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Governing role of primary afferent drive in increased excitation of spinal nociceptive neurons in a model of sciatic neuropathy

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ABSTRACT

Previously we reported that the cuff model of peripheral neuropathy, in which a 2 mm polyethylene tube is implanted around the sciatic nerve, exhibits aspects of neuropathic pain behavior in rats similar to those in humans and causes robust hyperexcitation of spinal nociceptive dorsal horn neurons. The mechanisms mediating this increased excitation are not known and remain a key unresolved question in models of peripheral neuropathy. In anesthetized adult male Sprague–Dawley rats 2–6 weeks after cuff implantation we found that elevated discharge rate of single lumbar (L_{3-4}) wide dynamic range (WDR) neurons persists despite acute spinal transection (T9) but is reversed by local conduction block of the cuff-implanted sciatic nerve; lidocaine applied distal to the cuff (i.e. between the cuff and the cutaneous receptive field) decreased spontaneous baseline discharge of WDR dorsal horn neurons ~40% ($n=18$) and when applied subsequently proximal to the cuff, i.e. between the cuff and the spinal cord, it further reduced spontaneous discharge by ~60% ($n=19$; $P<0.05$ proximal vs. distal) to a level that was not significantly different from that of naive rats. Furthermore, in cuff-implanted rats WDR neurons ($n=5$) responded to mechanical cutaneous stimulation with an exaggerated afterdischarge which was reversed entirely by proximal nerve conduction block. These results demonstrate that the hyperexcited state of spinal dorsal horn neurons observed in this model of peripheral neuropathy is not maintained by tonic descending facilitatory mechanisms. Rather, on-going afferent discharges originating from the sciatic nerve distal to, at, and proximal to the cuff maintain the synaptically-mediated gain in discharge of spinal dorsal horn WDR neurons and hyperresponsiveness of these neurons to cutaneous stimulation. Our findings reveal that ectopic afferent activity from multiple regions along peripheral nerves may drive CNS changes and the symptoms of pain associated with peripheral neuropathy.

Introduction

Neuropathic pain is a dysfunctional and debilitating pain that remains intractable to most current treatment strategies (Finnerup et al., 2007; Hawksley, 2006; Vissers, 2006). The etiology of neuropathic pain is varied in that the causes can include infection (e.g. varicella-zoster virus leading to post-herpetic neuralgia, neuritis, HIV), metabolic disorders (e.g. diabetes, vitamin deficiency), toxins/drugs (e.g. chemotherapy), autoimmune disease (e.g. Guillain–Barre syndrome), and physical trauma to the nerve and other structures. Neuropathic pain is distinguishable from acute pain in that it may be exaggerated (hyperalgesia), provoked by innocuous stimulation (dysesthesia, allodynia), and/or occurs spontaneously. It is commonly thought that pain resulting from peripheral neuropathy is triggered by discharge in the freshly injured nerve that generates multiple pathophysiological, transcriptional, translational, neurochemical, and structural changes in sensory processing in pain pathways. The current state of understanding of this type of pain is that ectopic discharge from both damaged and also neighboring intact/surviving primary afferent fibers, produced by the effects of Wallerian degeneration (Obata et al., 2004), develops (Devor and Wall, 1990; Kovalsky et al., 2008; Lee et al., 2003; Li et al., 2000; Obata et al., 2003; Wu et al., 2002; Yoon et al., 1996), and elevates discharge of spinal nociceptive neurons (Pitcher and Henry, 2000, 2004; Sotgiu et al., 1995a), inducing changes in sensory processing in the spinal dorsal horn and in supraspinal structures.

