Experimental Neurology 240 (2013) 157–167

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Differential effects of activity dependent treatments on axonal regeneration and neuropathic pain after peripheral nerve injury

Stefano Cobianchi, Laura Casals-Diaz, Jessica Jaramillo, Xavier Navarro *

Group of Neuroplasticity and Regeneration, Institute of Neurosciences and Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain
Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

ARTICLE INFO

Article history:
Received 31 August 2012
Revised 16 November 2012
Accepted 21 November 2012
Available online 30 November 2012

Keywords:
Nerve regeneration
Neuropathic pain
Neurotrophins
Electrical stimulation
Treadmill training

ABSTRACT

Activity treatments are useful strategies to increase axonal regeneration and functional recovery after nerve lesions. They are thought to benefit neuropathy by enhancing neurotrophic factor expression. Nevertheless the effects on sensory function are still unclear. Since neurotrophic factors also play a fundamental role in peripheral and central sensitization, we studied the effects of acute electrical stimulation and early treadmill exercise on nerve regeneration and on neuropathic pain, and the relation with the expression of neurotrophins. After sciatic nerve section and suture repair, rats were subjected to electrical stimulation (ES) for 4 h after injury, forced treadmill running (TR) for 5 days, or both treatments combined. Sciatic nerve section induced hyperalgesia in the medial area of the plantar skin in the injured paw. TR and ES differently but positively reduced adjacent neuropathic pain before and after sciatic reinnervation. ES enhanced motor and sensory reinnervation, and combination with TR induced strong agonistic effects in relieving pain. The differential effects of these activity treatments were related to changes in neurotrophic factor mRNA levels in sensory and motor neurons. ES speeded up expression of BDNF and GDNF in DRG, and of BDNF and NT3 in the ventral horn. TR reduced the levels of pro-nociceptive factors such as BDNF, NGF and GDNF in DRG. Combination of ES and TR induced intermediate levels suggesting an optimal balancing of treatment effects.

Introduction

Although peripheral neurons have the intrinsic capacity to grow and sprout new axons after injury, recovery after severe lesions such nerve transection is limited by incomplete and non-specific regeneration, variable clinical results and the development of neuropathic pain disorders. Activity-dependent therapies have promising potential for enhancing axonal regeneration and for modulating neuropathic pain after neural injuries (Udina et al., 2011). Several studies have demonstrated that treadmill running (Marqueste et al., 2004; Sabatier et al., 2008), and immediate electrical stimulation of the injured peripheral nerve (Al-Majed et al., 2000, 2004; Brushart et al., 2005; Geremia et al., 2007; Vivó et al., 2008) have positive effects on nerve regeneration and functional recovery. However, their effects on neuropathic pain symptoms have not been well investigated. Particularly, it is not yet clear how the activity treatments could be applied, depending on the trauma and the subsequent pathology, in order to allow not only a better recovery of muscle function, but also the normalization of patient's sensitivity. Since neuropathic pain is probably the most disabling condition of patients with severe nerve injuries (Berger et al., 2004; Breivik et al., 2006), rehabilitation treatments increasing the activity of the injured limb should take into account potential positive or negative effects on the sensory system. Thus, the general objective of our studies was to assess activity-dependent therapies for accelerating both functional recovery and remission of neuropathic pain symptoms after peripheral nerve injury.

Interventions to surgically repair the damaged nerve and to stimulate functional recovery are more effective when administered early after the lesion (Dahlin, 2008; Gordon et al., 2003). We hypothesized that activity treatments, if administered early after injury, may influence the intrinsic growth capacity of peripheral axons, and improve the recovery of motor as well as sensory function. We recently showed that acute electrical stimulation speeds up the initial recovery of both muscular reinnervation and nociceptive responses after sciatic nerve section and repair, and that additional training on a treadmill may enhance recovery of motor function (Asensio-Pinilla et al., 2009). Moreover, in a separate study we demonstrated that an intense and short-lasting treadmill exercise significantly reduced mechanical allodynia in the chronic constriction injury (CCI) model of neuropathic pain, and it resulted also in better recovery of sensorimotor function (Cobianchi et al., 2010). On the other hand, prolonged treadmill exercise or electrical stimulation was detrimental for regeneration and neuropathic pain (Asensio-Pinilla et al., 2009; Cobianchi et al., 2010). These previous experiments suggested that, applied at certain intensity and at specific times after injury, these activity treatments could prevent maladaptive plasticity of nociceptors that contribute to trigger neuropathic

