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Research paper

Monoaminergic descending pathways contribute to modulation of neuropathic pain by increasing-intensity treadmill exercise after peripheral nerve injury

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ABSTRACT

This study characterizes the impact of increasing-intensity treadmill exercise (iTR) on noradrenergic (NE) and serotonergic (5HT) modulation of neuropathic pain. Following sciatic nerve transection and repair (SNTR) rats developed significant mechanical and thermal hyperalgesia that was partially prevented by iTR performed during the first 2 weeks after injury.

Marked decrease in the expression of $5HT_{2A}$ and α_{1A} and β-, but not α_{2A} adrenergic receptors in the spinal cord dorsal horn was associated to SNTR and recovered by iTR, particularly in lamina II. iTR significantly increased $5HT_{2A}$ in periaqueductal grey (PAG), raphe magnus (RM) and dorsal raphe nucleus (DRN), with a pattern suggesting reorganization of serotonergic excitatory interconnections between PAG and DRN. iTR also increased the expression of α_{1A} in locus coeruleus (LC) and DRN, and β_2 in LC, indicating that exercise enhanced activity of NE neurons, likely by activating autologous projections from DRN and PAG.

iTR hypoalgesia was antagonized by blockade of β_2 and $5HT_{2A}$ receptors with administration of butoxamine and ketanserin. The neurotoxin DSP4 was injected to induce depletion of NE projections from LC before starting iTR. DSP4 treatment worsened mechanical hyperalgesia, but iTR hypoalgesia was similarly produced. Moreover, $5HT_{2A}$ expression in LC further increased after DSP4 injection, all these results suggesting an intrinsic regulation of 5HT and NE activity between PAG, DRN and LC neurons activated by iTR.

Finally, iTR significantly reduced microglial reactivity in LC and increased non-microglial BDNF expression, an effect that was reverted by butoxamine, implicating BDNF regulation in central 5HT/NE actions on neuropathic pain.

1. Introduction

Increasing-intensity treadmill exercise (iTR), performed during the first days after injury, has been shown to significantly reduce hyperalgesia in neuropathic pain models, such as sciatic nerve chronic constriction injury (CCI) (Cobianchi et al., 2010) and section and suture repair (SNTR) (Cobianchi et al., 2013). iTR decreased the expression of BDNF and other neurotrophins although without impairing nerve regeneration (Cobianchi et al., 2013; Lopez-Alvarez et al., 2015). iTR-induced decrease of BDNF expression was associated to reduction of microgliosis and restoration of the expression of chloride transporters in the primary sensory neurons and along the central pain pathways (Lopez-Alvarez et al., 2015; Modol et al., 2014). Little is known, however, on the central mechanisms by which iTR induces hypoalgesia, and we hypothesize that it may act by activating descending pathways for inhibition of pain transmission at the spinal level.

Brain areas can modulate the ascending pain signals by serotonergic and noradrenergic projections to spinal cord neurons, which can facilitate or inhibit the afferent sensory neurons. Periaqueductal grey (PAG) areas receive pain and temperature fibers and activate defensive and stress responses by sending axons to both locus coeruleus (LC) and raphe magnus nucleus (RM), whose antinociceptive output trigger descending inhibition (Millan, 2002). Serotonergic RM and noradrenergic LC projections normally activate spinal enkephalinergic and

Abbreviations: iTR, increasing-intensity treadmill exercise; 5HT, serotonergic; NE, noradrenergic; SNTR, sciatic nerve transection and repair; i.p., intraperitoneal; RM, raphe magnus; DRN, dorsal raphe nucleus; PAG, periacqueductal grey; LC, locus coeruleus; Bu, butoxamine; Ke, ketanserin; DSP4, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine; BDNF, brain-derived neurotrophic factor

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GABA/glycinergic interneurons. Enkephalin released from terminals of enkephalinergic dorsal horn interneurons acts on the opioid receptors located on the central processes of nociceptive primary afferents, reducing Ca^{2+} entry into their terminals and decreasing the release of nociceptive neurotransmitters such as glutamate and substance P (Ossipov et al., 2010). Similarly, activation of dorsal horn interneurons containing GABA or glycine also inhibit the spinal transmission of noxious sensory signals, and previous studies indicate that spinal GABAergic inhibition is reduced after experimental nerve injury (Moore et al., 2002). The loss of tonic inhibition by spinal interneurons is also associated with dysregulation of NKCC1/KCC2 chloride cotransporters expression, inducing an inversion of GABAergic depolarizing currents in neuropathic conditions (Modol et al., 2014), that is prevented by iTR (Lopez-Alvarez et al., 2015).

Besides the demonstrated peripheral effects (Cobianchi et al., 2010, 2013; Lopez-Alvarez et al., 2015; Udina et al., 2011), we hypothesized that iTR may activate pain central inhibition normally gating the nociceptive input to supraspinal, medullary and cortical areas, which are decreased after peripheral nerve injury. By stimulating the descending noradrenergic and serotonergic projections to the dorsal horn, specific exercise training may result in the activation of inhibitory circuits in the dorsal horn and in the consequent inhibition of second-order spinothalamic neurons by presynaptic and postsynaptic mechanisms.

