Experimental Neurology 234 (2012) 39–49

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Intraspinal transplantation of GABAergic neural progenitors attenuates neuropathic pain in rats: A pharmacologic and neurophysiological evaluation

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ARTICLE INFO

Article history:
Received 29 July 2011
Revised 27 October 2011
Accepted 5 December 2011
Available online 13 December 2011

Keywords:
Neuropathic pain
GABAergic progenitors
GABA receptors
Wind-up
Post-discharges

ABSTRACT

Dysfunctional γ-aminobutyric acid (GABA)-ergic inhibitory neurotransmission is hypothesized to underlie chronic neuropathic pain. Intraspinal transplantation of GABAergic neural progenitor cells (NPCs) may reduce neuropathic pain by restoring dorsal horn inhibition. Rat NPCs pre-differentiated to a GABAergic phenotype were transplanted into the dorsal horn of rats with unilateral chronic constriction injury (CCI) of the sciatic nerve. GABA signaling in antinociceptive effects of NPC grafts was tested with the $GABA_A$ receptor antagonist bicuculline (BIC), $GABA_B$ receptor antagonist CGP35348 (CGP) and GABA reuptake inhibitor SKF 89976A (SKF). NPC-treated animals showed decreased hyperalgesia and allodynia 1–3 week post-transplantation; vehicle-injected CCI rats continued displaying pain behaviors. Intrathecal application of BIC or CGP attenuated the antinociceptive effects of the NPC transplants while SKF injection induced analgesia in control rats. Electrophysiological recordings in NPC treated rats showed reduced responses of wide dynamic range (WDR) neurons to peripheral stimulation compared to controls. A spinal application of BIC or CGP increased wind-up response and post-discharges of WDR neurons in NPC treated animals. Results suggest that transplantation of GABAergic NPCs attenuate pain behaviors and reduce exaggerated dorsal horn neuronal firing induced by CCI. The effects of GABA receptor inhibitors suggest participation of continuously released GABA in the grafted animals.

Introduction

Chronic pain often accompanies injury to the peripheral and central nervous systems. The mechanisms responsible for this type of pain are still not completely understood. Conventional therapies have low efficacy for neuropathic pain. Neither pharmacological treatment nor surgical intervention is optimal, since drug tolerance and addiction, untoward side effects and worsening pain emerge over time. Thus, there is a critical need to identify alternative approaches based on the pathophysiology of neurotraumatic pain syndromes.

Processing of somatosensory information in relation to pain occurring in the superficial laminae of the spinal dorsal horn is modulated by local and descending inhibitory circuits, in which γ-aminobutyric acid (GABA) and glycine play key roles as inhibitory neurotransmitters. The importance of GABA signaling has been shown by blockade of spinal GABAergic neurotransmission with intrathecally applied GABA receptor antagonists, which produce hypersensitivity to innocuous tactile stimuli (Gwak et al., 2006; Hao et al., 1994; Loomis et al., 2001; Malan et al., 2002; Sivilotti and Nistri, 1991). Also, transgenic mice that lack specific subunits of GABA receptors develop hyperalgesia and allodynia (Schuler et al., 2001; Ugarte et al., 2000). Decreases in GABA immunoreactivity (GABA-IR) and the GABA synthesizing enzyme GAD65/67 accompanied by the development of hyperalgesia and allodynia have been shown in rats after peripheral nerve injury (Castro-Lopes et al., 1993; Eaton et al. 1998; Gwak et al., 2006; Ibuki et al., 1997; Lee et al., 2008). Correspondingly, administration of GABA into the spinal cord alleviates nerve injury-induced nociceptive behavior (Eaton et al., 1999a). Intrathecal administration of $GABA_A$ or $GABA_B$ receptor agonists has been shown to produce a dose-dependent analgesia in animals with peripheral nerve injury, that is blocked by GABA receptor antagonists (Hwang and Yaksh, 1997; Malan et al., 2002). One electrophysiological study on spinal cord slices from rats has confirmed a deficit in GABAergic inhibitory neurotransmission in spinal dorsal horn after peripheral nerve injury (Moore et al., 2002). Such observations suggest that loss of inhibitory neural circuitry could play a role in allodynia and hyperalgesia developing after nerve injury (Eaton et al., 1999a). Although there is controversy with regard to actual overt loss of GABAergic interneurons in the spinal cord (Polgar et al., 2004), the above observations combine to suggest that there is a dysfunction of inhibitory processes in the spinal cord after peripheral nerve injury.

