Neuroscience Letters 505 (2011) 52–57

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Electrical stimulation at distinct peripheral sites in spinal nerve injured rats leads to different afferent activation profiles

Fei Yang[a,1], Chih-yang Chung[b,1], Paul W. Wacnik[c], Alene F. Carteret[a], Alvin D. McKelvy[a], Richard A. Meyer[d], Srinivasa N. Raja[a], Yun Guan[a,*]

[a] Anesthesiology and Critical Care Medicine, Johns Hopkins University, Baltimore, MD, USA
[b] Department of Anesthesiology, Far Eastern Memorial Hospital, Taipei, Taiwan
[c] Neuromodulation Research, Medtronic Inc., Minneapolis, MN, USA
[d] Neurosurgery, The Johns Hopkins University, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 7 April 2011
Received in revised form 20 August 2011
Accepted 28 September 2011

Keywords:
Peripheral nerve stimulation
Pain
Compound action potential
Spinal nerve injury

ABSTRACT

The neurophysiological basis by which neuromodulatory techniques lead to relief of neuropathic pain remains unclear. We investigated whether electrical stimulation at different peripheral sites induces unique profiles of A-fiber afferent activation in nerve-injured rats. At 4–6 weeks after subjecting rats to L5 spinal nerve injury (SNL) or sham operation, we recorded the orthodromic compound action potential (AP) at the ipsilateral L4 dorsal root in response to (1) transcutaneous electrical nerve stimulation (TENS, a patch electrode placed on the dorsum of the foot), (2) subcutaneous electrical stimulation (SQS, electrode inserted subcutaneously along the dorsum of the foot), (3) peroneal nerve stimulation (PNS, electrode placed longitudinally abutting the nerve), and (4) sciatic nerve stimulation (SNS). The area under the Aα/β compound AP was measured as a function of the bipolar, constant-current stimulus intensity (0.02–6.0 mA, 0.2 ms). In both nerve-injured and sham-operated groups, the stimulus-response (S-R) functions of the Aα/β compound APs differed substantially with the stimulation site; SNS having the lowest threshold and largest compound AP waveform, followed by PNS, SQS, and TENS. The S-R function to PNS was shifted to the right in the SNL group, compared to that in the sham-operated group. The Aα/β-threshold to PNS was higher in the SNL group than in the sham-operated group. The S-R functions and Aα/β-thresholds to TENS and SQS were comparable between the two groups. Electrical stimulation of different peripheral targets induced distinctive profiles of A-fiber afferent activation, suggesting that the neuronal substrates for the various forms of peripheral neuromodulatory therapies may differ.

1. Introduction

Neuropathic pain is often resistant to conventional pharmacotherapies and neurosurgical interventions. Increasing clinical and experimental evidence suggests that peripheral nerve stimulation may represent a simple, effective, and minimally invasive procedure in the treatment of neuropathic pain [1,7,8,10,11]. The commonly used peripheral neuromodulatory techniques include transcutaneous electrical nerve stimulation (TENS), peripheral nerve field stimulation (PNFS), and peripheral nerve stimulation [1,5,9]. For each of these treatment modalities, low-stimulus currents are applied at a wide range of frequencies (e.g., 2–100 Hz) by means of electrodes placed on the skin of the affected dermatome (TENS) or implanted subcutaneously in the vicinity of (PNFS) or in close association with the peripheral nerve that innervates the painful area. Although the gate control theory of pain may offer a partial explanation for the analgesic effects obtained by different peripheral nerve stimulation techniques [14], the neurophysiological basis for their beneficial clinical effects on chronic pain remains largely unknown [1,8].

The differences in the type and location of stimulation electrode, among the various peripheral neuromodulatory techniques, may affect the pattern of peripheral nerve activation substantially and hence the therapeutic outcome. Electrophysiological studies in animal models of neuropathic pain are essential for understanding the biological and neurophysiological mechanisms for the beneficial clinical effects of neuromodulatory techniques [4,16,20]. We sought to determine whether different peripheral nerve stimulation techniques for pain induce unique profiles of primary afferent fiber activation in a well-established rat model of neuropathic pain. Our observations indicate that electrical stimulation of different

* Corresponding author at: Division of Pain Medicine, Department of Anesthesiology/CCM, Johns Hopkins University, 720 Rutland Ave., Ross 351, Baltimore, MD 21205, USA. Tel.: +1 410 502 5511; fax: +1 410 614 2109.
E-mail address: yguan1@jhmi.edu (Y. Guan).
[1] These authors contributed equally to this work.

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.09.065

peripheral targets induces distinctive profiles of A-fiber afferent activation that may contribute to the different therapeutic features of various peripheral neuromodulatory techniques.

2. Materials and methods

To model neuropathic pain observed in humans, we subjected adult male Sprague-Dawley rats (300–400 g, Harlan, Indianapolis, IN) to the tight ligation and transection of the L5 spinal nerve (SNL) [2,3]. The animals were deeply anesthetized with isoflurane (2.0%, Abbott Laboratories, North Chicago, IL). The left transverse process of the L6 vertebra was removed, and the left L5 spinal nerve was tightly ligated with a 6-0 silk suture and cut distally [2,3]. In sham operation, the L6 transverse process was not removed and the L5 spinal nerve was not injured. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee (Baltimore, MD, USA) as consistent with the National Institutes of Health Guide for the Use of Experimental Animals to ensure minimal animal use and discomfort.