There is a lack of consensus, however, regarding the locus of the governing change that maintains neuropathic pain and, in fact, different components of the somatosensory axis have been proposed to maintain this pain. One hypothesis suggests that central sensitization at the level of the spinal dorsal horn is maintained independently of primary afferent input (Sandkuhler and Liu, 1998) since input from the periphery is reportedly insufficient to maintain spinal modifications in

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sensory processing and ultimately neuropathic pain (Burgess et al., 2002; Sun et al., 2005; Xie et al., 2005). Another view suggests that mechanisms at the spinal dorsal horn level require continuous facilitatory input from supraspinal structures (Bian et al., 1998; Carlson et al., 2007; Gardell et al., 2003; Kauppila et al., 1998; Kovelowski et al., 2000; Ossipov et al., 2000; Pertovaara et al., 1997, 2001; Porreca et al., 2001; Saade et al., 2006a,b, 2007; Suzuki et al., 2002, 2004a,b; Vera-Portocarrero et al., 2006).

However, in both animal models and humans effects of peripheral neuropathy including peripheral neurodegeneration, altered Na^+ and K^+ ion channel expression (Coward et al., 2001a,b; Hong et al., 2004; Hong and Wiley, 2006; Joshi et al., 2006; Matthews et al., 2006; Shembalkar et al., 2001), and abnormal impulse discharge (Nordin et al., 1984; Nystrom and Hagbarth, 1981) in sensory afferents have been shown to persist several weeks/months (Casula et al., 2004; Coward et al., 2000; Fried et al., 1991; Kretschmer et al., 2002; Lin et al., 2001; Pan et al., 2001; Pertin et al., 2005; Roytta et al., 1999; Seltzer et al., 1991b). This raises the question what role, if any, do such multiple and long lasting modifications in peripheral sensory input play in maintaining the persistent changes in the central nervous system and in neuropathic pain?

In earlier studies on a rat model of neuropathic pain, in which a polyethylene cuff had been placed around the sciatic nerve, we observed spontaneous pain and tactile hypersensitivity that lasted several weeks (Pitcher et al., 1999a). Electrophysiological recordings made in the same neuropathic animals showed a gain in rate of spontaneous on-going discharge of wide dynamic range (WDR) dorsal horn neurons and gain in magnitude and duration of the after-discharge of these neurons in response to stimulation of the peripheral cutaneous receptive field at the same time points weeks after cuff implantation correlating with neuropathic pain behaviors (Pitcher and Henry, 2000, 2004). This increased excitation of WDR neurons was not mediated by descending supraspinal input as the rats in these experiments were spinalized. What was not clear was whether this increased excitation was due to post-synaptic changes in the spinal dorsal horn or whether it was maintained by abnormal input from primary afferents. Thus, the present study was aimed to answer whether primary afferent input at time points well after the onset of peripheral neuropathy (i.e. several weeks) plays a role in mediating increased excitation of spinal dorsal horn neurons. We investigated this by recording from single lumbar nociceptive dorsal horn neurons in spinalized cuff-implanted rats and applying lidocaine directly to the sciatic nerve during spontaneous on-going activity or during the afterdischarge provoked by mechanical cutaneous stimulation of the cuff-implanted hind paw. We provide evidence that hyperexcited nociceptive dorsal horn neurons in rats with chronic peripheral neuropathy is reversed by application of lidocaine to the sciatic nerve proximal as well as distal to the cuff.

Materials and methods

Experiments were done using adult, male Sprague–Dawley rats (375–425 g) from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana, USA). They were housed in plastic cages containing wood chip bedding (Hardwood Laboratory Bedding, Northeastern Products Corp., Warrensburg, NY, USA) and maintained on a 12:12 h light:dark cycle (lights on at 07:00 h) with access to food and water *ad libitum*. Guidelines in *The Care and Use of Experimental Animals* by the Canadian Council on Animal Care were followed and all experiments were approved by the *McGill University Animal Care Committee*. At the end of the experiment, anesthetized rats were euthanized.

