LIMBURGS
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CENTRUM

Faculteit Geneeskunde
Departement Medische Basiswetenschappen

The Intrinsic Nerve Supply of the Human Lumbar Spine

A light- and electronmicroscopic and immunohistochemical study

Frank Vandenabeele

Proefschrift voorgedragen tot het bekomen
van de graad van
Doctor in de medische wetenschappen

promotoren : Prof. em. dr. W. Robberechts
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" I have assumed that no demonstration is required of how necessary the knowledge of human parts is for us who have enlisted under the banner of medicine, since the conscience of each and all will bear full testimony to the fact that in the cure of illness the knowledge of those parts lays rightful claim to first, second and third place; and this knowledge is to be sought primarly from the affected portion ... ."

Andreas Vesalius, Epitom (1543)*

* Excerpt from the introductory letter written by Vesalius in which he dedicates his Epitome to King Philip II of Spain (In *The Epitome of Andreas Vesalius*, translated by LR Lind (1949). M.I.T. Press; Cambridge, Mass.).

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Frank Vandenabeele

Abbreviations

ABC: arachnoid barrier cell
Ach: acetylcholine
AchE: acetylcholinesterase
ALL: anterior longitudinal ligament
ARC: arachnoid reticular cell
CGRP: calcitonin gene-related peptide
CPON: C-flanking peptide of neuropeptide Y
DBC: dural border cell
DBH: dopamine-beta-hydroxylase
EM: electron microscopy
FAGV: flattened agranular vesicles
HRP: horseradish peroxidase
5HT: 5-hydroxytryptamine
IR: immunoreactive
IVD: intervertebral disc
LAB: labelled avidin-biotin
LGV: large granular vesicles
LM: light microscopy
LOV: large opaque vesicles
LSAB: labelled streptavidin-biotin
NA: Nomina Anatomica
NF: neurofilament
NOR: noradrenaline
NPY: neuropeptide Y
PLL: posterior longitudinal ligament
SAGV: small agranular vesicles
SGV: small granular vesicles
SP: substance P
SVN: sinu-vertebral nerve
SYN: synaptophysin
TH: tyrosine hydroxylase
VIP: vasoactive intestinal peptide

CHAPTER 1

Introduction

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Introduction

Recurrent or chronic low back pain is a major problem in daily medical practice, but the diagnosis and structural source of the pain are often uncertain. The mechanism of spinal pain is complex (Haldeman, 1990). There is substantial clinical evidence for the occurence of facet, disc and nerve root pain (Cavanaugh et al. 1996, 1997). Although they may follow each other chronologically, the combination of disc pain and facet pain is uncommon (Schwarzer et al. 1994).

Physical signs are often aspecific and the radiologic imaging does not always correlate with the complaints nor with the physical signs. So the anatomical substrate of the low back pain often remains uncertain, despite careful clinical examination, invasive diagnostic procedures, as well as extended radiologic imaging.

It is generally accepted that pain is *an expression of a disturbance of neurological function*. Only structures innervated by nerve fibres which transmit the pain message, can be a potential source of pain. Pain generators are usually the noncorpuscular (unencapsulated) 'free' nerve endings of the pain fibres. These fibres belong to the smallest somatosensory neurons, and are either small myelinated fibres (group III or Aδ, 1 – 5 µm in diameter) or unmyelinated fibres (group IV or C, 0.5 – 2 µm in diameter).

The exact knowledge of the neuromorphology of the vertebral column and associated structures is essential for the understanding of the neurological mechanisms involved in low back pain. Blockade of the extrinsic nerve supply of spinal structures is not only a useful diagnostic tool in determing the source of the spinal pain. A temporary blockade or permanent interruption of the sensory pathway from a nociceptive structure, is also a possible symptomatic treatment, targetting the 'source' and not the 'cause' of spinal pain (Stolker & Vervest, 1994; Stolker et al. 1994; van Kleef, 1996).

The clinical importance of spinal tissue innervation has prompted interest in defining the microscopic anatomy of the lumbar spine and the distribution of the nociceptive neural structures in the lumbar spine. Milestones in the study of the nerve supply to the lumbar spine have been the descriptions of the sinu-vertebral nerve (N.A.: ramus meningeus nervi spinalis) by von Luschka (1850), Roofe (1940), and Pedersen et al. (1956). Pedersen et al. described in detail the anatomy of the lumbar dorsal rami and also the nerve supply of the lumbar zygapophyseal (apophyseal or facet) joints.

The studies of Wiberg (1949), Hirsch et al. (1963), and Jackson et al. (1966) are fundamental for the present knowledge of spinal tissue innervation. The numerous other contributions to the sinuvertebral nerves and the lumbar dorsal rami are summarised in excellent reviews of the innervation of the lumbar spine (Edgar & Nundy, 1966; Lamb, 1979; Bogduk, 1983). The neuroanatomic work of Bogduk (1983) renewed interest in spinal innervation and focused on the role of the intrinsic nerve supply of lumbar spinal tissues in low back pain, and the topographic association with the sympathetic nervous system. Immunohistochemistry permitted a new 'functional' look at the neural elements in spinal tissues. This technique has the advantage of higher specificity in connection with the chemical nature of the neural structures.

Different classifications of pain originating in the lumbar spine have been proposed. Based on the nociceptor location and on the pain projecting pattern, Wyke (1980, 1987) classified spinal pain as primary, secondary, referred and psychogenic pain.

1. **Primary pain** originates from the vertebral column or from adjacent structures, by direct mechanical or chemical irritation of the nociceptors. The pain is experienced with varying degrees of precision.

2. **Secondary pain** is considered to be a consequence of a functional disturbance or lesion of the sensory pathway from the lumbar spine to the spinal cord (e.g. the emerging spinal nerves and nerve roots, the lumbar rami, or the sinu-vertebral nerves).

3. **Referred pain** is explained by the segmental relationship between the innervation of the superficial tissues of the low back in which the pain is perceived, and the innervation of the tissue or organ in which the actual pain source is located.

4. **Psychogenic pain** is unrelated to any structural or functional disturbance or lesion of the lumbar spine, but caused by psychological disturbances. However, it is now generally accepted that this term should no longer be used, as it rather describes an associated emotional disturbance that would aggravates the perception of spinal pain (van Kleef, 1996).

Wyke (1987) classifies primary pain, according to its source, as:

1. *cutaneous* and *subcutaneous pain* , originating in the skin, subcutaneous and adipose tissues of the back;
2. *myofascial pain* , which originates in the muscles, their tendinous attachments, their fascia and intramuscular septa;
3. *articular* and *ligamentous pain,* which is caused by pathologic conditions of the facet joint capsules and spinal ligaments;
4. *osseous pain* , which originates in the vertebral bodies, their growth plate and periosteum;
5. *vascular pain*;
6. *dural pain,* originating in the ventral spinal dura or in the dural sleeve into the intervertebral foramen.

O'Brien (1984) gives particular attention to the importance of spinal biomechanics and the plurisegmental distribution of spinal sensory pathways in spinal pain , and classifies primary pain, according to its source, into:

1. pain originating from the *mobile segment of the spinal column and its associated structures* (intervertebral discs, facet joints);
2. pain originating from *tissues that surround the vertebral column* (spinal ligaments, muscles);
3. pain caused by *involvement of the segmental nerves* associated with the vertebral column.

Bogduk (1983, 1992), but originally Steindler & Luck (1938), defined a virtual coronal plane through the intervertebral foramina, separating a ventral and dorsal compartment in the lumbar spine. The ventral compartment contains the ventral dura mater, intervertebral discs, both longitudinal ligaments, paravertebral sympathetic trunks, rami communicantes, and prevertebral muscles. The dorsal compartment contains the dorsal dura mater, zygapophyseal joints, ligamentum flavum, interspinous and supraspinous ligaments, the lumbodorsal fascia, and the deep muscles of the back. Medially, the spinal cord and emerging spinal nerves and nerve roots lie between the two compartments. Laterally, the two compartments are separated by the transverse processes and the intertransverse ligaments. Bogduk classifies spinal pain on the topography of the source of pain within the ventral and dorsal compartments. He emphasizes the deep structures of the lumbar spine as possible sources of primary spinal pain. Cutaneous and subcutaneus sources of spinal pain are not under consideration in his classification.

Stolker et al. (1994) classifies chronic spinal pain clinically, into:

1. *dorsal compartment syndrome*: lumbar facet syndrome;
2. *ventral compartment syndromes*: syndrome of anular tear (leakage of nucleus pulposus material into the annulus fibrosus), and the syndrome of instability of the motion segment;
3. *myogenic pain syndrome* (often secondary);
4. *segmental pain syndrome*, caused by a herniated disc with compression and irritation of the spinal nerve;
5. *pain from epidural adhesions*;
6. *spinal stenosis (Verbiest's) syndrome*.

The present manuscript attempts to provide a comprehensive description of the microscopical anatomy and the innervation of the lumbar spine, based on Bogduk's classification. Particular attention is given to origin, terminal arborisation and neuropeptides of the intrinsic nerves of the human lumbar spine using light- and electron microscopy and immunocytochemistry. An important statement of Bogduk (1983), "Whatever it is in the lumbar spine that causes pain, it must have a nerve supply", will always hold true. Only the deep structures of the lumbar spine, pertinent to the discussion of the anatomical basis of low back pain, are included in the present manuscript, meaning: the meningeal structures, intervertebral disc, facet joints, ligaments, vessels, meningeal structures, the vertebral bodies, and muscles. Hypothetical mechanisms of pain originating in the lumbar spine, are briefly discussed.

In this study we also documented the fine structural characteristics of human spinal dura mater and arachnoid mater, with special reference to their spatial relationships.

Parts of this manuscript are published papers, which are integrally included. Likely, this facilitates the reading of each individual paper as a separate entity within the manuscript, but causes a substantial overlap in the introduction sections.

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CHAPTER 2

Macroscopic and Microscopic Structure of the Lumbar Spine
A review of literature data and own observations

Before studying the neurohistology of the lumbar spinal soft tissues, a short review of the macroscopic and microscopic morphological characteristics of the tissues in the lumbar spine seems appropriate. The review is based on Romanes (1978), Bogduk &Twomey (1987), Peeters et al. (1991), Fawcett (1994), Williams (1995) and Yamashita et al. (1996) except when mentioned otherwhise.

The Joints of the Lumbar Spine

The basic anatomical and functional unit of the vertebral column is the articular triad consisting of the intervertebral joint and the two synovial joints between the vertebral articular processes (zygapophyseal, apophyseal or facet joints). The intervertebral joints are the joints of the vertebral bodies. The facet joints are the joints of the vertebral arches (Fig. 1).

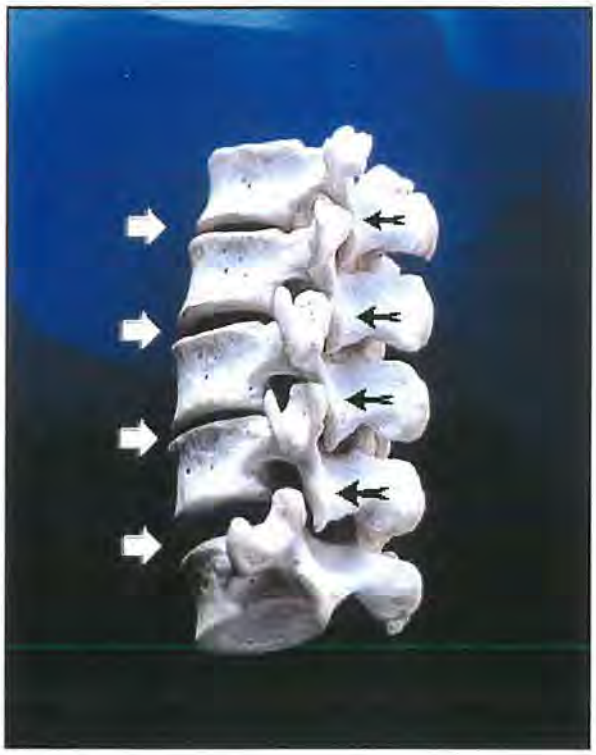


Fig. 1. The five lumbar vertebrae: left postero-lateral aspect. The vertebrae articulate to one another by a series of fibrocartilaginous joints (intervertebral discs) between the vertebral bodies (large arrows), and a series of synovial joints (zygapophyseal joints, facet joints) between the articular processes (zygapophyses) of the vertebral arches (small arrows).

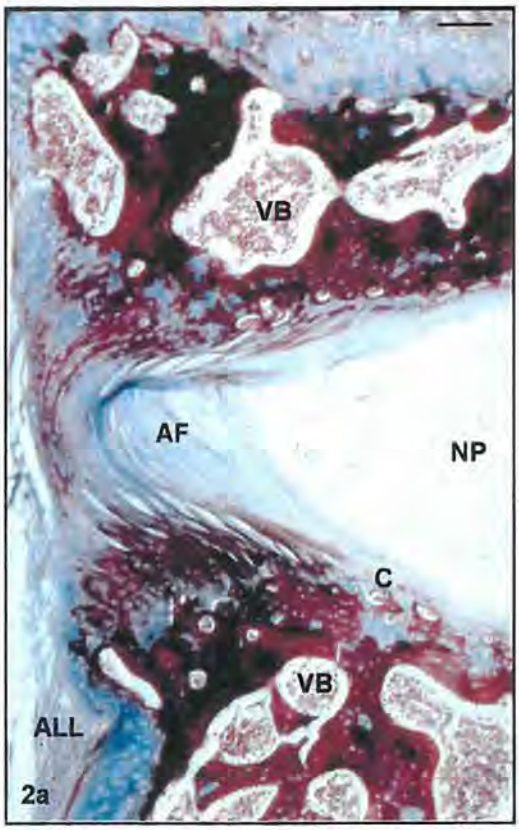
Fibrous Intervertebral Joint or Symphysis

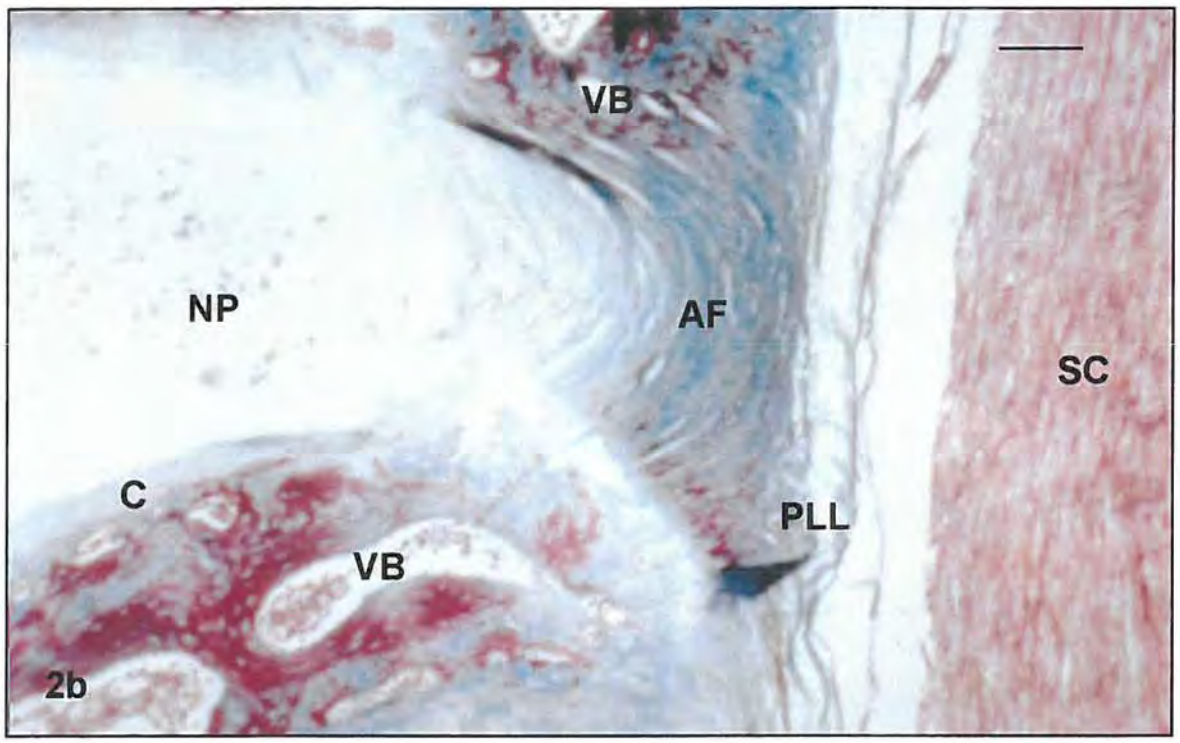
Macroscopy

Vertebral bodies are united by anterior and posterior longitudinal ligaments and by the intervertebral symphysis. The intervertebral symphysis is formed by the fibrocartilaginous intervertebral disc (IVD) and the thin layers of hyaline cartilage covering the superior and inferior surfaces of the adjacent vertebral bodies. IVDs are found in the entire vertebral column and are thickest in the lumbar region. They are adherent to the hyaline cartilage and to the anterior as well as the posterior longitudinal ligament. The PLL is more adherent to the IVD due to the broadening of this ligament in between two successive vertebrae. Each disc consists of two basic components: an inner core (nucleus pulposus) and a peripheral laminated fibrocartilaginous ring (annulus fibrosus). The annulus fibrosus consists of fibrocartilaginous laminae. The nucleus pulposus is a derivative of the notochord of the embryo. At birth, the nucleus pulposus is large and consists of gelatinous mucoid material. While aging, the nucleus pulposus is gradually replaced by fibrocartilage (Vanormelingen, 1997). In the adult, there is no clear-cut boundary between the nucleus pulposus and the annulus fibrosus.

Microscopy.

The annulus fibrosus consists of a narrow outer collagenous zone and wider inner fibrocollagenous zone. Within each lamina the collagen fibres run predominantly parallel and obliquely between two vertebrae, ending in the hyalin cartilage covering the vertebrae (Figs 2*a-c*). The collagen fibres in adjacent laminae run obliquely to each other, particularly in the deeper zones of the annulus fibrosus. At birth the nucleus pulposus contains a few multinucleated notochordal cells in a network of collagen fibrils embedded in a mucoid ground substance, rich in hyaluronic acid.





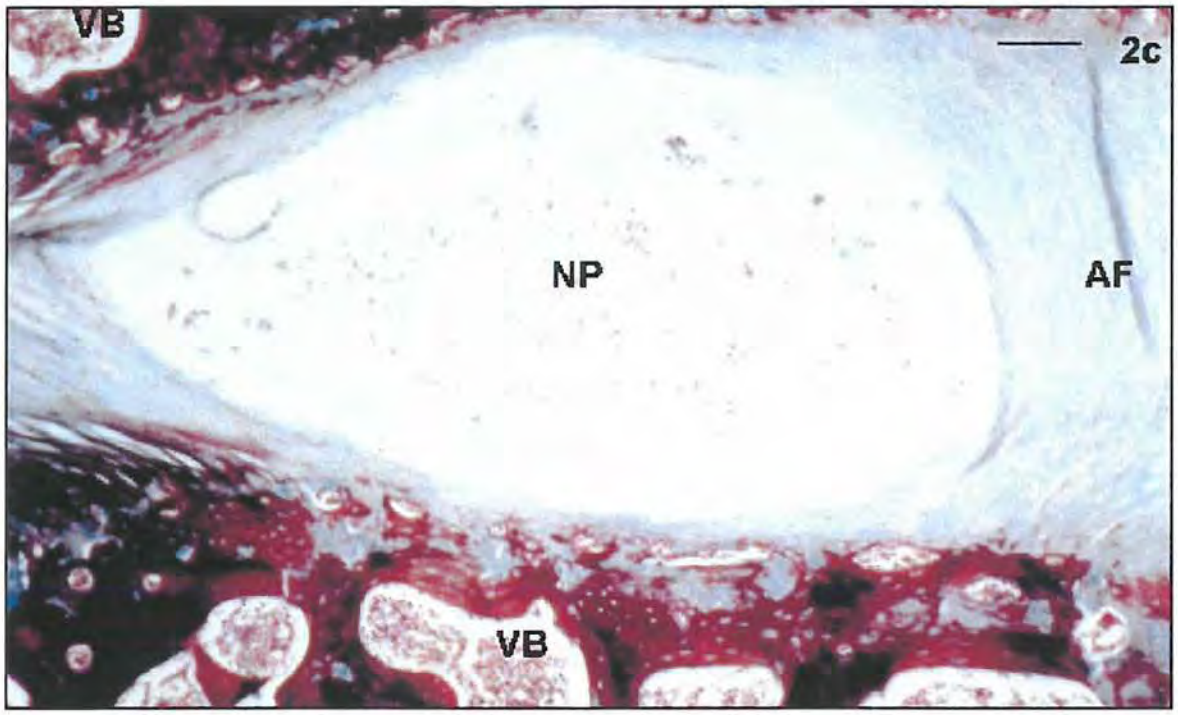


Fig. 2. Light micrographs of Masson's Trichrome-stained median sagittal sections through an intervertebral disc in the lumbar region of the vertebral column of the rat. Adjoining vertebral bodies (VB) are united by the anterior (ALL) and posterior (PLL) longitudinal ligaments and by the fibrocartilaginous intervertebral disc between laminae of hyaline cartilage, together forming symphyses. (a) The ALL is strongly adherent to the intervertebral disc, hyaline cartilage laminae (C) and margins of adjacent vertebral bodies. (b) The PLL lies in the vertebral canal. Its fibres attach to the intervertebral disc, laminae of hyaline cartilage and adjacent margins of vertebral bodies. Spinal cord (SC). (c) The nucleus pulposus (NP) is located centrally in the annulus fibrosus (AF). BAR = 250 µm.

Facet Joints (N.A.: articulationes zygapophysiales)

Macroscopy

The posterolateral joints of the vertebral column are paired and synovial. They are commonly called 'facet joints', and more accurately known as 'zygapophysial' or 'apophysial' joints. Each joint is formed by the superior articular processes (zygapophyses) of one vertebra with the inferior articular processes of the adjoining vertebra (Fig. 3). They join the vertebral arches and are of simple (cervical and thoracic) or complex (lumbar) synovial variety. In the lumbar area of the standing subject the facet joint plane is vertically oriented. The superior process presents a concave, cylindrical articular surface directed dorso-medially. The inferior articular process is convex and faces antero-laterally.

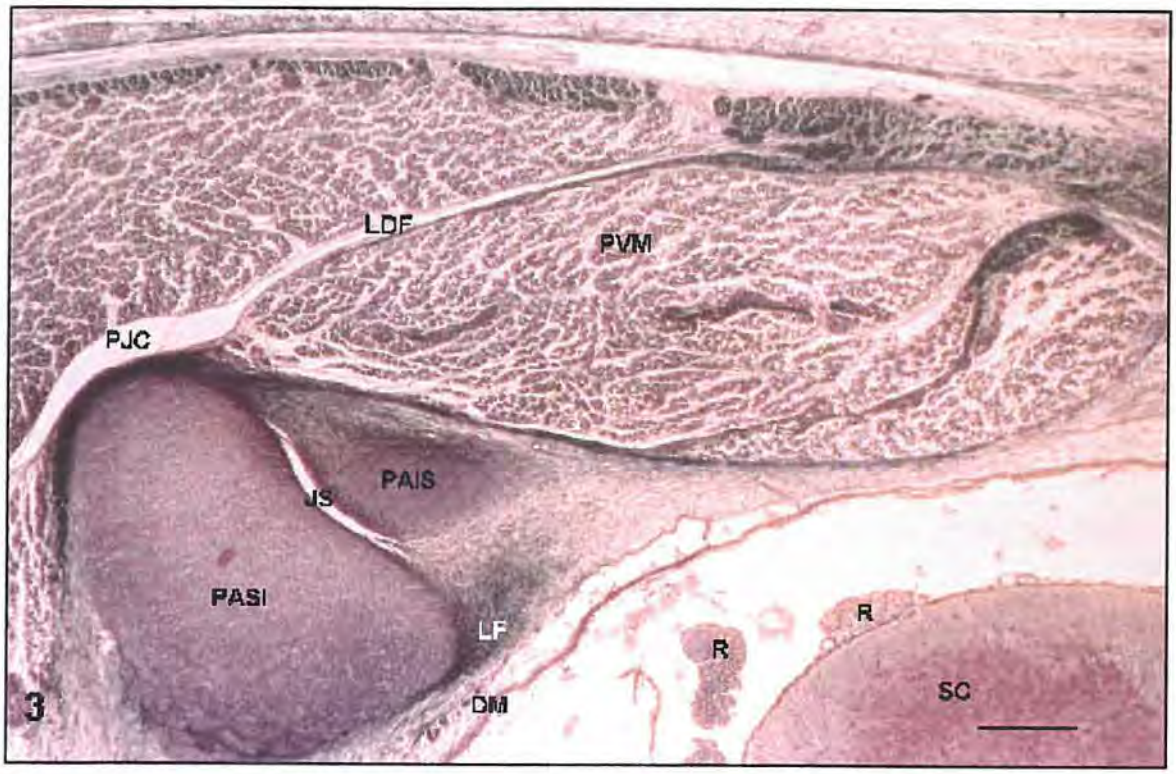


Fig. 3. Light micrograph of Masson's Trichrome-stained transverse section through the facet joint of the human fetal spine (30 weeks). The anterior joint capsule is formed by the ligamentum flavum (LF). Posterior joint capsule (PJC). Joint space (JS). Spinal dura mater (DM). Spinal cord (SC). Paravertebral musculature (PVM). Deep layer of the lumbodorsal fascia (LDF). Processus articularis superior of the inferior vertebra (PASI). Processus articularis inferior of the superior vertebra (PAIS). Spinal nerve roots (R). BAR = 330 µm.

The articular capsule consists of an outer fibrous layer and an inner synovial membrane. The medio-posterior part of the capsule is reinforced by a band of tendinous fibres of the rotatores muscle, i.e. the deepest layer of the multifidus muscle, running in inferior-superior direction from the posterior-lateral aspect of the lamina to the superior-medial aspect of the lamina one level above. The posterior capsule is attached close to or within the margin of the articular cartilage of adjacent articular processes. The anterior capsule is the ligamentum flavum, attaching close to the osteochondral junctions.

At the superior and inferior ends of the joint the joint capsule attaches further from the osteochondral junctions, creating sub-capsular pockets (recessus articularis superior and inferior, respectively) filled with fat. The fat in the subcapsular pockets communicates through thin foramina in the capsule, with extra-capsular fat located at the superior and inferior margins of the superior and inferior articular processes. The adipose tissue of the recessus articularis superior is continuous with the adipose tissue surrounding the spinal nerve in the intervertebral foramen. The adipose tissue in the articular recesses communicates with the adipose tissue of the synovial plical tissue lying in the joint cavity.

The synovial membrane lines the inner surface of the joint capsule and covers the intra-articular adipose tissue (synovial plical tissue, synovial fat pad, meniscoid), but not the articular cartilage. The synovial plicae are commonly thin and attenuated (Fig. 4). The surface of the synovial membrane has a smooth, glistening appearance.

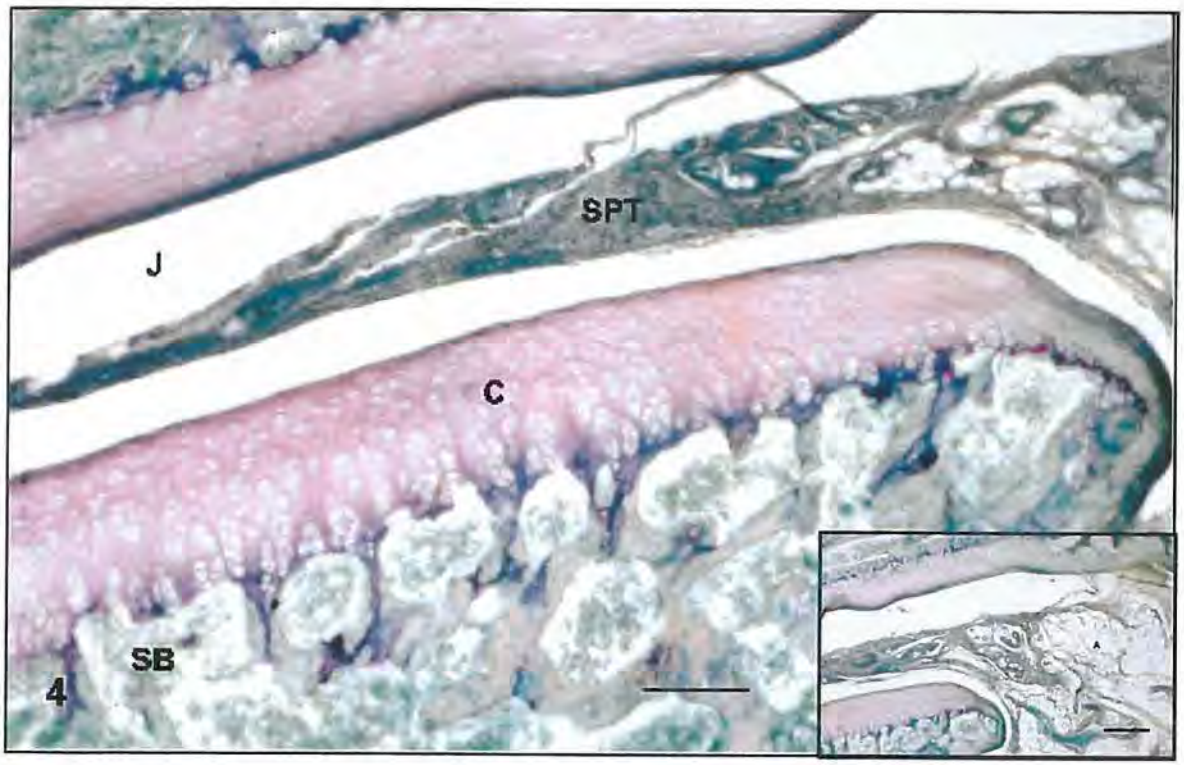


Fig. 4. Light micrograph of PAS and FAST Green-stained section of the human lumbar facet joint. A slender process of synovial plical tissue (SPT) lies in the joint space (J). Cartilage endplate (C). Subchondral bone (SB). BAR = 200 µm.

Insert: the intra-capsular adipose tissue of the lumbar facet joint, located in a subcapsular pocket (recessus articularis). Note that it is continuous with the subintimal layer of the synovial plica. BAR = 430 µm.

Microscopy

The fibrous capsule of the facet joint consists of an outer layer of dense connective tissue, and an inner loose fibro-elastic layer.

Outer layer of the fibrous capsule

The outer layer of the facet joint capsule consists of regularly arranged and densely packed parallel bundles of collagen fibres, considered to be a 'capsular ligament'. The fibres contribute to stabilize the joint in the transverse plane, while allowing mobility in the longitudinal plane. The tendinous fibres of the deepest layer of the multifidus muscle would limit the joint movement in the longitudinal direction.