Because of the evolving nature of injury-induced changes in the nervous system and because neuromodulatory techniques are used mostly to treat patients with established chronic pain, we examined rats during the maintenance phase of neuropathic pain (weeks 4–6); sham-operated rats were used as controls. On the experimental day, animals were anesthetized with pentobarbital (45–50 mg/kg, i.p.), and a tracheotomy was performed. The rats were ventilated mechanically (Kent Scientific Corporation, Litchfield, CT, 50–70 cycles/min). During the neurophysiological experiments, the rats were anesthetized with 1.5% isoflurane and paralyzed with pancuronium bromide (1–2 mg/kg, i.p., Elkins-Sinn Inc., Cherry Hill, NJ) via intermittent injections given as needed (1 mg/kg/h, i.p.). Core body temperature was kept in the normal range (36.0–37.0 °C).

2.1. Transcutaneous electrical stimulation of the dorsum of the foot (to mimic TENS)

The experimental setup is shown in the schematic diagram of Fig. 1A. We placed a TENS patch electrode of 1–1.2 cm in diameter on the dorsum of the foot and the return electrode on the plantar side of the foot. Dorsum was chosen because TENS is used mostly on hairy skin. The fur was removed with Nair (Church & Dwight Co., Princeton, NJ) to ensure better adhesion of the patch electrode. Constant current was used for transcutaneous stimulation (0.02–6.0 mA, 0.2 ms, model 2100, A-M Systems, Sequim, WA).

2.2. Subcutaneous electrical stimulation (SQS) to mimic PNFS of the foot dorsum

A deep brain stimulation (DBS) electrode produced by Medtronic (Minneapolis, MN) was used for SQS. Each electrode consists of a linear array of four contacts. The area stimulated was similar to that of the TENS site. The DBS electrode was inserted into the subcutaneous space by means of a needle. To optimally activate the field, we placed the DBS electrode longitudinal to the axis of the foot (i.e., along the limb). Constant current was used for bi-polar subcutaneous stimulation (0.02–6.0 mA, 0.2 ms). The most proximal and the most distal contacts of the lead were set as the cathode and the anode, respectively (Fig. 1A).

2.3. Peroneal nerve stimulation (PNS)

A DBS needle electrode was also used for PNS. To approximate an in vivo situation and mimic its potential clinical use, the DBS electrode was placed longitudinally parallel to and abutting the common peroneal nerve. Then, the surrounding muscle was sutured and the skin wound was closed to prevent the electrode from moving during the protocol. Parameters and lead configuration were the same as those for SQS.

2.4. Sciatic nerve stimulation (SNS)

As a positive control we also recorded the compound action potential (AP) obtained in response to SNS to better evaluate the relative proportion of fibers being activated at each site. The left sciatic nerve and its branches were exposed and dissected from the surrounding tissue. A pair of silver hook electrodes was placed on the sciatic nerve at mid-thigh level for recording compound APs. The parameters used were the same as those for PNS and SQS.

We recorded the orthodromic compound AP produced at the ipsilateral L4 dorsal root in response to the different types of peripheral electrical stimulation (Fig. 1A). This recording location was chosen because the dorsal root only includes sensory fibers and because conduction length would be sufficient to see separated Aα/β and Aδ waveforms. The left L4 dorsal root was exposed and lifted to separate it from surrounding tissue. A monopolar platinum hook electrode was placed on the dorsal root for recording compound APs. The reference electrode was placed in the nearby muscle. TENS was examined first. Then, the DBS electrode was inserted at the same location for testing SQS. Finally, we examined the effects of applying electrical stimulation to the common peroneal nerve and sciatic nerve. Since the compound AP waveforms were small, particularly from TENS and SQS, data from 25 trials of the same stimulus intensity (applied at 0.5 Hz) were added up to improve the signal-to-noise ratio (as noise will be averaged away).

We determined Aα/β-threshold (i.e., the lowest stimulus intensity to evoke an Aα/β-waveform) and Aδ-threshold of the compound AP and examined the profiles of Aα/β and Aδ afferent activations. Aα/β and Aδ compound AP waveforms were identified based on activation threshold and conduction velocities (CVs, Fig. 1B and C). The fastest and slowest CVs for Aα/β compound APs were calculated as the conduction length (i.e., distance between the stimulating electrode and recording site) divided by the latency of the earliest and latest Aα/β compound AP observed, respectively. The stimulus-response (S-R) functions, Aα/β-thresholds, and Aδ-thresholds of the compound APs produced by different types of stimulation were compared within groups by a two-way repeated measure ANOVA and between the SNL and sham-operated groups with a two-way mixed model ANOVA. The Tukey honestly significant difference post hoc test was used to compare specific data points. STATISTICA 6.0 software (StatSoft, Inc., Tulsa, OK, USA) was used to conduct all statistical analyses. Unless otherwise specified, two-tailed tests were performed, and data are expressed as means ± SEM; $p < 0.05$ was considered statistically significant.

3. Results

Examples of compound APs produced in response to increasing intensities of electrical stimulation applied ipsilaterally at the TENS, SQS, PNS, and SNS electrodes are shown in Fig. 1. At a suprathreshold intensity, two groups of waves were recorded in the compound AP, corresponding to Aα/β and Aδ fiber activation (Fig. 1C), which were distinguished on the basis of the threshold and calculated CVs of the different components (waves) of the compound APs. For example, the fast A-fibers gave a series of large peaks (the Aα/β compound AP) in response to SNS in SNL rats; the averaged CVs ranged from 63.4 ± 1.5 m/s to 14.8 ± 0.5 m/s and corresponded to the Aα/β-fiber activation. The CVs to SNS in the SNL group were similar to those in the sham-operated group and comparable to those reported previously [15]. The fastest CVs calculated from the earliest Aα/β compound AP to TENS (sham: 34.2 ± 0.3 m/s; SNL: