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Nociception originating from the crural fascia in rats

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ABSTRACT

Little is documented in the literature as to the function of muscle fascia in nociception and pain. The aim of this study was to examine the distribution of presumptive nociceptive nerve fibers, to characterize fascial thin-fiber sensory receptors, and to examine the spinal projection of nociceptive input from the rat crural fascia (CF). Nerve fibers labeled with specific antibodies to calcitonin gene-related peptide (CGRP) and peripherin were found to be densely distributed in the distal third of the CF. Thin-fiber receptors (Aδ- and C-fibers) responding to pinching stimuli to the CF with sharpened watchmaker's forceps, identified in vivo with the teased fiber technique from the common peroneal nerve, exist in the CF. Forty-three percent of the mechano-responsive fascial C-fibers were polymodal receptors (nociceptors) responding to mechanical, chemical (bradykinin), and heat stimuli, whereas almost all Aδ-fibers were responsive only to mechanical stimuli. Repetitive pinching stimulus to the CF induced c-Fos protein expression in the middle to medial part of superficial layers ie, laminae I–II of the spinal dorsal horn at segments L2 to L4, peaking at L3. These results clearly demonstrate the following: 1) peptidergic and non-peptidergic axons of unmyelinated C-fibers with nerve terminals are distributed in the CF; 2) peripheral afferents responding to noxious stimuli exist in the fascia, and 3) nociceptive information from the CF is mainly processed in the spinal dorsal horn at the segments L2 to L4. These results together indicate that the "muscle fascia," a tissue often overlooked in pain research, can be an important source of nociception under normal conditions.

1. Introduction

Muscle fascia is less documented in the literature except for the biomechanical properties, although it has long been considered not only an important source of nociception and pain but also a critical target for treatment of musculoskeletal pain [11]. Musculoskeletal pain is of greater clinical importance than cutaneous pain because of its higher prevalence, persistent nature, impact on activities in daily living, and other effects [16,19]. It is only recently, however, that musculoskeletal pain has come to be more appreciated. Indeed, the International Association for the Study of Pain (IASP) selected "musculoskeletal pain" as a theme for a Global Year Campaign (Oct. 2009–Oct. 2010). The "second skeleton," fascia, however, continues to be largely ignored even in the field of musculoskeletal pain research, and even after this campaign.

We have systematically investigated the spinal dorsal horn neurons receiving input from the thoracolumbar fascia (TLF) in rats [10,27], and found that the proportion of second-order neurons with fascial input from the TLF significantly increased in animals with chronic myositis in the low back. The TLF is presumably innervated by nociceptive nerve fibers both in rats and in humans [34]. In human subjects with delayed-onset muscle soreness (DOMS) after lengthening contraction of the leg, the fascia, presumably the crural fascia (CF) covering the tibialis anterior muscle, reportedly became more sensitive to noxious stimulus than the exercised muscle itself [9], although in an animal model of DOMS muscular nociceptors were also sensitized to mechanical stimulation [30]. It has been reported that a localized sensitive spot is formed at a restricted depth in fascia in experimental models of DOMS after lengthening contraction in rabbits and in humans [12,13]. In addition, nerve growth factor, a neurotrophic and survival factor for sensory neurons during development, and now

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known to be involved in inflammatory hyperalgesia in adulthood, induced a lasting increase in the sensitivity of the muscle fascia at the lumbar level to mechanical and chemical stimuli [5]. These recent articles suggest not only that the muscle fascia could be a source of nociception in a physiological condition, but also that it could play crucial roles in hyperalgesic conditions (eg, myofascial pain syndrome). However the role of fascia as a nociceptive organ has not been fully established. Only 1 article has addressed peripheral fascial nociceptors with receptive fields in the low back, and their conductive and receptive properties have not been clarified in detail [1].

Recently, ex vivo single-fiber recordings revealed that not only receptive properties but also axonal conductive properties of thin-fiber receptors, as shown by activity-dependent change of conduction velocity (ADCCV) and twin pulse difference (TPD), are useful in understanding peripheral mechanisms of nociception and pain [8,23,35]. ADCCV in particular exhibits a functional link to receptive properties of nociceptors [4,29].

Here we examined the following: 1) the distribution of presumptive nociceptive nerve fibers in the CF; 2) axonal and receptive characteristics of peripheral thin-fiber afferents with receptive fields in the CF; and 3) the spinal projection of nociceptive input originating from the CF.

A preliminary account appeared elsewhere [32].

2. Methods

2.1. Animals

A total of 42 male Sprague-Dawley rats (SLC Inc., Shizuoka, Japan), 8 to 14 weeks of age and 280 to 450 g, were used in this study. The animals were kept 1 to 3 per cage in a clean room with air-conditioning (temperature, 23°C) under a 12-hour light/dark cycle (light between 08.00/09.00 and 20.00/21.00 h). The animals had free access to food and filtered clean water until the final experiment. The present study was conducted in its entirety according to the Regulations for Animal Experiments in Nagoya University, the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions in Japan, and the Ethical Guidelines of the International Association for the Study of Pain [39].

