Neuroscience 194 (2011) 302–308

SENSORY INNERVATION OF THE THORACOLUMBAR FASCIA IN RATS AND HUMANS

J. TESARZ,[a] U. HOHEISEL,[b]* B. WIEDENHÖFER[c] AND S. MENSE[b]

[a]*Department of General Internal and Psychosomatic Medicine, Heidelberg University, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany*

[b]*Department of Neurophysiology, Heidelberg University, Medical Faculty Mannheim, CBTM, Ludolf-Krehl-Str. 13-17, D-68167 Mannheim, Germany*

[c]*Department für Orthopädie, Unfallchirurgie und Paraplegiologie, Orthopädische Universitätsklinik Heidelberg, Schlierbacher Landstraβe 200a, D-69118 Heidelberg, Germany*

Abstract—The available data on the innervation of the thoracolumbar fascia (TLF) are inconsistent and partly contradictory. Therefore, the role of the fascia as a potential source of pain in the low back is difficult to assess. In the present study, a quantitative evaluation of calcitonin gene-related peptide (CGRP) and substance P (SP)-containing free nerve endings was performed in the rat TLF. A preliminary non-quantitative evaluation was also performed in specimens of the human TLF. The data show that the TLF is a densely innervated tissue with marked differences in the distribution of the nerve endings over the fascial layers. In the rat, we distinguished three layers: (1) Outer layer (transversely oriented collagen fibers adjacent to the subcutaneous tissue), (2) middle layer (massive collagen fiber bundles oriented obliquely to the animal's long axis), and (3) inner layer (loose connective tissue covering the paraspinal muscles). The subcutaneous tissue and the outer layer showed a particularly dense innervation with sensory fibers. SP-positive free nerve endings—which are assumed to be nociceptive—were exclusively found in these layers. Because of its dense sensory innervation, including presumably nociceptive fibers, the TLF may play an important role in low back pain.

Key words: thoracolumbar fascia, low back pain, immunohistochemistry, sensory innervation, nociception, peptidergic free nerve endings.

The thoracolumbar fascia (TLF) is the most extensive aponeurosis in both humans and rats. It has been suggested that the TLF plays a role in low back pain (Yahia et al., 1992; Schleip et al., 2007). Furthermore, the TLF is a key structure in the mechanics of the lumbar spine and entire back area (Vleeming et al., 1995; Vleeming and Stoeckart, 2007). However, although low back pain has become a major problem in our health care system and often the origin of low back pain cannot be determined (so called non-specific low back pain), very few data are available regarding the TLF as a potential source of pain. In the past, the interest was mainly focused on spinal structures (vertebrae, intervertebral discs, annulus fibrosus, facet joints, and spinal ligaments). The TLF was largely ignored as a potential source of low back pain, even though soft tissues of the low back are assumed to play a key role in low back pain of unknown origin. Another fascia, the tibial anterior fascia of the lower leg, has been shown to be important for the pain of delayed onset muscle soreness (Gibson et al., 2009). Recently, an electrophysiological study showed that lumbar dorsal horn neurons receive nociceptive input from the TLF (Hoheisel et al., in press), indicating that the TLF is a possible source of pain. However, to date, little is known about the distribution and density of sensory nerve fibers in the TLF. This knowledge is a prerequisite for assessing the role of the TLF in low back pain. Another problem is that the few histological studies performed so far are partly contradictory: Bednar and colleagues (1995) did not find any sensory receptors in TLF specimens and stated that the TLF of low back pain patients "is deficiently innervated," whereas the results of a histological study by Yahia and colleagues (1992) did show that the TLF is innervated. Benetazzo and colleagues (in press) found that only the superficial layer of the human TLF is innervated, and Corey and colleagues (in press) showed a sensory innervation of the collagen matrix in the low back of the rat. Various staining techniques have been used to study the innervation of ligamentous structures in the low back. Hirsch and colleagues (1963) investigated the supraspinous and intraspinous ligaments with Methylene Blue, an intravital stain. Yahia and colleagues (1992) impregnated these ligaments with gold chloride and reported the presence of mechanoreceptors in humans. Immunostaining for the S-100 protein has demonstrated free nerve endings in the same ligaments (Rhalmi et al., 1993). A comprehensive visualization of all nerve fibers can be obtained with protein gene product 9.5 (PGP 9.5) immunohistochemistry. PGP 9.5 is a universal marker for all neural elements (Lundberg et al., 1988; Danielson et al., 2006). Sensory free nerve endings can be identified with antibodies to neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P (SP; Danielson et al., 2006), with SP-positive fibers being assumed to supply nociceptors (Lawson et al., 1997; Tsukagoshi et al., 2002; Danielson et al., 2006). A disadvantage of this staining technique is that it does not visualize non-peptidergic af-

*Corresponding author. Tel: +49-621-383-9637; fax: +49-621-383-9921.
E-mail address: ulrich.hoheisel@medma.uni-heidelberg.de (U. Hoheisel).

Abbreviations: CGRP, calcitonin gene-related peptide; 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; SCT, subcutaneous tissue; SP, substance P; TH, tyrosine hydroxylase; TLF, thoracolumbar fascia.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.07.066

ferent fibers. Nevertheless, neuropeptide immunohistochemistry gives a good overview over a large fraction of sensory fibers (Ohtori et al., 2007).

The aim of the present study was to obtain data on the density and distribution of nerve fibers in the rat TLF. Therefore, PGP 9.5-immunoreactivity (IR) was used as a universal marker for all nerve fibers, tyrosine hydroxylase (TH)-IR as a marker for sympathetic nerve fibers, and CGRP-IR/SP-IR as markers for sensory peptidergic nerve fibers. Moreover, a quantitative evaluation of CGRP-immunoreactive (ir) and SP-ir nerve fibers and sensory endings was performed. Such an investigation is important for two reasons: (1) The density of neuropeptide-containing fibers (such as SP and CGRP) has been shown to vary from one tissue to the other (McMahon et al., 1984, 1989; Brismee et al., 2009; Szadek et al., 2010). Therefore, these data cannot be simply transferred to the TLF. (2) Information on the density and distribution of the sensory fibers in the various layers of the TLF is essential for a better understanding of the role the TLF may play in low back pain. For reasons of comparison, a preliminary non-quantitative evaluation of nerve endings was also performed in specimens of the human TLF.

EXPERIMENTAL PROCEDURES

The experiments were performed on eight adult male Sprague–Dawley rats (body weight 280–470 g). In five animals immunohistochemistry was performed on transversal sections of the TLF. In three further animals whole mount preparations of the TLF were studied. All experiments were carried out in accordance with the German law on the protection of animals. The experimental design was approved by the local ethics authority responsible for animal experimentation.

Immunohistochemistry

The animals were killed 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 superficial lamina of the left thoracolumbar fascia including subcutaneous tissue (SCT) and the underlying multifidus muscle were removed at the level of the lumbar vertebrae L4, L5, and L6 close to the spinous processes. The tissue was passed through PBS containing 30% sucrose for cryoprotection and snap frozen. Serial cryostat cross sections were made at a thickness of 40 μm. The sections were processed for immunohistochemistry as follows:

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

TH-IR: Primary antiserum: sheep anti-TH (Chemicon International, USA), dilution 1:200 in PBS, incubation for 24 h at room temperature. Secondary antiserum: biotinylated anti-sheep IgG (Vector Lab., USA), 1:200, 60 min at room temperature.

CGRP-IR: Primary antiserum: rabbit anti-CGRP (Peninsula Lab., Bachem, USA), 1:4000 in PBS, 48 h at room temperature. Secondary antiserum: biotinylated anti-rabbit IgG (Vector Lab., USA), 1:200, 60 min at room temperature.

SP-IR: Primary antiserum: rabbit anti-SP (Chemicon International, USA), 1:1000 in PBS, 24 h at room temperature. Secondary antiserum: biotinylated anti-rabbit IgG (Vector Lab., USA), 1:200, 60 min at room temperature.

All immunoreactive fibers were visualized using the avidin–biotin complex method and 3,3-diaminobenzidine tetrahydrochloride as the chromogen. The quantitative evaluation of SP/CGRP fibers and free nerve endings required a stable, non-fading staining. Therefore, 3,3-diaminobenzidine tetrahydrochloride staining was performed over fluorescent techniques.

To exclude unspecific staining, control experiments were performed by omission of the primary or secondary antibody. No staining could be seen under these conditions.

Quantitative evaluation of immunoreactive nerve fibers

Every tenth section—approximately 35 sections per animal—was chosen for immunohistological processing. In each of the selected sections, immunoreactive nerve fibers were identified at 400 times magnification under the light microscope and their length reconstructed using an imaging software (analySIS B, Soft imaging System, Olympus Company, Germany). Only neural elements outside of nerve fiber bundles were evaluated.

For scanning specifically free nerve endings, neural elements were subdivided into fibers of passage and free nerve endings. Free nerve endings were distinguished from fibers of passage in that they showed a chain of at least three varicosities. These axonal expansions are assumed to be the site where stimuli act on sensory endings.

In each section, an area of 5 mm in length starting at the spinous process was analyzed. The length of all fibers and nerve endings was measured within that area and the mean fiber length calculated. The quantitative evaluation of CGRP- and SP-ir was performed separately for each layer of the TLF (see Fig. 1). For PGP 9.5-IR, the outer layer of the fascia was evaluated together with the SCT because of the difficulties to determine the exact border between the two structures and the finding that fibers often crossed the border.

Whole mount preparations

Whole mount preparations of the TLF were removed close to the spinous processes L4, L5, and L6 (10×10 mm in length, thickness approximately 0.5–1 mm). They were fixed for 2 h in 4% paraformaldehyde in 0.1 M PBS and rinsed in PBS for 24 h. Immunohistochemistry for PGP 9.5 and CGRP were performed on free-floating preparations as described for immunohistochemistry of tissue sections.

Immunohistochemistry of the human TLF

After approval by the ethical committee at Heidelberg University, specimens of the TLF roughly 10×10 mm in size were removed during surgery of three patients (two males, one female) undergoing an intervertebral disk surgery. The specimens were removed at the L4-L5 level, stored in 0.1 M PBS, and fixed in 4% paraformaldehyde for 2 h. Cryostat cross sections (thickness 40 μm) were processed for immunohistochemistry as described for the rat TLF.

