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Research paper

L5 spinal nerve axotomy induces sensitization of cutaneous L4 Aβ-nociceptive dorsal root ganglion neurons in the rat *in vivo*

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HIGHLIGHTS

- Cutaneous L4 Aβ-nociceptors became sensitized 7 days after L5-spinal nerve axotomy (SNA). They exhibited:
- Decreased mechanical and electrical thresholds and enhanced responsiveness to suprathreshold mechanical stimulus.
- An apparent increase in incidence of spontaneous activity.
- These changes in L4 Aβ-nociceptors may contribute to peripheral neuropathic pain.

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ABSTRACT

Partial nerve injury often leads to peripheral neuropathic pain (PNP), a major health problem that lacks effective drug treatment. PNP is characterized by ongoing/spontaneous pain, and hypersensitivity to noxious (hyperalgesia) and innocuous (allodynia) stimuli. Preclinical studies using the L5 spinal nerve ligation/axotomy (SNL/SNA) model of PNP suggest that this type of chronic pain results partly from sensitization of ipsilateral L4C-and Aδ-fiber nociceptive dorsal root ganglion (DRG) neurons, but whether L4 β-nociceptors, which constitute a substantial group of DRG neurons, also become sensitized remains unanswered. To address this issue, intracellular recordings from somata of cutaneous Aβ-nociceptors (classified according to their dorsal root conduction velocities (>6.5 m/s), and physiologically based on their responses to noxious (but not innocuous) mechanical stimuli) were made from L4-DRGs in normal (control) rats and in rats seven days after L5 SNA *in vivo*. Compared with control, cutaneous L4 Aβ-nociceptive DRG neurons in SNA rats (that developed mechanical hypersensitivity) exhibited sensitization indicated by: a) decreased mean mechanical threshold (from 57.8 ± 7.1 to 10.3 ± 1.7 mN), b) decreased mean dorsal root electrical threshold (from 11.4 ± 0.7 to 4.3 ± 0.4 V), c) increased mean response to a suprathreshold mechanical stimulus (from 18.5 ± 1.8 to 34 ± 3.7 spikes/sec) and d) an obvious, but non-significant, increase in the incidence of ongoing/spontaneous activity (from 3% to 18%). These findings suggest that cutaneous L4 Aβ-nociceptors also become sensitized after L5 SNA, and that sensitization of this subclass of A-fiber nociceptors may contribute both directly and indirectly to nerve injury-induced PNP.

1. Introduction

Chronic peripheral neuropathic pain (PNP) often results from partial injury of a peripheral nerve. It affects 6–8% of the general population [1], and is inadequately controlled by currently available drugs. PNP is characterized, in humans, by ongoing/spontaneous pain and hypersensitivity to normally painful (hyperalgesia) and non-painful (allodynia) stimuli [2]. Several animal models have been developed to investigate the pathophysiology of PNP including the widely used L5 spinal nerve ligation/axotomy (SNL/SNA) model [3]. The advantage of this model is that the directly axotomized L5-dorsal root ganglion (DRG) neurons are separated from the spared "*uninjured*" L4-DRG neurons, thereby allowing investigation of the relative contributions to SNL/SNA-induced PNP of directly injured L5-DRG neurons and adjacent ipsilateral L4-neurons (with conducting fibers intermingling with the degenerating axotomized L5-fibers in the same peripheral nerve).

Preclinical studies using the SNL/SNA model suggest that not only injured/axotomized L5-DRG neurons are involved in the development of PNP, but the adjacent L4-DRG neurons also

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play an important role (see e.g. [4]). Indeed, there is growing and compelling evidence, from animal studies using the 5 SNL/SNA model, for a major contribution of L4-neurons to PNP including: (1) the necessity of the L4-afferent neurons for transmission of evoked pain signals to the CNS; (2) spontaneous firing in L4C- and A-fiber neurons [5–9]; (3) up-regulation and phenotypic changes in expression of neuropeptides and neurotransmitters/neuromodulators in L4-DRG neurons [10–12]; 4) attenuation of mechanical hypersensitivity following elimination of L4C-afferents with local capsaicin treatment [13]; 5) desensitization of L4 Aβ-low threshold mechanoreceptive (LTM) neurons to mechanical stimuli [14] and 6) sensitization of L4C-and Aδ-fiber nociceptive DRG neurons [14,15]. However, whether cutaneous L4 Aβ-nociceptive DRG neurons also become sensitized after L5 spinal nerve injury remains unanswered.

Most Aβ-fiber DRG neurons are LTMs, but Aβ-nociceptors constitute a substantial group of these neurons (see [16]). Axotomized L5 Aβ-fiber neurons have been implicated in tactile allodynia following L5-spinal nerve injury, but these are believed to be Aβ-LTMs [6]. L4C-and Aδ-fiber nociceptive DRG neurons have also been implicated in development of PNP associated with spinal nerve injury [14,15], but it is possible that the conducting/*uninjured* L4 Aβ-nociceptors also become sensitized after L5 SNL/SNA and thereby contribute to the symptoms of PNP. Therefore, the primary aim of the present study was to examine this hypothesis. It should be pointed out that sensitization of primary afferent nociceptors (peripheral sensitization) is characterized by spontaneous activity (SA), lowered stimulus threshold and/or enhanced response to suprathreshold stimuli (*e.g.* [17].