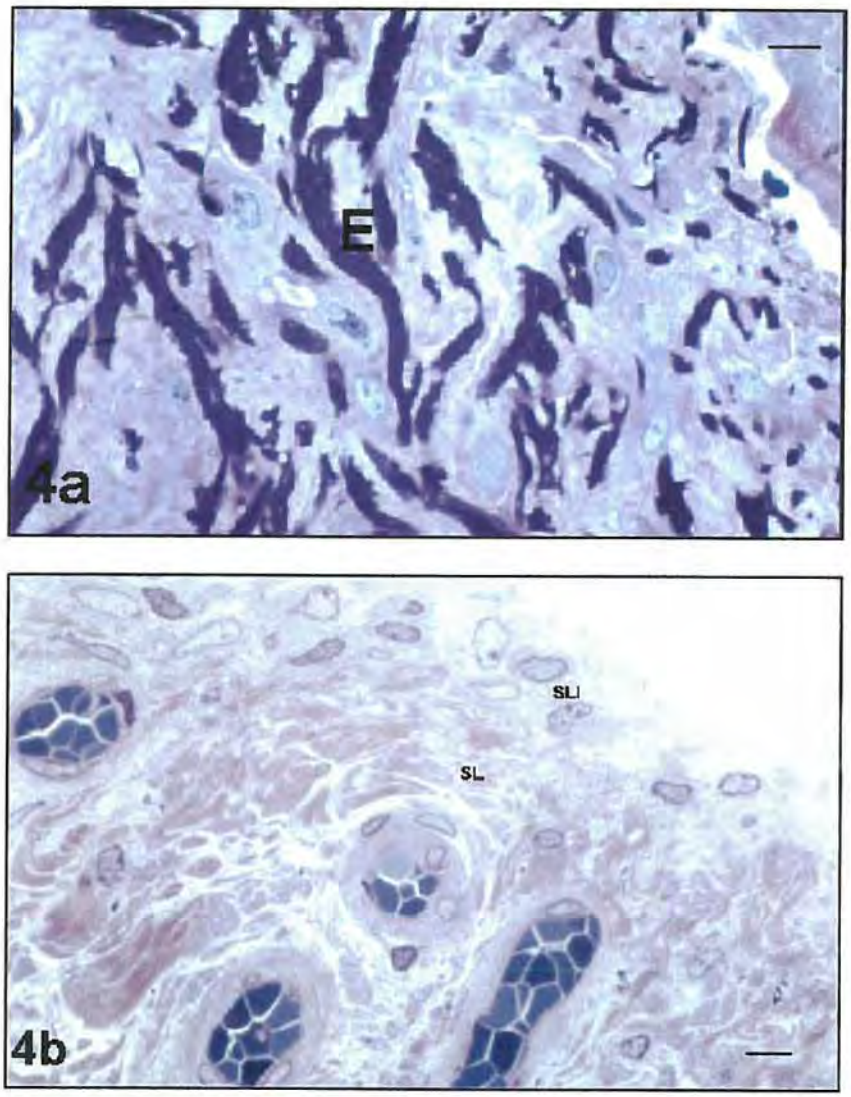


Fig. 4. Light micrographs of thionin-methylene blue-stained semithin sections of the capsule. (a) Detail of the elastic inner layer of the fibrous capsule: elastic fibres (E) intermingled with collagen. BAR = 10 µm. (b) Detail of the synovial membrane: discontinuous multilayered sheath of synovial cells (synovial lamina intima, SLI) and underlying vascular fibro-adipose subintimal layer (SL). BAR = 10 µm.

Inner layer of the fibrous capsule

The inner layer of the joint capsule was found to be a soft fibro-elastic connective tissue (Fig. 4*a*). Round or ovoid nuclei of fibroblasts are seen between interwoven elastic fibres. The elastic fibres run mostly in the same direction as the collagen fibres of the outer layer. The elastic fibres might constitute an 'elastic ligament', the typical form of which is found in the ligamentum flavum, that forms the ventral part of the joint capsule. The inner layer of the capsule cannot always be easily distinguished from the ligamentum flavum.

Synovial membrane

The synovial membrane consists of an inner intimal layer (synovial lamina intima), and an outer vascular fibro-adipose layer (subintimal layer). Locally, the light microscopic appearance of the intimal layer varies considerably. Synovial intimal cells vary in shape and distribution; usually they form a discontinuous single or multilayered sheath (Fig. 4*b*). The morphology of the subintimal tissue varies from adipose, areolar to fibrous. Mast cells are regularly found in the subintimal tissue. Ultrastructurally, the synovial intima comprises two main cell types or 'synovial intimal cells': a macrophage –like cell type ('type A or M'), and a fibroblast-like cell type ('type B or F') (Table1;Figs 5 *a-d*). Intermediate cell types have been described. Literature data on origin , function, and distribution of synovial intimal cells are contradictory.

Table 1.

Differentiating ultrastructural characteristics of the two major types of synovial intimal cells: the type A (M) or macrophage-like cell-type and the type B (F) or fibroblast-like synoviocyte.

Organelles	Type A(M)	Type B(F)
chromatin	heterochromatin-rich nucleus	euchromatin-rich nucleus
rough endoplasmic reticulum	poorly developed	well developed wide cisternae
golgi apparatus	moderate multiple	large
vacuolar apparatus	well-developed	poorly developed
lysosomes	numerous	few or absent
surface projections	large uneven	slender uniform
micropinocytotic caveolae	numerous	few

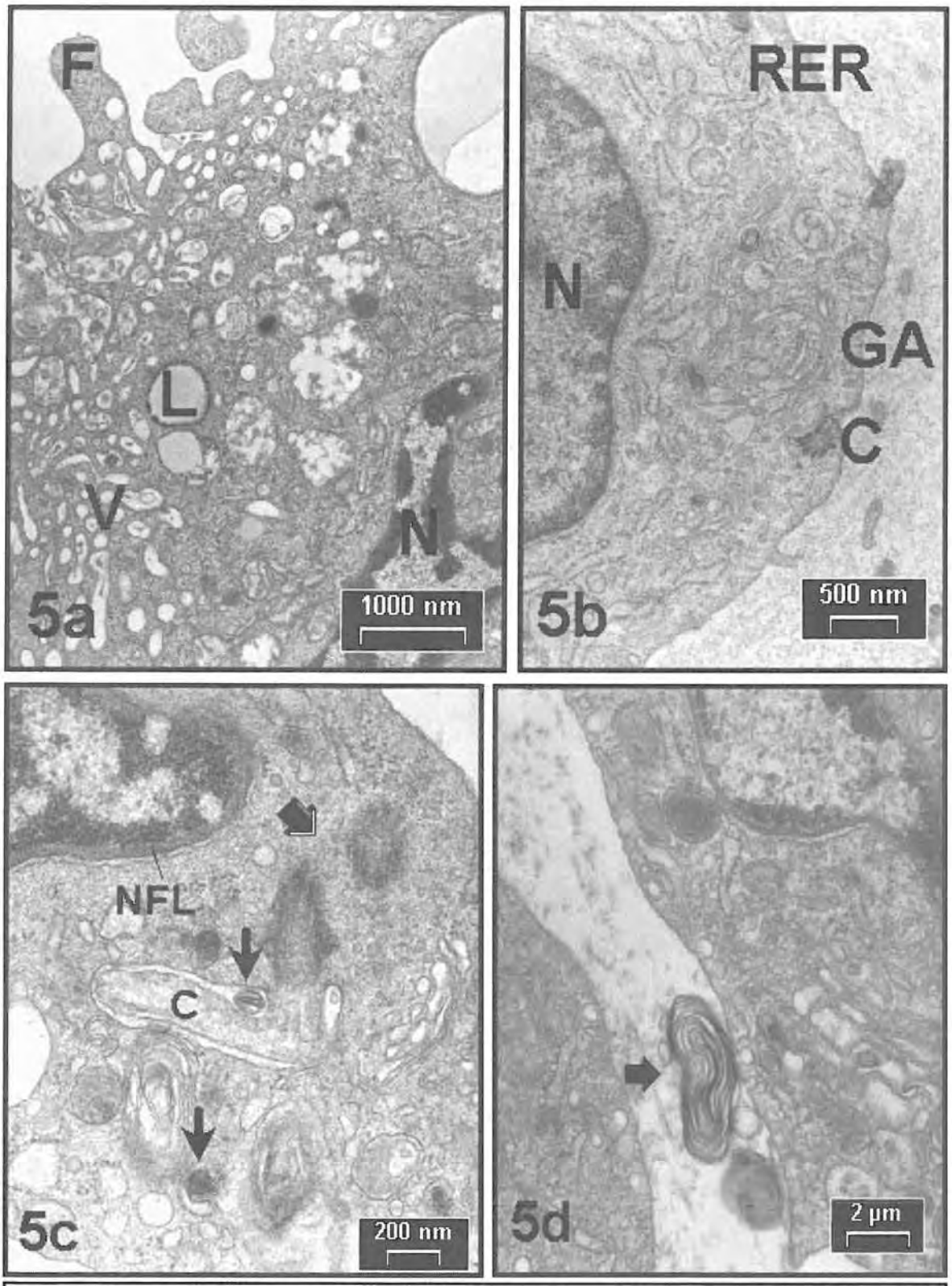


Fig. 5. Electron micrographs of synovial intimal cells in the human lumbar facet joint. (a) Type A(M) synovial cell, primarly characterized by filopodia (F), a heterochromatin-rich nucleus (N), lysosomes (L) and a well-developed vacuolar apparatus (V). (b) Type B (F) synovial cell, primarly characterized by a well-developed Golgi apparatus (GA) and rough endoplasmic reticulum (RER), and a euchromatin-rich nucleus (N). Cilium (C). (c) Detail of a synovial intimal cell demonstrating a nuclear fibrous lamina (NFL), intracellular lamellar bodies (thin arrows), and a cilium (C) arising from one centriole of a centriole-pair (thick arrow). (d) Extracellular lamellar body (arrow) in the immediate vicinity of a synovial intimal cell.

The Accessory Ligaments of the Lumbar Spine

(intervertebral syndesmoses)

The bodies, laminae, spines and transverse processes of the lumbar spine are connected through accessory ligaments (Fig. 6). Topographically, the ligaments of the lumbar vertebral column are classified into two groups:

1. Accessory ligaments of the intervertebral joints (*intervertebral symphysis*), interconnecting the vertebral bodies:
   (a) anterior longitudinal ligament,
   (b) posterior longitudinal ligament.

2. Accessory ligaments of the zygapophyseal joints, interconnecting
   (a) the vertebral arches (ligamentum flavum),
   (b) the spinous processes (interspinous ligaments, supraspinous ligament),
   (c) the transverse processes (intertransverse ligaments).

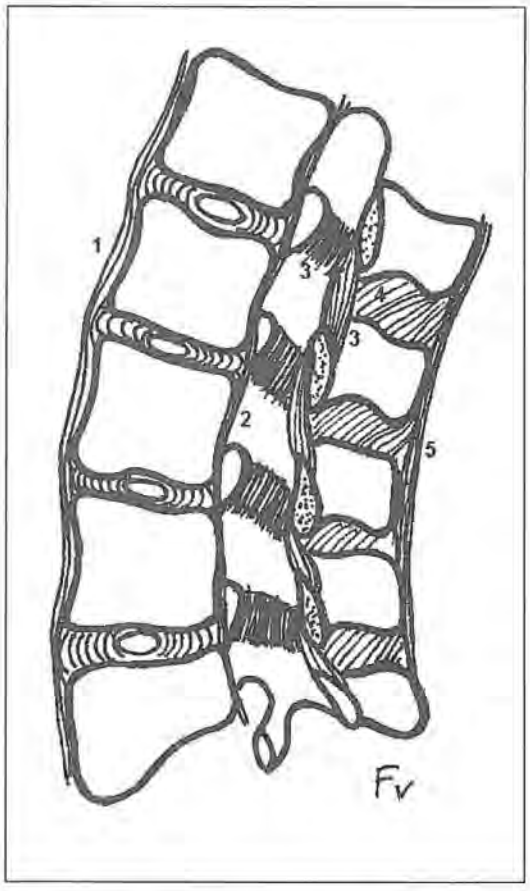


Fig. 6. Diagram showing a median sagittal section through the lumbar region of the vertebral column : anterior longitudinal ligament (1), posterior longitudinal ligament (2), ligamentum flavum (3), interspinous ligament (4) and supraspinous ligament (5).

Anterior longitudinal ligament

The anterior longitudinal ligament is a strong band extending along the entire vertebral column. It covers the intervertebral discs and anterior surfaces of the vertebral bodies. The deepest fibres of the ligament attache to the margins of the vertebral bodies, cover the intervertebral disc and focally adhere to the annulus fibrosus. They bridge the concave anterior surface of the vertebral body that is filled with loose areolar tissue. Some of these fibres insert into the vertebral bone or the overlying periosteum. The superficial fibres of the ligament are the longest and extend over three to four vertebrae. The intermediate fibres extend between two or three vertebrae. The anterior longitudinal ligament mainly serves to resist vertical separation of the anterior ends of the vertebral bodies during extension of the spinal column.

Posterior longitudinal ligament

Like the anterior longitudinal ligament, the posterior longitudinal ligament covers the entire vertebral column. The ligament is localized inside the vertebral canal, covering the posterior surfaces of the vertebral bodies. It has a polysegmental disposition. The deepest and shortest fibres span two intervertebral discs from the superior margin of one vertebra to the inferior margin of the vertebra two levels above. Longer, more superficial fibres span up to five vertebrae. The fibres of the posterior longitudinal ligament expand laterally over the intervertebral discs and thereby blend with those of the annulus fibrosus to attach to the posterior margins of the vertebral bodies. The posterior longitudinal ligament mainly serves to resist separation of the posterior ends of the vertebral bodies in flexion of the vertebral column.

Ligamenta flava

The ligamentum flavum connects the laminae of adjacent vertebrae (Fig. 7). The ligament arises from the inferior anterior surface of the lamina and the inferior edge of the pedicle, and divides into a medial and a lateral portion. The medial portion inserts on the posterosuperior surface of the lower lamina. The lateral portion passes in front of the neighbouring zygapophysial joint and forms its anterior capsule. It consists of 80% elastin and 20% collagen and acts as an elastic band. The elastic nature of the ligamentum flavum is well suited to cushion the effect of stretching and deforming forces, and in keeping the spinal cord and the spinal nerve roots free from compression. The unique nature of the ligamentum flavum has also been implicated biomechanically in restoring the flexed lumbar spine to its extended position, while its lateral portion is said to prevent the anterior capsule of the zygapophysial joint being nipped within the joint space during movement.

Interspinous ligaments

The interspinous ligaments are thin and membranous ligaments that connect adjoining spinous processes. They run obliquely in the interspinous spaces. They meet the ligamenta flava anteriorly and the supraspinous ligament posteriorly.

Supraspinous ligament

The supraspinous ligament is a strong fibrous cord connecting the tips of the spinous processes. It intimately blends with neighbouring fascia and prevents separation of the spinous processes. It is consistently absent at L5-S1, frequently so at L4-L5, and poorly developed at L3-L4.

Intertransverse ligaments

They connect adjoining transverse processes. The intertransverse ligament is thin and membranous and does not constitute a true ligament.

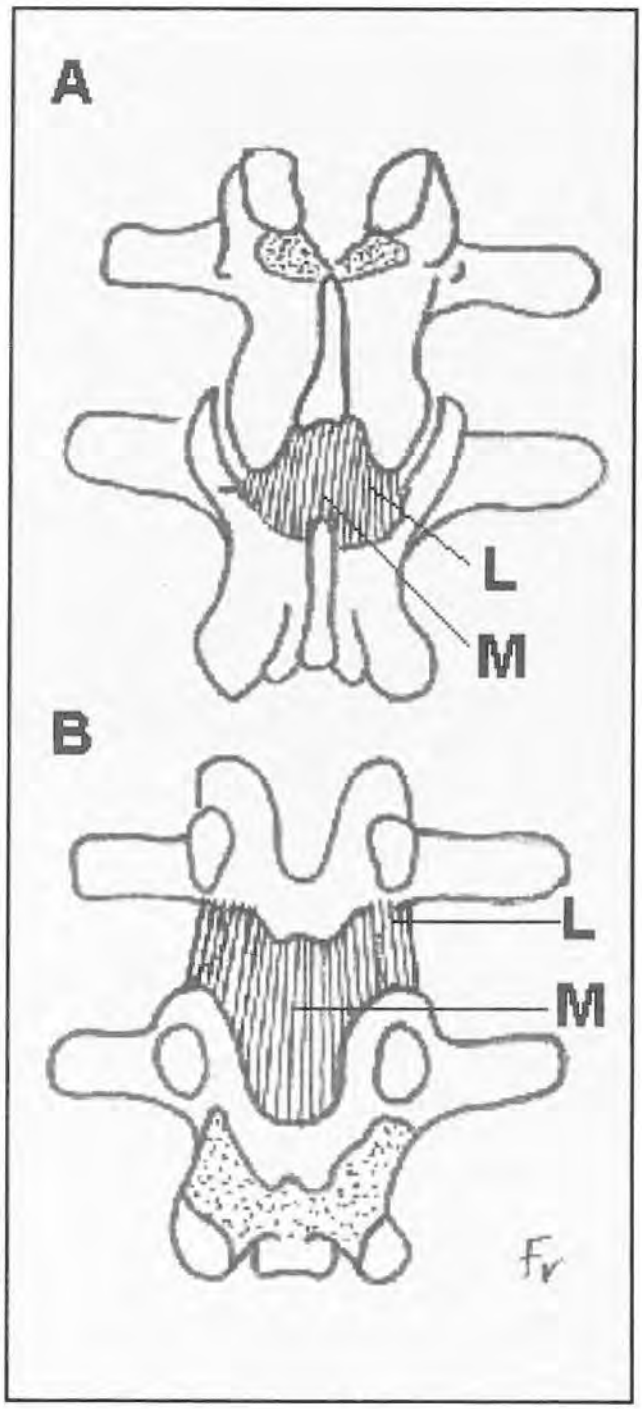


Fig. 7. The ligamentum flavum at the L2-L3 level. A: posterior view. B: anterior view. The medial (M) and lateral (L) divisions of the ligament are labelled. The shaded areas depict the sites of attachment of the ligamentum flavum at the levels above and below L2-L3.

The Deep Muscles of the Lumbar Spine

The deep or intrinsic muscles of the back control the vertebral column, and consist of a complex group of muscles extending from the pelvis to the skull.

In the lumbar region they include the short segmental muscles (interspinales and intertransversarii), and the complex extensor and rotatores masses (erector spinae or sacrospinalis, and the transversospinalis).

In the lumbar region are four pairs of interspinales between the spines of the five vertebrae. Each pair is attached above and below to the apices of contiguous spines, one on either side of the interspinous ligament. The intertransversarii are placed between the transverse processes of the vertebrae.

The erector spinae forms a thick muscle that begins on the sacrum and ascends to the lumbar region, lying between the spines and transverse processes of the vertebrae, deep to the lumbar fascia. In the lumbar region its inserts to the spines of the vertebrae, and consists from lateral to medial of the iliocostalis, longissimus, and spinalis.

In the lumbar region, the transversospinal muscles include the rotatores and multifidus. Part of the transversospinal muscles connect the sacrum with the transverse processes, laminae, and spines. They are classified as multifidus, rotatores, interspinales, and intertransversarii. The multifidus muscle lies deep to the erector spinae and covers the intervertebral joints.

The Vertebral Canal

The vertebral canal is a continous channel that is formed by the alignment of consecutive vertebral foramina, and encloses the spinal cord and its sheaths. In transverse section, the lumbar vertebral canal varies in shape, from oval at the upper end to triangular more caudally (Figs 8 *a, b*).

Anterior the vertebral canal is formed by the posterior surfaces of the lumbar vertebrae, the intervertebral discs, and the posterior longitudinal ligament. Its posterior wall is formed by the laminae of the vertebrae and the ligamenta flava. The lateral walls of the vertebral canal are formed by the pedicles of the lumbar vertebrae.

In the lateral wall two consecutive pedicles form the intervertebral foramen, through which the spinal nerve roots run. Each intervertebral foramen is bounded anteriorly by an intervertebral disc, and the adjacent parts of the posterior surfaces of the two consecutive vertebral bodies. Posteriorly, the intervertebral foramen is bounded by the lateral portion of the ligamentum flavum, covering the anterior aspect of the lamina and the zygapophysial joint.

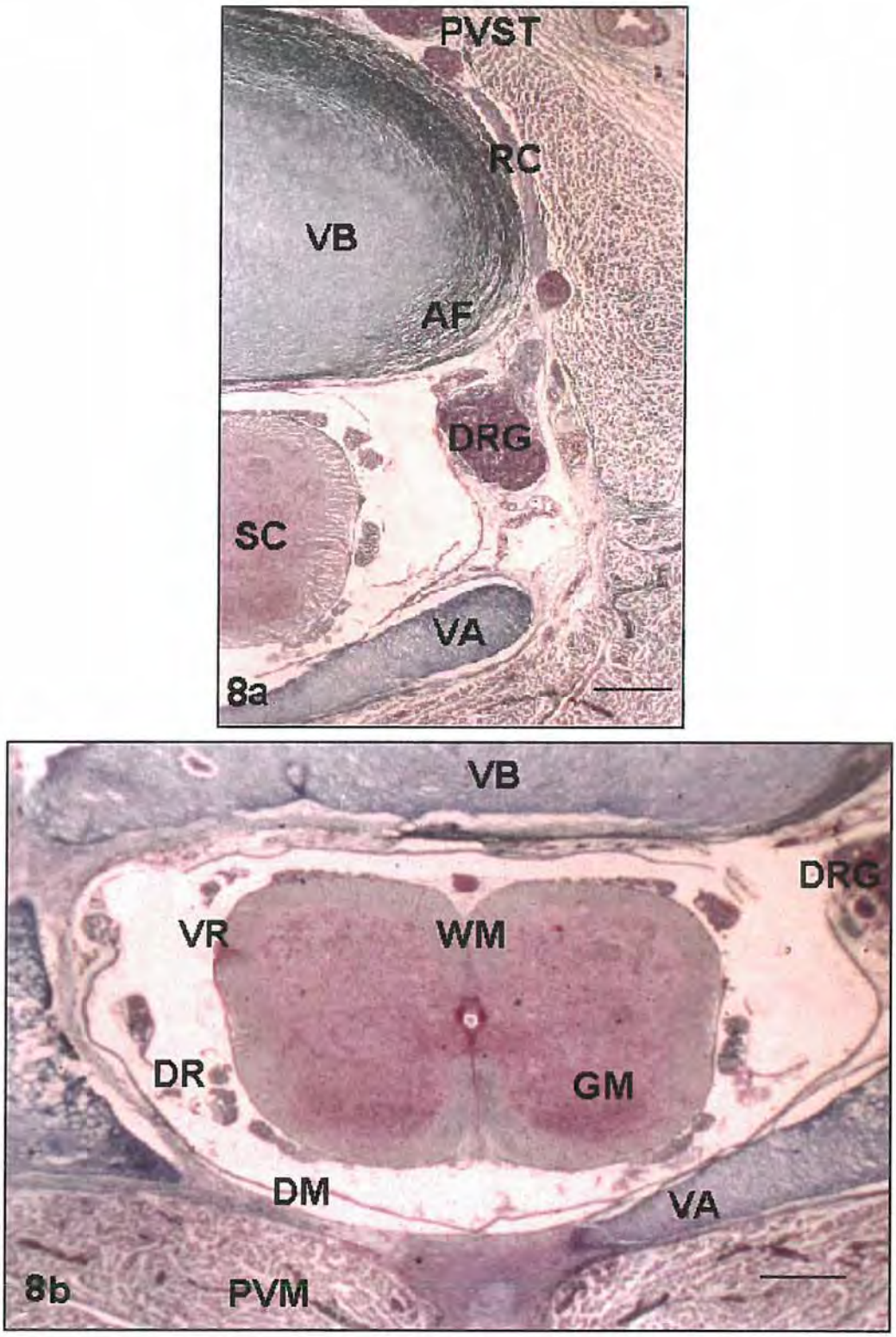


Fig. 8. Light micrographs of Masson's trichrome- stained transverse sections of the human fetal lumbar spine.

(a) vertebral body (VB), vertebral arch (VA), spinal cord (SC), dorsal root ganglion (DRG), ramus communicans (RC), annulus fibrosus (AF). BAR = 400 µm.
(b) vertebral body (VB), vertebral arch (VA), grey matter (GM), white matter (WM), dorsal root ganglion (DRG), ventral root (VR), dorsal root (DR), paravertebral musculature (PVM), dura mater (DM). BAR = 300 µm.

The Extradural (Epidural) Space

The spinal and cranial dura mater differ in their relationship to the surrounding bone tissue. The outer layer of the cranial dura functions as periosteum and adheres to the inner surface of the skull.

The spinal dura is separated from the ligaments and the periosteum of the vertebral canal by a wide extradural (epidural) space (Fig. 9). It contains loose connective tissue, adipose cells, a venous plexus (internal vertebral venous plexus) and small arteries that supply the structures in the vertebral canal. The adipose tissue of the extradural space extends laterally along the spinal nerves through the intervertebral foramina.

In the lumbar region the loose connective tissue and adipose tissue in the extradural space permit displacement of the dura mater during movement and venous engorgement.

The Subdural Space

In the classical anatomical textbooks and clinical literature, frequent references are made to a so called 'subdural' space located between the dura mater and arachnoid mater. A subdural space is included in the Nomina Anatomica. There is no such space in the the normal human spine. The spinal dura and arachnoid mater are closely apposed in man (Vandenabeele et al., 1996).

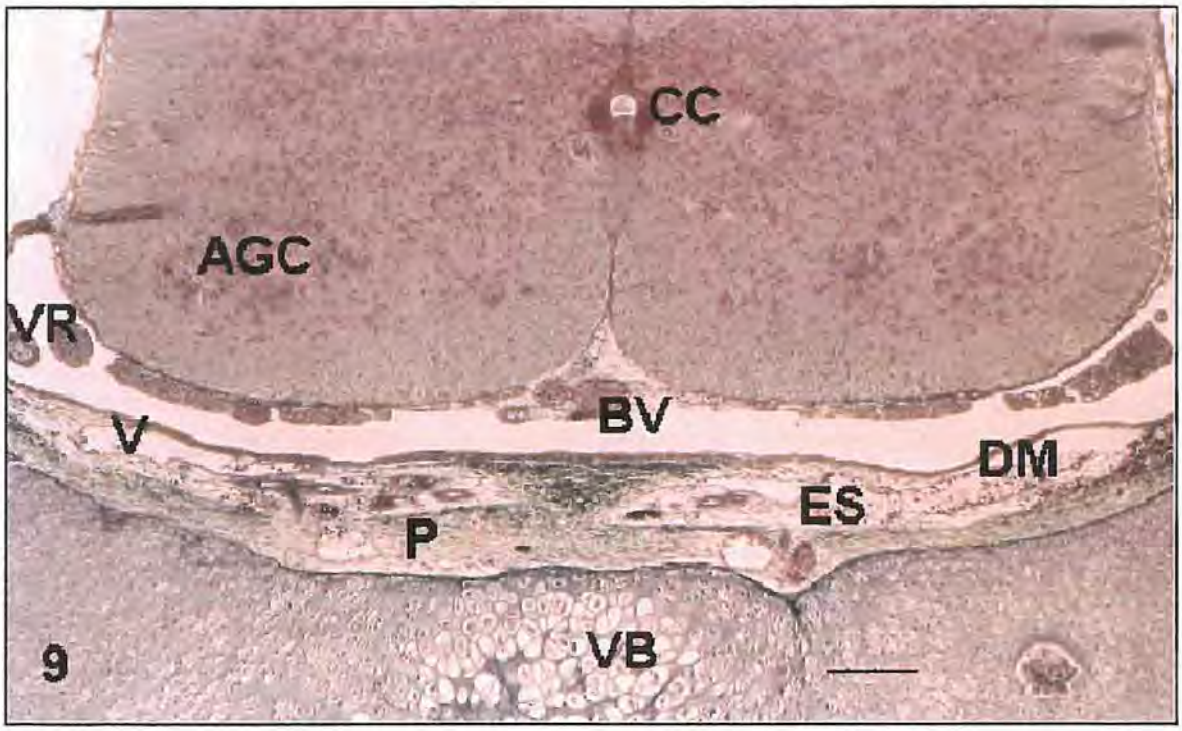


Fig. 9. Light micrograph of a Masson's Trichrome-stained transverse section of the anterior half of human fetal spine at a lumbar level. The periosteum (P) lines the vertebral canal and is separated from the spinal dura mater (DM) by the extradural (epidural) space (ES) containing loose connective tissue, fat and a venous plexus (V). Anterior spinal vein and artery (BV). Ventral root (VR). Vertebral body (VB). Anterior grey column (AGC). Canalis centralis (CC). BAR = 180 µm.

The Spinal meninges

The central nervous system (brain and spinal cord) is enveloped in three membranes or meninges, composed of connective tissue: the outermost *dura mater* (or *pachymenínx*), the intermediate layer or *arachnoid mater* and the innermost *pia mater* (or l*eptomeninges*). The dura and arachnoid extend down the vertebral canal as tubular sheaths till the level of the second sacral vertebra.

Macroscopy

Spinal dura mater

The spinal dura mater is continuous with the cerebral dura at the foramen magnum, and is a strong and dense fibrous sheet. The dural tube narrows at the lower border of the second sacral vertebra and invests the spinal filum terminale, while descending to the coccyx were it blends with the periosteum. The spinal dura has separate tubular sheaths or 'sleeves' around the spinal roots as they pass out of the intervertebral foramina. The sleeves fuse with the epineurium, within or slightly beyond the intervertebral foramina.

Spinal arachnoid and pia mater

There are two major layers of leptomeninges, the pia mater and the archnoid mater, often referred to as the *pia - arachnoid*. They are thin, delicate and transparent membranes, are separated by the subarachnoid space and bound to each other by numerous trabeculae.

The spinal arachnoid mater loosely surrounds the spinal cord. It is continuous with the cranial arachnoid mater. It is closely applied to the deep aspect of the spinal dura mater. Deep to the arachnoid lies a relatively wide subarachnoid space, filled with cerebrospinal fluid and contains small blood vessels. The trabeculae in the subarachnoid space are attached to the arachnoid mater and form a delicate network. They fuse with the pia mater. The arachnoid trabeculae are well developed in some mammals but in man they may be locally absent.

The spinal pia mater is closely adherent to the contours of the spinal cord. It contains a large number of blood vessels which supply the underlying nervous tissue. Beyond the apex of the *conus medullaris* (second lumbar vertebra), the pia mater continues as the coating of the *filum terminale*. The filum terminale is a delicate median connective tissue filament that descends from the apex of the conus medullaris, between the roots of the lumbar and sacral nerves (*cauda equina*). The final five centimeters of the filum terminale (*filum terminale externum*) fuse with the dura mater. The upper part of the filum terminale (*filum terminale internum*) is surrounded by extensions of the dural and arachnoid meninges until the second sacral vertebra.

The pia - arachnoidea ensheathes the spinal roots along their course in the subarachnoid space. At the subarachnoid angle were the spinal nerves pass through the dura into the intervertebral foramina, the leptomeningeal layers fuse and become continuous with the perineurium of the spinal nerves.

The subarachnoid space is crossed on each side of the spinal medulla by the denticulate ligaments, that are thin fibrous ridges of pia mater. They connect the spinal cord to the dura mater, midway between the points of emergence of the ventral and dorsal nerve roots, and suspend the spinal cord in the subarachnoid space. The last denticulate ligament descends laterally from the conus medullaris between the exits of T12 and L1.

Microscopy

The spinal dura mater is a strong membrane of dense connective tissue. Collagen fibres predominate, and are intermingled with elastic fibres. The pia and archnoid mater or 'pia-arachnoid', consist of collagen and reticulin fibres, and flattened cells (Figs 10 *a, b*).

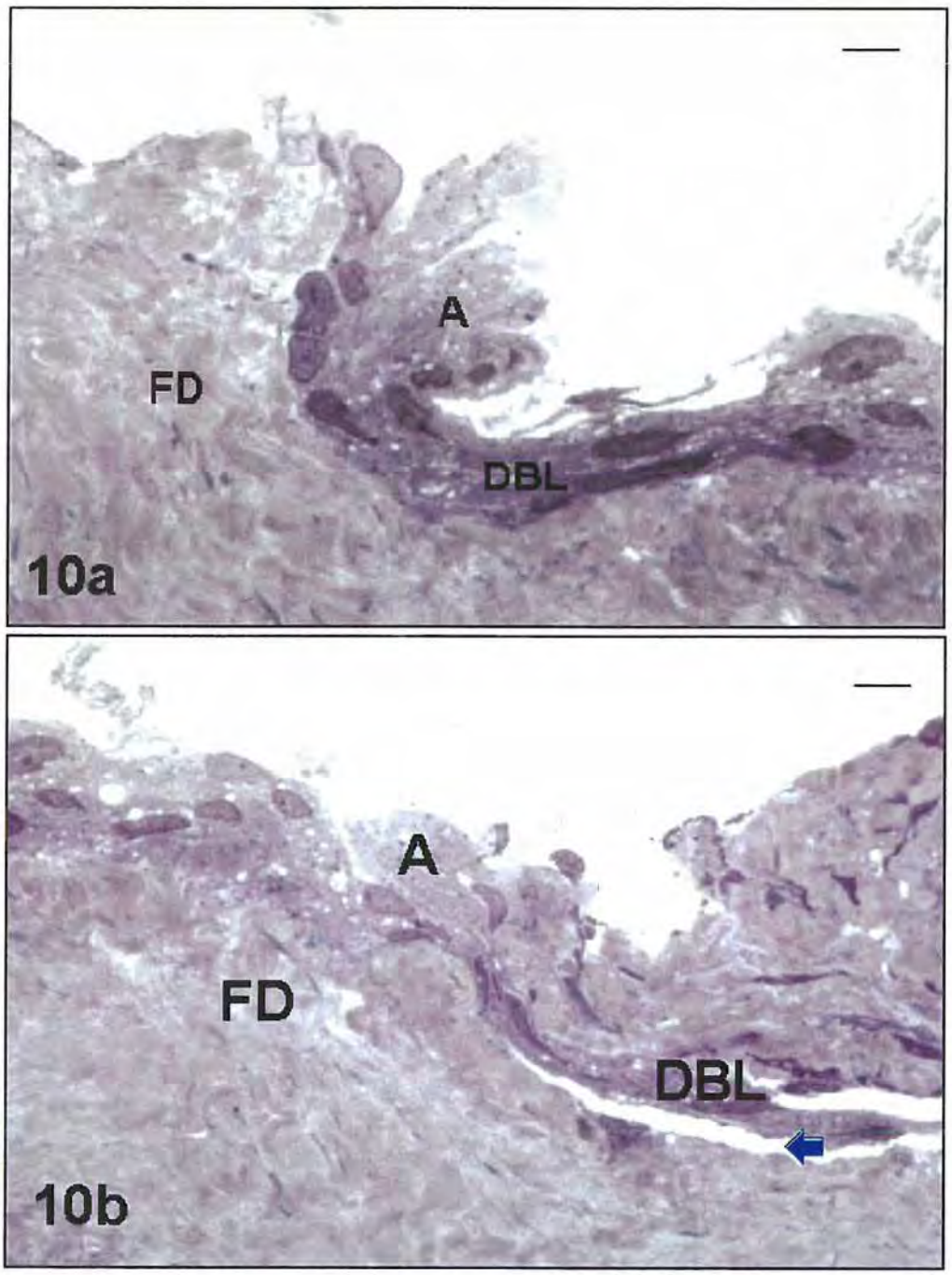


Fig. 10. Light micrographs of thionin-methylene blue-stained semithin sections of the dura-arachnoid interface in the human lumbar spine. Fibrous dura (FD). Dural border cell layer (DBL). Arachnoid mater (A). (a) Intact spatial relationships. (b) Disrupted spatial relationship in the dura mater: the dura is splitted throught the DBL (arrow). BAR = 3 µm.

