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INNERVATION CHANGES INDUCED BY INFLAMMATION OF THE RAT THORACOLUMBAR FASCIA

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Abstract—Recently, the fascia innervation has become an important issue, particularly the existence of nociceptive fibers. Fascia can be a source of pain in several disorders such as fasciitis and non-specific low back pain. However, nothing is known about possible changes of the fascia innervation under pathological circumstances. This question is important, because theoretically pain from the fascia cannot only be due to increased nociceptor discharges, but also to a denser innervation of the fascia by nociceptive endings. In this histological study, an inflammation was induced in the thoracolumbar fascia (TLF) of rats and the innervation by various fiber types compared between the inflamed and intact TLF. Although the TLF is generally considered to have proprioceptive functions, no corpuscular proprioceptors (Pacini and Ruffini corpuscles) were found. To obtain quantitative data, the length of fibers and free nerve endings were determined in the three layers of the rat TLF: inner layer (IL, adjacent to the multifidus muscle), middle layer (ML) and outer layer (OL). The main results were that the overall innervation density showed little change; however, there were significant changes in some of the layers. The innervation density was significantly decreased in the OL, but this change was partly compensated for by an increase in the IL. The density of substance P (SP)-positive – presumably nociceptive – fibers was significantly increased. In contrast, the postganglionic sympathetic fibers were significantly decreased. In conclusion, the inflamed TLF showed an increase of presumably nociceptive fibers, which may explain the pain from a pathologically altered fascia. The meaning of the decreased innervation by sympathetic fibers is obscure at present. The lack of proprioceptive corpuscular receptors within the TLF does not preclude its role as a proprioceptive structure, because some of the free nerve endings may function as proprioceptors.

Key words: fascia innervation, sympathetic fibers, inflammation, fasciitis, non-specific low back pain.

INTRODUCTION

The mechanical properties of fascia tissue have been and still are studied by many scientific groups (Macintosh et al., 1987; Vleeming et al., 1995; Benetazzo et al., 2011; Langevin et al., 2011; Corey et al., 2012; Schleip et al., 2012). Recently, the fascia innervation has become an important issue, particularly the existence of nociceptive fibers (Corey et al., 2011; Tesarz et al., 2011; Taguchi et al., 2013). Fascia can be a source of pain in fasciitis, non-specific low back pain, and also in cases of adhesions between the fascia and adjacent tissues (muscle, nerve; Rowe et al., 2013). Experiments on human volunteers showed that noxious stimulation of fascia tissue – including the thoracolumbar fascia (TLF) – evokes pain (Gibson et al., 2009; Deising et al., 2012; Schilder et al., 2014). There is also experimental evidence showing that injections of algesic agents into the fascia are more painful than the same injections into the skin or muscle (Gibson et al., 2009; Deising et al., 2012; Schilder et al., 2014).

An anatomical structure functions as a pain source only if it is equipped with nociceptors. Recently, several publications have described a dense innervation of the TLF including putative nociceptors in rats and humans (Corey et al., 2011; Tesarz et al., 2011). Also other fasciae are known to possess nociceptors (Taguchi et al., 2013). The nociceptive nature of the nerve endings was mainly identified with immunohistochemical techniques (e.g. immunoreactivity (IR) for substance P (SP) and calcitonin gene-related peptide (CGRP; Corey et al., 2011; Tesarz et al., 2011), but also in electrophysiological experiments (Taguchi et al., 2013).

In this article, the term fascia is used in the sense of a thickening of the epimysium, i.e. as a layer of dense connective tissue covering a muscle. The studied structure was the posterior lamina of the TLF, and the covered muscles are the genuine back muscles (erector spinae muscle). Additionally, the posterior lamina serves as an aponeurosis for the internal oblique, the latissimus

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Abbreviations: CGRP, calcitonin gene-related peptide; CFA, Complete Freund's Adjuvant; IL, inner layer; ir, immunoreactive; IR, immunoreactivity; ML, middle layer; OL, outer layer; PBS, phosphate-buffered saline; PGP 9.5, protein gene product 9.5; SP, substance P; TH, tyrosine hydroxylase; TLF, thoracolumbar fascia.

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dorsi, and (indirectly) for the transverse abdominal muscles (Gray's Anatomy et al., 2005). Other groups use the term fascia in a more general way including e.g. perimysium and endomysium (Langevin and Huijing, 2009).

It is well known that fascia tissue is plastic (Langevin and Sherman, 2007) and adapts to changing requirements, for instance mechanical loads. Repeated mechanical strain has been found to lead to microlesions in connective tissue that are repaired by a sterile inflammatory process (Solomonow, 2012). However, nothing is known about another aspect of fascia plasticity, namely possible changes in the innervation due to inflammation. Such changes may occur in clinical cases of pain where fascia tissue is involved.