RESULTS

Composition of the rat TLF

The rat TLF had three layers that were well visible medially close to the spinous processes and less distinct laterally (Fig. 1a):

(1) The thin outer layer (OL) adjacent to the SCT and consisting of densely packed collagen fibers oriented in the transversal plane. (2) The middle layer (ML) composed of massive bundles of densely packed collagen fibers oriented obliquely to the long axis of the

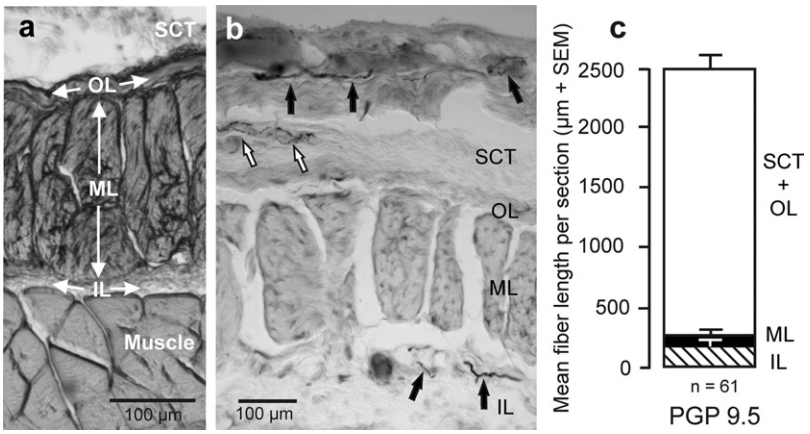

Fig. 1. Structure of the rat thoracolumbar fascia (TLF) close to the spinous processes L4/L5. (a) Transversal section showing the three layers of the TLF (hematoxylin and eosin staining): OL, outer layer with transversely oriented collagen fibers; ML, middle layer composed of collagen fiber bundles oriented diagonally to the long axis of the body; IL, inner layer of loose connective tissue covering the multifidus muscle (muscle). SCT, subcutaneous tissue. (b) PGP 9.5-ir nerve fibers in the layers of the TLF. Black arrows, fibers on passage; open arrows, nerve endings. (c) Mean fiber length of PGP 9.5-ir fibers in the TLF. The great majority of all fibers were located in the outer layer (OL) of the fascia and in the subcutaneous tissue (SCT). White part of the bar: subcutaneous tissue plus outer layer of the TLF; black: middle layer; hatched: inner layer. n, number of sections evaluated.

rat. This layer was the thickest one. (3) The thin inner layer (IL) of loose connective tissue separating the middle layer from the underlying paraspinal muscles. The IL consisted of irregularly oriented collagen fibers with relatively few elastic fibers in between.

Innervation of the rat TLF

Fibers immunoreactive to PGP 9.5. Fibers immunoreactive for PGP 9.5 were mainly found in the OL plus SCT and the IL of the fascia (Fig. 1b, c). The neuronal structures included fibers of passage and nerve endings characterized by chains of varicosities close to the nerve terminal. In whole mount preparations, the TLF had the appearance of a densely innervated tissue exhibiting an extensive net of nerve fibers (Fig. 2a). The dense innervation appears to be a general feature of the TLF, at least we did not find any difference in innervation density between the lumbar levels L4, L5, or L6. In the SCT close to the OL of the fascia, often fibers were found that accompanied blood vessels (Fig. 2b). Nerve endings were present in all layers; however, no corpuscular receptors such as Pacinian and paciniform corpuscles or Golgi tendon organs were found. In one specimen of the human TLF (see below) there were cross sections of a corpuscular receptor, which may have been a Ruffini ending, but no clear identification was possible, because the adjacent tissue sections were not available.

Fibers immunoreactive to tyrosine hydroxylase (TH). The majority of TH-ir nerve fibers were found in the SCT and the OL of the fascia. Most fibers appeared to accompany blood vessels (Fig. 2c). TH-ir fibers—at a lower frequency—were also found in the IL of the fascia but not in the ML. Interestingly, the TH-ir fibers in IL often had no association with blood vessels but terminated freely in the connective tissue (Fig. 2d).

Peptidergic sensory nerve endings. CGRP- as well as SP-ir fibers were densely stained thin fibers of passage or looked like strings of beads with numerous varicosities (Fig. 3a, c). Of these endings, not a single one exhibited branches. Generally, the varicosities of SP-ir nerve endings were smaller than those of CGRP-ir endings (compare Fig. 3a, b). CGRP-ir nerve fibers were present in all layers of the TLF, but the majority was located in the OL plus SCT (Fig. 4a). SP-ir nerve fibers were found mainly in the OL and the SCT with some isolated fibers in the IL (Fig. 4b). No SP-ir structures were present in the ML of the fascia. CGRP- as well as SP-ir nerve fibers were also found in lateral regions of the TLF where muscle fibers are attached to the TLF (Fig. 3d).