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Ultrastructure of the Human Spinal Arachnoid Mater and Dura Mater

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- Vandenabeele F, Creemers J, Lambrichts I (1995) The spinal arachnoid mater in man, a potential meningeal barrier: ultrastructural observations. *Jaarboek van de Nederlandse Vereniging voor Microscopie*, 123 – 124.
- Vandenabeele F, Lambrichts I, Creemers J (1996) The spinal arachnoid in man: ultrastructural observations. *Proceedings Royal Microscopical Society* **31**, 148 – 149.
- Vandenabeele F, Creemers J, Lambrichts I (1996) Fine structure of the dura-arachnoid interface in human spinal meninx. *Jaarboek van de Nederlandse Vereniging voor Microscopie*, 173 – 175.
- Vandenabeele F, Creemers J, Lambrichts I (1998) Morphological observations on the Question of a naturally occuring 'subdural space' in the human spinal meninx. *Cell Biology International*, **21** (in press).

ABSTRACT

Human spinal dura and arachnoid, obtained during neurosurgical operations, were studied by transmission electron microscopy. The ultrastructure of spinal meninges largely conformed to the morphology of the cranial meninges, but some minor differences were detected.

The dura was composed of an outermost loosely arranged fibroelastic layer, a middle basically fibrous portion, and an innermost cellular layer (dural border cell layer). The dural border cell layer was characterized by multiple interdigitating cell processes, no extracellular collagen, significant extracellular spaces, and few cell junctions. Para-vascular vesiculated nerve profiles were encountered within the fibroadipose epidural tissue.

The arachnoid was composed of an outermost portion (arachnoid barrier cell layer), presenting tightly packed cells, numerous tight junctions, and no extracellular collagen. In view of its numerous tight junctions, the arachnoid barrier cell layer is considered to represent an effective morphological and physiological meningeal barrier between the cerebrospinal fluid in the subarachnoid space and the blood circulation in the dura. The arachnoid barrier cell layer was always characterized by a distinct continuous basal lamina on its inner surface toward the innermost collage-nous portion of the arachnoid (arachnoid reticular cell layer). The interweaving arachnoid trabecular cells within this layer possessed numerous mitochondria and anchored to the inner surface of the arachnoid barrier cell layer by desmosomes. An additional layer of flattened branching cells was demonstrated along the inner surface of the arachnoid reticular cell layer and assumed to be an "arachnoid border cell layer". Morphological data suggest that the dura and arachnoid closely adhere at spinal levels in man without any naturally occuring 'subdural space'. However, structurally, the dural border cell layer forms a weak cell layer at the dura-arachnoid continuum that is easily disrupted. The creation of an artifactual subdural space at spinal levels is discussed.

Key words: Meninges; cerebrospinal fluid.

INTRODUCTION

Since the initial studies of Key & Retzius (1876), Weed (1938) and Millen & Woollam (1961, 1962), the meninges enclosing the central nervous system have been considered to consist of 3 layers: the outermost dura mater, the intermediate arachnoid mater, and the innermost pia mater (Peeters et al., 1991). Recently, an additional intermediate layer has been described between the arachnoid and the pia mater by means of scanning electron microscopy (Nicholas & Weller, 1988).

The ultrastructure of the cranial and spinal meninges has been studied in different animals by Pease & Schultz (1958), Nelson et al. (1961), Andres (1966 and 1967 *a,b*), Waggener & Beggs (1967), Klika (1967,1968), Himango & Low (1971), Cloyd & Low (1974), Nabeshima et al. (1975), Malloy & Low (1976), Oda & Nakanishi (1984), Orlin et al. (1991), and Frederickson (1991). Particular attention was given to the presence of tight junctions by Andres (1967 *a,b*), Waggener & Beggs (1967), Tripathi (1973), Cloyd & Low (1974), and Nabeshima et al. (1975), in order to determine the basis for the meningeal barrier (Broman, 1949; Rodriguez-Peralta, 1957) between the blood circulation in the dura and the cerebrospinal fluid in the subarachnoid space.

Electron microscopic investigations of the human meninges have been exclusively devoted to the cranial meninges (Ramsey, 1965; Thomas, 1966; Klika, 1967, 1968; Anderson, 1969; Lopes & Mair, 1974; Rascol & Izard, 1976; Schachenmayr & Friede, 1978; Dobrovol'skii, 1984; Yamashima & Friede, 1984; Hutchings & Weller, 1986; Alcolado et al. 1988; Nicholas & Weller, 1988). In general, the spinal and cranial meninges are assumed to have a similar fine structural organization in man (Williams & Warwick, 1980). Only minor modifications are to be expected at the spinal level (Orlin et al. 1991).

The nerve supply of spinal meninges has been extensively studied (Bridge, 1959; Pedersen et al. 1956; Stilwell, 1956; Bridge, 1959; Kimmel, 1961; Edgar & Nundy, 1966; Edgar & Ghadially, 1976; Wyke, 1980; Bogduk, 1983; Groen et al. 1988). Recent immunohistochemistry revealed the presence of autonomic (Ahmed et al. 1991, 1993), and sensory (Kumar et al. 1996) innervation of spinal dura mater.

It is generally known that difficulties are encountered in obtaining intact biopsy specimens during cranial surgery. In particular, the continuity between dura and arachnoid is difficult to preserve.

The dura, being intimately adherent to the inner surface of the skull, is usually separated from the underlying arachnoid by surgical manipulation of the dura when opening the skull. However, using an in situ perfusion fixation technique immediately post-mortem, Schachenmayr & Friede (1978) obtained intact anatomical relationships in the human cranial meninges and demonstrated continuity between the dura and arachnoid. In contrast, at spinal levels, the dura is separated from the periosteum lining the vertebral canal by an interval, termed the extradural space or the 'clinical epidural space', that contains fibroadipose tissue (Williams & Warwick, 1980). The extradural space and its fibroadipose tissue form a weak plane when opening the vertebral bone during surgery. The spatial anatomical relationships between the different meningeal layers are expected to be less easily disrupted during spinal surgery.

The aim of the present account is to demonstrate the continuity between dura and arachnoid in the spinal meninges as was demonstrated for the cranial meninges using a postmortem in situ perfusion fixation technique (Schachenmayr & Friede, 1978). In addition, the present study concentrates on possible neural structures, the distribution of specialised intercellular contacts and on possible differences compared with the cranial meninges. The creation of an artifactual 'subdural space' is discussed.

MATERIAL AND METHODS

Surgical specimens from the mediodorsal portion of normal spinal meninges were obtained from 5 patients (2 men and 3 women, aged 7 - 45 y) undergoing neurosurgery at the thoracolumbar, and lumbosacral spinal levels. Clinical diagnoses included epidermoid cysts, arachnoid cyst, congenital malformation, and disc herniation. The vertebral bone and underlying periosteum were easily removed from the dura. In order to preserve intact anatomic relationships between dura and arachnoid, biopsy specimens were obtained before the dura was opened. In addition, separated specimens of dura and arachnoid were obtained when opening the dura in the surgical field, and separating the dura from the underlying arachnoid.

The specimens were immediately fixed with a solution of 2% glutaraldehyde in 0.05 M cacodylate buffer (pH=7.3), washed in 0.05 M cacodylate buffer for 10 min, and then postfixed in 2% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH=7.3) for 1 h. Following dehydration through graded concentrations of aceton, the specimens were embedded in Araldite. Semithin sections were cut on a Reichert Ultracut E microtome and stained with thionin-methylene blue (0.1% aqueous sulution) for light microscopy. Serial ultrathin sections (0.06 µm) from selected tissue blocks were mounted on 0.7% formvar-coated grids, contrasted with uranyl acetate followed by lead citrate and examined in a Philips EM 400 electron microscope.

RESULTS

Dura Mater

The dura was basically composed of fibroblasts interspersed with extracellular collagen and elastic fibres (Fig. 1a). Three distinct layers could be identified throughout its breadth. The middle and largest portion of the dura was richly vascularised and basically made up of extracellular collagen. The collagen was interwoven in laminae and oriented in various directions (Fig. 2). Individual collagenous fibrils were fairly uniform in diameter in cross section (20 - 55 nm). In longitudinal section they were cross-striated with a periodicity of 67 nm along their length. As seen in Figure 2, the extracellular collagen was intermingled with microfibrils, elastic fibres (0.2 - 1 µm), and infrequent fibroblasts. Each elastic fibre was composed of an amorphous core surrounded by a thin fuzzy layer of 10 nm microfibrils. Some parallel microfibrils were commonly seen within the amorphous substance. The fibroblasts were mainly oriented parallel to the flat axis of the dura and presented sinuous cytoplasic extensions, a flattened dense nucleus, variable amounts of mitochondria, rough endoplasmic reticulum, and pinocytotic vesicles at their surface membrane.

The middle portion of the dura lacked any type of innervation. The thin outermost portion of the dura (termed the 'outer dural border layer') with a thickness of on average 2 µm, was made up of less densely arranged extracellular collagen, intermingled with few elastic fibres and long, sinuous parallel cell processes that extended for considerable distances parallel to the flat axis of the dura (Fig. 1a). Similar cell extensions were noted along the outer aspect of this layer, forming a clear-cut boundary towards the epidural space.

The adjacent fibroadipose tissue located in the extradural space was richly vascularised. Occasionally paravascular vesiculated nerve profiles were observed in the extradural tissue (Fig. 1*b*).

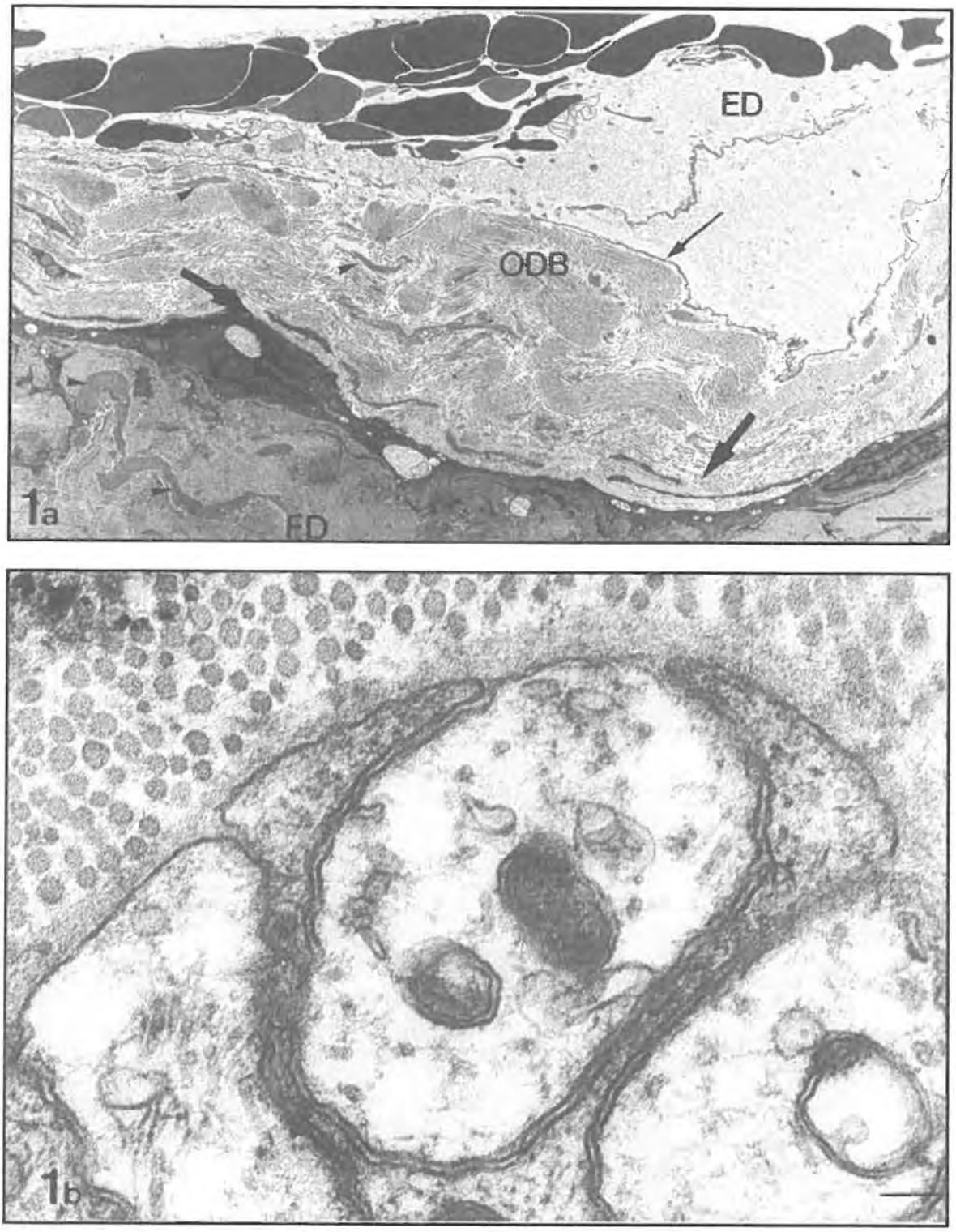


Fig. 1. (a) Outer border of the dura. Fibroelastic layer with flattened cells (large arrows), fibrous dura (FD) and elastic fibres (arrowheads). Note that the outer dural border (ODB) is bounded by thin, and long cell extensions (small arrows) towards the epidural space (ED). Bar, 20.5 µm. (b) Epidural tissue. Paravascular vesiculated nerve profile with few mitochondria, neurotubules and neurofilaments. Note the accumulation of vesicles in the naked part of the axilemma. Bar, 95 nm.

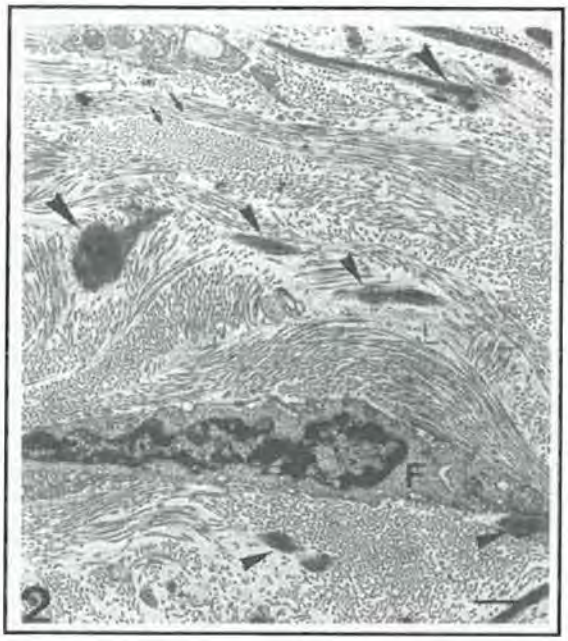
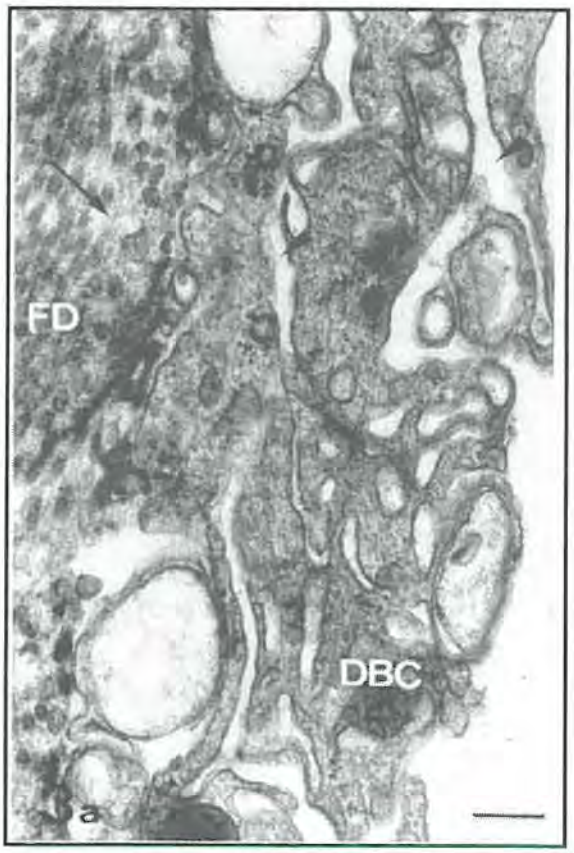


Fig. 2. Fibrous dura, composed of collagen fibrils, fibroblasts (F), and elastic fibres (arrowheads). Note lamellar organization of the collagen (small arrows). Bar, 1 µm.



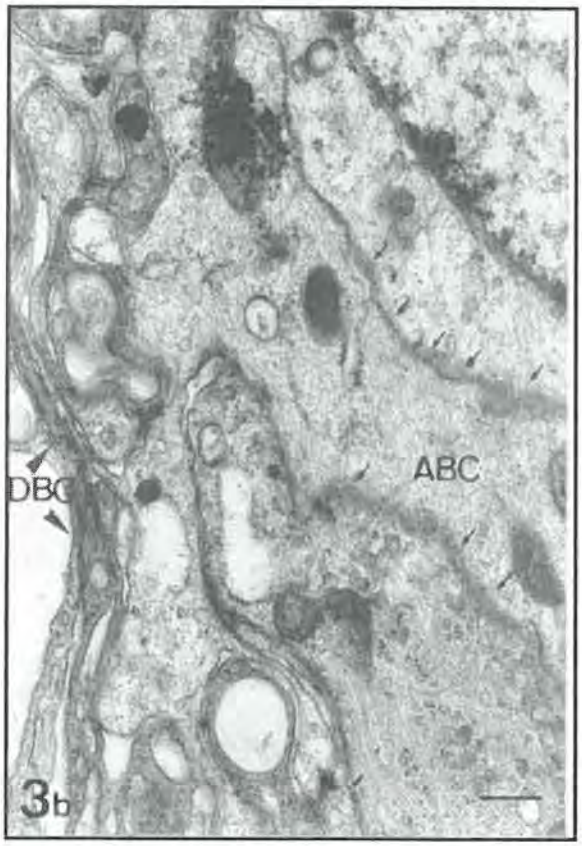


Fig. 3. Dura and archnoid separated during dissection. (a) Dura removed from the underlying arachnoid. Dural border cells (DBC), continuous with the innermost part of the fibrous dura (FD). Enlarged extracellular spaces (arrowheads) between dural border cells. Filamentous material situated in the inner dural border (arrow). Bar, 270 nm. (b) Outermost part of the arachnoid, after the dura has been removed. Cells with comparable morphology (DBC), closely apposed to the arachnoid barrier cells (ABC), are seen. Note the continuity between dural border cells and arachnoid barrier cells, and the absence of any significant intercellular space between both layers. Segments of cytoplasmic densification along the intercellular space between neighbouring arachnoid barrier cells (small arrows). Bar, 350 nm.

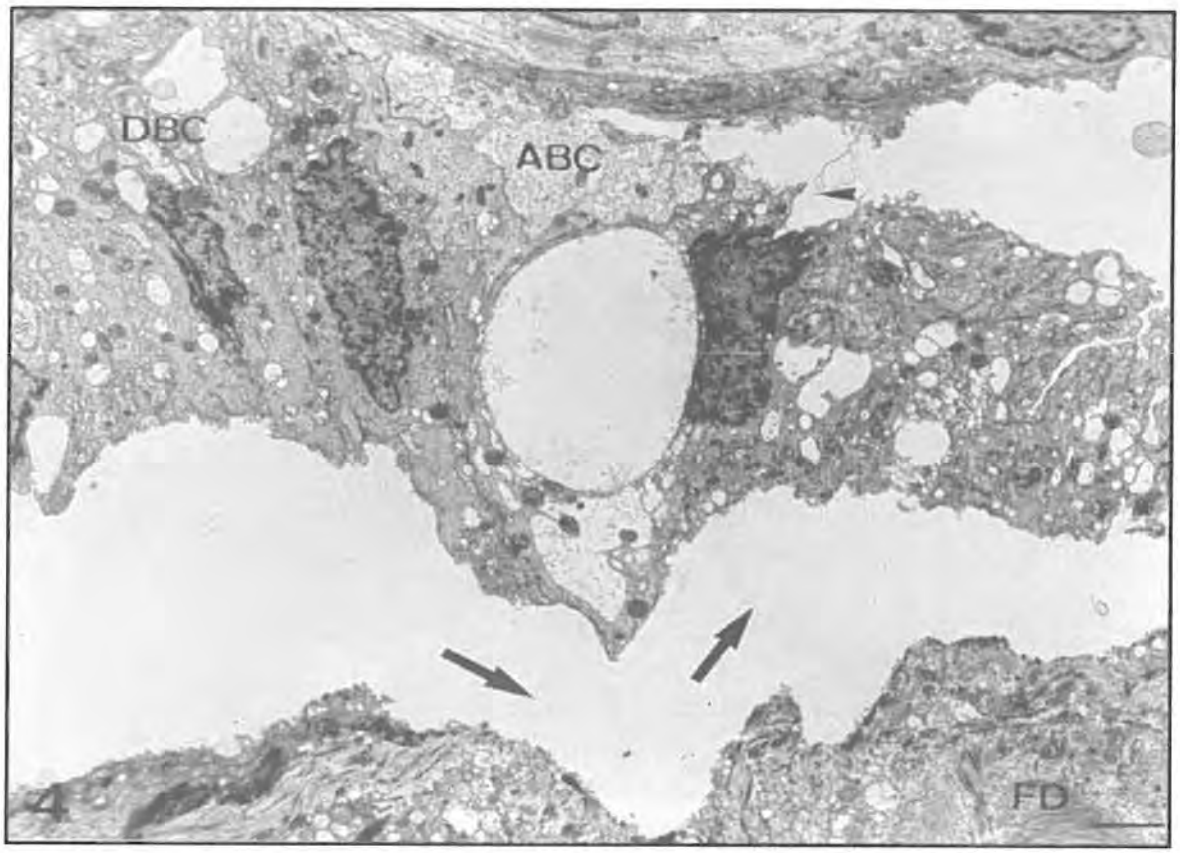


Fig. 4. Survey view of dura and arachnoid left in juxtaposition demonstrating the continuity between the dural border cells (DBC) and the underlying arachnoid barrier cells (ABC). The dura-arachnoid junction has been sheared open (large arrows) between the innermost part of the fibrous dura (FD) and the dural border cell layer. In addition, the dura-arachnoid junction has been sheared open (arrowhead) throughout the dural border cell layer, towards the archnoid barrier cell layer. Bar, 1.6 µm.

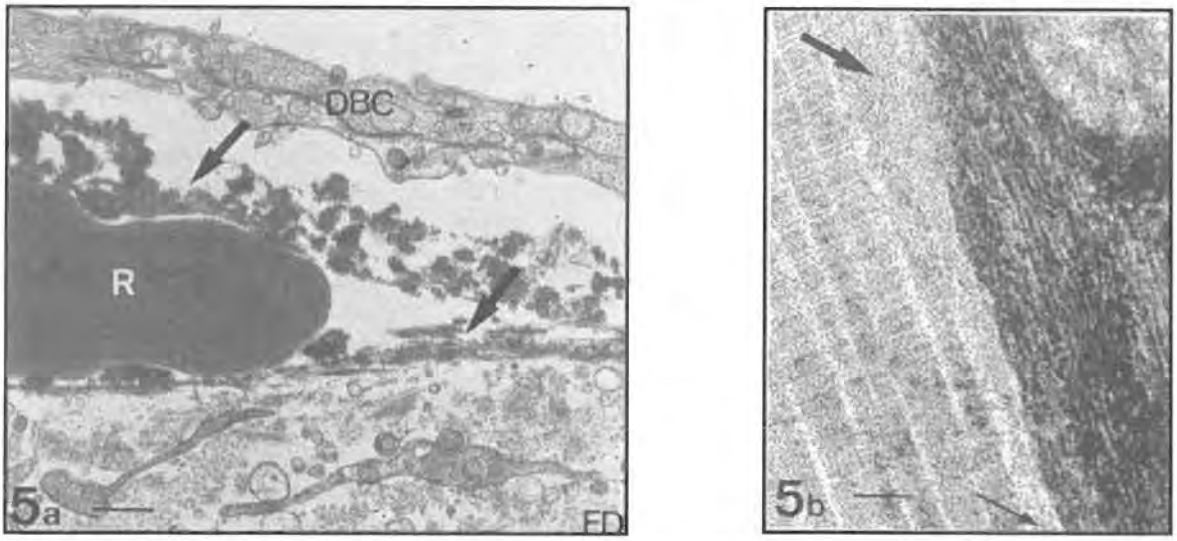


Fig. 5. Dura removed from the underlying arachnoid. (a) Fibrous dura (FD) separated from the underlying dural border cells (DBC). Detail view at the point of separation; note the presence of extracellular electron-dense filamentous material (large arrows) accompanied by red blood cells (R). Bar, 470 nm. (b) Dural border cells left in continuity with the fibrous dura. Detail view of the transition zone; filamentous material (large arrow), locally organised into basal lamina-like material (small arrow), is present. Bar, 470 nm.

Dural Border Cell Layer

When the dura was opened and separated from the underlying arachnoid its inner aspect commonly displayed one to several layers of sinuous cell processes (Fig. 3*a*). Cell processes with comparable morphology were observed at the outer aspect of the arachnoid when the dura has been removed (Fig. 3*b*), and at the dura-arachnoid junction in biopsy specimens obtained before opening of the dura (Fig. 4). Cells with similar morphological features have been variously described in different animal species and in man (Haines, 1991; Haines et al., 1993). We referred to the terminology used by Nabeshima et al. (1975), and called them dural border cells (DBC).

The cells of the DBC layer (on average 8 µm thick) were commonly closely apposed to the underlying arachnoid and the extracellular collagen of the middle fibrous portion of the dura without any significant extracellular spaces (Figs 3 *a,b*, 7). Secondly, the cells of the DBC layer firmly adhered to the arachnoid barrier cell layer (see below) by morphological distinct cell junctions (Fig. 7). However, as demonstrated in Figure 4, anatomical relationships between dura and arachnoid were often locally disrupted throughout the transition zone between the middle fibrous portion of the dura and the DBC layer, or throughout the DBC layer proper. At the point of separation between dura and arachnoid, we regularly observed extracellular filamentous electron-dense material accompanied by red blood cells (Fig. 5*a*). When the dura-arachnoid junction was disrupted throughout the DBC layer, we occasionally observed damaged cell membranes or cleaved cell junctions at the point of separation among cells (Fig. 3*a,b*).

The transition zone between the DBC layer and the fibrous middle portion of the dura was commonly characterised by the presence of filamentous material of medium electron density (Figs 5*b*, 6). Some specimens apparently lacked a DBC layer, presenting arachnoid cells in contact with the innermost part of the fibrous dura (Fig. 6). The cells of the DBC layer were dense and exhibited long flattened interdigitating cytoplasmic extensions. Their nuclei were ovoid to elongated and fairly dense. The cytoplasmic extensions of the cells in the DBC layer created multiple various-sized extracellular spaces which contained an amorphous non-fibrillar material (Figs 3 *a,b*, 7). Extracellular collagen, elastic fibres, and microfibrils were consistently absent within this layer. Specialised cell junctions (desmosomes and infrequent tight junctions) were less numerous in the DBC layer, in contrast to the innermost arachnoid. DBCs were typically attached by desmosomes and infrequent tight junctions.

A distinct basal lamina or specialised junctional structures were not observed at the interface between the middle fibrous portion of the dura and the DBC layer, nor along the extracellular spaces of the DBC layer proper. However, the filamentous material at the transition zone between the extracellular collagen of the middle portion of the dura and the DBC layer appeared regularly organised into basal lamina-like material with an average thickness of 20 - 40 nm (Fig. 5*b*).

In summary, the DBC layer is characterised by dark cells, interdigitating sinuous cell extensions, relatively few cell junctions, the presence of multiple enlarged extracellular spaces, and the absence of a collagen reinforcement.

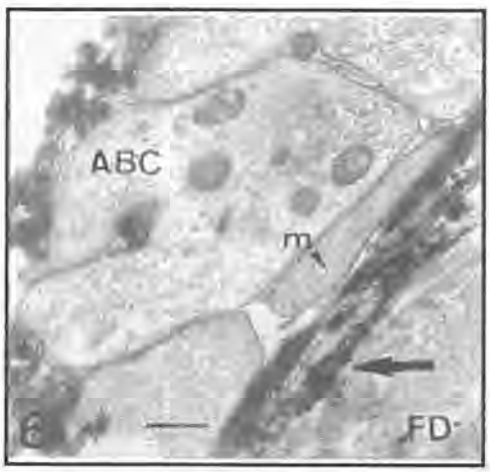


Fig. 6. Dura-arachnoid junction. In some specimens, the dural border cell layer was absent: arachnoid barrier cells (ABC) were apparantly in contact with the innermost part of the fibrous dura (FD). Large arrow, extracellular electron-dense material. m, microtubules. Bar, 360 nm.