2.2. Immunohistochemical labeling of nerve fibers in crural fascia

Under deep anesthesia with sodium pentobarbital (80 mg/kg, i.p.), the animals were perfused with 0.1 M phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde in PBS. The CF was quickly excised and removed from anterior lower legs. Whole-mount fascia preparations (n = 4–5) were made to examine the distribution of nerve fibers and the terminals in the CF. Transverse sections of the CF (n = 6) were also made to measure the length and number of labeled nerve fibers for quantitative analysis. The whole mount preparations were incubated overnight at room temperature (RT) with primary antibodies against protein gene product 9.5 (PGP 9.5, n = 4, 1:1,000, RA95101, UCL, Isle of Wight, UK), CGRP (n = 5, 1:1,000, polyclonal rabbit antibody to CGRP, C8198, Sigma, St. Louis, MO), or peripherin (n = 5, 1:1,000, polyclonal rabbit antibody to peripherin, AB1530, Millipore, MA). After washing, the preparations were incubated with a secondary antibody (1:200, BA-1000, Vector Laboratories, CA) for 3 hours at RT. In the transverse sections, the primary antibodies were incubated for 2 days at 4°C. For quantitative analysis, transverse sections 20 μm in thickness were cut in the proximal, middle, and distal third of the CF. Six sections from the 6 different preparations (ie, 1 section from each preparation), were analyzed in each part of the CF. Immunoreactive nerve fibers and their terminals were traced and counted with a light microscope–equipped and computer-aided imaging system at a magnifying power of ×200, or in some cases ×400 (NeuroLucida, MicroBrightfield, Williston, VT). The most final end or the finest tip of a nerve fiber at the peripheral terminal in the CF, which was obviously different from a cut end with a relatively broader diameter, was considered to be a nerve terminal.

2.3. Electrophysiology

2.3.1. General surgical procedures

The animals were deeply anesthetized with sodium pentobarbital (50 mg/kg i.p. initially), followed by an i.v. infusion of the same anesthetic as above at a constant rate (approximately one-third of the initial dose per hour) using an infusion pump (ESP-64, Eicom Corp., Kyoto, Japan) to maintain a deep and constant level of anesthesia during the experiment. A catheter was inserted into the right external jugular vein for administration of the anesthetic and a muscle relaxant. The anesthesia was kept deep enough to abolish flexor reflexes and marked blood pressure changes (exceeding 10 mmHg) to noxious pinching of the tail. The electrocardiogram was monitored, and the depth of the anesthesia was adjusted so that the heart rate did not exceed 430 beats per minute. The muscle relaxant (pancuronium bromide, Mioblock, Schering-Plough, Osaka, Japan) was used to immobilize the animals (0.6–0.8 mg/h/rat i.v.). Another catheter was inserted into the right common carotid artery to measure blood pressure (mean arterial blood pressure >80 mmHg). The animals were put on a thermal blanket with feedback regulation (ATB-1100, Nihon Kohden Corp., Tokyo, Japan), left-side up, to monitor and maintain rectal temperature at a physiological level (37–38°C). Artificial ventilation was performed via a tracheal cannula with a gas mixture of 47.5% O_2, 2.5% CO_2, and 50% N_2 (arterial pO_2 of >100 mmHg, pCO_2 of 32–40 mmHg, pH close to 7.4) [10,27]. The skin was cut laterally along the femur and the fibula between the iscial tuberosity and the lateral malleolus. The skin flap from this was used to make a pool with paraffin oil to cover the recording site and the exposed CF (Fig. 1).

2.3.2. Recordings of thin-fiber receptors

A teased fiber technique was used to obtain the activities of a single fascial thin-fiber receptor from the common peroneal nerve in vivo (Fig. 1). First a mechanically sensitive receptive field (RF) was roughly searched by manual pinching of the CF with blunt forceps. Then the exact distribution (size and location) of the RF was defined with sharpened watchmaker's forceps, and mapped on a standardized chart. The CF alone could be stimulated separately from the muscle underneath because it has sufficient thickness and is connected to the muscle with a loose connective tissue. The size of the RF was measured by calculating the number of pixels in the RF that was drawn on the chart with ImageJ software from the National Institutes of Health (http://rsbweb.nih.gov/ij/index.html available online). Action potentials were analyzed on a computer with the DAPSYS data acquisition system (http://www.dapsys.net; Brian Turnquist, turnquist@bethel.edu) [38].

When a single fiber was identified, the RF was stimulated with twin rectangular electric pulses via a bipolar stimulating electrode placed beside the center of the RF (pulse duration: 500 μs, interstimulus interval: 50 ms, frequency: 0.5 Hz, repetitions: 6, and stimulus intensity: 1–2 times the threshold (range: 1.8–40 V) (Fig. 2) to observe axonal conductive properties of fascial thin-fiber receptors using the indices of activity-dependent change in conduction velocity (ADCCV) and twin pulse differences (TPD). The usefulness and method for calculation of these indices have been reported in recent articles [6,23,29].