By scanning specifically for free nerve endings, it became clear that SP-containing free nerve endings existed exclusively in the SCT and OL. Fig. 4c, d shows the proportion of immunoreactive endings exhibiting a chain of at least three varicosities (Fig. 3b, c) and therefore fulfilled the criteria for a free nerve ending. SP-ir endings were found in both the dense collagenous tissue of the OL and in the loose connective tissue of the SCT (Fig. 4d). In the IL of the TLF, only SP-ir fibers of passage were found. CGRP-ir free nerve endings were present in the SCT in the OL, and a few also in the IL (Fig. 4c). The ML was free of CGRP-ir free nerve endings, only some isolated CGRP-ir fibers of passage were found.

Quantitative evaluation

The quantitative evaluation of the distribution of CGRP- and SP-ir nerve fibers showed that the great majority of these fibers were situated in the SCT and the OL of the TLF (Fig. 4a, b) and thus exhibited a distribution pattern similar to that of all PGP 9.5 fibers (Fig. 1d). CGRP- and SP-ir fibers represented only a small fraction of the total innervation visualized with PGP 9.5 (compare Figs. 1d and 4a, b). In the IL, only a small fraction of both fiber types were found. In contrast to CGRP fibers, SP-ir fibers were not present in the ML (Fig. 4a, b). SP-containing free nerve

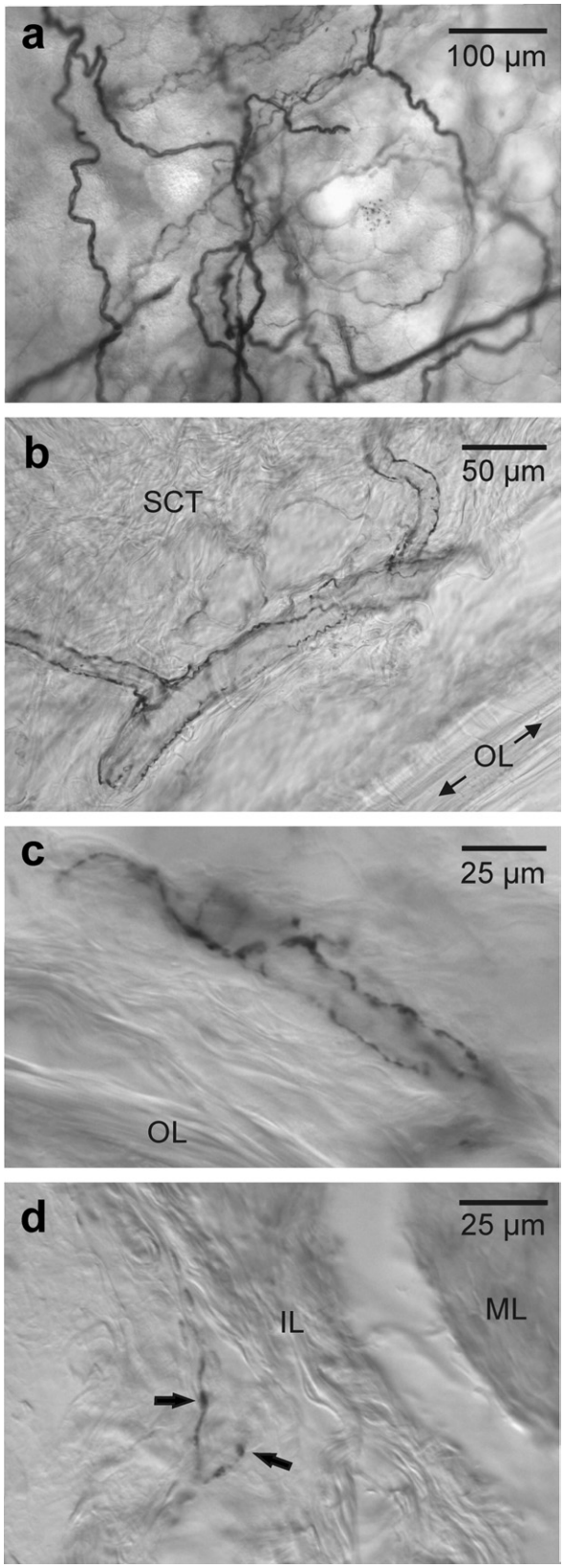

Fig. 2. PGP 9.5- and TH-ir nerve fibers in the rat thoracolumbar fascia. (a) A dense network of PGP 9.5-ir fibers in a whole mount preparation of the TLF close to the L4/L5 spinous processes (dorsal view). (b) PGP 9.5-ir fibers in the subcutaneous tissue closely associated with blood vessels (transversal section). (c) TH-ir nerve fiber in the subcutaneous tissue surrounding a blood vessel. (d) TH-ir nerve fibers (arrows) not associated with blood vessels in the inner layer (IL) of the fascia (arrows mark varicosities). SCT, subcutaneous tissue; OL, outer layer of the TLF; ML, middle layer; IL, inner layer.