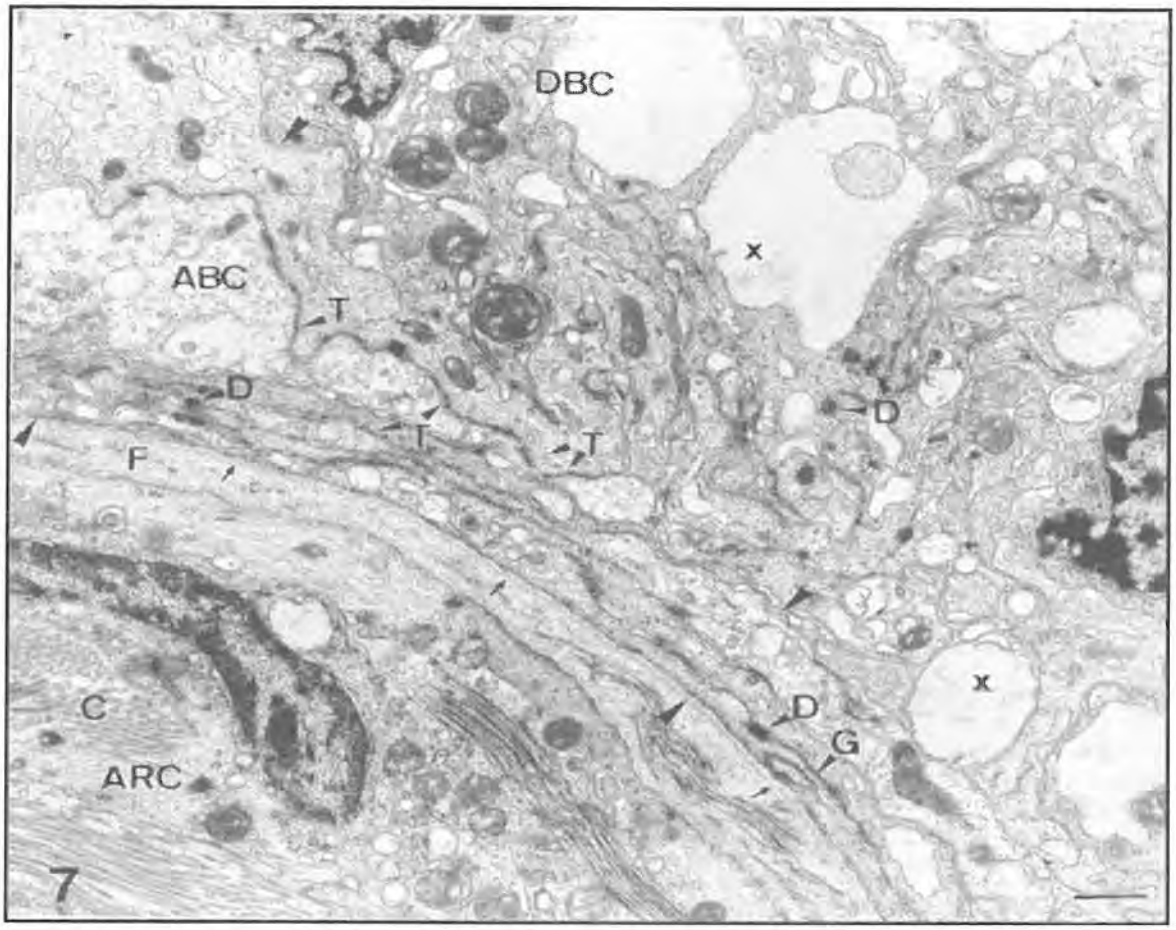


Fig. 7. Survey view demonstrating the continuity between the dural boder cell layer (DBC), the arachnoid barrier cell layer (ABC; between arrowheads) and the arachnoid reticular cell layer (ARC). Enlarged intercellular spaces between DBCs containing an amorphous nonfibrillar material (x) are present. D, desmosome; G, gap junction; T, Tight junction; small arrows, basal lamina; C, collagen. Bar, 0.5 µm.

Arachnoid Barrier Cell Layer

As seen in Figure 7, the arachnoid could be divided into an outermost layer of tightly packed interlocking cells that represents the arachnoid barrier cell (ABC) layer (Nabeshima et al., 1975), and an innermost loosely organised collagenous innermost portion that represents the arachnoid reticular cell layer (Orlin et al., 1991).

The cells of the ABC layer (average thickness 2 - 5 µm) showed relatively large amounts of electron-lucent cytoplasm in contrast to the elongated, fairly dense cells of the DBC layer (Figs 7, 8*a*). The cytoplasm of the ABCs contained vesicles, a few mitochondria, lysosomes, and varying numbers of intermediate filaments oriented at random. The mitochondria of the cells of the ABC layer were commonly smaller compared with the DBCs. Smooth-walled pinocytotic invaginations of the plasma membranes were regularly seen. Their nuclei were large, round to oval, and rather euchromatic with peripherally distributed heterochromatine (Fig. 9*c*). Their cytoplasmic extensions were compactly arranged without any significant extracellular spaces, in contrast to the enlarged extracellular spaces between the loosely arranged cells in the DBC layer. Secondly, the intercellular spaces between neighbouring cells of the ABC layer, with a width of on average 20 nm, contained no extracellular collagen, elastic fibres or microfibrils.

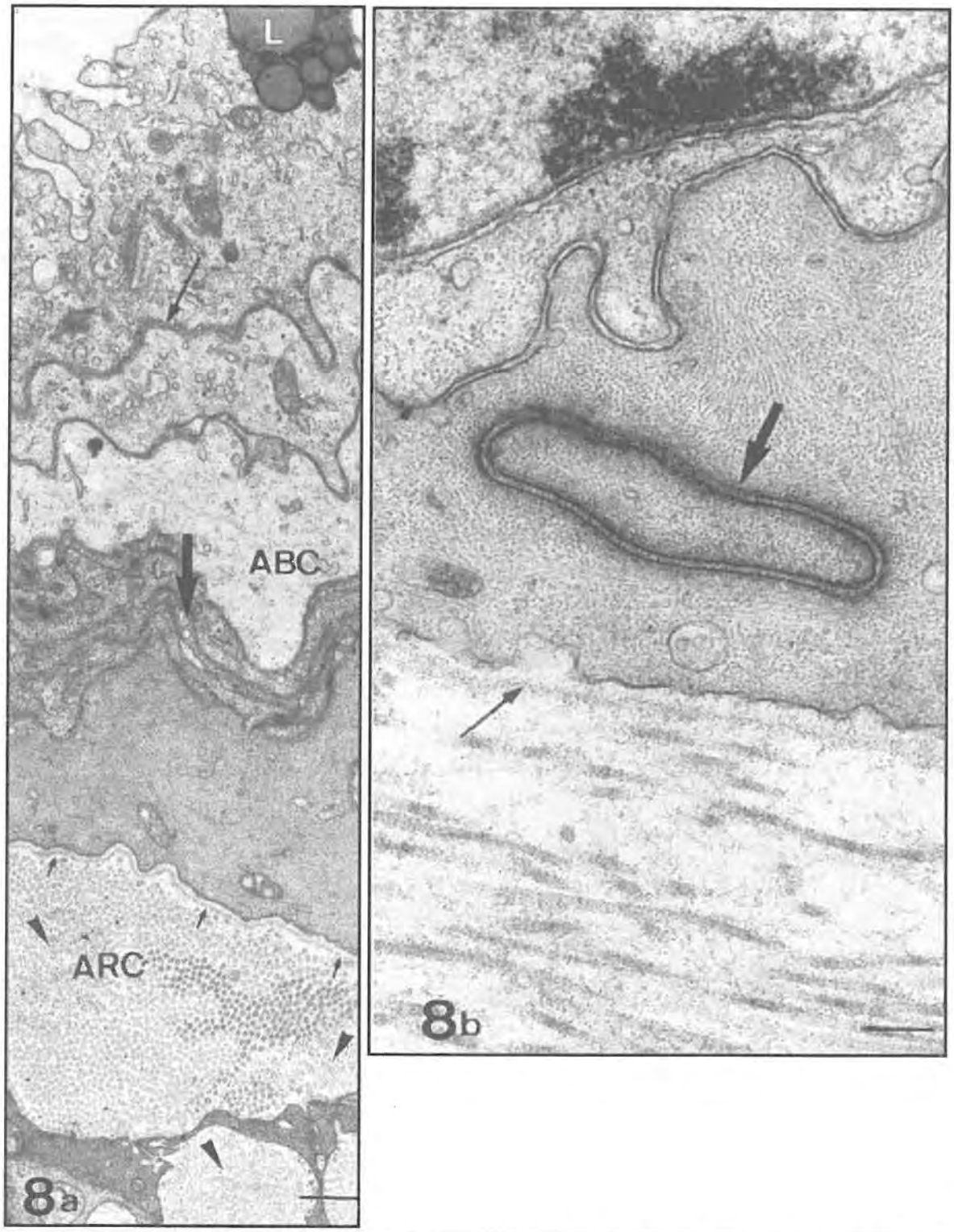


Fig. 8. Arachnoid after the dura has been removed. (a) Survey view of the arachnoid barrier cell layer (ABC). The interlocking arachnoid barrier cells present an electron-lucent cytoplasm, few cell organelles and varying numbers of intermediate filaments. The innermost cells contain numerous microfilaments and have a denser cytoplasm (large arrow). A continuous basal lamina (small arrows) forms a clear-cut demarcation for the arrachnoid barrier cell layer towards the innermost collagen-containing arachnoid reticular cell layer (ARC). Arrowheads, groups of microfibrils; L, lysosome. Note the increased density along intercellular spaces (arrow). Bar, 0.5 µm. (b) High power electron micrograph of an innermost arachnoid barrier cell presenting numerous intermediate filaments, denser cytoplasm, and pinocytotic vesicles. Note the intimate contact with a neighbouring cell (large arrow) and continuous basal lamina (small arrow). Bar, 160 nm.

A characteristic feature of the ABC layer was the presence of numerous desmosomes and tight and gap junctions. Segments of alternating cell junctions with a random pattern were regularly observed (Fig. 9*a*). In particular, the innermost tier of the ABC layer appeared well endowed with individual tight junctions and a series of morphologically distinct cell junctions between their closely apposed cell membranes. Occasionally, individual hemidesmosomes were found between the inner aspect of the ABC layer and the collagenous inner portion of the arachnoid (see below).

Commonly, the intermediate cytoplasmic filaments of the cells in the ABC layer were related to the adhering junctions and anchored within the plate-like condensed paradesmosomal cytoplasm. The condensed parajunctional cytoplasm of alternating cell junctions commonly presented as dense lines between neighbouring cells of the ABC layer (Figs 3*b*, 8*a*).

Significant variation in cytoplasmic density, cellular constitution and stratification pattern was noticed between specimens. In particular, the structural pattern of the innermost cells of the ABC layer varied considerably. Usually they presented a dense cytoplasm with numerous intermediate filaments (Fig. 8*b*). Others appeared electron lucent and contained few intermediate filaments.

The internal aspect of the ABC layer was always lined by a continuous basal lamina of about 40 nm thick (Figs 7, 8*a,b*), that formed a clear-cut demarcation towards the collagenous innermost arachnoid reticular cell layer (see below).

Arachnoid reticular Cell Layer

The cells of the arachnoid reticular cell (ARC) layer were loosely arranged and anchored by desmosomes to the inner aspect of the ABC layer (Fig. 9a). Collagen fibrils varied in diameter from 15 to 35 nm and were usually smaller compared with the extracellular collagen of the dura.

The loosely arranged, interweaving cell processes of the cells in the ARC layer extended from the ABC layer towards the inner aspect of the ARC layer and were joined to each other by infrequent desmosomes. Commonly, the cells of the ARC layer presented fairly dense cytoplasm, intermediate filaments, numerous small mitochondria with dense matrices, and elongated nuclei (Fig 7, 9*b*). The extracellular collagen of the ARC layer was organised into randomly oriented bundles and intermingled with groups of microfibrils (Fig. 9*a,b*). Infrequent thick electron-lucent cell extensions anchored by desmosomes to the ABC layer were also readily identified within the ARC layer (Fig. 9*c*). Both types of cells were lined by segments of basal lamina. Elongated cells with dense nuclei, a few mitochondria, lysosomes and long pseudopod-like cytoplasmic extensions regularly lined the inner aspect of the ARC layer (Fig. 10).

In summary, in contrast to the DBC layer, the outermost portion of the arachnoid (arachnoid barrier cell layer) lacked extracellular collagen and significant extracellular spaces, and was characterised by closely packed electron-lucent cells, numerous intermediate filaments, desmosomes, tight and gap junctions. The collagenous innermost portion of the arachnoid (arachnoid reticular cell layer) was characterised by loosely arranged cells associated with extracellular collagen and groups of microfibrils and was separated from the ABC layer by a continuous basal lamina. The DBC layer appeared firmly attached to the underlying arachnoid by morphologically distinct cell junctions, without significant extracellular spaces.

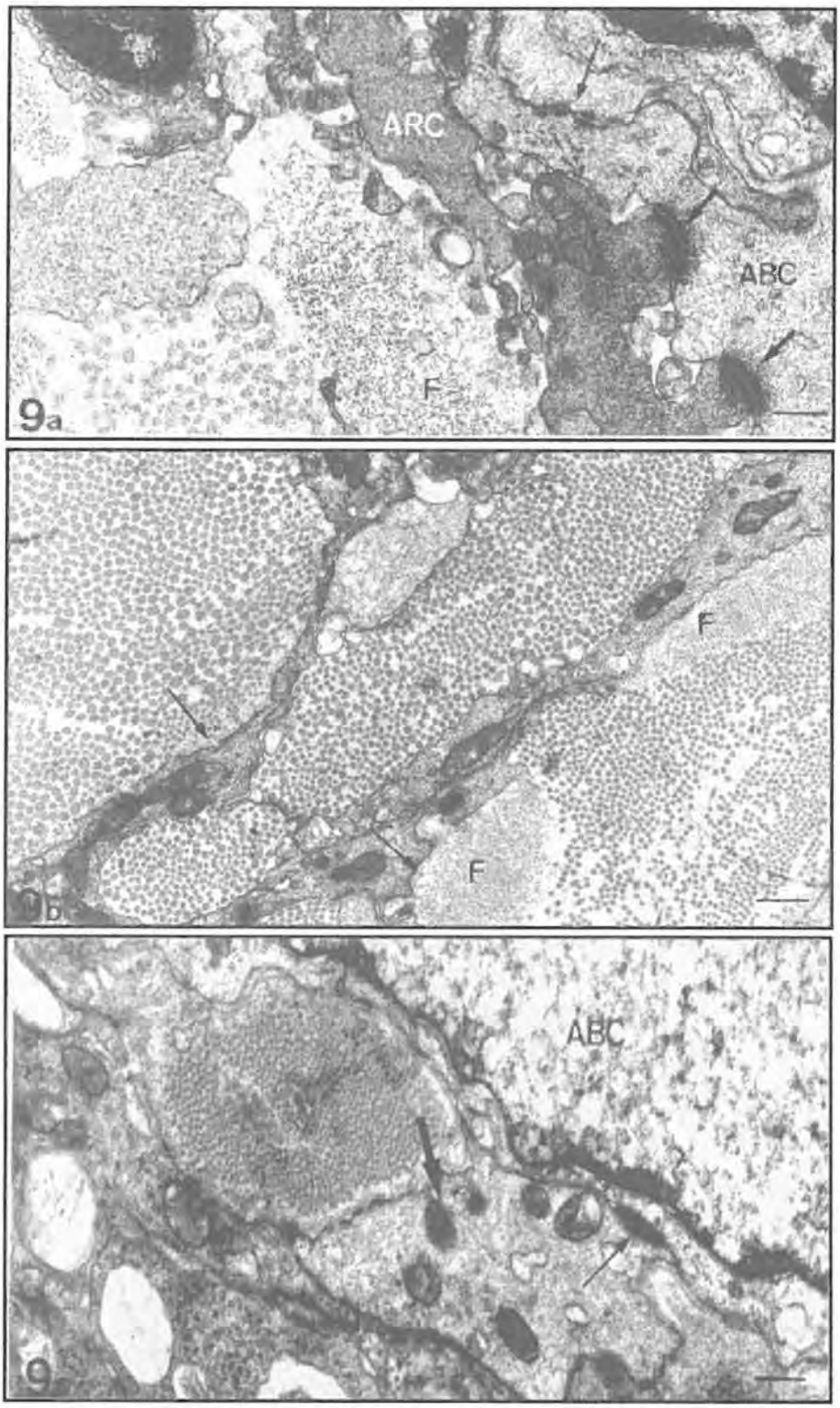


Fig. 9. Detail view of the arachnoid reticular cell layer. (a) Electron-dense arachnoid reticular cell (ARC), anchored by desmosomes (large arrows) towards electron-lucent arachnoid barrier cells (ABC). Note group of microfibrils (F) and sequence of alternating junctions between neighbouring arachnoid barrier cells (small arrow). Bar, 270 nm. (b) Network of long electron-dense cytoplasmic extensions, traversing the innermost collagenous part of the arachnoid. Note the numerous mitochondria, segments of basal lamina (arrows) and groups of microfibrils (F). Bar, 450 nm. (c) Thick, electron-lucent cytoplasmic extension (large arrow), anchored by a desmosome (small arrow) towards an innermost electron-lucent arachnoid barrier cell (ABC), presenting small mitochondria and a basal lamina. Bar, 430 nm.

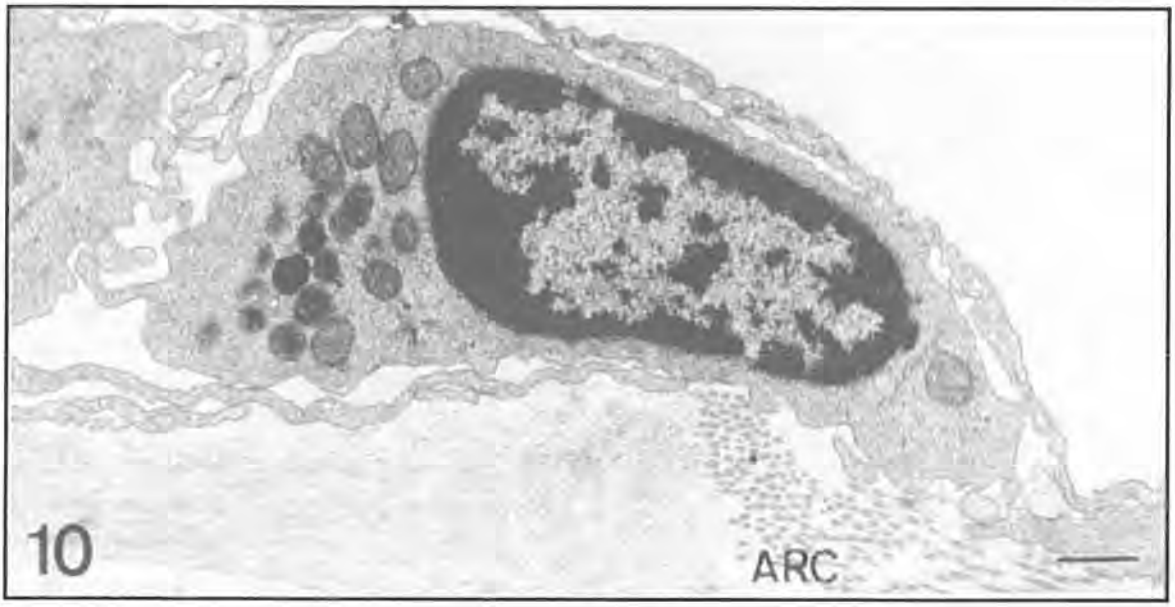


Fig. 10. Inner surface of the arachnoid reticular cell layer 5ARC). Fairly dense cell, presenting pseudopod-like cytoplasmic extensions, mitochondria, and lysosomes. Bar, 590 nm.

DISCUSSION

The spinal meninges form a tubular sheath around the spinal cord, anchored on each side to the dura by the dentate ligaments (Nicholas & Weller, 1988).

The dura is known to displace during flexion and extension movements of the spine (Lang, 1983; Tencer et al. 1985). Furthermore, it is tensed during limb movement as a result of the displacement of the spinal nerves and their dural cones in the intervertebral foramina (Sunderland, 1980). On morphological grounds, the presence of numerous elastic fibres within the fibrous dura implicates functionally considerable flexibility and elasticity when subjected to stretching and deforming forces during movements and postural changes. Secondly, the extracellular collagen, being for the most part organised into interwoven laminae and assumed to be helically wrapped around the spinal axis, provides additional tensile strenght and protects the spinal cord. Furthermore, the effect of deforming forces onto the spinal cord and its meninges is cushioned and dispersed by the subarachnoidal space and the supporting fibroadipose tissue within the extradural space.

We found infrequent paravascular vesiculated nerve profiles within the epidural fibroadipose tissue. These nerve endings are probably sensory in function. However, on morphological grounds the functional interpretation of vesiculated nerve profiles is uncertain (Vandenabeele et al. 1995). No neural structures could be identified in the present material of dorsal spinal dura. Indeed, recent histochemical studies demonstrated that the dorsal spinal dura does not have a rich innervation in contrast to the cranial dura (Groen et al. 1988; Kumar et al. 1996). Groen et al. (1988) suggested that the paucity of dorsal dural nerves correlates with clinical reports of the painlessness of dural puncture (Cyriax, 1978; Wyke, 1980). Furthermore, the specimens studied by EM are small and came from circumscribed regions of the dorsal dura. According to Kumar et al. (1996), the spinal dura serves primarily as a protective membrane enclosing the spinal cord and cerebrospinal fluid. They suggested that the spinal dura plays a limited role in the pathogenesis of pain, in contrast to the cranial dura which is known to be richly innervated.

The DBC layer (in the terminology of Nabeshima et al. 1975) forms the innermost portion of the dura. It has been extensively studied and variously classified by others (Haines, 1991; Haines et al. 1993). In studying cranial meninges, attention was first focused by Schachenmayr & Friede (1978) on the complete absence of formed extracellular connective tissue within this layer. Our study clearly demonstrates that the spinal DBC layer is composed of sinuous and interdigitating cells that create significant extracellular spaces. Structurally, it closely resembles the DBC layer of cranial meninges in man (for review, see Haines et al. 1993). While the DBC layer in most animal species has been described as an orderly thin layer composed of parallel flattened cells (see Introduction), it is more substantial in man. Furthermore, the present account clearly demonstrates the continuity between dura and arachnoid. Secondly, morphological data suggest that the spinal meninges do not include a 'naturally occuring' subdural space at the dura-arachnoid continuum. Our morphological data conform to the morphology of the cranial dura-arachnoid in man (Schachenmayr & Friede, 1978).

The DBC layer, being characterised by relatively few cell junctions, no extracellular collagen and multiple enlarged extracellular spaces, has been suggested as being the structurally weakest plane at the dura-arachnoid continuum (Haines & Frederickson, 1991; Haines et al. 1993). Indeed, our study clearly demonstrates that, even at spinal levels, the DBC layer is easily disrupted during surgery, creating an artifactual 'subdural space'. Similar difficulties were encountered by others when obtaining surgical specimens of cranial meninges from living subjects (see Introduction). Furthermore, recent morphological, clinical and experimental data (Frederickson, 1991; Haines & Frederickson, 1991; Orlin et al. 1991; Haines et al. 1993; Ralph & Williams, 1996) demonstrated that such an 'artifactual subdural space' can be created by a pathological process (e.g., by a 'subdural' haematoma), by an accidental process (e.g., by an accidental 'subdural' injection of a local anaesthetic during epidural analgesia) or by a traumatic insult (e.g., manipulation of the dura in the surgical field).

The present morphological data suggest that the dura and arachnoid were usually cleaved by a shearing open of the enlarged extracellular spaces within the structurally weak DBC layer. The electron dense fibrillar material found within the artifactual subdural space at the point of separation of dura and arachnoid, is most probably fibrin as it is closely associated with red blood cells. Desmosomes and tight junctions were not found in any significant numbers between DBCs. This is in agreement with previous reports by Nabeshima et al. (1975) and Haines et al. (1993).

In contrast to the DBC layer, the ABC layer presented closely packed interdigitating cells with numerous tight junctions and without significant extracellular spaces. The most characteristic feature of the ABC layer is the presence of varying numbers of intermediate filaments. It is generally accepted that this layer, with its numerous tight junctions, represents an effective morphological and functional meningeal barrier between the blood circulation in the dura and the cerebrospinal fluid in the subarachnoid space (Nabeshima et al. 1975; Schachenmayr & Friede, 1978; Haines, 1991; Haines et al. 1993). The organisation of the arachnoid has been variously described (Lopes & Mair, 1974; Nabeshima et al. 1975; Schachenmayr & Friede, 1978; Oda & Nakanishi, 1984; Alcolado et al. 1988; Frederickson et al. 1991). As classically identified, it consists of 2 cellular portions: an outermost compact ABC layer and a loosely organised innermost portion that represents the 'arachnoid trabeculae' of the subarachnoid space that transverse the subarachnoid space from the arachnoid to the pia mater (Williams & Warwick, 1980; Alcolado et al. 1988; Haines & Frederickson, 1991).

The subarachnoid space contains extracellular collagen, situated free in the subarachnoid space or associated with the 'arachnoid trabecular cells' (Haines & Frederickson, 1991; Haines et al. 1993). The trabecular cells are assumed to form trabeculae that transverse the subarachnoid space from the arachnoid to the pia mater (Williams & Warwick, 1980; Alcolado et al. 1988). Our study, however, clearly demonstrates that the collagen content of the arachnoid is more substantial at spinal levels in man and structurally forms a distinct layer. According to Orlin et al. (1991), we emphasized this collagenous innermost portion of the arachnoid as the arachnoid reticular cell (ARC) layer, implicating structurally the extracellular collagen as a mechanical reinforcement of the arachnoid.

The sinuous cells found along the inner surface of the ARC layer in the present account have been termed 'arachnoid border cells'. Although similar cells have been described by Alcolado et al. (1988) in the cranial arachnoid, the exact nature of this layer is uncertain. It is not known whether these flattened cells actually represent arachnoid cells or cells of the pia that remained adherent to the trabecular cells. On morphological grounds, it has been suggested that cells of the arachnoid trabeculae are indistinguishable from cells of the pia mater (Haines, 1991).

The organisation of the loosely arranged leptomeningeal cells that traverse the subarachnoid space structurally conforms to the 'intermediate leptomeningeal layer' demonstrated by scanning electron microscopy on the dorsal aspect of the spinal cord in man (Nicholas & Weller, 1988; Orlin et al. 1991).

The distinct continuous basal lamina found along the entire inner surface of the ABC layer formed a clearcut boundary between the ABC layer and the ARC layer. The latter is known to be a characteristic morphological feature of the ABC layer (Haines et al. 1993). The basal lamina is described as discontinuous in several animal species (see Introduction).

The arachnoid and DBC layer lacked blood vessels. According to Orlin et al. (1991) they are nourished either via the cerebrospinal fluid in the subarachnoid space or via the blood circulation in the dura. An electron-dense line has been observed at the dura-arachnoid interface in different animal species (Pease & Schultz,1958; Waggener & Beggs, 1967; Nabeshima et al. 1975). No comparable line could be identified in the human cranial meninges (Rascol & Izard, 1972; Schachenmayr & Friede, 1978;). However, we regularly observed segments of densified para-junctional cytoplasm associated with alternating cell junctions between neighbouring cells of the ABC layer. This could conform to the erroneous impression of a dense line mentioned by others (see Introduction).

In summary, the fine cytostructural characteristics and organization of the spinal meninges presented in this study largely conform to the structure of cranial meninges in man. Furthermore, the collagen content of the arachnoid appeared more substantial at spinal levels. Its possible role as a mechanical reinforcement of the arachnoid is discussed. Morphological data suggest an intimate fusion between the innermost portion of the dura (DBC layer) and the outermost portion of the arachnoid (ABC layer). The dural border cell layer is characterised by multiple enlarged extracellular spaces, absence of a collagenous reinforcement and few intercellular contacts, implicating structurally a lack of cohesion within this layer. This weak zone is easily disrupted, creating an artefactual subdural space. The ABC layer is considered to be the only effective meningeal barrier in view of its numerous tight junctions.

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CHAPTER 3

The Extrinsic and Intrinsic Nerve Supply of the Human Lumbar Spine

Chapter 3.1

The Extrinsic Nerve Supply of the Human Lumbar Spine

The tissues in the lumbar spine have a complex pattern of innervation. The extrinsic nerve supply is triple and includes: 1) the sinu-vertebral nerves, 2) branches derived from the lumbar plexus associated with the anterior and the posterior ligaments and 3) the lumbar spinal rami. This chapter is predominantly based on Williams (1995), Bogduk (1980, 1983), Bogduk et al. (1982), Groen et al. (1990), Kojima et al. (1990).

Origin and Distribution

Sinu-vertebral nerves

Sinu-vertebral nerves (SVN's) were first described by Luschka (1850). Descriptions on the origin, branching pattern and extension of the sinu-vertebral nerves are contradictory. SVN's have been reported to be recurrent branches of the ventral ramus of the spinal nerve (Roofe, 1940; Wyke, 1970, 1980; Kojima et al. 1990), but others consider that they originate from the union of a somatic root from the ventral ramus of the spinal nerve and an autonomic root from the grey ramus communicans (Wiberg, 1949; Pedersen et al. 1956; Stilwell, 1956; Bridge, 1959; Edgar & Nundy, 1966; Edgar & Ghadially, 1976; Bogduk et al. 1981; Bogduk, 1983). According to Groen et al. (1988) the SVN's originate from the rami communicantes (RC) just anterior to the spinal nerve. The RC's form (by definition) the connections between the spinal nerve and the sympathetic ganglia.

Lumbar Plexus (see Fig. 1)

The entire column of vertebral bodies and intervertebral discs is surrounded by a continuous interlacing network of nerve fibres. This network consists of a ventral and dorsal portion, interconnected by branches of rami communicantes at the level of the intervertebral foramina. The *ventral nerve plexus* is associated with the anterior longitudinal ligament (and innervates the ligament throughout its entire length.) The *dorsal nerve plexus* is associated with the posterior longitudinal ligament.

In the lumbar region, the nerve plexus of the anterior longitudinal ligament recieves bilateral contributions from 1) the sympathetic trunk, 2) the rami communicantes, 3) interconnections between both sympathetic trunks, and 4) from the perivascular nerve plexuses of segmental arteries. The contributing nerves to the anterior lumbar nerve plexus run deeply to the psoas major muscle.

The nerve plexus of the posterior longitudinal ligament receives bilateral contributions from the sinu-vertebral nerves over the entire length of the vertebral column.

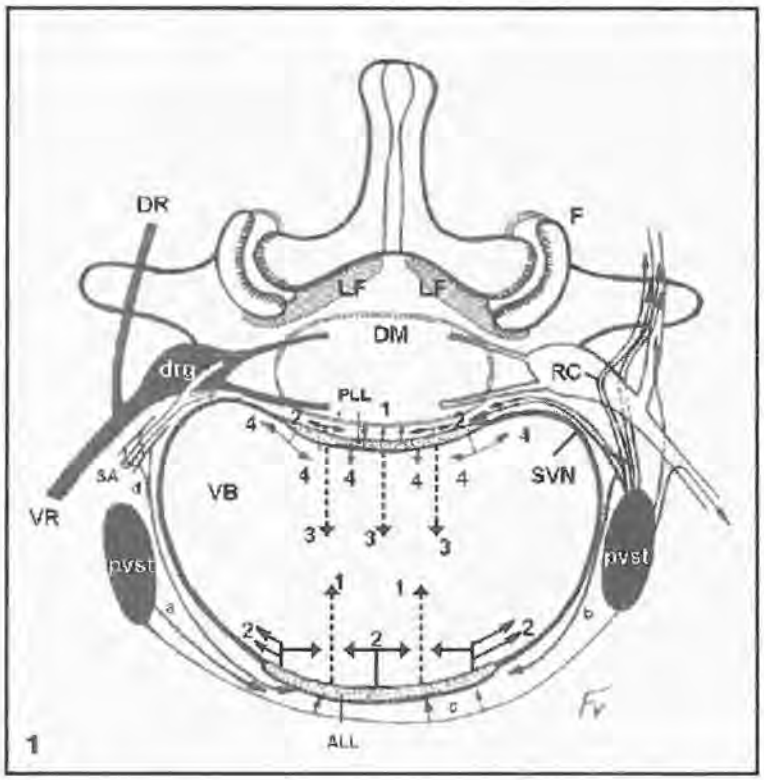

Fig. 1. Diagram illustrating the origin and the termination pattern of the extrinsic nerve supply to the human lumbar spine, based on Groen et al. (1990).
The dorsal nerve plexus associated with the posterior longitudinal ligament (PLL) is supplied bilaterally by the sinu-vertebral nerves (SVN).
The ventral nerve plexus associated with the anterior longitudinal ligament (ALL) receives bilateral contributions from (a) the paravertebral sympathetic trunks (pvst), (b) the rami communicantes (RC), (c) interconnections between pvst, and (d) from the nerve plexus associated with the segmental artery (SA).
The nerve plexus associated with the ALL gives of branches towards (1) the cancellous bone of the vertebral body (VB) and (2) the outer annulus fibrosus.
The nerve plexus associated with the PLL gives branches to (1) the ventral spinal dura mater (DM), (2) the epidural blood vessels, (3) the cancellous bone of the VB, and (4) the outer annulus fibrosus. Ligamentum flavum (LF), facet joint (F), dorsal root ganglion (drg), ventral ramus (VR), dorsal ramus (DR).

Rami of the Spinal Nerves (see Fig. 2)

Each spinal nerve divides in a thick ventral ramus and a thinner dorsal ramus in the intervertebral foramen. The anatomy of the L1-4 dorsal rami and L5 dorsal ramus are different. The L1-4 dorsal rami are about 5 mm long and run dorsocaudally through the intervertebral foramina. They enter the dorsal compartment through a foramen in the dorsal leaf of the intertransverse ligament and divide in a medial, intermediate and lateral branch. The L5 dorsal ramus lacks a lateral branch. It is much longer, follows the rostral and dorsal surfaces of the ala of the sacrum, and divides into a medial and an intermediate branch. Each medial branch passes dorsally and caudally through the intertransverse space, curves around the subadjacent superior articular process in a groove between the mamillary process and accessory process that is bridged by the mamillo-accessory ligament, and finally crosses the vertebral lamina medially and caudally, deep to the multifidus muscle.