However, systemic pharmacological targeting of the GABAergic system has proven unsatisfactory for relieving such pain (Robert et al., 2010; Slonimski et al., 2004), perhaps because of the widespread distribution and multifunctional roles of GABA in the central nervous system (CNS). Novel ways to obviate the problems of ubiquitous

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0014-4886/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2011.12.005

GABA receptor distribution in the CNS and the temporary effects of pharmacologic treatment include direct delivery of GABA to spinal pain processing sites via cell-based or gene therapy. These are also better suited to the long-term management of chronic pain. Chronic pain behavior in rodent models has been previously reported to be improved by transplantation of GABAergic neurons or of cells bioengineered to secrete GABA (Eaton et al., 2007, 1999a, 1999b; Stubley et al., 2001). In particular, pre-differentiated GABAergic neural progenitors (NPCs) have been shown to attenuate peripheral nerve injury-induced allodynia when transplanted intraspinally (Mukhida et al., 2007). Our laboratory has shown that primary neurospheres generated from E14 rat telencephalic NPCs, when intraspinally transplanted, attenuate spinal cord injury-induced neuropathic pain in rats. These neurospheres contain numerous GABAergic neurons when pre-differentiated by transient withdrawal of mitogenic factor FGF-2 (Furmanski et al., 2009). In the present study, pre-differentiated GABAergic NPCs were intraspinally transplanted into rats with peripheral nerve injury and their effects on symptoms of neuropathic pain were assessed. To further characterize the effect of the NPCs, responses of dorsal horn neurons in close vicinity of the graft to peripheral stimulation were evaluated by electrophysiology. Pharmacologic evaluations in both behavioral and electrophysiological outcomes were used to determine the involvement of GABAergic signaling in the effect of grafts. Preliminary accounts of these findings have been reported previously (Jergova et al., 2009, 2010)

Materials and methods

Animals

Male Sprague–Dawley rats (140–160 g at the time of the first surgery) were used for peripheral nerve injury and intraspinal injections; pregnant female Sprague–Dawley rats were used for E14 embryo harvesting (Harlan Lab, IN). Animals were housed two per cage with free access to food and water in 12 h light/dark cycle. Experimental procedures were reviewed and approved by the University of Miami Animal Care and Use Committee and followed the recommendations of the "Guide for the Care and Use of Laboratory Animals" (National Research Council).

E14 cell isolation

Pregnant females were deeply anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). E14 embryos were harvested and placed in ice-cold Hank's Balanced Salt Solution (HBSS). The cortical lobes and underlying lateral ganglionic eminences were removed with fine forceps and collected in fresh ice-cold HBSS. Tissues were pooled, centrifuged at 4 °C, resuspended in HBSS, dissociated by trituration and single cell suspensions were collected. Dissociated NPCs were pelleted and resuspended in growth medium DMEM/F12 with 1% N2 supplement and 10 ng FGF-2/ml (R&D Systems). NPCs cultures were seeded in tissue culture flasks at 1×10^5 cells/cm^2. NPCs were allowed to grow and form neurospheres over 3 to 4 days in vitro. Withdrawal of FGF-2 one day prior transplantation was used to induce pre-differentiation of NPC to a GABAergic phenotype, as shown in our lab previously (Furmanski et al., 2009). NPCs were then used for transplantation.

