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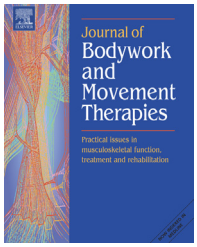

FASCIA SCIENCE AND CLINICAL APPLICATIONS: PROCEEDINGS NOTE: 4TH INTERNATIONAL FASCIA RESEARCH CONGRESS. WASHINGTON DC, SEPTEMBER 2015

Evidence for the existence of nociceptors in rat thoracolumbar fascia

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Summary Recently, the existence of nociceptive fibers in fascia tissue has attracted much interest. Fascia can be a source of pain in several disorders such as fasciitis and non-specific low back pain. However, little is known about the properties of fascia nociceptors and possible changes of the fascia innervation by nociceptors under pathological circumstances.

In this histologic study, the density of presumably nociceptive fibers and free nerve endings was determined in the three layers of the rat TLF: inner layer (IL, covering the multifidus muscle), middle layer (ML) and outer layer (OL). As markers for nociceptive fibers, antibodies to the neuropeptides CGRP and SP as well as to the transient receptor potential vanilloid 1 (TRPV1) were used. As a pathological state, inflammation of the TLF was induced with injection of complete Freund's adjuvant. The density of CGRP- and SP-positive fibers was significantly increased in the inner and outer layer of the inflamed fascia. In the thick middle layer, no inflammation-induced change occurred.

In additional experiments, a neurogenic inflammation was induced in the fascia by electrical stimulation of dorsal roots. In these experiments, plasma extravasation was visible in the TLF, which is clear functional evidence for the existence of fascia nociceptors. The presence of nociceptors in the TLF and the increased density of presumably nociceptive fibers under chronic painful circumstances may explain the pain from a pathologically altered fascia. The fascia nociceptors probably contribute also to the pain in non-specific low back pain.

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Introduction

The biomechanical properties of fascia tissue have been studied by many scientific groups (Vleeming et al., 1995; Langevin et al., 2011; Schleip et al., 2012; Corey et al., 2012). Recently, the existence of nociceptive fibers has become an important issue (Tesarz et al., 2011; Taguchi et al., 2013). Fascia can be a source of pain in fasciitis and probably also in non-specific low back pain (Langevin and Sherman, 2007). Experiments on human volunteers showed that noxious stimulation of the thoracolumbar fascia (TLF) evokes pain. Moreover, injections of pain-producing agents into the fascia have been shown to be more painful than the same injections into skin or muscle (Gibson et al., 2009; Deising et al., 2012; Schilder et al., 2014).

Not only the TLF but also other fasciae possess nociceptors (Taguchi et al., 2013). The nociceptive nature of the nerve endings was identified with immunohistochemical (Tesarz et al., 2011) or electrophysiological techniques (Taguchi et al., 2013).

In this article, the studied structure was the posterior lamina of the TLF, which covers the genuine back muscles (erector spinae muscle).

The article has two aims, namely 1. to describe inflammation-induced changes in the TLF innervation, and 2. to present immunohistochemical and functional data on the properties of fascia nociceptors. Generally, pain from fascia can be due not only to higher discharges in nociceptors but also to an increase in the innervation density by nociceptive endings.

Methods

All data were obtained from adult male Sprague—Dawley rats. The experiments were carried out in accordance with the German law on the protection of animals and the ethical proposals of the International Association for the Study of Pain (Zimmermann, 1983).

Immunhistochemistry

The experiments with fascia inflammation were carried out on deeply anesthetized rats (Ketamine 100 mg/kg i.p. and Xylazine 7.5 mg/kg i.p.; Essex Pharma, Germany and Alverat, Germany, respectively). To induce an experimental fasciitis, 50 μl complete Freund's adjuvant (CFA, Difco Lab., USA) were injected into the TLF in five animals (CFA group; cf. Hoheisel and Mense, 2015). Five naïve rats served as a non-injected control (control group). The experimenter was blinded to the experimental groups.

The CFA injection was made 3 mm lateral to the spinous processes L4 and L5. The cannula was inserted horizontally into the TLF under control of a dissecting microscope. The immunohistochemical evaluation was carried out 12 days after the CFA injection.

In histologic sections the inflamed fascia showed marked leukocyte infiltrations that were largely restricted to the fascia (middle and inner layer). Only minor infiltrations were seen in the multifidus (MF) muscle underlying the fascia.

Nociceptive fibers were visualized with antibodies to calcitonin gene-related peptide (CGRP) and to substance P (SP; Danielson et al., 2006). SP-containing fibers are assumed to be nociceptive (Lawson et al., 1997). The same applies to many of the CGRP-positive fibers (Levine et al., 1993). Moreover, the nociceptive nature of free nerve endings in the fascia was tested with antibodies to the transient receptor potential receptor subtype V1 (TRPV1), one of the main receptor molecules in the membrane of nociceptors (Caterina et al., 1997).

Visualization of calcitonin gene-related peptide (CGRP)
Primary antiserum: rabbit anti-CGRP (Bachem), 1:4000 in PBS, 48 h. Secondary antiserum: biotinylated anti-rabbit IgG (Vector), 1:200, 60 min.

Visualization of substance P (SP)
Primary antiserum: rabbit anti-SP (Chemicon) 1:1000 in PBS, 24 h. Secondary antiserum: biotinylated anti-rabbit IgG (Vector) 1:200, 60 min.

Visualization of transient receptor potential vanilloid 1 (TRPV1)
Primary antiserum: rabbit anti- TRPV1 (Alomone Labs) 1:500 in PBS, 24 h. Secondary antiserum: Cy3-anti-rabbit IgG (Dianova GmbH) 1:500, 60 min.

The histologic staining techniques visualized fibers of passage and nerve endings alike. All nerve endings had the appearance of free nerve endings. A free nerve ending consists of several unmyelinated terminal axons that exhibit axonal expansions (so-called varicosities; Stacey, 1969). The decisive criterion for a free nerve ending was the presence of more than 3 axonal expansions.

Twelve days after induction of the inflammation, the animals were euthanized with an overdose of thiopental sodium i.p. (Trapanal®, Altana Pharma, Germany), and transcardially perfused with a fixative. A piece of TLF containing the site of the CFA injection together with the surrounding tissue was removed close to the spinous processes and snap frozen. Serial cryostat cross sections were made at a thickness of 40 μm and processed for immunohistochemistry.

The quantitative evaluation of immunopositive nerve fibers was carried out on sections in which the fibers were stained with the antibody-avidin-biotin complex method using 3,3-diaminobenzidine tetrahydrochloride as a chromogen. Only TRPV1-positive fibers were visualized with fluorescence staining. To quantify the innervation density, the length of the stained fibers in the tissue sections was determined using an imaging software (analySIS B, Soft imaging System, Olympus Company).

In the medial part of the fascia, three layers can be distinguished in the rat (outer layer underneath the subcutaneous tissue; middle layer with thick collagen fiber bundles orientated obliquely to the axis of the spine, and a thin inner layer of loose connective tissue between the TLF and the underlying MF (Tesarz et al., 2011)). In each layer, the length of the immunopositive fibers was measured, and for each layer the fiber length per 1000 μm^2 area was calculated. Comparisons between the groups were made