2. Materials and methods

2.1. Experimental animals

The experiments were conducted on young female Wistar rats (180–220 g, Charles River, U.K.). Prior to the electrophysiological experiments, the rats were housed in a room maintained at room temperature between 22 and 24 °C while under a 12 h (h) dark and light cycle, with soft bedding and access to food and water *at libitum*. All experimental procedures were approved by the University of Liverpool Ethical review group, and complied with the 1986 UK Scientific Procedures Animals Act.

2.2. Animal model of peripheral neuropathic pain (PNP)

The L5 SNA model of PNP, which is a modified version of the original SNL (Chung) model [3] was used. It was produced as described previously [8,9].

2.3. Behavioral testing experiments

The behavioral sign of mechanical allodynia was examined in rats 7 days after L5 SNA as reported previously [18,19] using the dynamic plantar esthesiometer touch stimulator (Ugo Basile, Comerio, Italy). Mechanical allodynia was indicated by decreased withdrawal thresholds to pressure applied with a blunt metal filament, through an elevated mesh, to the mid-plantar surface of the hind paw (L4 dermatome).

2.4. In vivo *electrophysiological experiments*

As described previously [8,9,18] the electrophysiological experiments were conducted under deep anesthesia induced with an initial dose of sodium pentobarbitone (60 mg/kg, i.p.). Core temperature, the end tidal CO_2, and blood pressure were maintained throughout at ~36 ± 0.5 °C, 3% and 4%, and 80–100 mm Hg respectively. Intracellular voltage recordings were made from neuronal somata of L4 Aβ-fiber nociceptive DRG neurons (see below) in rats 7 days after L5 SNA, and in normal (control) rats of similar age/weight, using sharp glass microelectrodes filled with 1 M KCl (see [8,9,18]). The temperature in the paraffin pool measured near L4 DRGs was between 30 and 32 °C.

2.4.1. Conduction velocity (CV) classification of L4-DRG neurons as Aβ-fiber neurons

Somatic action potentials (APs) evoked antidromically by stimuli applied to the dorsal root, as well as any spontaneously occurring APs were recorded on line (using the CED Spike II program (see [8,9,18]) from somata of L4-DRG neurons classified as Aβ-fiber neurons according to dorsal root CV of >6.5 m/s (see [20]). The CV for each neuron was estimated (offline) by dividing the conduction distance (in mm) by latency (in ms) from the stimulus artefact to the AP onset. The CV values in the present and other previous studies by this author and his colleagues are relatively low because utilization time was not excluded (see Ref. [16]).

2.4.2. Functional classification of L4-DRG neurons as Aβ-nociceptors

To evaluate the sensory modality of recorded neurons, mechanical stimuli were applied to their receptive fields in the glabrous skin of the ipsilateral (left) hindpaw using hand-held natural stimulators. As described previously [21], cutaneous Aβ-nociceptive neurons were those that failed to respond to low-intensity (non-painful) stimuli (*e.g.* light pressure or stroking the skin with a fine paint brush), but responded to noxious mechanical stimuli. These noxious stimuli included pressure applied with a sharp object (*e.g.*, needles), and pinching the superficial skin and lifting it away from the underlying tissue with very fine forceps (see Ref. [21]). These were thought to have receptive terminals in the epidermis or the superficial dermis. It should be pointed out that Aβ-nociceptors were distinguished from low threshold Aβ-units (LTMs) on the basis of their responses to noxious mechanical stimuli and not on the basis of their mechanical thresholds. Unlike Aβ-LTMs which respond to non-noxious stimuli (including light brushing, skin contact and light pressure with blunt objects, light tap, tuning forks vibrating at 100 or 250 Hz), Aβ-units were classed as nociceptors only if they failed to respond to these non-noxious mechanical stimuli but responded to noxious mechanical stimuli mentioned above (see Ref. [21]). Aβ LTM units including rapidly adapting (RA) or slowly adapting (SA) units as well as Aβ moderate pressure units (see Ref. [21]) which have lower thresholds than Aβ-nociceptors were not included in the present study. Cutaneous Aβ-nociceptors were not tested with noxious or innocuous thermal stimuli because they normally do not respond to such stimuli (see Ref. [21]). Before sensory testing, any spontaneous APs were recorded for approximately 2 min to avoid any influence on spontaneous firing generation of the natural search stimuli as previously described [8]. Neurons with at least 1 spontaneous AP during this time were classed as displaying spontaneous activity (SA). Short-lasting (a few seconds) injury discharge due to electrode impalement was excluded.

2.5. Excitability and response properties of Aβ-nociceptors

To determine whether Aβ-nociceptors become sensitized after L5 SNA, the followings were measured in these neurons in both control and SNA rats: (1) the mechanical threshold which was determined using calibrated von Frey filaments (VFF) applied perpendicularly to the most sensitive spots in the receptive fields of the neurons for about 2 s. It was defined as the lowest VFF force (in mN) needed to evoke at least 2 APs during the application period