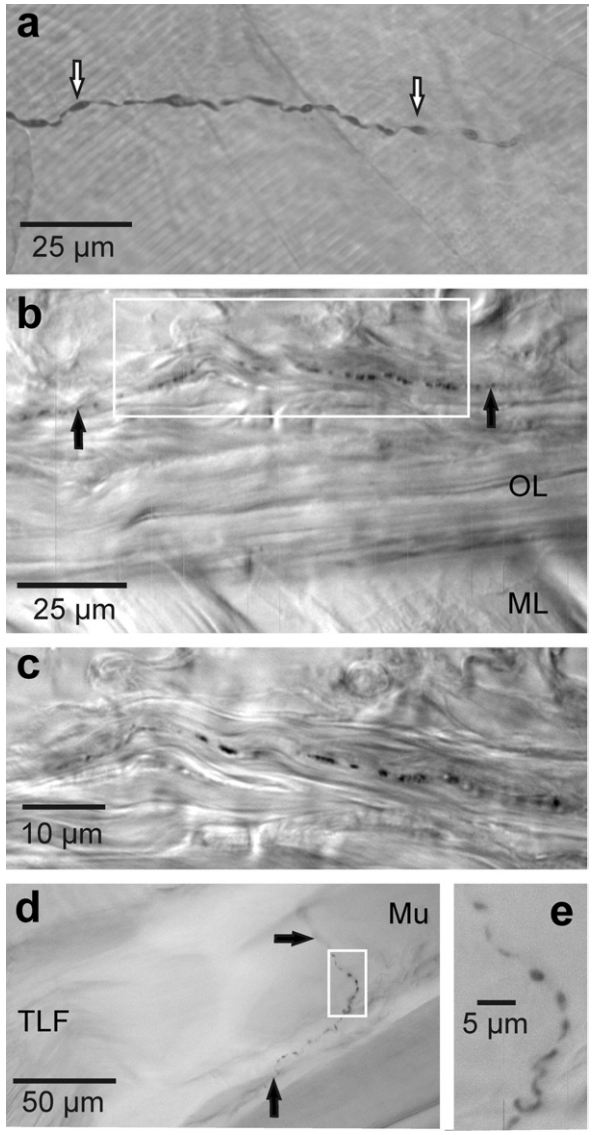

Fig. 3. Peptidergic sensory fibers and nerve endings in the rat thoracolumbar fascia. (a) Whole mount preparation showing a CGRP-ir receptor with varicosities in the deep layer of the fascia (open arrows). The cross striation of the underlying multifidus muscle is faintly visible. (b) SP-containing free nerve ending/terminal axon with a chain of varicosities close to the outer layer (OL; ML, middle layer). (c) Area boxed in (b) at a higher magnification. (d) SP-ir free nerve ending in a region where cells of a low back muscle (Mu) made contact with collagen fibers of the fascia (TLF). (e) Area boxed in (d) at a higher magnification.

endings—which are assumed to be nociceptive—were located only in the SCT and the OL of the fascia (Fig. 4b), whereas CGRP-containing endings were also found in the IL.

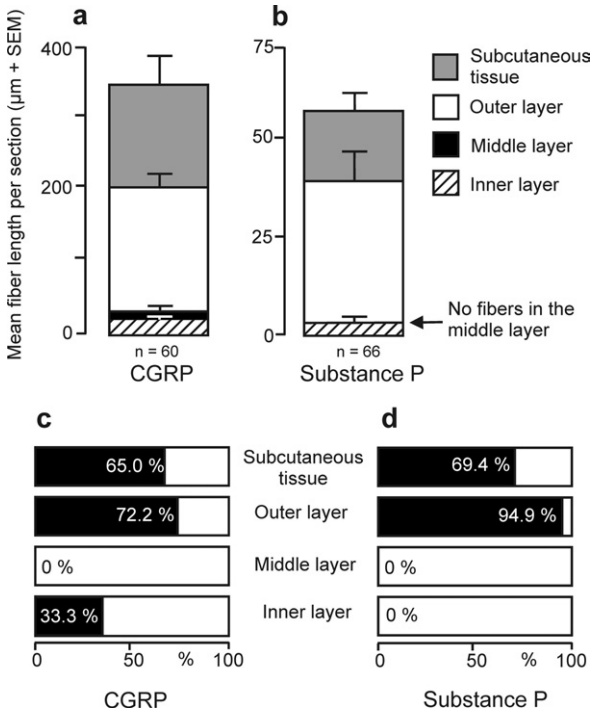

Fig. 4. Distribution of CGRP and Substance P (SP)-immunoreactive nerve fibers in the TLF. (a) Mean fiber length of CGRP-ir nerve fibers. (b) Mean fiber length of SP-ir nerve fibers. Almost all fibers were found in the outer layer of the fascia and the subcutaneous tissue. The middle layer was free of SP-positive fibers. Gray part of the bars: subcutaneous tissue; white: outer layer of the TLF; black: middle layer; hatched: inner layer. n=number of sections evaluated. (c, d) Distribution of CGRP- (c) and SP-containing receptive free nerve endings (d) expressed as percent of the total number of CGRP- or SP-containing fibers in each layer. For classification as receptive endings, the structures had to exhibit at least three varicosities. SP-containing free nerve endings were restricted to the outer layer of the thoracolumbar fascia and the subcutaneous connective tissue while CGRP-containing free nerve endings were also found in the inner layer of the thoracolumbar fascia.