Surgical procedures

All surgical procedures were conducted under 2–3% Isoflurane/O2 anesthesia. Chronic constriction of the sciatic nerve was used to induce peripheral neuropathic pain (Bennett and Xie, 1988). The common sciatic nerve was exposed on the right side at the mid-thigh level using aseptic surgical techniques. Four 4–0 chromic gut ligatures spaced about 1 mm apart were loosely tied around the sciatic nerve proximal to the trifurcation. The wound was closed in layers and animals (total n = 72, utilized in various studies as described in relevant sections below) were left to recover in heated cages.

One week after CCI animals showing changes in the reaction to thermal or mechanical stimuli, (behavioral testing details below) were used for intraspinal injection of cells (n = 38) or equal volume of saline vehicle (n = 34). A T13–L1 laminectomy was done aseptically to expose L3–L4 lumbar spinal cord. Cells were loaded into a glass micropipette (tip diameter ~50 μm) attached to a Hamilton syringe, and injected into the right (ipsilateral) lumbar gray matter using a stereotaxic stage (Stoelting, Wood Dale, IL). The glass pipette was placed 0.5 mm right from the central vein and an injection was made at depth of 1 mm from the dorsal lumbar spinal surface. A volume of 3 μl (100,000 cells/μl) was injected at a rate of 1 μl/min; 3 μl of saline was injected in control CCI rats. The injection of saline vehicle was chosen as control to avoid any potential confounding effects that may result from release of trophic factors or other active agents by control cellular transplants. The use of vehicle or cell culture media as control has been previously employed in other transplantation studies (Hendricks et al., 2006; McDonald et al., 1999; Mitsui et al., 2003; Park et al., 2010; Zhao et al., 2004). After injection, the pipette was kept in place for additional 1 min. Muscles were sutured in layers, overlying skin was closed with wound clips and animals were transferred to heated cages for recovery. All transplanted rats received cyclosporine A (IP, 10 mg/kg; Bedford Labs, OH) from 1 day until sacrifice.

Some of these animals at 1–2 weeks post intraspinal injection of either saline (n = 10) or NPC (n = 10) were used for pharmacologic evaluation of grafted cells by intrathecal injection of GABAergic drugs. An intrathecal catheter (7.5–8 cm; ReCathCo, PA) was threaded through a slit in the atlanto-occipital membrane down the intrathecal space and secured to the neck muscles with sutures under 2–3% isoflurane/O2 anesthesia as described previously (Hama and Sagen, 2009; Yaksh and Rudy, 1976). This procedure brings the tip of catheter over the lumbar spinal segments. Rats were allowed to recover at least three days following intrathecal surgery prior to use in experiments.

Behavioral tests

Rats were tested weekly up to 5 weeks post injury. All behavioral tests were performed by a trained person blinded to the experimental treatment. The same person always performed a given test to reduce variability. On each testing day, animals were moved in the testing room and left to acclimate for 30 min before testing. When testing chambers were used, animals were placed in the chamber for another 20 min or until exploratory behavior ceased prior to sensory assessments. For each test the positive response was considered as the withdrawal of the paw during the stimulation. Such a response was usually a part of a more complex behavior such as licking and shaking the paw or vocalization.

Heat hyperalgesia

Sensitivity to noxious heat stimulation was evaluated according to the method of Hargreaves (Hargreaves et al., 1988). Animals were placed in plastic chambers with the heat source underneath. A heat source was positioned under the middle of the plantar side of the hind paw and was controlled by a timer that was stopped by paw withdrawal and time latency was recorded. To avoid tissue damage in the absence of withdrawal, a cut-off time was set at 20 s. Latency was tested for left and right hind paws with at least one minute interval between each paw. Three trials were conducted with 5 minute interval between each trial on the same paw. Difference scores were calculated by subtracting withdrawal latencies on the intact side from the injured side; thus negative difference scores are indicative of heat hyperalgesia.