Peripheral neuropathy was induced in the anesthetized rat (sodium pentobarbital; 50 mg/kg, i.p.; Abbott Laboratories Ltd., Montreal, Quebec, Canada) by placing a 2 mm section of split polyethylene tubing (Intramedic PE-90, Fisher Scientific Ltd., Whitby, Ontario, Canada) around the left sciatic nerve (Pitcher et al., 1999a; Pitcher and Henry, 2000, 2004). To confirm that this procedure produced neuropathic pain, the von Frey filament test was used to determine whether the withdrawal threshold to mechanical cutaneous stimulation of the cuff-implanted hind paw was significantly reduced (tactile hypersensitivity) compared to baseline levels of the same animals preoperatively. Details of this pain model and the procedure of von Frey filament testing are reported elsewhere (Pitcher et al., 1999a,b; Pitcher and Henry, 2000, 2004).

For the acute electrophysiological experiments animals were anesthetized (sodium pentobarbital; Abbott Laboratories Ltd.; 50 mg/kg, i.p. followed by supplements of 10 mg/kg/h, i.v.). Details for spinal cord transection (at the T_9 vertebral level), extracellular recording of spinal dorsal horn neurons, and for data acquisition are described elsewhere (Pitcher and Henry, 1999; Pitcher and Henry, 2000, 2004).

Surgical preparation of the cuff-implanted hind paw for peripheral nerve block was done immediately after laminectomy in anesthetized rats and was similar to that described elsewhere (Pitcher and Henry, 2002). Briefly, the left sciatic nerve was exposed via blunt dissection through the biceps femoris muscle and was carefully isolated from surrounding connective tissue. Melted agar (37.5 °C) was poured to cover all exposed tissue but leaving approximately a 10 mm segment of the sciatic nerve exposed. The agar was allowed to solidify and the isolated sciatic nerve was bathed in 0.9% saline (1.5 ml) at 37.5 °C. The purpose of agar in this procedure was to prevent access of lidocaine (2%, Astra Pharma Inc., Mississauga, Ontario, Canada), in experiments in which lidocaine (1.5 ml at 37.5 °C) was applied to the sciatic nerve (see below), from the bath into surrounding tissue and into the circulation.

Functional classification of lumbar spinal dorsal horn neurons (L_3–L_4) was based on their responses to both innocuous and noxious stimuli applied to the cutaneous receptive field of the plantar surface of the cuff-implanted hind paw. The protocol used in this study to identify and classify single dorsal horn neurons is identical to that reported previously (Pitcher and Henry, 1999; Pitcher and Henry, 2000, 2004). Only dorsal horn neurons that responded to both innocuous and noxious stimuli were examined in this study and were considered WDR neurons. The response of these neurons to the noxious range of mechanical stimulation (pinch; 21 N for 3 s) showed the characteristic exaggerated slowly decaying afterdischarge (Pitcher and Henry, 2000).

The effect of lidocaine block of the cuff-implanted sciatic nerve was determined on cutaneous stimulation-elicited discharge and on elevated spontaneous on-going discharge of WDR neurons in cuff-implanted rats. In experiments in which the effect of peripheral nerve block was determined on pinch stimulation-evoked discharge of WDR neurons, lidocaine was administered to the exposed cuff-implanted sciatic nerve (for a duration of 4 min) beginning four minutes after the pinch stimulus followed immediately by saline flush three times. This nerve block experiment was done approximately 60 min after a control experiment in which the sciatic nerve was bathed in saline during the afterdischarge. This 60 min period provided adequate time for the control pinch-elicited afterdischarge to dissipate completely. The control experiment in which the sciatic nerve was perfused with saline, beginning 4 min after the 3 s pinch stimulus and lasting for 4 min, was without effect on the discharge of WDR neurons (Pitcher and Henry, 2000). Quantification of the pinch-induced afterdischarge consisted of the number of spikes beginning immediately following saline administration to the cuff-implanted sciatic nerve and ending once the firing rate returned to the pre-stimulus discharge level (i.e. spontaneous on-going discharge), minus the background on-going discharge of the same duration. Data are expressed as the number of spikes during the respective sample periods. In experiments in which the effect of peripheral nerve block was determined on exaggerated spontaneous on-going discharge of WDR neurons in cuff-implanted rats, lidocaine was administered to the exposed cuff-implanted sciatic