Termination Pattern

Ventral Compartment

The deep tissues of the ventral compartment of the lumbar spine (ventral dura mater, intervertebral discs, longitudinal ligaments, prevertebral and intertransverse muscles), are innervated by branches derived from the ventral rami or from the plexus associated with the longitudinal ligaments (Fig. 1).

The *lumbar ventral rami* supply the prevertebral muscles (psoas major, psoas minor, and quadratus lumborum muscles) and the intertransverse muscles. Notably, motor branches to the psoas muscles have been suggested as a possible sensory pathway innervating the posterolateral portion of the IVD (Jinkins et al. 1989; Takahashi et al. 1993).

The *nerve plexus of the anterior longitudinal ligament* gives of branches 1) that penetrate the vertebral bodies along radially arranged blood vessels and 2) towards the outer annulus fibrosus.

The *nerve plexus of the posterior longitudinal ligament* gives of branches to 1) the ventral spinal dura, 2) the epidural blood vessels, 3) the vertebral bodies and 4) the outer annulus fibrosus.

The ventral spinal dura is also directly innervated by sinu-vertebral nerves. The entire vertebral column and associated structures is thus supplied with sympathetic fibres, known as the paravertebral autonomic nerve plexus (Jinkins et al. 1989).

Dorsal Compartment

The dorsal compartment is innervated by branches of the *lumbar dorsal rami*. They have a muscular and skeletal distribution (Fig. 2).

Muscular distribution

The lateral branches of the L1-4 dorsal rami, innervate the iliocostalis lumborum muscle, whereas the intermediate branches innervate the lumbar portions of the longissimus thoracis muscle. Both muscles have separate attachments to the lumbar vertebrae. They are separated by an intermuscular aponeurosis and constitute the lumbar erector spinae muscle, which arises from the erector spinae aponeurosis. The lateral branches of the L1-3 dorsal rami innervate the lumbodorsal fascia, pierce its dorsal layer and become cutaneous, innervating the skin over the lateral buttock. The L4 lateral branch remains intramuscular. The medial branches of the lumbar dorsal rami innervate the multifidus, interspinales, and intertransversarii mediales muscles. The multifidus muscle is the third major longitudinal muscle system in the lumbar spine. The L5 dorsal ramus lacks a lateral branch. Its second branch is interpreted as an intermediate branch and innervates the lumbar portion of the longissimus thoracis muscle, whereas its medial branch innervates the multifidus muscle.

Skeletal distribution

Besides their muscular distribution while crossing the vertebral lamina, the medial branches innervate the rostrally and caudally related facet joints and the adjacent interspinous muscle and ligament. The rostrally related facet joint is innervated at its caudal aspect, whereas the caudally related facet joint is innervated at its rostral aspect. Each facet joint thus receives medial branches from at least two consecutive spinal segments. Some investigators also describe a medial branch to the facet above. The presence of nerve endings in the ligamentum flavum, forming the anterior capsule of the facet joint, is uncertain. If present, they most likely would originate from the medial branches of the dorsal rami.

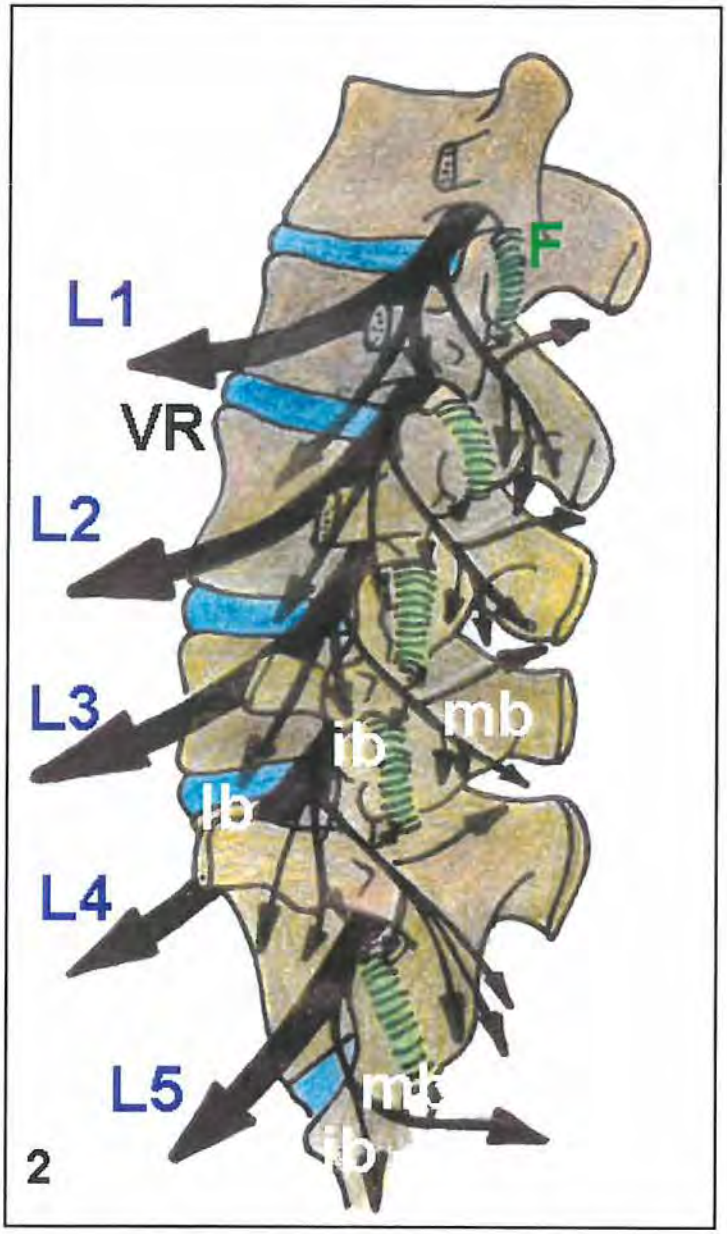

Fig. 2. Termination pattern of the lumbar dorsal rami.
Each spinal nerve divides in a thick ventral (VR) and a thinner dorsal ramus. The L1-4 dorsal rami divide in a medial (mb), intermediate (ib) and lateral (lb) branch. The L5 dorsal ramus lacks a lateral branch.
The facet joint (F) is innervated by the medial branches of the dorsal rami from at least two consecutive spinal nerves.

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Chapter 3.2

The Intrinsic Nerve Supply of the Human Lumbar Spine

Morphology, distribution, and neuropeptides

This chapter contains the light-, and electron microscopic and immunocytochemical observations on the occurence, distribution and morphological characteristics of the intrinsic nerves in the ventral and dorsal compartment of the lumbar spine. Literature data are complemented with personal observations, except for our ultrastructural and immunocytochemical studies of the facet joints and supra- and interspinous ligaments, that are described in Chapter 4.
The functional implications of important neurohistological observations are briefly discussed.

VENTRAL COMPARTMENT

The following section reports on the neurohistology of spinal tissues anterior to the virtual plane through the intervertebral foramina, referred to as the ventral compartment (Bogduk, 1983). The intervertebral disc (IVD), the posterior longitudinal ligament (PLL), the ventral spinal dura mater, the vertebral bone and periosteum and the anterior longitudinal ligament (ALL), are successively described.

Intervertebral Disc

Occurrence, distribution, morphology and origin of neural elements

The early reports on the innervation of intervertebral discs are contradictory. Some authors found that the annulus fibrosus lacks any type of innervation (Jung & Brunschwig, 1932; Wiberg, 1949; Ikari, 1954; Pedersen et al. 1956). Roofe (1940) and Ehrenhaft (1943) concluded that the superficial layers of the annulus fibrosus are richly innervated. Tsukada (1938,1939) stated that the nucleus pulposus is innervated. It is generally agreed that the lumbar intervertebral disc (IVD) is innervated in its outer fibrocartilagenous zone (Roofe, 1940; Malinsky, 1959; Hirsch et al. 1963; Jackson et al. 1966; Shinohara, 1970; Yoshizawa et al. 1980; Bogduk et al. 1981; Bogduk, 1983; Groen et al. 1990; Kojima et al., 1990). Noncorpuscular 'free' nerve endings, as well as corpuscular (encapsulated) nerve endings have been shown in the IVDs (Malinsky, 1959; Hirsch et al. 1963; Jackson et al. 1966; Shinohara, 1970; Yoshizawa et al. 1980; Coppes et al. 1990; Kojima et al. 1990)

Light microscopical immunohistochemistry showed noncorpuscular 'free' nerve endings reacting with sensory and autonomic neuronal markers in the outer annulus fibrosus of lumbar spine in rats (Ahmed et al. 1991; McCarthy et al. 1991; Ahmed et al. 1993), and also in man (Yoshizawa et al. 1980; Coppes et al. 1990; Konttinen et al. 1990; Ashton et al. 1994; Palmgren et al. 1996; Brown et al. 1997). Konttinen et al. (1990) demonstrated a co-localization of the sensory neuronal markers substance P (SP) and calcitonin gene-related peptide (CGRP).

Roberts et al. (1995) found Golgi tendon-like and Ruffini-like endings in the outer annulus fibrosus of human IVD's, showing immunoreactivity to CGRP, neuropeptide Y (NPY) and SP. Immunoreactivity to CGRP was most frequently seen, with occasional staining for SP and NPY. CGRP mediates sensory modalities, including nociception. Brown et al. (1997) noted an increase in the density of sensory nerve fibres (especially for CGRP), as well as of sympathetic nerve fibres (NPY), in the outer annulus fibrosus, of degenerated IVD's, accompanying neovascularization. The outer annulus fibrosus of lumbar IVD's in human fetal spine – likely prototypes of healthy, non-degenerated discs- has been reported to be scarcely innervated (Vandenabeele et al., 1998).

The innervation of the nucleus pulposus is also debated. Occasional reports are found in the literature on the innervation of the nucleus pulposus, using classical neurohistological staining techniques (Tsukada, 1938,1939). In various classical neuroanatomic studies the nucleus pulposus has been described as devoid of any type of innervation (Wiberg, 1949; Pedersen et al. 1956; Yoshizawa et al. 1980). However, Shinohara (1970) reported nerve endings in infiltrated granulation tissue in the nucleus pulposus of patients with degenerative disc disease, supporting studies of Hirsch & Schajowicz (1952), and Hirsch et al. (1963). Palmgren et al. (1996) demonstrated immunohistochemically, the presence of sensory and autonomic nerve terminals in herniated lumbar disc tissue, in close proximity to disc cells (Palmgren et al. (1996). Palmgren and coworkers suggested that the nucleus pulposus - normally without neural elements, is infiltrated by neural elements accompanying vascularized granulation tissue in degenerative disc disease. Most neuroimmunohistochemical studies failed to demonstrate neural elements in the human nucleus pulposus, in the adult (Coppes et al. 1990; Konttinen et al. 1990; McCarthy et al. 1991; Ashton et al. 1994; Brown et al. 1997), and in fetal spine (Vandenabeele et al. 1998),

Fig. 1. Light micrograph of a Mayer's hematoxylin-stained transverse paraffin section of the annulus fibrosus in the lumbar region of the human fetal spine. The arrows point to S100-immunoreactive nerves (general neuroglial marker) in the outer lamellae of the annulus fibrosus (AF). Paravertebral sympathetic trunk (PVST). BAR = 200 µm.

The origin of nerves supplying the lumbar IVD is of particular interest. Different nerves innervate specific portions of the lumbar IVD. The posterior portion is innervated by the SVNs and the plexus associated with the PLL, originating in the sympathetic trunks (Groen et al. 1990; Nakamura et al. 1996a). The anterior portion receives a 'dual' peripheral innervation: from (1) the paravertebral sympathetic nerve plexus associated with the ALL (Groen et al. 1990; Gilette et al. 1994) and (2) anterior branches from the lumbar ventral rami, probably of minor importance (Takahashi et al. 1993). The lateral portion is innervated by posterolateral branches from the ventral ramus of the spinal nerve (Jinkins et al. 1989).

The innervation of the lumbar IVDs has been reported to be multisegmental and bilateral (Groen et al. 1990; Morinaga et al. 1996; Nakamura et al. 1996a,b).

Functional significance

The concept of discogenic pain is accepted by many clinicians and has long been regarded as the main source of low back pain (Mooney, 1987). Electrophysiological and clinical studies corroborate this concept (Hirsch, 1949; Wiberg, 1949; Smyth & Wright, 1958; El Mahdi et al. 1981; Vanharanta et al. 1987; Kuslich et al. 1991; Gilette et al. 1993; Kääpä et al. 1994; Schwarzer et al. 1994; Nakamura et al. 1996b). It is generally accepted that discogenic pain in the lower back is evoked by stimulation of the outer annulus fibrosus and the PLL, whereas leg pain or sciatica is induced by mechanical stimulation causing inflammatory reactions in the nerve roots (Wiberg, 1949; Smyth & Wright, 1958; Kuslich et al. 1991).

The vasomotor and vasosensory innervation of the IVD are also involved in neurogenic inflammation, tissue repair processes and local vasoregulation, especially when the discs are degenerated and neovascularized (Palmgren et al. 1996, Brown et al. 1997). Neuroanatomical studies suggest that focal increases in the innervation of vascularised degenerated discs make them highly sensitive to mechanical stimuli, causing abnormal painful responses to normal stimuli (Hirsch & Schajowicz, 1952; Hirsch et al. 1963; Grönblad et al. 1991; Palmgren et al. 1996). The normal IVD is avascular (Crock & Goldwasser, 1984) and nourished by diffusion from vessels in the vertebral endplates (Brown et al. 1997). According to Brown and coworkers, disturbances in the neural control of vasomotor nerves and/or cartilage defects in the vertebral endplate might limit disc nutrition, followed by disc degeneration and neovascularisation. In most studies on the innervation of human IVDs, study material was obtained from patients with severe degenerative disc disease undergoing neurosurgery. It would be interesting to know the innervation of healthy, non-degenerated IVDs in young human subjects. Vandenabeele et al. (1998) demonstrated a scarce innervation of the outer annulus fibrosus and the absence of any neuronal elements in the nucleus pulposus in fetal lumbar spine (Fig. 1). Further neurohistological studies of the IVD are necessary, particularly in 'normal' human material and at different ages.

The corpuscular endings found in the outer annulus fibrosus of the lumbar spine are assumed to be primarly involved in sensation of posture, in movements and in maintaining muscle tone in the vertebral column (Roberts et al. 1995). As Roberts and coworkers found a greater incidence of mechanoreceptors in patients with low back pain compared with pain-free patients, they were tempted to speculate that corpuscular nerve endings in the IVDs might have a nociceptive function and are involved in spinal pain transmission. The involvement of the corpuscular endings in monitoring pain transmission in the IVD is not apparent.

Patient's complaints and physical signs in lower lumbar disc lesions are variable. Low back pain is often diffuse and ill-defined. Afferent pathways of discogenic low back probably follow 'sympathetic' routes, and the mechanism of spinal pain transmission may be similar to that of visceral pain, that is also poorly localised (Malliani et al. 1986; Procacci et al. 1986; Ness & Gebhart, 1990).

Discogenic low back pain can be referred pain (Nakamura et al. 1996a,b), as illustrated in diagram 1. The L1 and L2(L3) dermatomes of Foerster (1933) are the dermatomes mainly corresponding to the lower back. Indeed, the dorsal rami of L4 and L5 do not have any dorsal cutaneous branches (Bogduk 1983; Williams 1995). Clinical and experimental studies demonstrated that afferent pathways of lower lumbar discs run nonsegmentally through sympathetic afferents in the sympathetic trunk connecting with L2 or higher levels (White, 1955; Takahashi et al. 1993,1995; Morinaga et al. 1996; Nakamura et al. 1996a,b). Electrophysiological (Jänig and McLachlan 1986; Gilette et al. 1994) and immunohistochemical (Suseki et al. 1996; Vandenabeele et al. 1998) studies also prove that paravertebral sympathetic nerves in the lumbar region are nociceptive in function.

Discogenic low back pain arising from the lower lumbar IVDs, is thus transmitted through the second lumbar spinal nerve and perceived as referred pain in the L2 dermatome: (1) commonly in the skin of the low back and buttock, which is supplied by cutaneous branches of the dorsal rami of L1L2(L3) spinal nerves (superior clunial nerves), (2) sometimes in the groin supplied by the anterior cutaneous branches of iliohypogastric nerve, anterior scrotal branches of ilioinguinal nerve, and femoral branches of genitofemoral nerve, derived from the ventral rami of the L1L2 spinal nerve, or (3) in the anterolateral thigh supplied by the lateral femoral cutaneous nerve, derived from the ventral ramus of the L2 spinal nerve. Only seldom is the pain referred to the medial side of the thigh and leg, at the site of the L3 and L4 dermatomes.

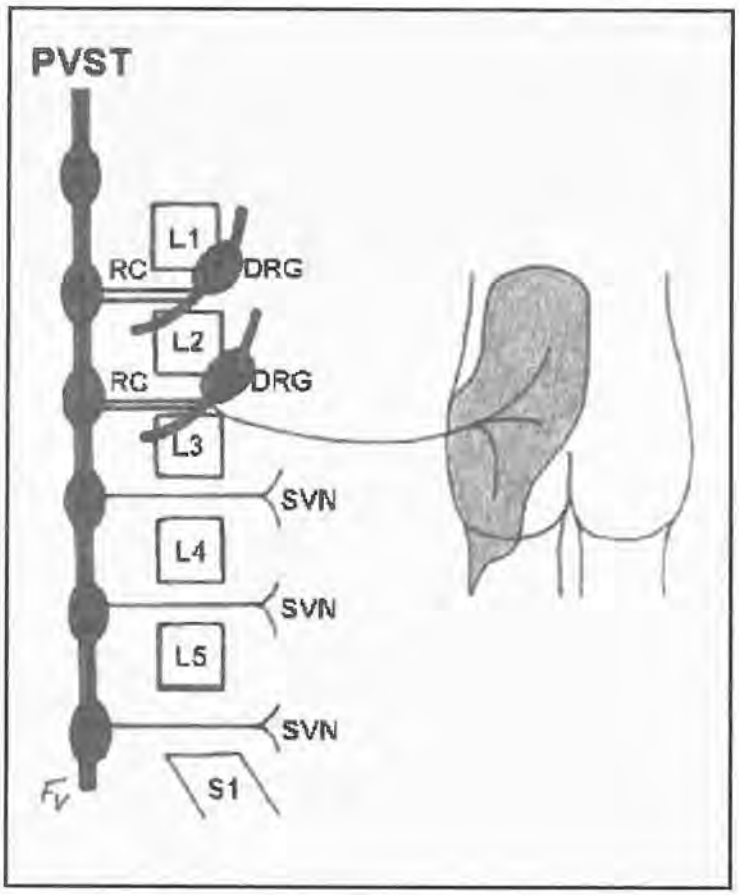

Diagram 1, illustrating the afferent pathway of discogenic pain in the lower back, based on Nakamura et al. (1996). Pain from a lower lumbar disc is transmitted nonsegmentally by visceral sympathetic afferent fibres mainly through the L2 spinal nerve root. It may be perceived as referred pain in the L2 dermatome (DRG, dorsal root ganglion; SVN, sinuvertebral nerve; PVST, paravertebral sympathetic trunk; RC, rami communicantes).

Posterior Longitudinal Ligament

Occurrence, distribution, morphology and origin of the neural elements

Most studies on the innervation of the lumbar spine, demonstrate that the posterior longitudinal ligament (PLL) is richly innervated, including encapsulated and unencapsulated nerve endings (Roofe, 1940; Stilwell, 1956; Hirsch et al. 1963; Jackson et al.,1966). The nerve plexus of the PLL in human fetal spine is wider at the level of the intervertebral discs, more irregular and denser than that of the ALL, and exclusively derived from the SVNs (Groen et al. 1990).

Kojima et al. (1990a) described a superficial and deep nerve plexus in the PLL of rat lumbar spine. The superficial plexus is supplied by the sinu-vertebral nerves and found throughout the entire length of the ligament, but primarily at the level of the intervertebral discs. The deeper plexus is absent at the level of the vertebral bodies and is supplied by nerve fibres out of the posterolateral portion of the annulus fibrosus.

Furthermore, Kojima et al. (1990b) noticed regional differences in density and distribution between the superficial and deep plexus of the PLL, that were assumed to correlate with the extensibility of the ligament at the different spinal levels.

A substantial sensory innervation (SP and CGRP), remote from blood vessels, was demonstrated by Korkala et al. (1985) and Konttinen et al. (1990) in man and by Ahmed et al. (1991) in rat. Recently, Kumar et al. (1996) identified a large network of CGRP- immunoreactive nerve fibres in the PLL of the lumbar spine in rats. Immunohistochemical studies of Düring et al. (1995) demonstrated that the PLL of rat has a poor sensory (SP and CGRP), but a dense sympathetic (NPY and DBH) innervation, thus supporting Ahmed et al. (1993). Notably, both Düring et al. (1995) and Ahmed et al. (1993) observed numerous predominantly nonvascular VIP-immunoreactive nerve fibres in the intervertebral portions of the PLL in rat.

Functional implications

Düring et al. (1995) and Ahmed et al. (1993) proposed that the VIP-immunoreactive nerve fibres of PLL regulate the function of local connective tissue and/or bone metabolism, supporting Hohmann et al. (1983).

The SP- and CGRP- immunoreactive nerve fibres of PLL are assumed to mediate sensory perception including nociception and might be involved in back pain (Korkala et al. 1985; Konttinen et al. 1990).

The PLL plays a major role in 'discogenic pain', because its is anatomically the first structure to become impinged by a herniated nucleus pulposus (Edgar & Ghadially, 1976; Groen et al. 1990). Since the PLL is anatomically not separable from the annulus fibrosus, it would not be legitimate to consider the PLL separately as a source of spinal pain (Bogduk, 1992). The role of the peripheral layers of the annulus fibrosus and the ventral spinal dura should not be neglected as causes of discogenic pain.

Ventral Spinal Dura Mater

Occurrence, distribution, morphology and origin of the neural elements

The spinal pia mater and arachnoid mater are generally accepted to lack corpuscular as well as noncorpuscular 'free' nerve endings. The only fibres being present are autonomic fibres in the adventitia of blood vessels (Bridge, 1959).

Studies on the innervation of the spinal dura have been controversial. Purkinje (1845), Luschka (1850), Pedersen et al. (1956), and Bridge (1959) failed to demonstrate nerve fibres in the spinal dura. Stilwell (1956), Kimmel (1961), Edgar & Nundy (1966), and Jackson et al. (1966) on the contrary demonstrated intrinsic nerves in the spinal dura but did not mention their origin.

Studies of Groen et al. (1988, 1990) irrefutable demonstrated evidence for spinal dural innervation. They identified a large plexus of Acetylcholinesterase (AchE) positive fibres in the ventral spinal dura and in the dural sleeves around the nerve roots, while the dorsal spinal dura is scarcely innervated and does not reach the midline. Ach E positive nerve fibres were found perivascular, as well as unrelated to blood vessels. Part of the nerve plexus of the ventral dura is derived from the sympathetic chain and likely vasomotor in function. The ventral dural plexus, receives contributions from 1) the sinu-vertebral nerves, 2) the nerve plexus associated with the posterior longitudinal ligament, and 3) the nerve plexus associated with segmental arteries. Nerve fibres of the dorsal dura are exclusively derived from the intrinsic innervation of the ventral spinal dura via the dural sleeve around the spinal nerve roots (Groen et al. 1988), supporting Cuatico et al. (1988). In agreement with Kimmel (1961) and Jackson et al. (1966), nerve endings in the spinal dura are not encapsulated and predominantly nonvascular in nature (Groen et al. 1988).

Immunohistochemical studies in rats demonstrated an autonomic (Ahmed et al. 1993) and sensory (Kumar et al. 1996) innervation. However, Kumar and coworkers found that the spinal dura is scarcely innervated. They found relatively few CGRP- and SP-IR nerve fibres, that were primarily located in the ventral parts of the dura and unrelated to blood vessels. Ahmed et al. (1993) did not mention on differences in the innervation pattern of NPY- and Tyrosine Hydroxylase (TH)-IR nerves between ventral and dorsal dura.

The occurrence of immunoreactivity to the general neuronal-glial markers S-100 in the ventral spinal dura of the human fetus (Vandenabeele et al. 1998) agrees with groen et al. (1988).

Functional significance

Clinical and experimental studies demonstrated that the dura is sensitive to both mechanical and chemical stimulation, causing back pain and somatic referred pain in the buttock (Smyth & Wright, 1959; Walton, 1977; El Mahdi et al. 1981; Spencer et al. 1983).

The studies of Groen et al. (1988, 1990) in the human fetus emphasized the importance of spinal dura as a source of spinal pain and suggested that part of the spinal dural nerves are sympathetic in nature, in agreement with Ahmed et al. (1993). Sensory innervation of spinal dura, including pain transmission, is thus likely comparable to pain perception in the viscera, sharing common sympathetic pathways.

However, Kumar et al. (1996) disputed the existence of a rich ventral spinal dural innervation in rats, in contradiction with the other studies in rat (Ahmed et al. 1993), monkey (Stilwell, 1956) and humans (Kimmel, 1961; Edgar & Nundy, 1966; Groen et al. 1988). They concluded that the spinal dura is relatively insensitive and plays a limited role in pain transmission, but acts primarily as a protective covering. Likely, observations of Kumar and coworkers on spinal dural innervation relate to interspecies-variety or regional differences.

Epidural Plexus

The spinal dural sac is separated from the vertebrae and spinal ligaments by the extradural (epidural) space, containing fibro-adipose tissue and blood vessels (Batson's venous plexus). Purkinje (1845) and Luschka (1850) were the first to describe lightmicroscopically, the nerve supply of the epidural space. Further studies by Bridge (1959), Edgar & Nundy (1966) also focussed on the innervation of the epidural space. The epidural veins are innervated by branches of the nerve plexus associated with the PLL, receiving contributions from the lumbar sinuvertebral nerves (Pedersen et al. 1956; Groen et al. 1990). The epidural veins are a possible source of pain (Boas, 1980; Bogduk, 1992).

While studying the ultrastructure of spinal meninges, we found paravascular vesiculated nerve profiles in the fibroadipose tissue of the epidural space and in the dorsal fibrous dura (Vandenabeele et al. 1996)

Vertebral Bone and Periosteum

Occurrence, distribution, morphology and origin of the neural elements

Neural structures have been described in normal bone, including both sensory and sympathetic nerves (Bjurholm, 1988; Davidovitch et al. 1988; Lambrichts et al. 1998a,b). A possible innervation of osteocytes is uncertain (Thurston, 1982). CGRP-immunoreactive nerves have been described in direct contact with metaphyseal osteoblasts and osteoclasts (Imai, 1997).

The vertebral bodies of the lumbar vertebrae are the weight-bearing elements of the lumbar spine and consists of trabecular and cortical bone covered by periosteum at their sides. Branches of the sinu-vertebral nerves, associated with the posterior longitudinal ligament (PLL), and branches of the plexus associated with the anterior longitudinal ligament (ALL) penetrate deeply into the vertebral bodies (Pedersen et al. 1956; Edgar & Nundy, 1966; Groen et al. 1990) and provide a possible substrate for bone pain. It is not known whether the intraosseous nerves are exclusively vascular (vasomotor or vasosensitive) or whether the vertebral bone itself receives an innervation (Bogduk, 1992). Most likely, vertebral pain is evoked by stimulation of perivascular sensory nerves (e.g. inflammation, space occupying processes, intra-osseous hypertension, ...). We found SP-immunoreactive nerves in the periosteum of the vertebral body in the human fetal spine (Vandenabeele et al. 1998). The vertebral periosteum is likely pain-sensitive (Bogduk, 1992).

In rat lumbar spine a predominantly nonvascular sensory (SP and CGRP) and a predominantly vascular autonomic (NPY and TH) innervation of the vertebral endplates, bone marrow, and periosteum has been demonstrated (Ahmed et al. 1991, 1993). The same authors also demonstrated substantial nonvascular immunoreactivity to VIP in the vertebral bodies, thought to be involved in bone cell physiology.
Recently, Brown et al. (1997), reported on the innervation and histopathological changes of the vertebral endplate and underlying subchondral bone in patients with degenerative disc disease. Their observations can be summarised as follows: (1) medullary cavities are innervated by both perivascular sensory (SP and CGRP) and sympathetic nerve fibres (NPY), (2) the vertebral endplates were commonly affected by focal cartilage defects, (3) evidence of local increases in vascularity and innervation (SP and CGRP) adjacent to the endplate cartilage defects, (4) the endplate is devoid of sympathetic (NPY) fibres. According to Brown and coworkers, IVD's are avascular in physiologic conditions, and mainly nourished by diffusion from the microcirculation in the vertebral endplates, that is under control of vasomotor nerve fibres.

Functional implications

SP- and CGRP-containing sensory nerves in the cartilage endplates and underlying vertebral bodies are assumed to be involved in pain transmission (Ahmed et al. 1991, 1993; Brown et al. 1997). The mechanism of pain transmission is uncertain. Ahmed and coworkers reported that the sensory innervation is activated by increased intra-osseous pressure in the subchondral bone. According to Brown and cowokers, disturbances in vasomotor control and/or defects in the cartilage endplates, lead to failure of disc nutrition and disc degeneration. Local increases in vascularity as well as in CGRP-containing nerves are induced chemotactically by degeneration products.

Anterior Longitudinal Ligament

Occurrence, distribution, morphology and origin of the neural elements

The ALL is richly innervated, forming a more-or-less regularly arranged longitudinal nerve plexus (Jung & Brunschwig, 1932; Stilwell, 1956; Hirsch et al. 1963; Jackson et al. 1966; Groen et al. 1990). At the level of the vertebral bodies, the nerve plexus of the ALL is arranged in a more dense and irregular transverse nerve plexus (Groen et al. 1990).

The intrinsic nerve supply of the ALL is segmental and connected by branches of the rami communicantes and direct branches of the sympathetic trunk (Stilwell, 1956; Edgar & Ghadially, 1976; Bogduk et al. 1981, Groen et al. 1990). The latter are likely postganglionic sympathetic nerve fibres, as proved by neuronal tracer studies (Groen et al., unpublished observations) and by immunohistochemistry (Ahmed et al. 1993).

Immunoreactivity to the sympathetic neuronal markers neuropeptide Y (NPY) and tyrosine hydroxylase (TH), as well as to the sensory neuronal markers SP and CGRP, has been demonstrated in the ALL of the rat lumbar spine, but was sparse compared to the posterior longitudinal ligament (Ahmed et al. 1991, 1993).

Functional implications

SP and CGRP-immunoreactive nerve fibres in the ALL may prove to be involved in spinal pain transmission. NPY and TH likely mediate autonomic modalities and play a role in vasoregulation.

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DORSAL COMPARTMENT

Facet Joint

The biomechanical, neuroanatomical and neurophysiological basis for facet joint pain has been scientifically established (Cavanaugh et al. 1996; Dreyer & Dreyfuss, 1996). Clinically, facet joint pain is often aspecific (Bogduk, 1995). The only reliable criterion for diagnosing lumbar facet joint pain is a diagnostic block (Bogduk, 1995; Dreyfuss et al. 1995).

To date it is generally accepted that the lumbar facet joint is richly innervated, ipsilaterally and segmentally by the somatic sensory nervous system (Pedersen et al., 1956; Edgar & Ghadially, 1976; Bogduk, 1983; Cavanaugh et al. 1989; Gilette et al. 1993).

We investigated the innervation pattern in the joint capsules of the human lumbar facet joints by light and transmission electron microscopy, with special reference to the fine structural characteristics and distribution of sensory nerve endings. We examined peroperative tissue samples of joint capsules obtained from patients with chronic low back pain and sciatica, undergoing neurosurgery for recurrent disc herniation (with partial or total discectomie).

Light microscopically, nonmyelinated and myelinated nerve fibres (Figs 2 c, d, e), as well as their nerve endings have been identified in the joint capsules and synovial plical tissues of the facet joints in man, using routine, more-or-less specific, neurohistological staining methods (Hadley, 1964; Hirsch et al. 1963; Jackson et al. 1966; Ivachenko 1971, 1973; Nade et al. 1980; Giles et al. 1986; Giles, 1988).

The cited authors described three different types of nerve endings: (1) simple lamellated or paciniform corpuscles, (2) complex non-encapsulated Ruffini spray-like endings, and (3) noncorpuscular 'free' nerve endings. This innervation pattern has been classically described in joint capsules of mammals (Polacek, 1966), and referred to as the 'proprioceptive receptor triad' (Stilwell, 1957).