* Corresponding author at: Facultat de Medicina, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain. Fax: +34 935812986.
E-mail address: xavier.navarro@uab.cat (X. Navarro).

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http://dx.doi.org/10.1016/j.expneurol.2012.11.023

pain after injury. Thus, we made a comparative study of the effects of brief acute electrical stimulation and of early short-lasting treadmill training treatments, alone or combined, on the functional outcomes of regeneration and pain following section and repair of the sciatic nerve in rats.

Since the pro-regenerative effects of exercise and electrical stimulation have been related to the increased expression of neurotrophic factors, we raised the possibility that specific activity protocols could differently modulate early expression of neurotrophins in motor and sensory neurons. After nerve injury the expression of neurotrophic factors increases during the first days, in an attempt to support neuronal survival and axonal regeneration (Allodi et al., 2012). Several works demonstrated that a few days of exercise induce increased production of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) in muscles (Gomez-Pinilla et al., 2001; Perreau et al., 2005), in dorsal root ganglia (DRG) (Molteni et al., 2004) and in spinal cord (Gomez-Pinilla et al., 2002; Macias et al., 2007; Skup et al., 2002), enhancing survival and regeneration of injured axons. Other neurotrophic factors have also been involved in the exercise-dependent signal for growth and innervation, such as nerve growth factor (NGF) (Chae and Kim, 2009; Neeper et al., 1995) and glial-derived neurotrophic factor (GDNF) (Wehrwein et al., 2002). On the other hand, electrical stimulation also showed beneficial effects on nerve regeneration, mainly by accelerating the upregulation of BDNF and its receptor TrkB in motor and sensory neurons (Al-Majed et al., 2000; Brushart et al., 2002; Geremia et al., 2007). Thus, activity treatments may play a critical role in modulating and redistributing the expression of neurotrophic factors within the nervous system.

Despite their involvement in axonal regeneration, NGF and BDNF are well-known mediators and modulators of pain (Pezet and McMahon, 2006), and have several roles in neuropathic pain (Obata and Noguchi, 2006; Ro et al., 1999). Anti-NGF and anti-TrK receptor treatments significantly reduced the severity of autotomy, prevented the spread of collateral sprouting into the denervated skin, and reduced hyperalgesia in sciatic nerve injury models (Diamond et al., 1987; Ro et al., 1999; Ugolini et al., 2007; Wild et al., 2007). GDNF has trophic effects on non-peptidergic nociceptive neurons following axotomy (Bennett et al., 1998). On the other hand, NT3 seems to have an important role for the survival of proprioceptive and mechano-receptive sensory neurons (Airaksinen et al., 1996; Ernfors et al., 1993). We also analyzed the changes in these neurotrophic factor expressions in each of the activity-dependent treatment conditions, in order to shed light on the possible mechanisms of regulation of pain and regeneration.

Materials and methods

Animals and surgery

Adult female Sprague–Dawley rats (240 ± 30 g) were housed in standard cages (four per cage) and kept on standard laboratory food and water ad libitum with a light–dark cycle of 12 h. All experimental procedures were approved by the Ethics Committee of our institution, and followed the EU Directive 2010/63/EU for animal experiments. Rats were anesthetized with pentobarbital (40 mg/kg i.p.), and lidocaine was applied at the transection site prior to incising the skin to minimize discomfort. The right sciatic nerve was exposed at the mid thigh, transected at 92 mm from the tip of the third toe, and repaired by epineurial sutures (10–0), maintaining the fascicular alignment of tibial, peroneal and sural branches. The wound was closed in two layers. Rats were kept in a warm environment until their complete recovery from anesthesia.

