Neuroscience 132 (2005) 193–201

MECHANICAL AND HEAT SENSITIZATION OF CUTANEOUS NOCICEPTORS IN RATS WITH EXPERIMENTAL PERIPHERAL NEUROPATHY

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Abstract—This study examined whether or not the properties of cutaneous nociceptive fibers are altered in the neuropathic state by comparing lumbars 5 and 6 spinal nerve ligation (SNL) rats with sham-operated controls. The rats with the unilateral SNL developed mechanical allodynia in the ipsilateral hind limb, whereas the sham group did not. Two to 5 weeks after the neuropathic or sham surgery, rats were subjected to single fiber-recording experiments to examine the properties of afferent fibers in the sural and plantar nerves. A total of 224 afferents in the C- and Aδ-ranges were characterized in the neuropathic and sham groups. Spontaneous activity was observed in 16 of 155 fibers in the neuropathic group and one of 69 fibers in the sham group. The response threshold of both the C- and Aδ-fibers to mechanical stimuli was lower in the neuropathic group than the sham group. The afferent fibers responsive to heat stimuli were all C-fibers, and none were Aδ-fibers. The response threshold of the C-fibers to the heat stimuli was lower in the neuropathic group than the sham group. The magnitude of the responses of both C- and Aδ-fibers to the suprathreshold intensity of the mechanical stimulus was greater in the neuropathic group than the sham group. However, the magnitude of the responses of C-fibers to the suprathreshold intensity of the heat stimulus in the neuropathic group was not different from that in the sham group. These results suggest that after a partial peripheral nerve injury, the nociceptors on the skin supplied by an uninjured nerve become sensitized to both mechanical and heat stimuli. This nociceptor sensitization can contribute to neuropathic pain. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: neuropathic pain, nerve injury, nociceptive fibers, spinal nerve ligation, single fiber recording.

A peripheral nerve injury leads to neuropathic pain consisting of spontaneous and abnormally evoked pain, such as allodynia and hyperalgesia (Bonica, 1990). There is evidence suggesting that neuropathic pain is due to changes in the primary afferent neurons (Campbell et al., 1992; Noguchi et al., 1995; Sato and Perl, 1992). One important change is the development of the ongoing or ectopic activity in the primary afferents, which results in changes in the CNS, i.e. central sensitization. Kim and Chung (1992) developed a spinal nerve ligation (SNL) model of a nerve injury, in which a tight ligation of lumbars 5 and 6 (L5 and L6) spinal nerves induced the behavioral signs of neuropathic pain. Based on studies using this model, it was hypothesized that the ectopic activity causes the sensitization of the spinal neurons, and that the evoked activity in the uninjured afferents making contact with the sensitized spinal neurons results in abnormally evoked pain. It was reported that the ectopic activity arises mainly from the rapidly conducting A-fibers in the injured and uninjured spinal nerve following SNL, but not from the C-fibers, and that the time course of the changes in this ectopic activity well correlated with the changes in the behavioral changes (Liu X et al., 1999, 2000; Han et al., 2000; Liu CN et al., 2000; Ma et al., 2003). However, whether or not the ectopic activity in the rapidly conducting A-fibers is sufficient to trigger and maintain central sensitization remains unclear.

An alternative hypothesis emphasizes that the changes in the uninjured afferents adjacent to the injured afferents underlie the initiation and maintenance of the SNL-induced pain behaviors. Single nerve fiber recording studies have demonstrated that following a SNL, a subpopulation of uninjured C-afferents develops ongoing ectopic activity (Ali et al., 1999; Wu et al., 2001). It was reported that an antisense treatment against Na_V 1.8, a tetrodotoxin-resistant Na channel protein expressed mainly in the unmyelinated afferents, reduces the SNL-induced pain behavior (Lai et al., 2000). A previous study demonstrated that a capsaicin treatment on the sciatic nerve, under the conditions where the L5 and L3 dorsal roots had been transected prior to the L5 SNL, reversed the L5 SNL-induced mechanical hyperalgesia (Jang et al., 2003). This suggests that the activity of the uninjured C-afferents contributes to the SNL-induced neuropathic pain behavior.

Another important change that can occur in the uninjured afferents after SNL, other than the development of ectopic activity, is the sensitization of the nociceptive afferents. It is well known that peripheral inflammation sensitizes the nociceptors in the inflamed skin to heat and mechanical stimuli (Andrew and Greenspan, 1999; Kidd and Urban, 2001). However, there is little evidence to suggest that nociceptor sensitivity changes in a nerve injury-induced neuropathic

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Abbreviations: CV, conduction velocity; RF, receptive field; SNL, spinal nerve ligation; TNF-α, tumor necrosis factor-α; VFF, von Frey filament.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2004.12.036

state. This study investigated whether or not the properties of the cutaneous nociceptors are altered in the SNL-rats compared with the controls. The property changes examined included 1) whether or not spontaneous activity develops, 2) whether or not the response threshold to the mechanical or thermal stimuli is lowered, and 3) whether or not the magnitude of the responsiveness to mechanical or thermal stimuli at the supra-threshold intensity is elevated.

EXPERIMENTAL PROCEDURES

Animals

A total of 78 young adult male rats (Sprague–Dawley; Harlan) weighing 150–350 g were used in this study. The animals were housed in groups of three to four, with food and water available *ad libitum*, and were allowed to acclimate under a light/dark cycle for approximately 1 week prior to surgery and behavioral test. Behavioral testing was started with a habituation period, in which rats were placed in plexiglas cages for 30 min. The experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee of Yonsei University, Seoul, Korea. All efforts were made to minimize the number of animals used and their suffering.