Unlike the cited authors, however, we found no typical Ruffini spray-like endings (Lambrichts et al. 1992, 1993) in our material in accordance with Horackova & Malinovsky (1987). The corpuscular endings appeared sparsely distributed in our material, in accordance with McLain (1994). This investigator also suggested that mechanoreceptors in joint capsules of the human facet joint have a relatively large receptive field.

We basically found two distinct types of corpuscular nerve endings: glomerular and lamellated. The majority of corpuscular endings in our material were glomerular corpuscles (Fig. 2*a*), resembling the glomerular endings of Horackova & Malinovsky (1987). They classified the glomerular endings of the facet joint as 'Krause's bulboid' glomerular corpuscles. The lamellated (Paciniform) corpuscles are differentiated from glomerular nerve endings, by the presence of a thick perineural capsule around a single or a branching axon (Fig. 2*b*). Glomerular sensory nerve endings are generally found in the skin of the genital organs and are assumed to be involved in protopathic sensibility (Malinovsky, 1974; Halata & Munger, 1986). We distinghuished two subtypes of glomerular nerve endings in our material: (1) numerous small, spherical, and clustered glomerular endings, and (2) scarce large, fusiform, often solitarily, spindle-like glomerular endings, in accordance with Horackova & Malinovsky (1987).

More recently, comparable morphological data on human facet joint innervation have been reported by Ozaktay et al. (1991) and McLain (1994). These authors classified the sensory nerve endings of the facet joint according to their morphology and function (Freeman & Wyke, 1967), into (1) three basic types of mechanoreceptive corpuscular endings (type I or Ruffini endings, type II or Paciniform corpuscles, and type III or Golgi tendon organs), and (2) noncorpuscular 'free' nerve endings (type IV).

Likely the different types of sensory nerve endings in the classifications of Horackova & Malinovsky (1987), Ozaktay et al. (1991) and McLain (1994), represent morphological equivalents (Table 1).

McLain (1994) found that type II or Paciniform endings predominate in cervical facet joints. We found the small globular receptors (type I) to predominate in the joint capsule of the facet joint of lumbar spine, in accordance with Ozaktay et al. (1991).

Ultrastructurally the glomerular nerve endings found in our material have been referred to as encapsulated Ruffini-like endings (Vandenabeele et al. 1997; vide infra).

Table 1. Light microscopic equivalence of the different types of sensory nerve endings observed in the joint capsules of the human facet joints.

Horackova and Malinovsky's (1987) Vandenabeele et al. (1997)	Freeman and Wyke (1967); Ozaktay et al. (1991); McLain (1984)
Small spherical glomerular endings	type I (Ruffini-like endings)
Larger fusiform glomerular endings	type III (Golgi tendon-like corpuscles)
Lamellated corpuscles	type II (Paciniform corpuscles)

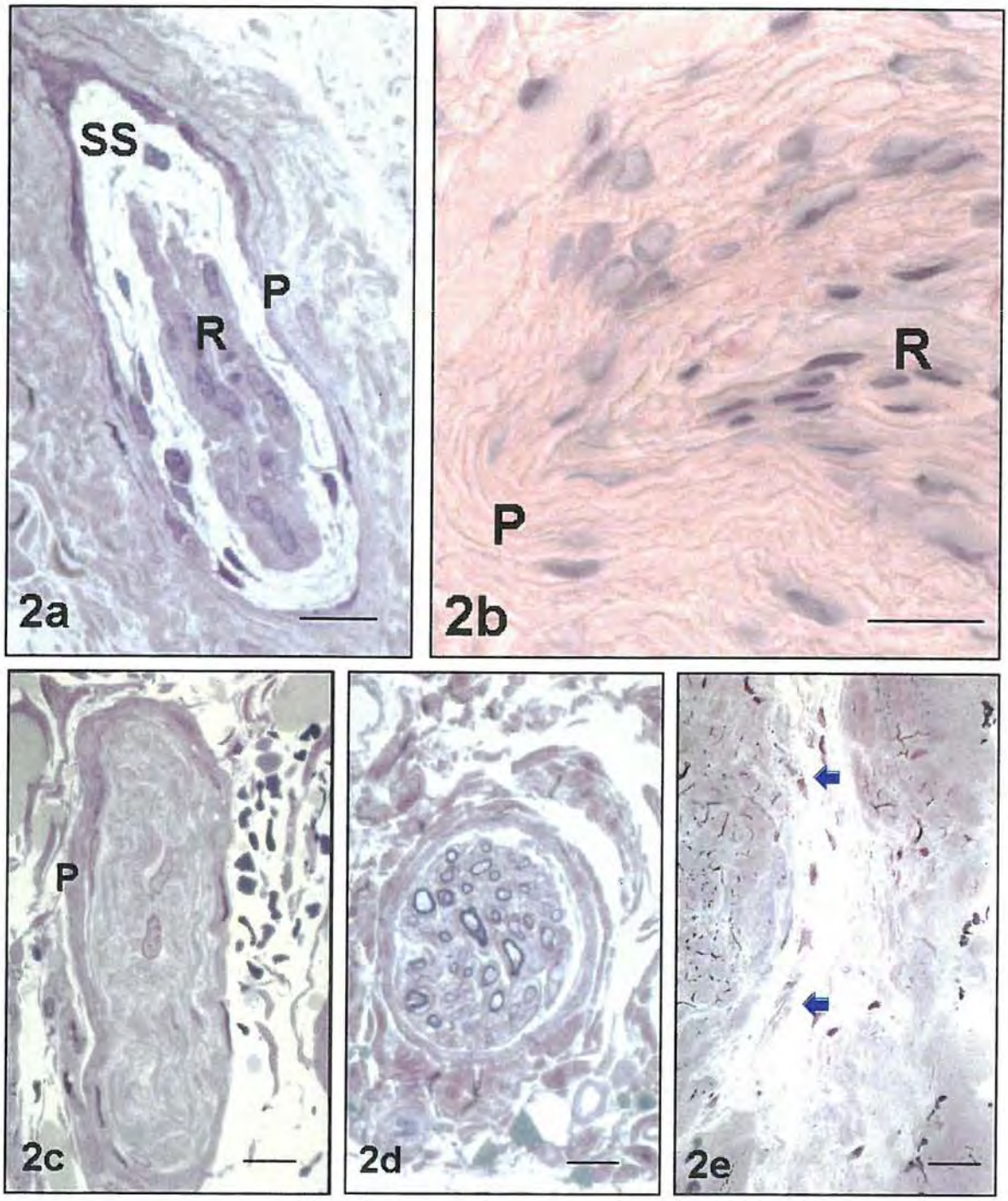

Fig. 2. Dense fibrous layer of the facet joint capsule. Light micrographs of thionin-methylene blue-stained sections. (a) Large glomerular encapsulated corpuscular ending. The receptive complex (R) lies centrally in an extensive subcapsular space (SS) enclosed by the perineurium (P). BAR = 40 µm. (b) Detail of the thick multilayered perineurium (P) surrounding the receptive complex (R) of a small lamellated corpuscle. BAR = 20 µm. (c) Large unmyelinated nerve bundle. Perineurium (P). BAR = 25 µm. (d) Large myelinated nerve bundle. BAR = 25 µm. (e) Small unmyelinated nerve fibre (arrow). BAR = 65 µm.

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Ligaments

The dorsal compartment of the lumbar spine contains several ligamentous structures, including:

- lumbodorsal fascia,
- outer layer of the facet joint capsules,
- paired ligamenta flava,
- interspinous ligaments,
- supraspinous ligament.

Gracovetsky et al. (1981) referred to the ligamentous structures of the lumbar spine as the posterior ligamentous system (PLS).

Lumbodorsal fascia

The thoraco-lumbar fascia encloses the muscles of the lumbar spine. The combined action of the thoracolumbar fascia and the posterior ligaments of the lumbar spine limit the flexion of the vertebral column (Gracovetsky et al. 1981).

Likely the lumbodorsal fascia is richly innervated. However, little is known about its innervation. Stilwell (1957) found Paciniform corpuscles and Hirsch et al. (1963) identified unencapsulated nerve endings. Recently, Yahia et al. (1992) and Rhalmi et al. (1993) demonstrated NF- ir and S100- ir nerve bundles, noncorpuscular 'free' nerve endings, Ruffini endings and Vater-Pacini corpuscles in the lumbodorsal fascia of man and suggested that it might have a neurosensory role.

We observed single or bundled small myelinated nerve fibres and unmyelinated nerve fibres (Figs 1 *a,c,f*), large nerve bundles (Fig 1*b*), and noncorpuscular 'free' nerve endings (Figs 1*d,e,g*) in the lumbodorsal fascia of patients undergoing surgery for recurrent disc herniation (unpublished observations).

Recently, we demonstrated NF- ir, S100- ir and SP- ir nerves in the lumbodorsal fascia of the human fetal spine (Vandenabeele et al. 1998).

Functional implications

The light-, electron microscopic and immunohistochemical observations suggest that the lumbodorsal fascia is likely involved in spinal proprioception (postural and/or protective muscular reflexes), and in low back pain. Pain presumably arises as a result of strain in the fascia (Bogduk, 1992). Compartment syndromes of the back muscles, and painful herniations of fat through the lumbodorsal fascia have once been suggested as possible causes of low back pain (Faille 1978; Peck et al., 1986).

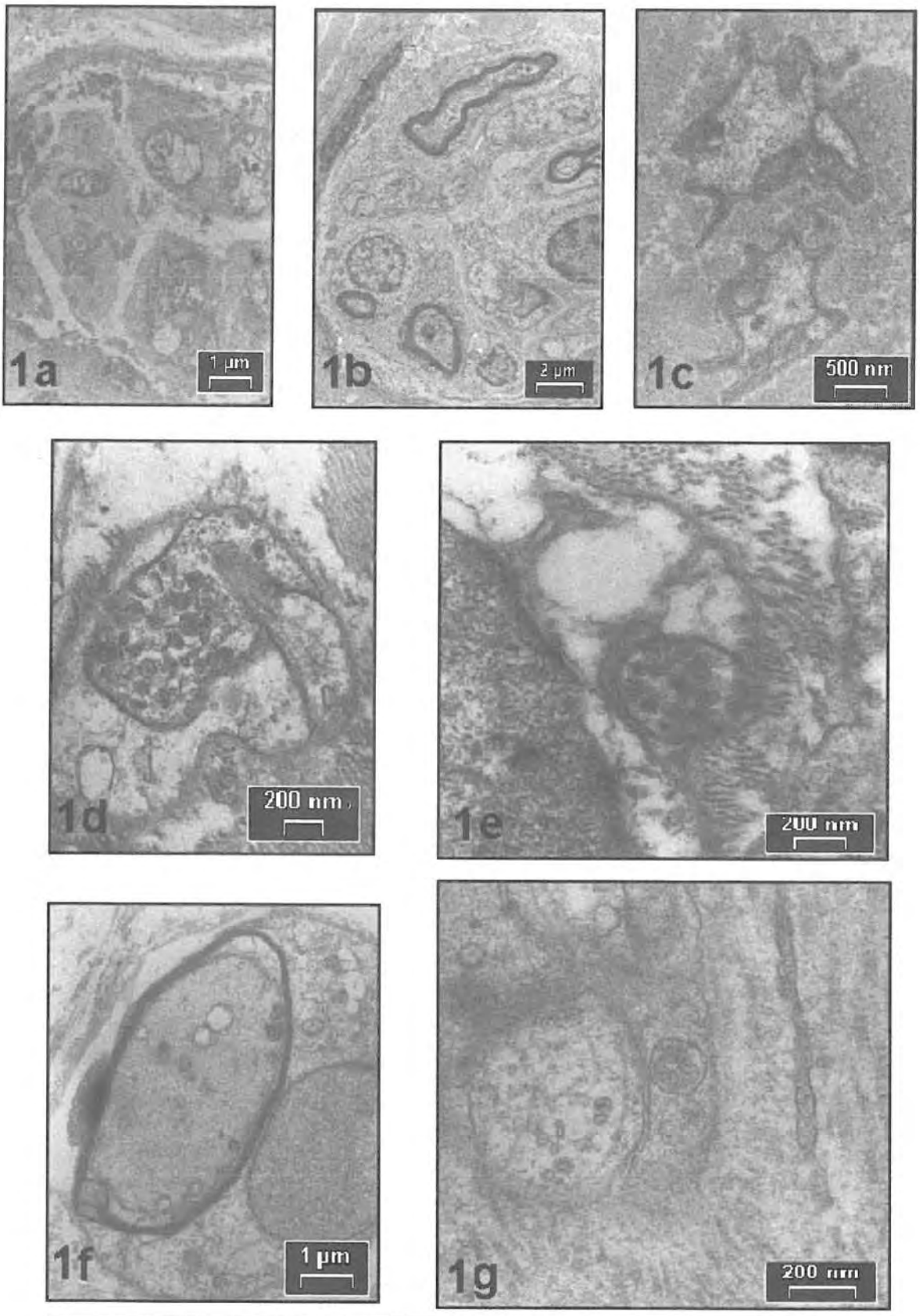

Fig. 2.Lumbodorsal fascia. (a) Bundle of unmyelinated nerve fibres. (b) Mixed nerve bundle. (c) Unmyelinated nerve fibres. (d, e) Vesiculated nerve profiles showing various-sized granular vesicles. (f) Small myelinated nerve fibre. (g) Detail of the receptive site of a 'free' nerve ending.

Ligamentum flavum

Electrophysiologically, mechanosensitive units were identified in the ligamentum flavum of rabbit lumbar spine (Yamashita et al. 1990). To our knowledge the results of neuroanatomical studies of ligamentum flavum are contradictory.

Bridge (1959) found nerves in the deepest and superficial layers of the ligamentum flavum. Hirsch et al. (1963), Yahia et al. (1988), and Yahia and Newman (1989), found neural structures in the outermost layer of the human ligamentum flavum by light microscopy.

Ahmed, identified nonvascular varicosal endings immunoreactive to calcitonin gene-related peptide (CGRP), and vasoactive intestinal polypeptide (VIP) in the ligamentum flavum of rat lumbar spine , and suggested that the ligamentum flavum may be involved in low-back pain (Ahmed et al., 1993). VIP has been demonstrated in postganglionic parasympathetic nerves (Hara et al. 1985; Lundberg et al. 1980) and is known to have a vasodilatory action (Laitinen et al. 1987; Malm et al. 1980), and to play a role in bone metabolism (Hohmann & Tashjian, 1984; Hohmann et al. 1983). CGRP has been implicated in nociception, inflammation , and vasoregulation (vide supra).

In accordance with Jackson et al. (1966), Korkala et al. (1985), Giles (1988), and Yahia et al. (1988), Ashton et al. (1992) could not demonstrate any immunoreactivity in the ligamentum flavum of human lumbar spine. Rhalmi and coworkers identified paravascular NF-immunoreactive nerves in human ligamentum flavum (Rhalmi et al. 1993).

Our electron microscopic findings on ligamentum flavum innervation (Vandenabeele et al. 1994) concur with the immunohistochemical findings of Ashton et al. (1992), as we could not find any neural elements in ligamentum flavum. However, our specimens of ligamentum flavum were small, and the areas investigated by electron microscopy even smaller.

Bogduk (1992) concluded that the ligamentum is poorly innervated and is therefore unlikely to be a source of pain. Furthermore, the ligamentum flavum is elastic and not susceptible to strain.

Supraspinous ligament and interspinous ligaments

Bogduk (1992) concluded that the so-called supraspinous ligament, is not a major source of low back pain since the ligament is locally absent at L5/L4/(L3), a common site of low back pain. Clinical and experimental studies demonstrated that the interspinous ligaments are involved in low back pain and referred pain in the lower limbs (Steindler & Luck, 1938; Kellgren, 1939; Feinstein et al. 1954).

As to Bogduk (1992), the existence of interspinous ligament pain is uncertain. He assumes that interspinous ligaments that are under strain following excessive flexion or rotation of the lumbar spine, as well as degenerated ligaments, might be painfull.

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Deep Back Muscles

It is generally accepted that the muscles of the lumbar spine are well innervated. Clinical and experimental studies indicate that the muscles of the lumbar spine might be a source of back pain and somatic referred pain (Bogduk, 1980 & 1992). Different mechanisms for muscular pain in the lower back have been suggested, including sprain, spasm, 'muscle imbalance', trigger points and fibromyalgia (Travell & Rinzler, 1952; Hirschberg et al., 1979; Wolfe et al., 1985; Roland, 1986; Jull & Janda, 1987; Bogduk, 1992). To our knowledge biopsy data on deep back muscles in humans have not been published. We identified NF-, S-100-, and SP- immunoreactive nerves in the perimysium of the intrinsic back muscles in human fetal spine (Vandenabeele et al., 1998).

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Chapter 3.3

Sensory Transmission in the Lumbar Spine

The Dorsal Root Ganglion

The mechanisms of low back pain are complex and largely unknown. For a deep structure of the lumbar spine to be a source of pain it must be innervated by nerve fibres that transmit the message of pain, namely unmyelinated group IV (C) fibres and probably small myelinated group III (Aδ) fibres. Under normal conditions, the noncorpuscular unencapsulated 'free' nerve endings of these fine afferents serve pain functions (nociceptors). They have high mechanical thresholds and only respond to noxious stimuli. In inflammed conditions the nociceptors are sensitized by chemical mediators and respond spontaneously and to low levels of stress and strain.

The cell bodies of the pain fibres are all located in the dorsal root ganglia of the spinal nerves. The dorsal root ganglion has been referred to as the 'brain' of the functional spinal unit, the 'vital link' between the intrathecal spinal nerve and the extrathecal peripheral nerve and the 'modulator of low back pain' (Grönblad et al. 1991). The emerging spinal nerves, nerve roots, and dorsal root ganglia lie between the two compartments of the lumbar spine (Bogduk, 1983).

Clinally (Weinstein et al. 1988a), experimentally (Rydevik et al. 1988, 1989; Weinstein et al. 1988b; Delamarter et al. 1990), and morphologically (Lindblom & Rexed, 1948; Bergmann & Alexander, 1941) there is substantial evidence that direct mechanical and/or chemical influences on the dorsal root ganglion might play a significant role in pain originating in the lumbar spine.

The dorsal root ganglion is known to be an important site of neuropeptide production, including the neurogenic modulators CGRP and SP. SP is produced in the small dark 'type B' neurons of the dorsal root ganglion, and transported by axonal transport to both the peripheral and central processes of the primary afferent neurons (Hökfelt et al. 1975; Otsuka & Yanagisowa, 1987; Nicoll, 1980; Pernow, 1983; Wessendorf & Elde, 1985).

Upon entering the spinal cord, the central processes of the primary afferents give off ascending and descending branches in the dorsolateral tract of Lissauer in the spinal white matter, and then penetrate into the dorsal horn of the spinal grey matter, where they synapse on relay neurons. The intraspinal distribution of lumbar nociceptive afferents and the nociceptive relays in the brain stem and thalamus (perception, experience, and memory of pain) are not under consideration here (see, Wyke, 1987). SP and CGRP mediate sensory modalities, including nociception and affect local blood vessels either directly or indirectly through the stimulation of mast cells and production of nonneurogenic mediators (Grönblad et al. 1991).

We demonstrated SP immunoreactivity in the dorsal root ganglion, spinal cord and spinal nerves of the lumbar spine in human fetus (Vandenabeele et al. 1988). Notably, immunoreactivity to SP appeared more pronounced in the dorsal nerve root.

The Afferent Autonomic Pathways of the Lumbar Spine

Much attention has been given over the years to the exact course of the sensory pathways from deep tissues of the lumbar spine. Histological, neuropathological and microdissection studies clearly demonstrate that the anterior spinal tissues are well innervated and that the nerves predominantly follow 'apparently sympathetic' routes.

Somatosensory patterns into the CNS serving the ventral compartment at all lumbosacral levels are dual. One is a bilateral, nonsegmental autonomic afferent inflow through sympathetic (visceral) nerves. These 'sympathetic afferents' ascend via the paravertebral sympathetic chain and enter the CNS at or above L2 (*'spinal somatic sympathetic nervous system'*). The other is an ipsilateral and segmental somatic afferent inflow into the CNS through somatic branches of ventral spinal rami (*'spinal somatic nervous system'*) (Jinkins et al. 1989; Morinaga et al. 1996).

The nociceptive afferents projecting from the deep somatic tissues in the dorsal compartment of the lumbar spine, mainly reach the dorsal root ganglia through the dorsal rami of the spinal nerves. The sensory afferents of the dorsal compartment also seem to follow sympathetic (visceral) routes (Suseki et al. 1997), so that the innervation of the dorsal compartment is also dual: ipsilateral and segmental by somatic (spinal) nerves and bilateral and nonsegmental by sympathetic (visceral) nerves).

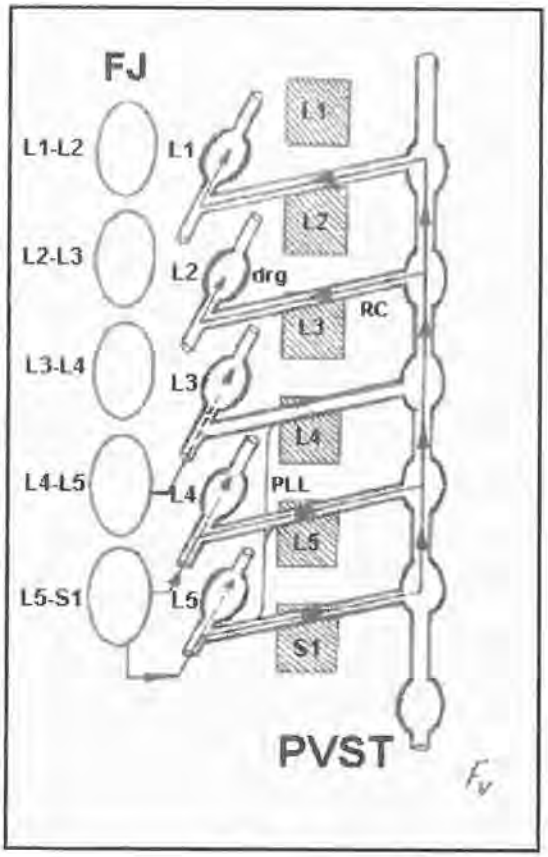

Figure 1. Diagram illustrating possible afferent pathways from the L5-S1 facet joint, modified from Suseki et al. (1997). Pain from the L5-S1 facet joint may be transmitted by sensory afferents through the dorsal rami of L4, and L5 spinal nerves, and by sympathetic afferents through the paravertebral sympathetic trunk (PVST). The sympathetic afferents enter the paravertebral sympathetic trunk through L4 and L5 rami communicantes (RC) and finally reach L1 and/or L2 dorsal root ganglia through L1 or L2 rami communicantes. Visceral sympathetic afferents may also ascend the nerve plexus associated with the posterior longitudinal ligament (PLL) and then enter the sympathetic trunk. (drg, dorsal root ganglion; RC, ramus communicans; FJ, facet joint.

Interestingly, immunoreactivity to the sensory neuronal marker CGRP has been demonstrated in rami communicantes of rat (Suseki et al. 1997), and to SP in rami communicantes of human fetuses (Vandenabeele et al. 1998), indicating that spinal pain pathways serving the low back region reach their destination by sympathetic routes. Likely the grey rami communicantes play a key role in spinal pain pathways. Indeed, they are present at all levels of lumbar spine, passing from the sympathetic ganglia to the corresponding spinal nerves, whereas white rami communicantes are only present till the first and second, and sometimes the third lumbar level (Williams, 1995).

A bilateral and plurisegmental distribution of pain fibres originating in the lumbar spine through sympathetic routes, might explain why low back pain is commonly poorly localized by the patient and accompanied by referred pain, autonomic dysfunction and muscular spasm.

'Sympathetic Modulation' of Pain Pathways in the Ventral Compartment

Clinically, there is substantial evidence for sympathetic involvement in low back pain (White & Sweet, 1955; Brena et al. 1980; El Mahdi et al. 1981; Jänig, 1985; Roberts, 1986; Sluijter, 1988). Gilette et al. (1992, 1994) tested dorsal horn neurons receiving nociceptor input from lumbar paraspinal tissues for activation by electrical stimulation of the paravertebral sympathetic trunk in anesthetized cats. They demonstrated a 'sympathetic' activation of spinal 'pain pathways', serving the low back region, by a direct and indirect mechanism.

The direct mechanism results from stimulation of somatic and/or visceral afferent fibres that ascend through the sympathetic trunk (spinal somatic sympathetic nervous system), and project directly or polysynaptically onto somatosensory 'low back' neurons (Gilette et al. 1994). Gilette et al. (1993), demonstrated that these spinal somatosensory neurons have large hyperconvergent receptive fields, and suggested that they thus likely serve nociceptive sensations that are poorly localised and experienced as dull or aching, as is the case with pain of visceral origin (Ness & Gebhart, 1990). Visceral afferent fibres and probably visceral pain fibres, also follow common peripheral sympathetic pathways together with efferent autonomic fibres to the viscera and blood vessels.

It would be of interest to know if the 'sympathetic' afferent and efferent pathways of the lumbar spine, actually project through different fibres within the sympathetic chain. Likely, sympathetic noradrenergic postganglionic efferents may be functionally afferent (vasosensory nerves) and might serve nociceptive modalities. Indeed, the concept of co-existence and co-transmission of functionally different neurotransmitters in the same nerve ending (Burnstock, 1976) is now generally accepted. Further investigations are necessary in proving the plurality of transmission in the 'sympathetic' spinal routes.

The indirect pathway results from the stimulation of primary sensory afferents in spinal tissues by noradrenergic sympathetic efferents via sympathetic-sensory interactions (Koizumi et al. 1970; Levine et al. 1986; Gilette et al. 1992, 1994).

Sato and Swenson (1984) demonstrated a sympathetic nervous system response to mechanical stress of the spinal column in rat. An indirect sympathetic modulation of the sensory transmission has been demonstrated electrophysiologically for mechanoreceptors (Cash & Linden; Roberts & Elardo, 1985b; Passatori & Filippi, 1983; Na et al. 1993), for nociceptors (e.g. Roberts & Elardo 1985a; Hu & Zhu, 1989; Sato & Perl, 1991; Gold et al. 1994; Bossut & Perl, 1995; Desmeules et al. 1995; Kinnman & Levine, 1995), and for dorsal horn neurons (e.g.Roberts & Foglesong, 1988a,b; Devor et al. 1994).

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CHAPTER 4

Ultrastructural and Immunocytochemical Identification of Nervous Structures in the Facet Joints and Supra- and Interspinous Ligaments.

Chapter 4.1

Electronmicroscopic Studies of Facet Joint Innervation

Electron microscopic studies of the sensory innervation of the joint capsules of facet joints have been scarce. Giles et al. (1986) and Giles & Taylor (1987a,b) identified paravascular and nonvascular small myelinated nerves in the human facet joint capsule by electron microscopy, but did not mention the finding of specific corpuscular end organs, nor noncorpuscular 'free' nerve endings.

We observed single and bundled unmyelinated nerve fibres, small myelinated nerve fibres, and larger nerves with a mixed population of unmyelinated and small myelinated nerve fibres. Particular attention has been given to the ultrastructure of their noncorpuscular 'free' nerve endings (Vandenabeele et al. 1994, 1995) and corpuscular nerve endings (Vandenabeele et al. 1997).

Ultrastructurally the glomerular nerve endings found in our material have been referred to as encapsulated Ruffini-like endings (Vandenabeele et al. 1997; vide infra). Notably, the largest Ruffini-like endings structurally closely resemble Golgi tendon-like endings (Strassman et al. 1987).

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Fine Structure of Vesiculated Nerve Profiles in the Human Lumbar Facet Joint

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ABSTRACT

The ultrastructural features of vesiculated nerve profiles were examined within a perivascular plexus of unmyelinated nerve fibres around small arteries and arterioles in the posterior facet joint capsuel. Such profiles were exclusively observed in the dense fibrous layer and the adjacent part of the subintimal layer. The ligamentum flavum lacked any type of innervation. The vesiculated nerve profiles were tentatively classified on the basis of the fine structural appearances of their vesicular content. Two major types of nerve profiles could be readily distinguished in the capsular tissue. Both displayed a variable number of mitochondria, neurotubules and neurofilaments. The first type, containing predominantly small vesicles with an electron-dense granule or core, was frequently encountered and considered to be adrenergic in function. Profiles similar in morphology were also observed in the synovial plical tissue. A second type of profile, found in the joint capsule, contained varying proportios of small agranular (clear) vesicles and mitochondria. Some of these profiles exhibited an accumulation of mitochondria and were considered to be sensory in function. Nerve profiles filled with predominantly small flattened vesicles were occasionally encountered.

Key words: Lumbar spine; lumbar facet joints; vesiculated nerve profiles.

INTRODUCTION

A detailed knowledge of the innervation of the lumbar spinal soft tissues is essential for a better understanding of the neurological mechanisms involved in low back pain syndromes. Disease of the intervertebral discs and related structures has long been considered a major cause, although operable disc disease accounts for only 1 % of patients presenting with back pain (Group & Stanton-Hicks, 1991). Other investigators have also implicated the facet joint in the aetiology of low back pain and defined a lumbar facet syndrome (Mooney & Robertson, 1976; Giles, 1992; Mehta & Parry, 1994) and a lumbar dorsal ramus syndrome (Bogduk, 1980). The dorsal compartment of the lumbar spine has been studied extensively (Pedersen et al. 1956; Hirsch et al. 1963; Mooney & Robertson, 1976). The nerve supply of the dorsal compartment was reviewed by Bogduk (1983) and Auteroche (1983). An additional nerve supply, derived from the sympathetic trunk and its ramifications, has been described by Groen et al. (1990. The morphology of the facet joint and its synovial plical tissue (synovial fold, synovial fat pad, fibro-adipose meniscoid) has been reviewed by Bogduk & Twomey (1987) and Giles et al. (1986). Neural structures have been demonstrated in the facet joint by light microscopy (e.g. Hirsch et al. 1963; Hadley, 1964; Jackson et al. 1966; Nade et al. 1980; Giles & Taylor, 1987 *a,b*; Giles, 1988; Ozaktay et al. 1991; Mc Lain, 1994). Wyke (1980) defined the distribution of a nociceptive receptor system in the lumbosacral tissues.

Immunohistochemistry has revealed the presence of an autonomic (Ashton et al. 1992; Ahmed et al. 1993) and a sensory (Giles & Harvey, 1987; Cavanaugh et al. 1989; Grönblad et al. 1991; Ashton et al., 1992; Ahmed et al, 1993; Beamen et al., 1993) innervation of the facet joint. Small myelinated nerve fibres were demonstrated by means of electron microscopy in the facet joint capsule and the synovial fold (Giles et al. 1986; Giles & Taylor 1987 *b*), but ultrastructural data concerning their nerve endings are lacking. Electrophysiological recordings (Cavanaugh et al. 1989; Yamashita et al. 1990; Avramov et al. 1992) demonstrated the presence of nociceptive units in the facet joint.

In order to obtain more precise basic morphological information concerning the innervation of the lumbar facet joint in man, this study was undertaken to investigate in detail the ultrastructural features of nerve endings present in the posterior facet joint capsule and the adjacent synovial plical tissue, as already demonstrated by classical neurohistology and immunohistochemistry. It is hoped that the study will contribute to a better understanding of the structural basis of low back pain. Neural structures are also sought in the medial facet joint capsule, part of the ligamentum flavum.

MATERIALS AND METHODS

Fragments of the posterior aspect of joint capsules, adjacent synovial plical tissue (synovial fold or fat pad) and ligamenta flava from 3 patients (aged 25, 40 and 55 y) were obtained during routine neurosurgical procedures at the L4-L5 and L5-S1 facet joint levels. The patients were healthy apart from their spinal pathology. The fresh surgical specimens were immediately immersed in a solution of 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3). Fine dissection in 1 mm3 blocks while immersed in the fixative was performed in the laboratory under a stereomicroscope.

The tissue was placed in fresh fixative and further fixed by immersion for 24 h. The small pieces were washed in cacodylate buffer, postfixed in 2% osmium tetroxide for 1 h, stained with 2% uranyl acetate in 10% aceton for 20 min, dehydrated through graded concentrations of acetone and embedded in Araldite. Semithin sections (0.5 µm) were stained with a solution of thionin and methylene blue (0.1% aqueous solution) and examined for capsular histology, vascular and neural structures. Ultrathin sections (0.06 µm) were stained with uranyl acetate and examined with a Philips EM 400 electron microscope.