Innervation of the human TLF

A preliminary non-quantitative evaluation of the human TLF indicates that the innervation is comparable with that of the rat TLF: (1) the innervation density of the human TLF visualized by PGP 9.5-IR (Fig. 5a) was likewise high and (2) the appearance of the free nerve endings was similar. A high number of fibers showed TH-IR (Fig. 5b) indicating that the human fascia has a rich innervation with sympathetic fibers. Again, peptidergic fibers represented only a small fraction of the total innervation. The stained neuronal structures included nerve endings characterized by chains of varicosities (Fig. 5c–e). The majority of peptidergic nerve endings were located in the SCT and in structures comparable with the OL and IL of the rat TLF.

DISCUSSION

Studies of the nerve supply to the human lumbar spine have been conducted by researchers for more than one century (Luschka, 1850; Yahia et al., 1992), but in these studies the TLF was not mentioned. To date, the knowledge about the innervation of the TLF is still scarce, and the existing data are partly contradictory. One study reported that no pain can be perceived from the TLF at all (Kuslich et al., 1991). However, from other anatomical (Budgell et al., 1997) and electrophysiological studies (Bove and Light, 1995), it is known that the thoracolumbar body region is innervated by the dorsal rami of spinal nerves. These rami have been shown to contain unmyelinated and myelinated afferents with conduction velocities

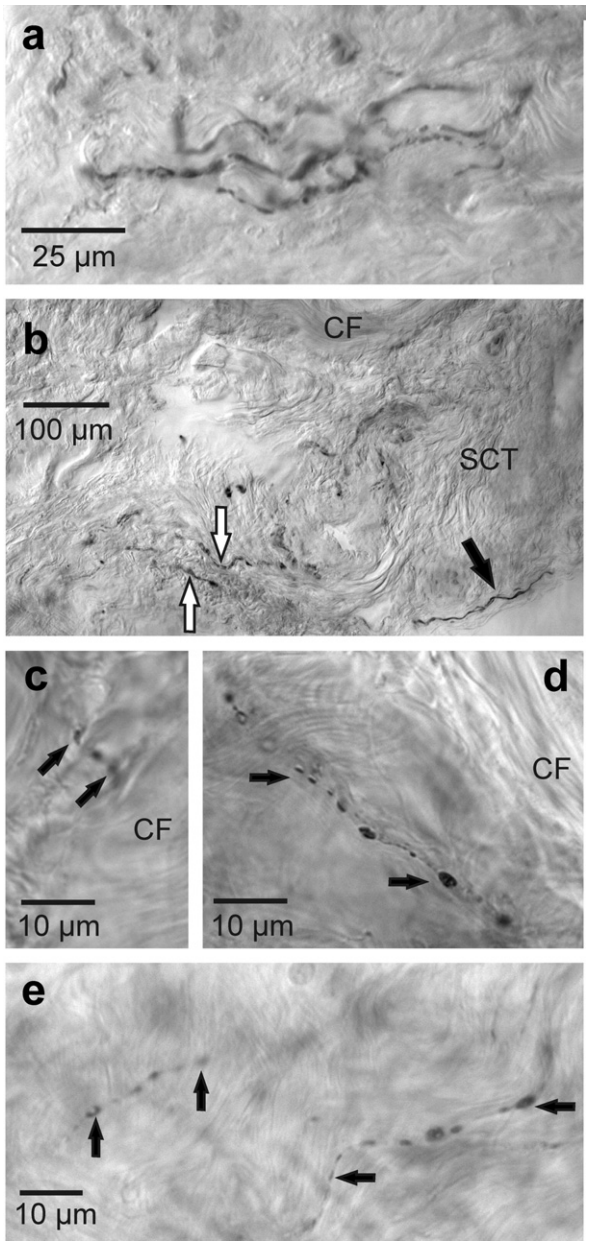

Fig. 5. Nerve fibers and endings in the human thoracolumbar fascia. (a) PGP 9.5-ir nerve fibers between collagen fibers and low back muscle corresponding to the inner layer of the rat. (b) TH-ir nerve fibers in the subcutaneous tissue (SCT) close to collagen fibers (CF) of the TLF. Black arrow, fiber on passage; open arrows, nerve endings. (c) SP-ir free nerve terminal in the subcutaneous tissue close to collagen fibers (CF). (d, e) CGRP-ir free nerve endings with varicosities (arrows) between collagen fibers (CF) and low back muscle (not visible).

in the range of C, A-delta, and A-beta fibers and that the tissues supplied by these fibers are sensitive to mechanical and noxious chemical stimulation (Bove and Light, 1995; Budgell et al., 1997). A recent immunohistochemical investigation showed both free and encapsulated nerve endings in the human iliolumbar ligament (Kiter et al., 2010). Studies using techniques other than immunohistochemistry suggested the presence of fine nerve fibers and complex unencapsulated endings in the supraspinous and intraspinous ligaments (Hirsch et al., 1963) but did not address fibers in the TLF. Yahia and colleagues (1992) reported the presence of encapsulated and free nerve endings in the thoracolumbar fascia suggesting for the first time a sensory role of the TLF in lumbar pain mechanisms. In the rat, Corey et al. (in press) recently described a dense innervation of the non-specialized connective low back tissue with PGP 9.5-ir fibers. They also confirmed the presence of CGRP-containing sensory nerve fiber terminations in the collagen matrix of the low back. However, a more detailed quantitative analysis of sensory nerve endings in distinct layers of the TLF was not undertaken.