In the present investigation, changes in the TLF innervation were studied in a rat model of fasciitis. As in a previous article on dorsal horn neurons (Hoheisel and Mense, 2015), the fasciitis was mimicked by an experimental inflammation induced by Freund's complete adjuvant. Inflammatory pain from fascia can be not only due to higher discharges in fascia nociceptors but also to an increase in the innervation by nociceptive endings. To our knowledge, the latter possibility has never been studied. Therefore, the aim of the present study was to obtain first data on changes in the innervation density of an inflamed fascia. For the determination of the overall innervation density, protein gene product 9.5 (PGP 9.5)-IR was used as a universal marker for all nervous structures (Lundberg et al., 1988). Tyrosine hydroxylase (TH-)-IR served as a marker for postganglionic sympathetic nerve fibers (Burgi et al., 2011), CGRP-IR and SP-IR as markers for sensory peptidergic nerve fibers (Danielson et al., 2006).

EXPERIMENTAL PROCEDURES

The experiments were performed on 10 adult male Sprague–Dawley rats (350–480 g). The experimental procedure was approved by the local ethics authority responsible for animal experimentation. The experiments were carried out in accordance with the German law on the protection of animals and with the ethical proposals of the International Association for the Study of Pain (Zimmermann, 1983).

The rats were divided into two groups:

1. Five animals received an intrafascial injection of Complete Freund's Adjuvant (CFA group).
2. Five naïve rats served as a control (control group).

The animals were housed in groups of 2–3 animals in standard plastic cages and maintained on a 12-h light/dark cycle. The experimenter was blinded to the experimental groups.

Intrafascial injection of CFA

To induce a chronic inflammation, 50-μl CFA (Difco Lab., USA) was injected into the TLF. In deeply anesthetized animals (Ketamine 100 mg/kg i.p. and Xylazine 7.5 mg/kg i.p.; Essex Pharma, Germany and Alverat, Germany, respectively) a small longitudinal skin incision (1–1.5 cm) was made about 2 cm caudal to the projected injection site. Because of the loose structure of the subcutaneous tissue in rats, the incised skin area could be pushed about 2 cm cranially to make the intrafascial injection at vertebral level L4–L5. Therefore, the skin incision did not overly the injection site, and the healing of the skin wound did not influence the course of the fascia inflammation. The intrafascial injection was made 3 mm lateral to the spinous processes L4 and L5 using a 27-gauge cannula. The cannula was inserted horizontally in the longitudinal direction (4–5 mm, Fig. 1A) into the TLF under control of a dissecting microscope. After injection the cannula was kept in place for 1 min. The immunohistochemical evaluation was carried out 12 days after the CFA injection.

In histological sections the inflamed fascia showed marked leukocyte infiltrations that were largely restricted to the fascia (middle (ML) and inner layer (IL), see 2.3). Only minor infiltrations were seen in the multifidus (MF) muscle underlying the fascia (Fig. 1C).

Immunohistochemistry

The histological staining techniques visualized fibers of passage and nerve endings alike. The nerve endings had the appearance of free nerve endings. According to the data of Stacey (1969) for muscle tissue, a free nerve ending consists of several unmyelinated terminal axons that are relatively straight and often course parallel to each other. For reasons of brevity, fibers of passage and terminal axons together were designated ''fibers'', except in places where the terminal axons were addressed specifically. The term terminal axon is preferred over free nerve ending because in our material it was sometimes hard to tell if a structure really formed the final ending or was a piece of the terminal axon. The decisive criterion for a terminal axon was the presence of several (more than 3) axonal expansions, so-called axonal varicosities.

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 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) followed by PBS containing 10% sucrose. The TLF at vertebral level L4–L5 (containing the injection site) together with the surrounding tissue was removed close to the spinous processes, passed through PBS containing 30% sucrose for cryoprotection and snap frozen. Serial cryostat cross sections were made at a thickness of 40 μm and processed for the immunohistochemistry of intra-axonal substances as follows:

PGP 9.5: Primary antiserum: rabbit anti-PGP 9.5 (Biotrend), dilution 1:1000 in PBS, incubation for 24 h. Secondary antiserum: biotinylated anti-rabbit IgG (Vector), 1:200, 60 min.

TH: Primary antiserum: sheep anti-TH (Chemicon), 1:200 in PBS, incubation for 24 h. Secondary antiserum: biotinylated anti-sheep IgG (Vector), 1:200, 60 min.