Neuropathy model and behavioral testing

Unilateral peripheral neuropathy was performed as described by Kim and Chung (1992). The left L5 and L6 spinal nerves were exposed and tightly ligated with a 6-0 silk thread under enflurane anesthesia (3% enflurane–O_2 mixture). The wound was sutured and maintained with adequate postoperative care. In the sham-operated group, the same procedure as used for the neuropathic rats was performed, but without the nerve ligation.

The rats were examined for their mechanical sensitivity using a filament (24.5 mN bending-force, 0.28 mm tip-diameter) taken from a set of von Frey filaments (VFF; Stoelting Co., Wood Dale, IL, USA), which was calibrated using a weighing balance. Each animal was placed in a plexiglas cage (8×8×20 cm) above a wire mesh bottom, which allowed full access to the paw, and the animal was acclimatized to the test cage for 30 min prior to testing. The mechanical sensitivity was measured as the incidence of paw withdrawals to 10 applications of the VFF stimulus to the plantar surface of the hind paw. Quick withdrawal of the paw in response to each VFF application was considered to be a positive response. When the paw withdrawn by a VFF application returned to the bottom, the next VFF was delivered. A trial of 10 applications of the VFF stimulus was carried out three times, which were separated by 3 min intervals, on each hind paw in a given test day. The frequency of paw withdrawals in a given test day was calculated as the percentage withdrawal [(number of paw withdrawals/30)×100]. The behavioral testing, which was carried out 2 and 3 days and once every week after surgery until the single fiber recording experiments commenced, was performed by the experimenter (D-W Kim) blinded to preceding surgeries. The average paw withdrawal frequency for all the test days was used to determine if animals had an increased mechanical sensitivity.

Single fiber recording experiments

The single fiber recording experiments were performed 2–5 weeks after the neuropathic surgery. The rats were initially anesthetized with urethane (i.p., 1.5 g/kg), and anesthesia was judged as being sufficiently deep when the eye-blink reflex to air-puffs as well as the withdrawal reflex to noxious limb stimulation were absent. An additional dose of urethane (100 mg/kg) was administered during the experiment when deemed necessary. A midline incision was made from the mid-thigh to the ankle of the left hind limb, which exposed the sural and plantar nerves. Under a dissecting microscope, each nerve was separated from the adjacent tissues and cut proximally at its junction with the sciatic nerve. The incised skin was retracted and bound around a frame to form a pool filled with warm mineral oil over the exposed tissue, and the hind paw was fixed on modeling clay. The rectal temperature was maintained at 37±0.5 °C using a servo-controlled heating blanket.

Single fiber recordings were made from the nerve filaments teased from the distal cut end of the nerves with a platinum recording electrode referenced by an electrode pinned to the nearby tissue. In order to locate the receptive field (RF) of the afferent fibers, a saline-soaked cotton ball-stimulating electrode was moved over the surface of the skin innervated by the nerve under study. Constant current pulses (0.5–2 ms, 0.5–10 mA, 0.33 Hz) were delivered for the cutaneous stimulation. The nerve filaments were subdivided into single fine filaments. Usually, a fine filament produced an evoked spike as a result of the electrical stimulation of the RF. The conduction velocity (CV) of the fibers with the single spikes was determined using two parameters: the propagation distance between the stimulating and recording electrodes, and the response latency to the single stimulating pulses. The single fibers were classified as being either Aδ- or C-fibers if their CV was between 2.5 and 25 m/s or slower than 2.5 m/s, respectively (Leem et al., 1993). The faster conducting Aβ-fibers were not examined. In some cases, when a fine nerve filament showed more than one evoked spike in the C-range, the fiber with the largest spike was examined because the larger spike was more durable during the recording period than the smaller one. The nerve activity of the single fibers was amplified, band pass filtered (300–10,000 Hz), and fed either directly or via a window discriminator (model 121, World Precision Instruments, New Haven, CT, USA) into the data acquisition unit (CED-1401 plus, Cambridge Electronic Design Ltd., Cambridge, UK) in order to display the wave forms or the peristimulus time histograms on a computer monitor, respectively. Single fiber recording experiments were not performed in a blind manner. However, the criteria used to identify the single fiber activity were the same for the lesioned and sham-operated animals.

Characterization of nociceptive fibers

After isolating the single fibers, the search stimuli were applied to the somatic RF in order to identify the receptor type. These stimuli included brushing with a camelhair brush and pinching the skin folds with blunt-tipped forceps. The recorded fibers were identified as cutaneous nociceptive afferents, when they showed a response to skin pinching. Skin pinching was delivered no more than twice in order to avoid the potential sensitization of nociceptors. In addition, when more than one afferent was examined in each rat, the fibers with the RFs that did not overlap were examined. The mechanically responsive afferents were subjected to an examination of the properties of the response to mechanical and heat stimuli, as described below. Since mechanical stimuli were the primary search stimuli in the present study, the search procedure was biased against finding mechanical insensitive afferent fibers. The ongoing spontaneous activity, if present, was recorded for each fiber over a 5-min period and averaged. An afferent fiber with a mean nerve activity of 0.1 Hz or higher was considered to be spontaneously active.

The mechanical sensitivity of afferents was examined by applying punctate mechanical stimuli to the center of the RFs with a set of calibrated VFFs (3.2, 5.7, 7.5, 12.3, 24.5, 35.3, 45.1, 56.9, 85.3, 101.1, 137.2, 274.4, 637.3, and 990 mN; 0.2–0.8 mm in tip-diameters). In some afferents with RFs consisting of several small mechanically sensitive spots, the most sensitive spot was identified with an appropriate VFF under a dissection microscope. This spot was initially used to roughly determine the response threshold with VFFs. With the use of a VFF having a one-step stronger force than the threshold, the RF of the afferent fiber was mapped. In these procedures, the VFF stimuli were applied by hand using a hand-held stick-holder. To obtain the response