Likewise, detailed anatomical studies concerning the macroscopic and microscopic characteristics of the rat TLF are still lacking. First anatomical analyses of the rat back musculature showed similarities with the multilayered structure of the human TLF (Brink and Pfaff, 1980) and associated muscles, but a detailed analysis in rats has not been performed so far.

Our study demonstrates that the rat TLF and the SCT overlying the fascia are densely innervated tissues, and therefore both the TLF and SCT, may play a role in low back pain. Most nerve fibers are located in the OL of the TLF and in the SCT, whereas in the ML nerve fibers are rare. Actually, no SP-ir fibers were found in this layer. Teleologically, the lack of fibers in the ML, particularly those containing SP, makes sense because each movement of the body causes shearing forces between the collagen fiber bundles, which might excite nociceptors. However, it has to be kept in mind that the peptidergic (CGRP- and SP-ir) fibers are not the only sensory fibers, because there are also non-peptidergic/lectin positive unmyelinated and thin-myelinated sensory fibers. In afferents innervating lumbar vertebral bodies, these non-peptidergic/lectin positive units represent 4% of all sensory neurons in the spinal ganglia, whereas 32% are peptidergic (CGRP-ir; Ohtori et al., 2007).

The relative abundance of the CGRP- and SP-ir nerve fibers in the TLF is similar to that of neuropeptide-ir fibers in skeletal muscle (Reinert et al., 1998). In the sections evaluated in the present study the overall number of nerve fibers appeared to be higher in the fascia than in the underlying muscle. The total number of PGP 9.5-ir fibers was five to six times higher than that of CGRP- and SP-ir fibers, indicating that only a small fraction of the TLF innervation is sensory. It has to be noted that a comparison of data from skeletal muscle and TLF is difficult, because the evaluation of both data sets was not identical. Furthermore, a dense innervation is only one prerequisite for a structure to play a role in nociception, central connections (e.g. convergence) are likewise important. On the other hand, results from the human anterior tibial muscle show that the surrounding fascia is more pain sensitive than the muscle itself (Gibson et al., 2009).

The preliminary non-quantitative data obtained in specimens of the human TLF show a first indication that the innervation of the human TLF is comparable with that of the rat TLF. As in the rat, the human TLF was densely innervated. Many fibers—especially in the SCT—expressed TH, an enzyme characteristic for postganglionic sympathetic fibers. This finding may explain why patients with low back pain report increased intensities of pain when they are under psychological stress (Chou and Shekelle, 2010). The location of most TH-ir fibers around blood vessels suggests that at least parts of them are vasomotor fibers. When activated, these fibers may cause ischemic pain.

The sensory TLF fibers appear to fulfill an important role as an input source for dorsal horn neurons. This interpretation is underpinned by recent studies of our group showing that many nociceptive dorsal horn neurons in the lumbar spinal segments receive a strong input from the lumbar TLF (Taguchi et al., 2008; Hoheisel et al., in press).

CONCLUSION

We show that the TLF possesses a dense network of nerve fibers including nociceptive ones. Therefore, the TLF may well be an important source for low back pain. The finding that most CGRP- and SP-ir (sensory) fibers are located in the outer layer of the fascia and the subcutaneous tissue may explain why some manual therapies that are directed at the fascia and the subcutaneous tissue (e.g. fascial release) are often painful.

Acknowledgments—The authors wish to thank M. Szymbara and B. Quenzer for excellent technical assistance. This work was supported by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung: LOGIN consortium), the Schweizer Ärztegesellschaft für Manuelle Medizin (SAMM), and Ärzteseminar für Manuelle Medizin (MWE).

REFERENCES

Bednar DA, Orr FW, Simon GT (1995) Observations on the pathomorphology of the thoracolumbar fascia in chronic mechanical back pain. A microscopic study. Spine 20:1161–1164.

Benetazzo L, Bizzego A, De Caro R, Frigo G, Guidolin D, Stecco C (in press) 3D reconstruction of the crural and thoracolumbar fasciae. Surg Radiol Anat, doi: 10.1007/s00276-010-0757-7.

Bove GM, Light AR (1995) Unmyelinated nociceptors of rat paraspinal tissues. J Neurophysiol 73:1752–1762.

Brink EE, Pfaff DW (1980) Vertebral muscles of the back and tail of the albino rat (Rattus norvegicus albinus). Brain Behav Evol 17:1–47.

Brismee JM, Sizer P, Dedrick G, Sawyer BG, Smith MP (2009) Immunohistochemical and histological study of human uncovertebral joints: a preliminary investigation. Spine 34:257–1263.

Budgell B, Noda K, Sato A (1997) Innervation of posterior structures in the lumbar spine of the rat. J Manipulative Physiol Ther 20: 359–368.