We studied the expression of noradrenaline (NE) and serotonin (5HT) receptors in sensory neurons of the spinal cord dorsal horn, that participate in pain modulation. Among NE receptors, α_{1A} receptors are expressed in GABAergic and glycinergic neurons of dorsal horn lamina II, where they may participate in endogenous inhibition of afferent pain by exciting inhibitory interneurons (Baba et al., 2000). α_{2A} receptors are expressed in the spinal cord predominantly on the terminals of primary C-fibers afferents, where they inhibit nociception (Stone et al., 1998). β_2 receptor is an excitatory adrenoreceptor expressed in dorsal horn neurons (Nicholson et al., 2005), which activation induces antinociceptive effects (Yalcin et al., 2009a, 2009b, 2010; Zhang et al., 2016). We also assessed changes in serotonergic $5HT_{2A}$ receptor since it is involved in spinal chloride homeostasis, which dysregulation is associated with spinal disinhibition and neuropathic pain (Gackiere and Vinay, 2014; Jacobs et al., 2002). Under hyperalgesic states the $5HT_{2A}$ receptor was found to be expressed in laminae I-III NK1R-positive projection neurons (Mantyh et al., 1997) and in lamina II galanin-containing neurons expressing GABAergic boutons (Tiong et al., 2011), receiving emerging interest as a potential target for treating nerve injury-induced pain and spasticity (Bos et al., 2013).

In this study, we investigated the changes induced by iTR on noradrenergic and serotonergic circuitry that may be related to antinociception. For this purpose, we analyzed the expression of adrenergic α_{1A}, α_{2A} and β_2 receptors, and serotonergic $5HT_{2A}$ receptor after injury to the sciatic nerve in rats, and their changes under increasing-intensity exercise when neuropathic pain is prevented. We also relate the exercise-induced hypoalgesia to the expression of β_2 receptor and the reduction of microgliosis in noradrenergic neurons.

2. Materials and methods

2.1. Animals and surgery

Adult female Sprague-Dawley rats (240 ± 30 g) were housed in standard cages with access to food and water ad libitum under a light–dark cycle of 12 h. All the experimental procedures were approved by the Ethics Committee of the Universitat Autonoma de Barcelona and followed the guidelines of the European Commission on Animal Care (EU Directive 2010/63/EU). Rats were anesthetized by intraperitoneal (i.p.) injection of ketamine (10 mg/kg, Imalgene 500; Rhone-Merieux, Lyon, France) and xylazine (1 mg/kg, Rompun; Bayer, Leverkusen, Germany).

Rats were submitted to a sciatic nerve transection and repair (SNTR), a well characterized model that allows the evaluation of neuropathic pain and nerve regeneration (Cobianchi et al., 2014). The right sciatic nerve was exposed at the mid thigh, transected at 92 mm from the tip of the third toe, and repaired by epineural sutures (10 − 0). The wound was closed in two layers and disinfected with povidone iodine. Rats were kept in a warm environment until their recovery from anesthesia.

2.2. Experimental design

Seven days before surgery, all the animals were habituated to the experimental device for treadmill locomotion (Treadmill LE 8706; LETICA, Barcelona, Spain) and pretrained to the task, by leaving them to explore the stopped treadmill for 5 min and then trained in a single iTR session. Each iTR session consisted of 1 h running, starting at a locomotion speed of 10 cm/s that was increased 2 cm/s every 5 min, until a maximal speed of 32 cm/s (Cobianchi et al., 2013; Lopez-Alvarez et al., 2015). All rats were evaluated during follow-up with sensory tests performed during the morning, whereas treadmill running sessions were performed during the afternoon.

At day 3 after surgery, animals were randomly selected to follow or not the iTR training. Training sessions were performed daily over 12 consecutive days from day 3 to 14 after injury.

SNTR rats were divided in several groups: a group of rats performed iTR (SNTR-iTR group, $n = 10$), and a second group remained sedentary (SNTR-sed group, $n = 8$). Other groups performed or not iTR with pharmacological blockade of β_2-receptors with butoxamine (Sigma-Aldrich, 8 mg/Kg in saline, i.p.; SNTR-iTR + Bu group, $n = 6$, and SNTR-sed + Bu group, $n = 6$), or blockade of $5HT_{2A}$-receptors with ketanserin (Sigma-Aldrich, 8 mg/Kg in saline, i.p.; SNTR-sed + Ke group, $n = 6$, and SNTR-iTR + Ke group, $n = 6$). These drugs were administered each day of training, 30 min before starting the exercise. Doses were chosen over the basis of previous studies using rats. Control groups for both drugs were injected with saline vehicle only. A naive group of rats was added for comparison with injured rats ($n = 6$).Finally, other two groups of animals were injected with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4, Sigma-Aldrich, 50 mg/kg in saline, i.p.), a neurotoxin that selectively induces degeneration of NE neurons in the LC, thus depleting NE projections originating from the LC (Jonsson et al., 1981; Prieto and Giralt, 2001). DSP-4 was injected within 10 min of preparation (Grzanna et al., 1989), and its administration was performed 4 days before the injury to ensure effect from the beginning of the training. One group of DSP4 injected rats followed iTR (SNTR-iTR + DSP4 group, $n = 8$), and another remained untrained (SNTR + DSP4 group, $n = 10$).

2.3. Nociceptive tests for pain threshold measurement

Three days before surgery, all the injured animals were habituated to the experimental devices, and then tested for baseline nociceptive thresholds recording. The nociceptive behavior tests for mechanical and thermal stimuli were performed on both hind paws before and at different days after injury (dpi), the experimenter being blind to assignment of rats to the different groups. Lateral and medial sites of the paw were tested to differentiate changes in sensory thresholds produced respectively by sciatic nerve injury from those due to saphenous nerve sprouting (Cobianchi et al., 2014).

Sensitivity 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. Then, a non-noxious pointed probe was gently applied to each test site, slowly increasing the pressure. The threshold was expressed as the force (in grams) at which rats withdrew the paw in response to the stimulus. A cutoff force was set at 40 g, when the stimulus lifted the paw without response. The mechanical nociceptive threshold was calculated as the mean of 3 measurements per test site, with a 3 min interval between each