Experimental design

One week before surgery, all the animals were habituated to experimental devices for nociceptive baseline thresholds measurement and treadmill locomotion. After surgery, animals were randomly divided in 4 groups following different activity treatments.

One group received acute electrical stimulation (group ES, n = 7) applied to the repaired nerve immediately after surgery for 4 h. With the wound open, the sciatic nerve was stimulated at the proximal stump with pulses of 0.1 ms duration and suprathreshold intensity (3 V) delivered at 20 Hz (Grass S44, Quincy MA). The cathode was a thin wire bared at the tip and gently twisted around the sciatic nerve near the sciatic notch, and the anode was a thin needle inserted in the near muscle. This pattern of stimulation has already been shown to promote motor nerve regeneration in rats and not produce harmful effects (Al-Majed et al., 2000; Asensio-Pinilla et al., 2009; Vivó et al., 2008). At the end of the procedure, the electrodes were removed and the wound was sutured and disinfected.

A second group was trained by treadmill locomotion (group TR, n = 8). We adapted to rats a forced treadmill protocol that was already shown to induce hypoalgesic effect in mice without detrimental effects (Cobianchi et al., 2010). Starting 3 days after surgery, rats were trained for 5 consecutive days for 1 h running on a motorized treadmill equipped with an electric shock grid (Treadmill LE 8706 LETICA, Spain). Inclination of treadmill was set at 0°. All rats were first acclimated to the treadmill belt for 5 min before starting the locomotion. Running started at a normal locomotion speed of 10 cm/s that was increased 2 cm/s every 5 min, until a maximal speed of 32 cm/s. During the training session before surgery, shock grid intensity was set at 0.4 mA to provide a mild negative stimulus. After surgery, during the experimental training the intensity was lowered to 0 mA.

In a third group, electrical stimulation was combined with the treadmill exercise (group ES + TR, n = 7), following the same procedures. A fourth group of injured rats was untreated and served as control (group C, n = 15). Additional rats were used for biochemical assays (n = 6 per group at each time point).

At each testing time point, behavioral and electrophysiological tests were performed during the morning (11:00 AM/1:00 PM) of two different days, while treadmill running sessions were carried out during the afternoon (3:00/5:00 PM). During the tests, experimenters were blind to rats' assignment to different groups.

Assessment of sensory function

The nociceptive threshold responses to mechanical and thermal stimuli were evaluated on both hindpaws by means of algesimetry tests at different days post-injury (dpi: 3, 7, 14, 21, 31, 40, 50 and 60). In both tests two different areas of the plantar surface of the hindpaw were tested: the lateral (innervated by tibial and sural nerves) and the medial (innervated by tibial and saphenous nerves) regions (Casals-Diaz et al., 2009). Sensibility to mechanical stimuli was measured by means of an electronic Von Frey algesimeter (Bioseb, Chaville, France). Rats were placed on a wire net platform in plastic chambers 30 min before the experiment for habituation. Mechanical nociceptive threshold was taken as mean of three measurements per paw region, with 5 min interval between each measurement, and expressed as the force (in grams) at which rats withdrew their paws in response to the stimulus. A cutoff force was set to 40 g at which stimulus lifted the paw with no response.

Thermal sensibility was assessed by using a plantar algesimeter (Ugo Basile, Comerio, Italy). Rats were placed into a plastic box with an elevated glass floor 30 min before the experiment for habituation. The beam of a projection lamp was focused onto the hindpaw plantar surface pointing at both the lateral and the medial paw test sites. Thermal nociceptive threshold was taken as mean of three trials per paw region, with 5 min resting between each trial, and expressed as the latency (in seconds) of paw withdrawal response. A cutoff time was set at 20 s to prevent tissue damage with no response. For both algesimetry tests, the contralateral paw of each rat was tested as control, to overcome possible variations between testing days conditions.