Chou R, Shekelle P (2010) Will this patient develop persistent disabling low back pain? JAMA 303:1295–1302.

Corey SM, Vizzard MA, Badger GJ, Langevin HM (in press) Sensory innervation of the nonspecialized connective tissues in the low back of the rat. Cells Tissues Organs, doi: 10.1159/000323875.

Danielson P, Alfredson H, Forsgren S (2006) Distribution of general (PGP 9.5) and sensory (substance P/CGRP) innervations in the human patellar tendon. Knee Surg Sports Traumatol Arthrosc 14:125–132.

Gibson W, Arendt-Nielsen L, Taguchi T, Mizumura K, Graven-Nielsen T (2009) Increased pain from muscle fascia following eccentric exercise: animal and human findings. Exp Brain Res 194:299–308.

Hirsch C, Ingelmark BE, Miller M (1963) The anatomical basis for low back pain. Studies on the presence of sensory nerve endings in ligamentous, capsular and intervertebral disc structures in the human lumbar spine. Acta Orthop Scand 33:1–17.

Hoheisel U, Taguchi T, Treede RD, Mense S (in press) Nociceptive input from the rat thoracolumbar fascia to lumbar dorsal horn neurones. Eur J Pain, doi: 10.1016/j.ejpain.2011.01.007.

Kiter E, Karaboyun T, Tufan AC, Acar K (2010) Immunohistochemical demonstration of free nerve endings in iliolumbar ligament. Spine 35:E101–E104.

Kuslich SD, Ulstrom CL, Michael CJ (1991) The tissue origin of low back pain and sciatica: a report of pain response to tissue stimulation during operations on the lumbar spine using local anesthesia. Orthop Clin North Am 22:181–187.

Lawson SN, Crepps BA, Perl ER (1997) Relationship of substance P to afferent characteristics of dorsal root ganglion neurones in guinea-pig. J Physiol 505:177–191.

Lundberg LM, Alm P, Wharton J, Polak JM (1988) Protein gene product 9.5 (PGP 9.5). A new neuronal marker visualizing the whole uterine innervation and pregnancy-induced and developmental changes in the guinea pig. Histochemistry 90:9–17.

Luschka H (1850) Die Nerven des menschlichen Wirbelkanales. Laupp und Siebeck, Tübingen.

McMahon SB, Lewin GR, Anand P, Ghatei MA, Bloom SR (1989) Quantitative analysis of peptide levels and neurogenic extravasation following regeneration of afferents to appropriate and inappropriate targets. Neuroscience 33:67–73.

McMahon SB, Sykova E, Wall PD, Woolf CJ, Gibson SJ (1984) Neurogenic extravasation and substance P levels are low in muscle as compared to skin the rat hindlimb. Neurosci Lett 52:235–240.

Ohtori S, Inoue G, Koshi T, Ito T, Yamashita M, Yamauchi K, Suzuki M, Doya H, Moriya H, Takahashi Y, Takahashi K (2007) Characteristics of sensory dorsal root ganglia neurons innervating the lumbar vertebral body in rats. J Pain 8:483–488.

Reinert A, Kaske A, Mense S (1998) Inflammation-induced increase in the density of neuropeptide-immunoreactive nerve endings in rat skeletal muscle. Exp Brain Res 121:174–180.

Rhalmi S, Yahia LH, Newman N, Isler M (1993) Immunohistochemical study of nerves in lumbar spine ligaments. Spine 18:264–267.

Schleip R, Vleeming A, Lehmann-Horn F, Klingler W (2007) Letter to the Editor concerning "A hypothesis of chronic back pain: ligament subfailure injuries lead to muscle control dysfunction" (M. Panjabi). Eur Spine J 16:1733–1735.

Szadek KM, Hoogland PV, Zuurmond WW, De Lange JJ, Perez RS (2010) Possible nociceptive structures in the sacroiliac joint cartilage: an immunohistochemical study. Clin Anat 23:192–198.

Taguchi T, Hoheisel U, Mense S (2008) Dorsal horn neurons having input from low back structures in rats. Pain 138:119–129.

Tsukagoshi M, Funakoshi K, Goris RC, Kishida R (2002) Differential distribution of nerve fibers immunoreactive for substance P and calcitonin gene-related peptide in the superficial and deep muscle layers of the dorsum of the rat. Brain Res Bull 58:439–446.

Vleeming A, Pool-Goudzwaard AL, Stoeckart R, van Wingerden JP, Snijders CJ (1995) The posterior layer of the thoracolumbar fascia. Its function in load transfer from spine to legs. Spine 20:753–758.

Vleeming A, Stoeckart R (2007) The role of the pelvic girdle in coupling the spine and the legs: a clinical-anatomical perspective on pelvic stability. In: Movement, stability and lumbopelvic pain (Vleeming A, Mooney V, Stoeckart R, eds), pp 113–137. Edinburgh: Churchil Livingstone Elsevier.

Yahia L, Rhalmi S, Newman N, Isler M (1992) Sensory innervation of human thoracolumbar fascia. An immunohistochemical study. Acta Orthop Scand 63:195–207.

(Accepted 26 July 2011)
(Available online 2 August 2011)