RESULTS

The facet joint capsule consists of an outer layer of dense connective tissue (fibrous capsule) and a inner discontinuous single or multilayered sheath of synovial cells (synovial lamina intima). Both layers are separated by vascular fatty connective tissue (subintimal layer) which could not always easily be distinguished from the fibrous layer of the capsule at the ulrastructural level. Small myelinated (1.5 - 5 µm) nerve fibres (Fig. 1*a*) and bundles of unmyelinated nerve fibres (Fig. 1*b*) were consistently found in the posterior joint capsule and the adipose synovial plical tissue. Although a systematic examination of serial ultrathin sections has been carried out, neural structures could not been demonstrated in the synovial lamina intima (posterior joint capsule) nor in the ligamentum flavum (medial part of the capsule).

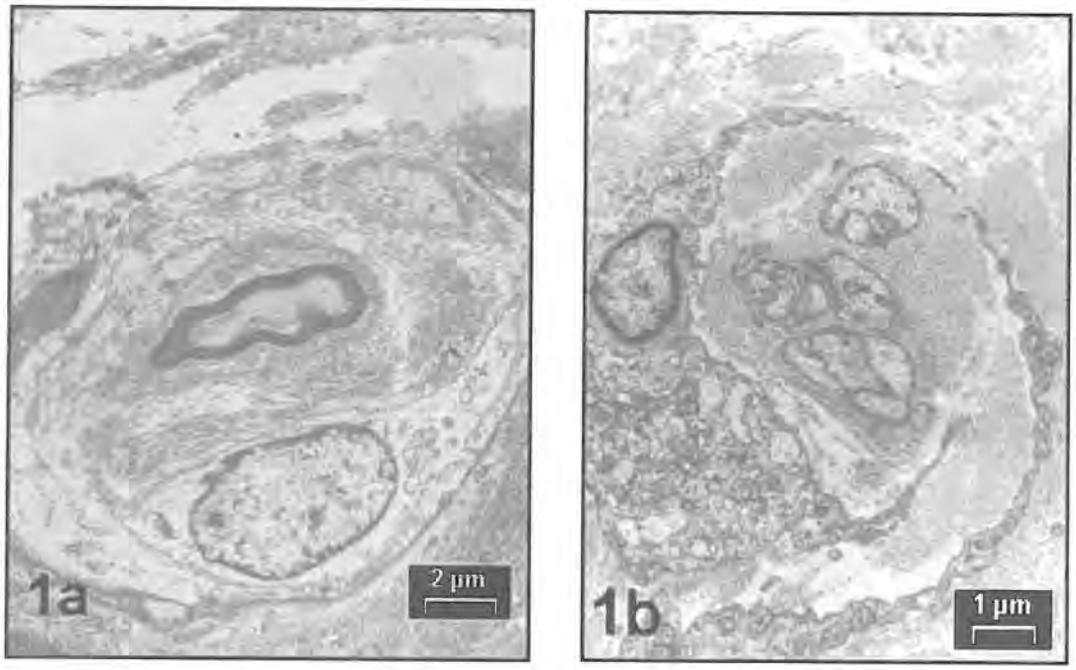

Fig. 1. Dense fibrous layer. (a) Small myelinated nerve fibre. (b) Bundle of unmyelinated nerve fibres.

Individual unmyelinated nerve fibres were also found, mainly perivascularly. The latter were made up of one or several axons enveloped by one accompanying Schwann cell. Within this perivascular plexus, we regularly observed axonal enlargements or beads containing mitochondria, neurotubules and neurofilaments in varying proportions (Figs 3 *a,b*, 5 *a,b*), in addition to a variable number of vesicles. Some of these nerve profiles were partly enclosed by the processes of their accompanying Schwann cell, and were classified as varicosities (Figs 2 *b-d*; 5 *a*; 6 *a,b*). The naked bulging part of the varicosity lay against the continuous basal lamina (25 - 55 nm) of the ensheathing Schwann cell. Occasionally we observed axonal profiles without a Schwann cell investment (Fig. 2 *d*). Some varicosities exhibited an accumulation of vesicles in close association with spots of electron-dense material located on the inner aspect of the naked axolemma (Figs 2 *b*; 6 *a*). Along the length of the terminal part of the axon, the varicosities alternated with thin axonal segments (Fig. 2 *a*) completely enclosed by the Schwann cell processes separated by a membrane-bounded cleft of approximately 25 nm. These thin axonal segments contained neurotubules and neurofilaments, and somethimes a few granular vesicles.

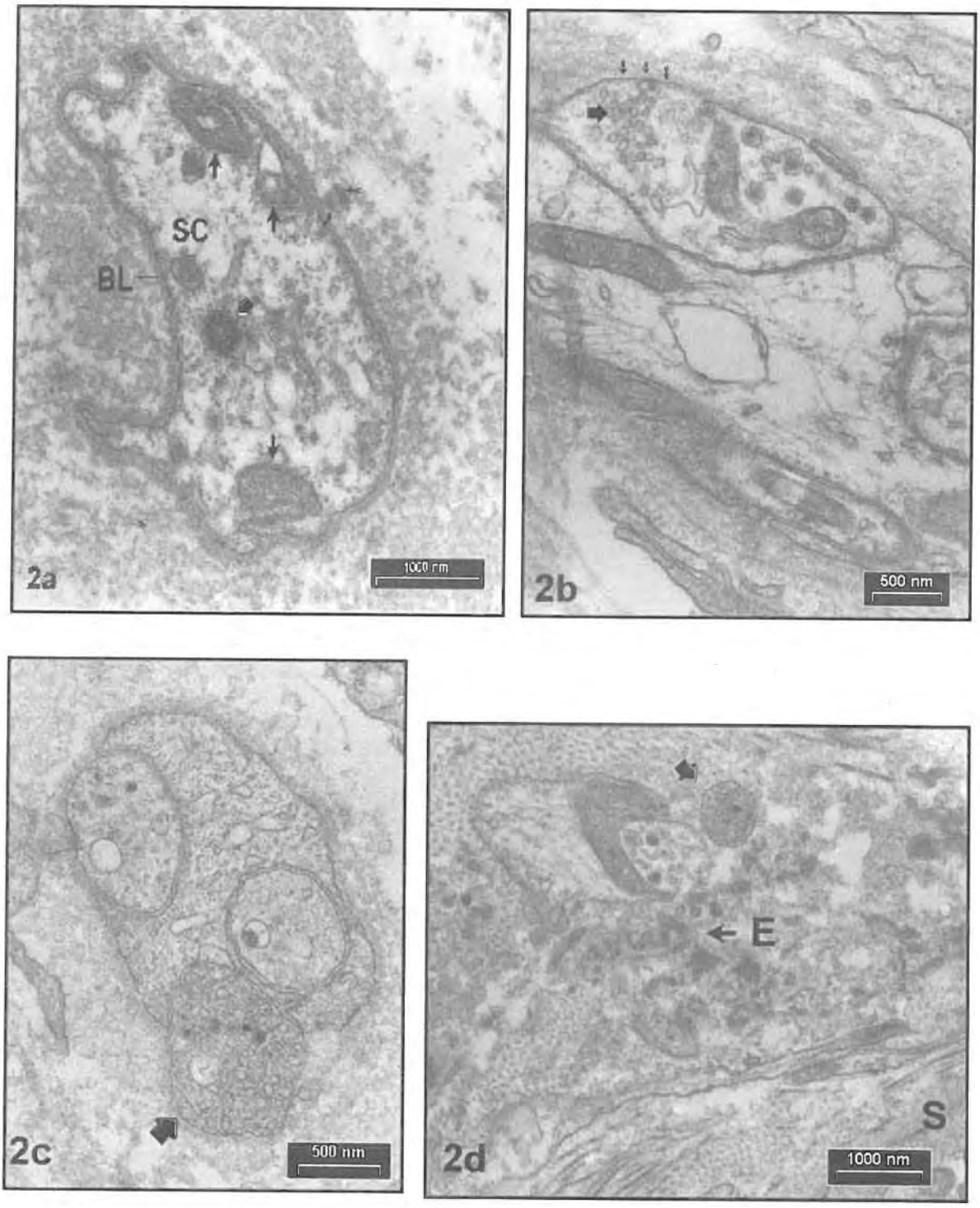

Fig. 2. Unmyelinated nerve fibres in the dense fibrous layer *(a-c)* and the synovial plical tissue *(d)*. *(a)* Thin axonal segments (thin arrows) enclosed by Schwann cell (SC) processes. Thick arrow, centriole; BL, basal lamina; small arrows, extracellular microfilaments. *(b)* Varicosity containing an accumulation of SAGV (thick arrow). Note the increased density of the axoplasm at the inner aspect of the axilemma (small arrows). *(c)* The naked axolemma of a varicosity abuts the basal lamina (arrow) and is in direct contact with the interstitium. *(d)* Synovial plical tissue. Perivascular naked nerve profile (thick arrow) located in the elastic tissue (E). S, smooth muscle cell.

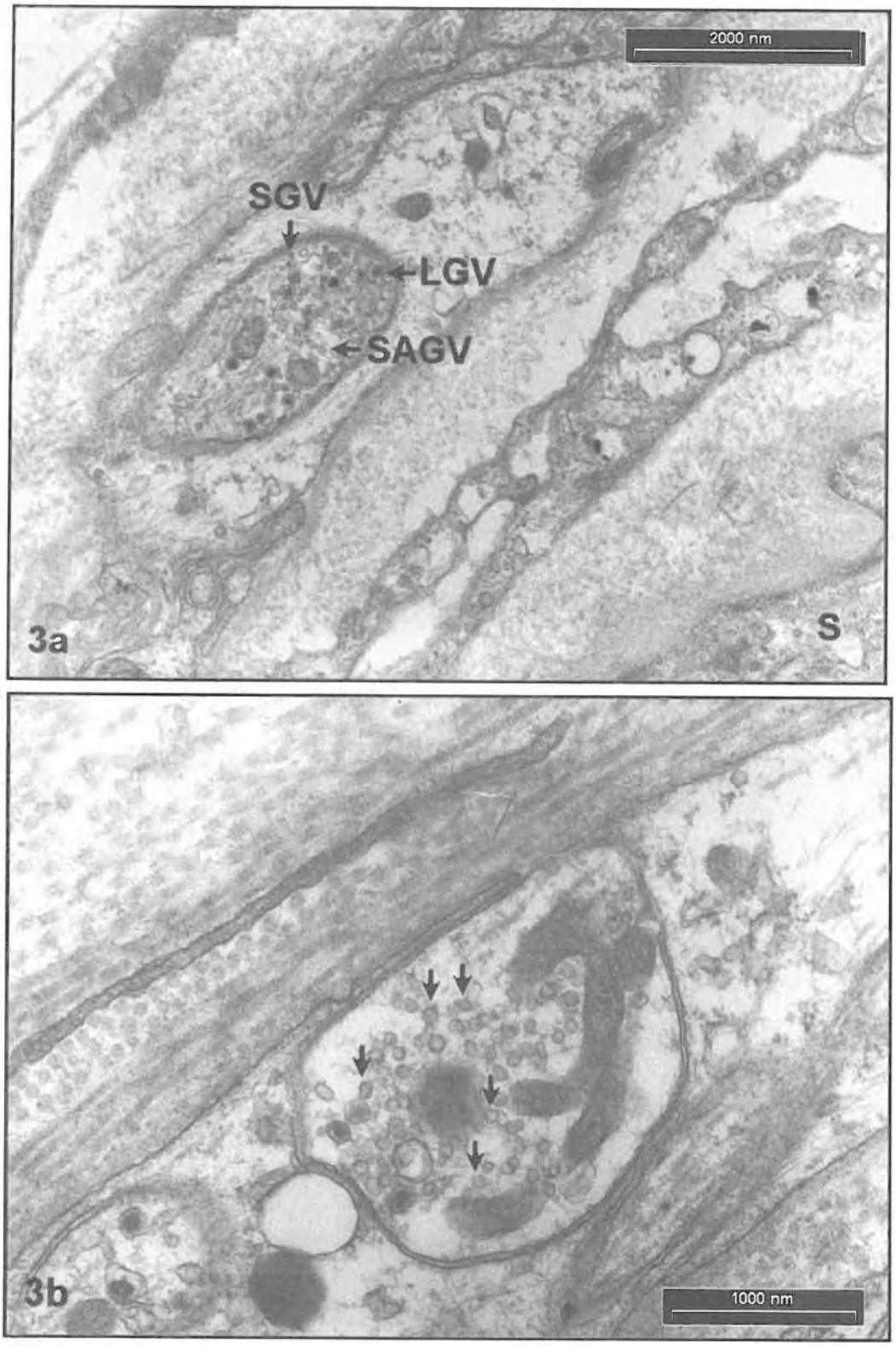

Fig. 3. Dense fibrous layer. (a) Type 1 nerve profile containing SGV, SAGV and LGV, located in the adventitial-medial boder of a small artery. S, smooth muscle cell. (b) Type 1 nerve profile containing SGV with indistinct granular cores (arrows).

Structure of the vesiculated nerve profiles.

Two morphologically distinct types of vesiculated nerve profiles were consistently identified and classified into 2 groups according to the staining characteristics of the small vesicles they contained.

Type 1 nerve profiles (n= 75) were frequently encountered in the specimens studied. They were filled with predominantly round small granular vesicles (SGV) 30-60 nm in diameter (Figs 3*a*, 4 *a,b*). About two-thirds of the SGV contained a electron-dense granule or core located centrally within the vesicle and separated from the vesicle membrane by an electron-lucent halo. The others contained multiple electron-dense spots. Some vesicles contained indistinct granular material (Fig. 3*b*). In addition type 1 nerve profiles contained varying numbers of small electron-lucent or agranular vesicles (SAGV) 30-60 nm in diameter and few large granular vesicles (LGV) 70-120 nm in diameter. Approximately 60 % of the type 1 nerve profiles also contained varying numbers of flattened agranular vesicles (FAGV; mean number 1.73, S.D. 0.83; n=22). Type 1 profiles contained approximately 14 % LGV, 67 % SGV; 19 % SAGV (mean number of vesicles per type 1 axonal profile 26.03, S.D. 14.53; n= 37). The type 1 nerve profiles frequently contained small elongated mitochondria (Fig. 4 *a*).

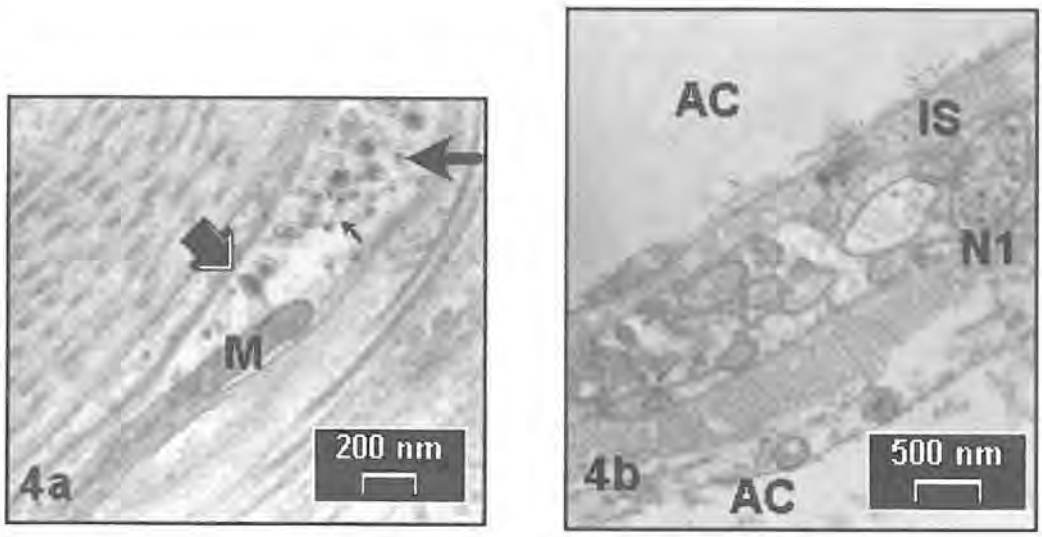

Fig. 4. *(a)* Small elongated mitochondrion (M) in a type 1 nerve profile situated in the dense fibrous layer. Thick arrow, LGV; thin arrow, SGV; small arrow, SAGV. *(b)* Type 1 nerve profile (N1) situated in the synovial plical tissue. Interstitial septum (IS) lies between adipose cells (AC).

Type 2 nerve profiles (n= 20) contained mainly SAGV 30-60 nm in diameter (Figs 5 *a,b*, 6 *a,b*), a few LGV 70-120 nm in diameter, and mitochondria. Considerable variation in vesicle numbers and proportions of SAGV to mitochondria was observed in the type 2 nerve profiles. One extreme of this variation was nerve profiles containing a large number of SAGV and few or no mitochondria (Fig. 5 *a*); another was nerve profiles filled with a larger number of mitochondria in addition to a few SAGV (Fig. 5 *b*).

The LGV observed in type 1 and 2 nerve profiles commonly contained a core of low electron-density ~ 50 nm in diameter. However, they were often variable in appearance: some were deformed, others lacked a clear-cut halo or were electron-lucent.

In addition to the above mentioned types of axonal profiles, a rare type of nerve profile was occasionally observed containing a heterogeneous population of FAGV ranging from 38 to 75 nm in length and 10 to 25 nm in width (Fig. 5 *c*). Vesicles were defined as flat, when they were approximately twice as long as wide. In addition, this type of nerve profile also contained varying numbers of LGV and round and/or deformed SAGV.

Distribution and orientation of the vesiculated nerve profiles.

Commonly, both types of nerve profiles were localised in a plexus of fine unmyelinated nerve fibres accompanying small capsular arteries and arterioles. The nerve fibres within this perivascular plexus, were mainly orientated along the axis of the blood vessels. The number of cross-sectioned perivascular nerve fibres varied from 4 to 7.

Type 1 nerve profiles were most frequent and were encountered in almost all vascularised capsular specimens studied. Commenly, this type was confined to the adventitial-medial border of small arteries and arterioles in the dense fibrous capsule and the adjacent part of the subintimal layer (Fig. 3 *a*). Similar profiles were observed related to arterioles within fibroelastic interlocular septa of the synovial plical tissue (Fig. 2 *d*). The bulging naked part of perivascular type 1 varicosities usually faced vascular smooth muscle cells (Fig. 2 *d*). No synaptic contacts could be demonstrated. The neuromuscular distance (average distance between the basal lamina of the smooth muscle cell and the axolemma of the varicosity) varied considerably. The closest distance was 0.95 µm, but, in some instances, type 1 nerve profiles were found remote from the capsular smooth muscle tissue, at a distance of 10 µm or more.

Some type 1 nerve profiles observed in the synovial plical tissue were located in narrow interstitial septa between adipose cells, unrelated to blood vessels (Fig. 4 *b*). The perivascular type 2 nerve profiles, especially those containing a larger number of mitochondria and only few SAGV, were usually confined to the outer adventitial border or encircled the tunica adventitia. The bulging naked part of type 2 varicosities frequently faced directly onto the surrounding perivascular capsular tissue, the Schwann cell cytoplasm being situated between the varicosity and the blood vessel (Fig. 6 *a,b*). Type 2 profiles containing mainly SAGV in addition to few mitochondria were most often localised in the adventitial-medial border.

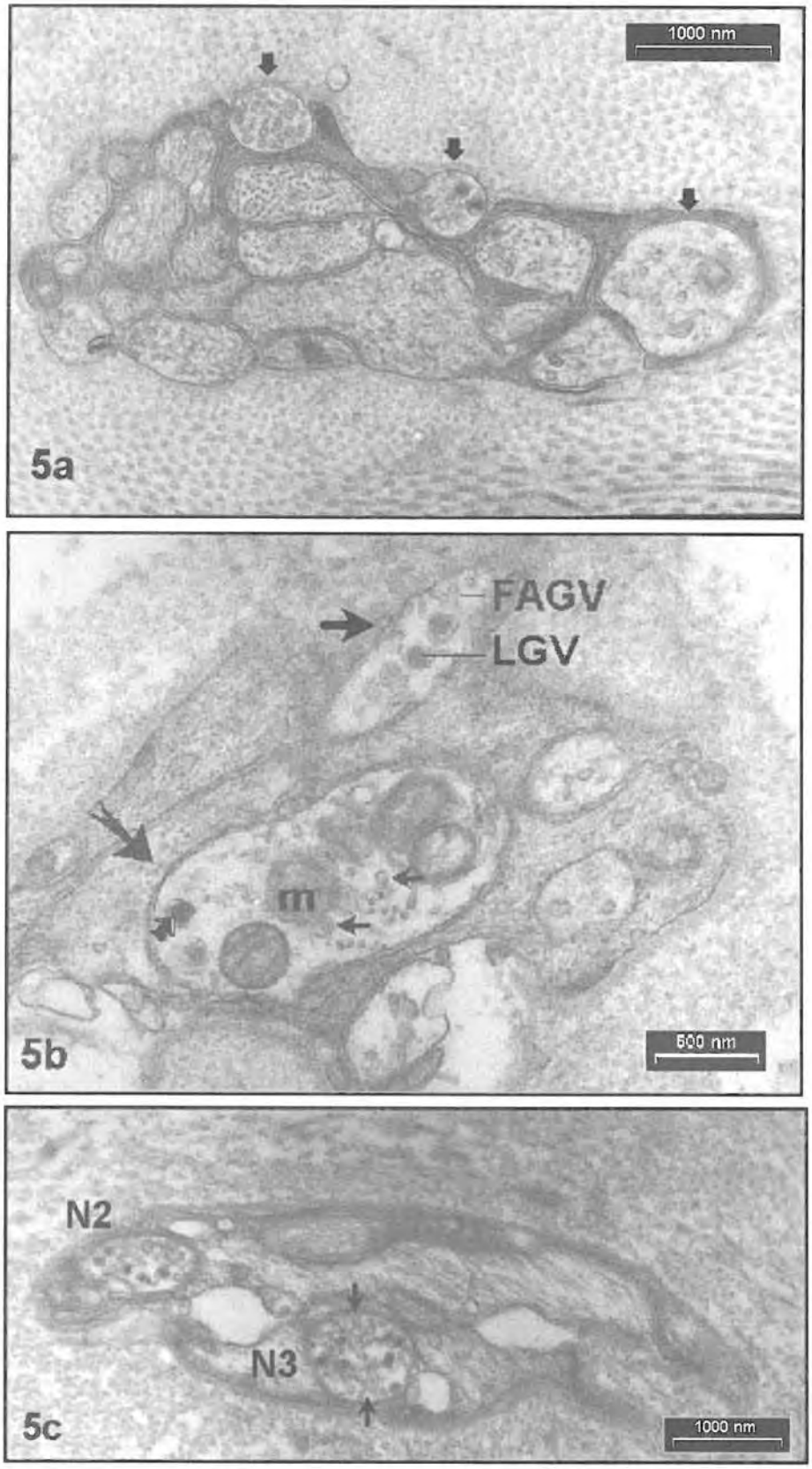

Fig. 5. Dense fibrous layer. (a) Type 2 nerve profiles (arrows) containing a predominance of SAGV. (b) Varicosity (thin arrow) containing FAGV and LGV. Type 2 nerve profile (large arrow) containing mitochondria (m), LGV (thick arrow) and a few SAGV (small arrows). (c) Nerve profile (N3) containing a predominance of FAGV (arrows) and another (N2) containing a heterogeneous population, predominantly of SAGV with a few FAGV and LGV.

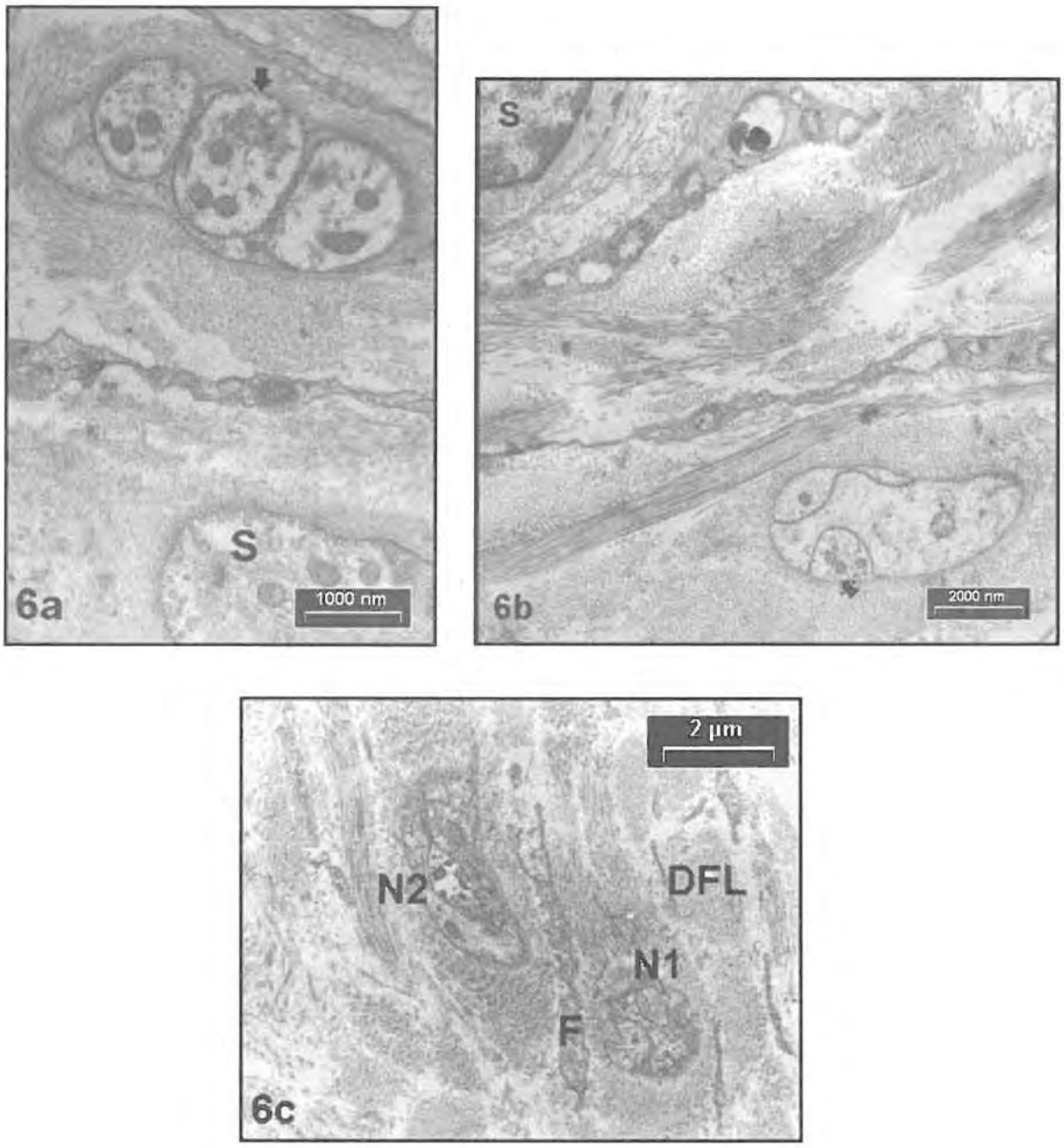

Fig. 6. Dense fibrous layer. (a) Three type 2 varicosities enclosed by the same Schwann cell in the tunica adventitia of an arteriole. Note that the varicosities are directly facing the surrounding outer connective tissue. Arrow, accumulation of small agranular vesicles; S, smooth muscle cell. (b) Type 2 nerve profile encircling the outer adventitial border of an arteriole, facing the surrounding interstitium (arrow). S, smooth muscle cell. (c) Type 2 nerve profile (N2) containing mainly mitochondria in the vicinity of a type 1 nerve profile (N1). Note the finger-like protrusion of the type 2 profile. DFL, dense fibrous layer; F, fibroblast.

DISCUSSION

According to Villaro et al. (1987) we defined the unmyelinated nerve fibre by the Schwann cell and used the morphological criteria for recognising true axons following Novotny et al. (1993). Unmyelinated nerve fibres presenting vesiculated axonal enlargements, were considered as nerve endings (Ruskell, 1994).

After glutaraldehyde fixation and staining with lead citrate and uranyl acetate, vesicles 30-60 nm in diameter with a electron-dense granule or core can be considered as characteristic vesicles of adrenergic nerve endings (Hökfelt, 1969; Geffen & Livett, 1971; Gabella, 1976). According to this classification, the perivascular type 1 nerve profiles observed in the joint capsule and the synovial plical tissue, are rather clearly distinguishable as varicosities along the length of the terminal part of sympathetic noradrenergic postganglionic fibres. Most probably they originate from sympathetic neurons in paravertebral ganglia of the sympathetic trunk, which are known to supply the vertebral column; nerve fibres connecting the dorsal ramus of the spinal nerve and rami communicantes of the sympathetic trunk were demonstrated by Groen et al. (1990) and considered as a possible sympathetic pathway (Stolker et al. 1994).

The presence of SGV with indistinct granular material and/or SAGV in the adrenergic profiles, is probably due to a inconsistent visualisation of the granular cores related to glutaraldehyde and osmium tetroxide fixation (Gabella, 1976). The presence of LGV in addition to the SGV is known as a characteristic ultrastructural feature of adrenergic nerve endings (Gabella, 1976).

According to Fried et al. (1985), SGV contain only noradrenaline (NOR), whereas the LGV contain both NOR and neuropeptide Y (NPY). However, in the enteric nerve plexus a type of SGV-containing varicosity is described, which is not noradrenergic (Gordon-Weeks, 1982). The adrenergic nerves encountered in the facet joint capsule are thought to form a perivascular autonomic ground plexus according to Burnstock (1986). Their localisation in the adventitial-medial border of capsular blood vessels suggests a major role in the regulation of capsular blood flow. On the other hand, a neuromuscular distance of 10 µm (or even more), as observed in our study, does not support claims for their function in vasoregulation. However, the minimal junctional cleft of adrenergic endings is known to vary considerably (Burnstock, 1986).

White adipose tissue is known to lack adrenergic fibres, except those related to the blood vessels (Gabella, 1976). In contrast, our study demonstrated adrenergic nerves unrelated to blood vessels in the adipose plical tissue of the facet joint.

Varicosities containing high proportions of SAGV 30-60 nm in diameter are conventionally regarded as cholinergic in the peripheral nervous system (Laitinen et al., 1985; Wang et al., 1994). Although the functional interpretation and origin of type 2 nerve profiles remains speculative, they fulfill this morphological criterion and could be regarded as cholinergic nerve endings most probably derived from the sacral outflow of the parasympathic nervous system.

According to Gibbins (1982) type 2 profiles could also represent a nonadrenergic, noncholinergic (NANC) population of autonomic nerves containing purinergic (adenosine 5'-triphosphate (ATP)), peptidergic (e.g. vasoactive intestinal peptide (VIP)) and/or serotonergic transmitters, stored in SAGV, LGV and/or large opaque vesicles (LOV). The identification of VIP-immunoreactive nerves in the facet joint (Ashton et al. 1992; Ahmed et al. 1993) could support this hypothesis. However, type 2 profiles- especially those containing an accumulation of mitochondria- most probably represent sensory nerve endings of type III (Aδ) and IV (C) fibres. The latter are involved in nociception and have a variable vesicular content (Heppelman et al. 1990). The presence of an accumulation of mitochondria is known to be a useful ultrastructural criterion for recognizing sensory nerve endings (Johnson & Halata, 1991; Lambrichts et al. 1993). The naked part of the varicosity is defined as the receptive area (Andres et al. 1985; Heppelman et al. 1990) and permits axonal contact with the interstitium (Novotny et al. 1993).

As described by Elfvin et al. (1994), we regularly observed accumulated SAGV in type 2 varicosities, in close association with electron-dense material located at the inner aspect of the naked axolemma. Tay & Wong (1992) localised substance P (SP) on the membranes of SAGV. This peptide-hormone is involved in nociception, vasoregulation and inflammation (Ahmed et al. 1993).

The perivascular localisation and orientation of the mitochondria-containing type 2 varicosities, facing the surrounding capsular tissue in most specimens studied, suggest a receptor function and is in agreement with the existence of a perivascular nociceptor as defined by Wyke (1980), and the demonstration of a sensory innervation by recent immunohistochemistry (see Introduction) in the facet joint.

Although two apparently distinct types of perivascular nerve profiles were observed in the facet joint, a conclusive morphofunctional identification of the vesiculated nerve profiles, using conventional electron microscopy, is impossible. Sectioning of an adrenergic profile through a clumped distribution of SAGV when the entire vesicle population is not visible (Cook & Burnstock, 1976), and the variability in ultrastructural appearance of SGV related to glutaraldehyde and osmiumtetroxide fixation (Gabella, 1976), could apparently lead to a misinterpretation of adrenergic profiles as type 2.

