the digital image. The counts and measurements were made using Image J software. Digital images of representative sections were obtained using a digital video camera (Nikon DXM1200).

2.6. Statistical analysis

Statistical analyses were performed using the analysis of variance (ANOVA) and significant interactions were followed-up with a Tukey and Bonferroni post-test comparison. All statistical analyses were performed using SPSS 22, and significance was set at $p < 0.05$.

3. Results

Photomicrographs in Fig. 1 illustrates the Nissl stained DRG neurons of animals without injury (Fig. 1).

Uninjured animals used as parameters had a mean area of neuronal cell body of 9.1 μm, compared to the saline group (6.86 μm^2), the nerve group (7.85 μm^2) and the nerve + FGF-2 group (8.54 μm^2). Considering the injured groups, the group that received sciatic nerve graft with FGF-2 added showed higher values