On the other hand, individual perivascular nerve profiles in the facet joint- especially those of the sympathetic nerves- regularly contained a mixture of various vesicle types, suggesting that more than one transmitter could be stored within the same nerve varicosity. Evidence for coexistence and/or cotransmission (Burnstock, 1976) of the classical transmitters and a variety of putative transmitters and/or pre- and postjunctional modulators, has been demonstrated within single nerve profiles of the autonomic and sensory nervous system (for review, see Burnstock, 1988, 1990) and implicates the presence of a mixture of vesicle types within a single nerve profile (Burnstock, 1981). Especially in sympathetic nerves, there is substantial evidence for the coexistence and/or cotransmission of NOR, ATP, NPY and 5-hydroxytryptamine (5-HT); in parasympathetic nerves a coexistence of acetylcholine (Ach) and VIP has been demonstrated; SP and calcitonin gene-related peptide (CGRP) have been shown to coexist in sensory perivascular nerves (Burnstock, 1990). A variety of putative transmitters and/or neuromodulators has already been demonstrated in the lumbar facet joint (see Introduction), and warrants further investigation by EM immunocytochemistry. Their localisation at the ultrastructural level is necessary, as untill now only speculations about the neurochemical content of the vesiculated nerve profiles can be made. They probably cooperate in the nervous controle of the capsular vasculature and/or could provide a morphological basis for low back pain originating in the lumbar facet joint. The presence of adrenergic endings, in the vicinity of nociceptive afferents in the joint capsule could also contribute to this hypothesis. The possibility of modulation of sensory inputs by the sympathetic system (Passatore & Filippi, 1983), the existence of a sympathetic response to C fibre afferent excitation (Koizumi et al.1970) and to mechanical stress of the spinal column (Sato et al. 1984) has been demonstrated.

The third type of nerve profile observed in our study, containing predominantly FAGV, resembled the noradrenergic axons described by others (Wilson et al. 1981; Wang et al. 1994). On the other hand, the flattening of the vesicles could be induced by the high osmolality of the aldehyde fixative solution (Jones & Cowan 1977).

In conclusion, the present study has revealed morphological distinct types of vesiculated nerve profiles in the facet joint, mainly in perivascular plexuses of the dense fibrous capsule and the synovial plical tissue. We have attempted to classify the profiles according to the size, shape and electron density of their vesicular content. The structure and distribution of the nerve endings probably represent different physiological conditions. In tissue conventionally fixed for electron microscopy, only speculations can be made as to their nature and putative neurochemical content, since they have no clearly distinguishing ultrastructural features (Gibbins, 1982; Gibbins et al. 1988). Immunocytochemistry at the ultrastructural level will be necessary to clarify their function.

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Encapsulated Ruffini-like Endings in Human Lumbar Facet Joints

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ABSTRACT

The innervation of the human lumbar facet joint capsule was studied by light and electron microscopy. Small numbers of encapsulated corpuscular endings were identified in the dense fibrous layer. Clusters of 2 types of endings have were found: small cylindrical corpuscles (type 1) and large fusiform corpuscles (type 2). The corpuscles were classified structurally as Ruffini-type endings. The 1st type was predominant and characterised by a compartmentalised receptor complex, a thin perineurial capsule and a narrow subcapsular space. The 2nd type was characterised by a thicker perineural capsule, a "spindle-like" receptive complex, and an extensive subcapsular space with capillaries and concentrically oriented fibroblast-like cells.

Both types of endings were innervated mainly by thinly myelinated group III (A delta) and unmyelinated group IV (C) nerve fibres that branched and terminated in the receptor complex. Their sensory endings were intimately related to the collagen fibre bundles as multiple enlarged axonal segments ('beads') with ultra-structural features which were characteristic of receptive sites: an accumulation of mitochondria and vesicles, and "bare" areas of axolemma lacking a Schwann cell investment but covered by a thin basal lamina. Some beads in the 2 nd type of ending contained granular vesicles, 30 - 60 nm in diameter, resembling sympathetic nerve endings.

Small diameter collagen fibrils situated within multilayered basal laminae were found among the multiple receptive sites in the receptive complex in both types of ending. Their possible functional significance in mechanoreception is discussed. Particular attention has been given to their apparent variable orientation to the mechanoreceptive site.

Key words: Spine; mechanoreception.

INTRODUCTION

The morphology and innervation of the facet joint has long been a subject of great interest in the orthopaedic and neurosurgical literature (Gilette et al. 1993; Mehta & Parry, 1994; Yamashita et al. 1996). Immunohistochemical investigations demonstrated the presence of an autonomic and sensory innervation (Giles & Harvey, 1987; El-Bohy et al. 1988; Cavanaugh et al. 1989; Gronblad et al. 1991; Ashton et al. 1992; Ahmed et al. 1993; Beamen et al. 1993). Recently, Vandenabeele et al. (1995) reported the ultrastructural characteristics of noncorpuscular nerve endings in the capsule of human lumbar facet joints. A variety of corpuscular endings have been observed in the fibrous capsule of the facet joints (Ozaktay et al. 1991; McLain, 1994). Their typification was based on the morphofunctional classification of articular receptors introduced by Freeman & Wyke (1967). Ultrastructural data on mechanoreceptive endings in the facet joint are completely lacking. Electrophysiologic studies identified mechanosensitive afferent units in the facet joint capsule (Cavanaugh et al. 1989; Yamashita et al. 1990; Avramov et al. 1992; Pickar & McLain, 1995). Pickar & McLain demonstrated that a majority of slowly adapting mechanosensitive units in the facet joint capsule of the human lumbar spine have a tonic discharge and are responsive to distraction and compression of the capsule in a graded fashion relative to the direction of force applied, and assumed that they probably represent a Ruffini-type of ending. Functionally, the Ruffini ending is believed to act as a slowly adapting stretch receptor with tonic discharge (Chambers, 1969; Chambers et al. 1972), that mediates in protective muscule reflexes (Johansson et al. 1991; Schenk et al. 1996).

The facet joint capsule of the human lumbar spine appears to be richly supplied by Ruffini-type nerve endings (Ozaktay et al. 1991). They probably ensure postural stability and are well suited to function as spinal column proprioceptors (Pickar & McLain, 1995). We investigated the ultrastructure of the Ruffini-type nerve endings present in the human lumbar facet joint and sought to determine whether they display specific features that could explain their directional sensitivity to spinal loading. Special attention was thereby given to the structural specialisations at their mechanoreceptive sites. Possible mechanisms for mechanoelectric transduction are discussed.

MATERIAL AND METHODS

Biopsy specimens of the facet joint capsule at lumbosacral spinal levels were obtained from 12 patients with significant low back pain and sciatica, undergoing posterior fusion operations. The patients were healthy apart from their spinal pathology. Tissue specimens of the inferolateral area of macroscopically intact capsules were excised with a sharp scalpel and fine forceps from both facets within each operative field. A careful examination and orientation within each sampled level were caried out in order to avoid harvesting adjacent tissue, and to make sure of recieving comparable samples from each joint. Care was taken to avoid tissue trauma during surgery and sampling.

The fresh surgical specimens were dissected in 1 mm^3 blocks and immediately immersed in a solution of 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3) for 24 - 48 hours. Tissue was postfixed in 2% osmium tetroxide for 1 h, contrasted with 2% uranylacetate in 10% acetone for 20 min, and embedded in Araldite. Semithin sections (0.5 µm) were stained with a solution of thionin and methylene blue (0.1% aqueous solution) for light microscopic examination to identify neural structures.

Presumptive corpuscular endings were often difficult to identify in semithin sections by light microscopy. Many tissue specimens were harvested and sectioned to find the areas illustrated in the present report. Serial sectioning avoided misinterpretation of size. Verification of presumptive corpuscular nerve endings on serial semithin sections by light microscopy was based on one or more of following criteria: (1) corpuscular structure with varying degree of encapsulation, (2) structure identifiable on consecutive sections, (3) inner core with parent nerve fibres and varying degree of compartmentalisation. Final verification of the mechanoreceptor ending was made by electron microscopy.

RESULTS

Small numbers of corpuscular nerve endings, structurally classified as a Ruffini-type ending, were identified in the dense fibrous capsule of the facet joint. They were found in fibroadipose septa and usually accompanied blood vessels. Neural structures were not found in the synovial membrane.

Light microscopy

Two types of encapsulated Ruffini-like corpuscles were readily identified. A small diameter cylindrical Ruffini-type ending was predominant (type 1). Type 1 endings were regularly clustered and easily recognised by light microscopy as compartimentalised entities with a minute subcapsular space (Fig. 1*a*). Their diameter measured about 20 - 25 µm. Additionally, a remarkably large encapsulated Ruffini-type corpuscle was found, up to 500 µm in total lenght (type 2). The latter was characterised by a distinct inner core and extensive subcapsular space (Figs 1*b*, 2*a, b*). Towards their polar region type 2 endings were fusiform in shape. Their main body measured 70 µm on average.

Electron microscopy

Ultrastructurally, attenuated cytoplasmic extensions of fibroblast-like 'septal cells' (Schoultz & Swett, 1972) compartmentalised the capsular space of the type 1 ending into 3-5 compartments (Fig. 3). The receptive complex was loosely arranged within these compartments.

The receptive complex of the type 2 ending was arranged as a 'spindle-like' inner core, about 40 µm in diameter. This core was separated from the perineural capsule by an extensive subcapsular space, measuring up to 25 µm width (Fig. 4). This subcapsular space was transversed by a web of delicate cell processes from concentrically oriented fibroblast-like cells, referred to as inner capsular cells. These inner capsular cells were only locally lined by a basal lamina, on average 50 nm (Fig. 5*a*). The subcapsular space enclosed several collagen bundles. Some of these collagen bundles were ensheated by loops of basal lamina, or by hook-like cell processes of inner capsular cells (Fig. 5*a*).

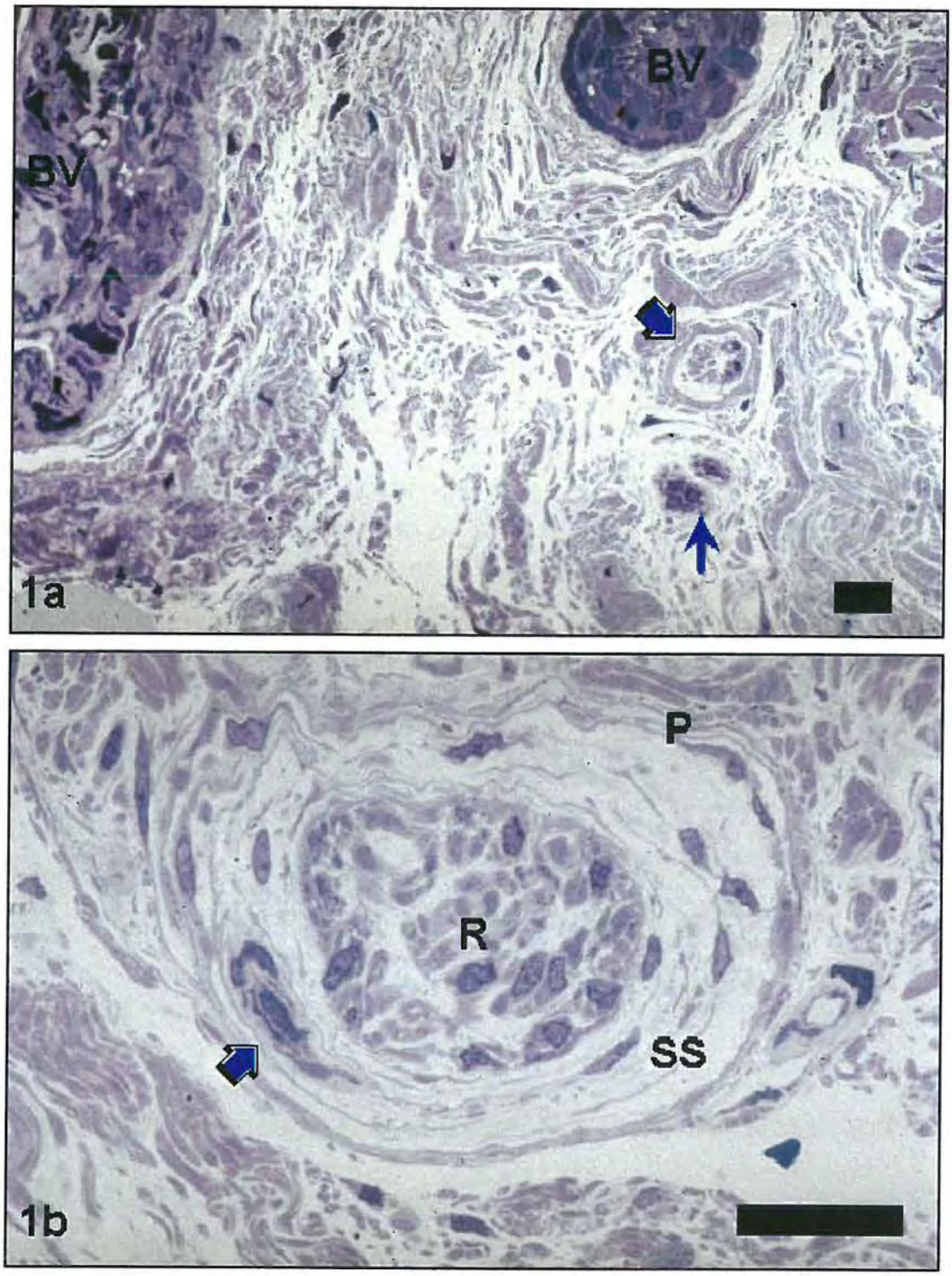


Fig. 1. Light micrographs of thionin-methylene blue-stained semithin sections of the dense fibrous capsule. (a) Type 1 encapsulated nerve ending (thick arrow) adjacent to blood vessels (thin arrow). Blood vessel (BV). (b) Type 2 ending. Note the blood vessels (arrow) in the extensive subcapsular space (SS). Perineurium (P). Receptive complex (R). Bar, 25 µm.

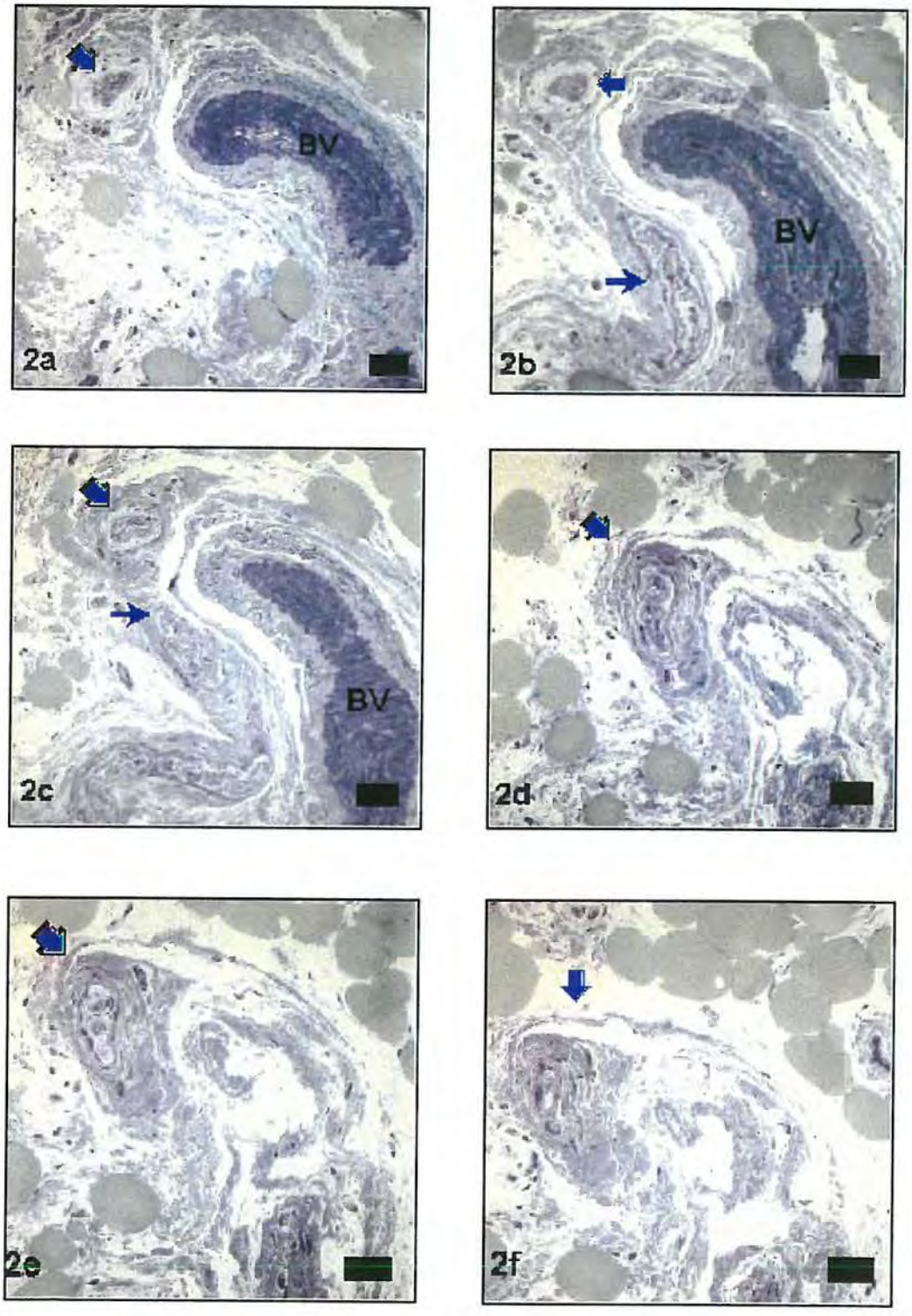


Fig. 2. Human lumbar facet joint capsule . Light micrographs of consecutive thionin-methylene blue-stained semithin sections. Blood vessel (BV). Bar, 25 µm. (a) Paravascular type 2 ending (thick arrow) in the dense fibrous capsule. (b and c) Afferent bundle (thin arrow) approaching the corpuscular ending. (d to f) After transition of the bundle and the ending, untill disappearing.

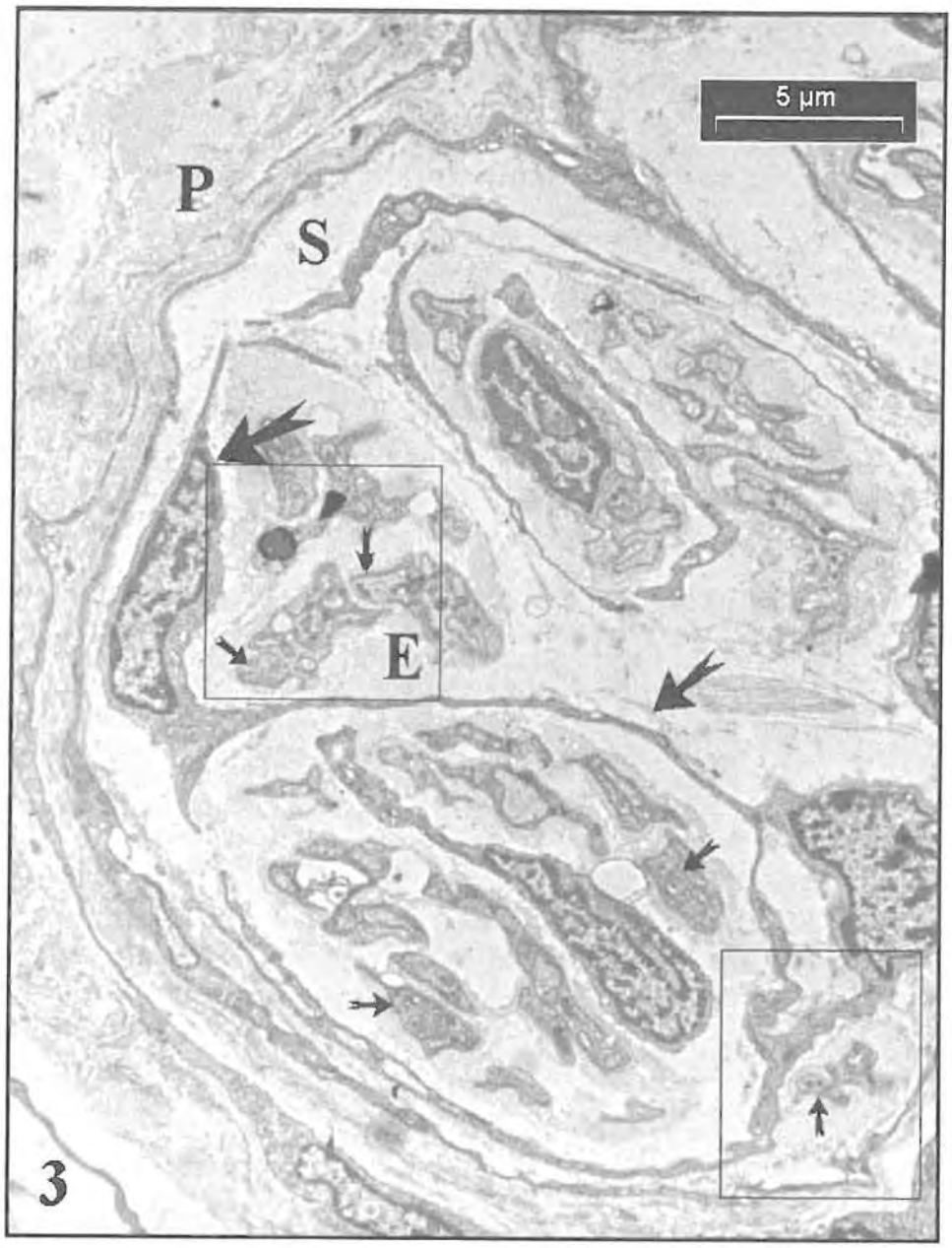


Fig. 3. Electron micrograph of a typical type 1 ending showing its dense fibrous capsule. The receptor complex is situated within several compartments of the capsular lumen, bounded by thin cytoplasmic processes of fibroblast-like septal cells (thick arrows). Note the small subcapsular space (S). Endoneurial connective tissue (E); nerve profiles (thin arrows); perineural capsule (P).

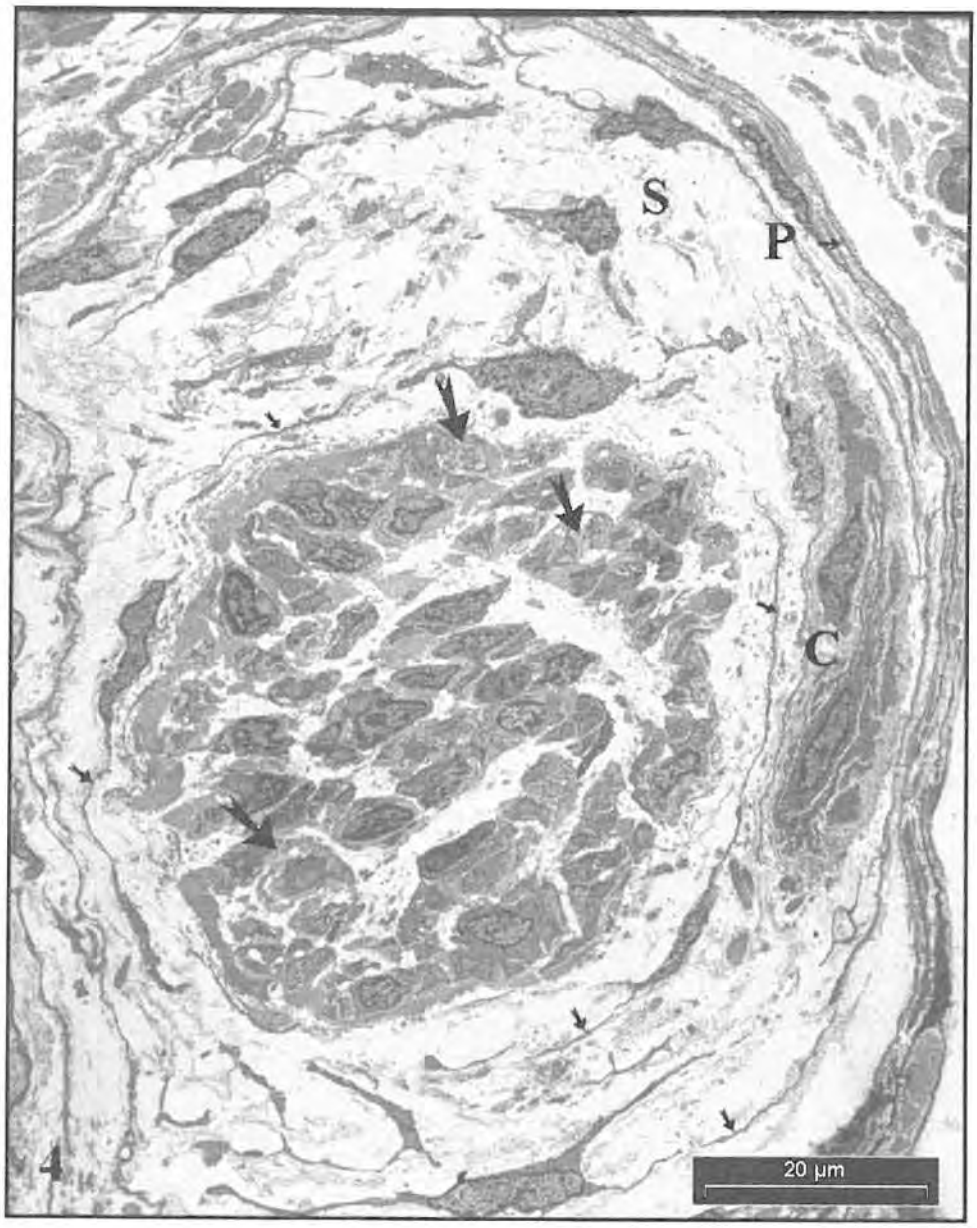


Fig. 4. Electron micrograph of a typical type 2 ending characterised by a dense fibrous capsule, an extensive subcapsular space (S), a 'spindle-like' receptive complex, and perineurial capillaries (C). Note that the subcapsular space is transversed by delicate cell processes (small arrows) of concentrically arranged inner capsular cells. P, outer (perineurial) capsular cells; large arrows, focally multilayered basal laminae within the receptor complex.

The capsule of both types of endings consisted of concentric layers of the flat perineurial cells (1-2 in type 1, 3-4 in type 2). Perineurial cells were lined on both sides by a continuous basal lamina and separated by narrow intercellular spaces. These intercellular spaces included collagen fibrils, a few scattered bundles of microfibrils, and some unmyelinated nerve fibres. The perineurial cells were refered to as outer capsular cells in the type 2 Ruffini ending. The overall capsule thickness of the type 2 ending ranged from 0.8 to 2.5 µm.

Adjacent cell processes of perineurial cells interlocked and presented specialised intercellular contacts. Tight junctions were prominent. The attenuated cell processes of the inner capsular cells in the type 2 ending also closely apposed to each other, forming cell contacts identified as tight junctions and gap junctions (Fig. 5*b*).

A peculiar feature of the type 2 ending was the presence of capillaries with a continuous endothelium in the periphery of the subcapsular space (Fig. *4*). The capillaries always carried a short extension of the perineurium with them, along their course in the subcapsular space.

Both types of corpuscular endings were supplied by a nerve bundle accompanying blood vessels. At the transition of this bundle and the corpuscle, the perineurial sheath of the nerve bundle fused with the receptor capsule. Type 1 endings were commonly supplied by a small bundle of unmyelinated group IV (C) nerve fibres. In its preterminal course the bundle divided over several cylindrical type 1 corpuscles.

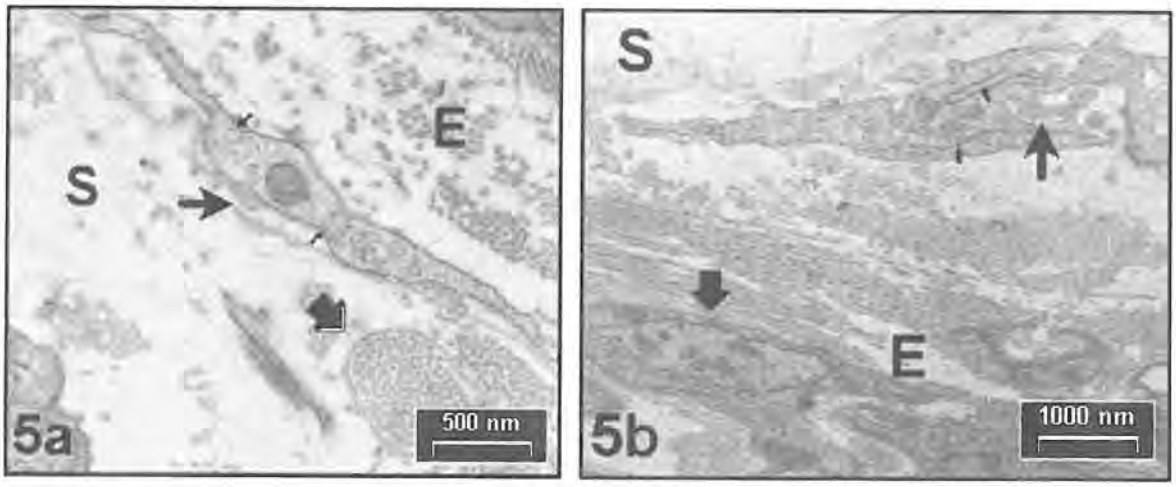


Fig. 5. Electron micrographs of inner capsular cells in the subcapsular space (S) of the 2nd type of ending. E, endoneurial connective tissue. (a) Cell process with detached basal lamina (thin arrow). Loops of basal lamina enclosing a collagen bundle (thick arrow). Small arrows, micropinocytotic caveolae. (b) Specialised intercellular contacts recognised as sequences of tight junctions (small arrows). Thin arrow, groups of microfibrils; thick arrow, group III axonal bead.

Type 2 endings are commonly supplied by a nerve bundle measuring on average 40 µm in diameter that comprised a mixed population of thinly myelinated group III (A delta) and unmyelinated group IV (C) nerve fibres (Fig. *6*). Group III (A delta) nerve fibres measured on average 1 - 3 µm, whereas Group IV (C) fibres measured on average 0.5 - 2 µm.

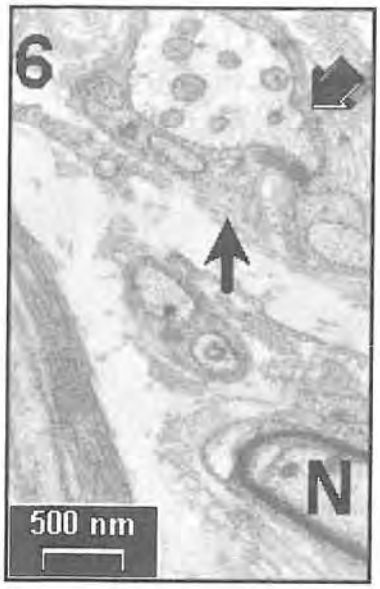
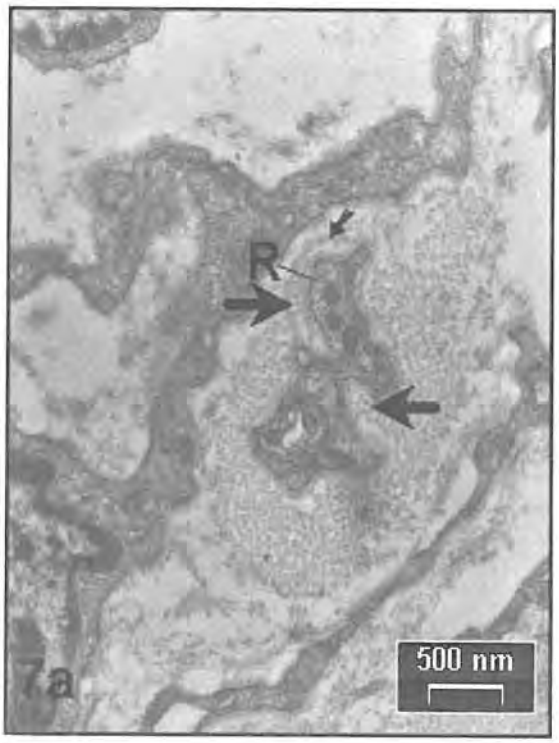


Fig. 6. Detail of the transition between the nerve bundle and a type 2 ending. Thinly myelinated group III (Ad) nerve fibre (N). Axonal bead with 'finger-like' extension (thick arrow). Note multilayered basal laminae enclosing small diameter collagen fibrils (thin arrow).



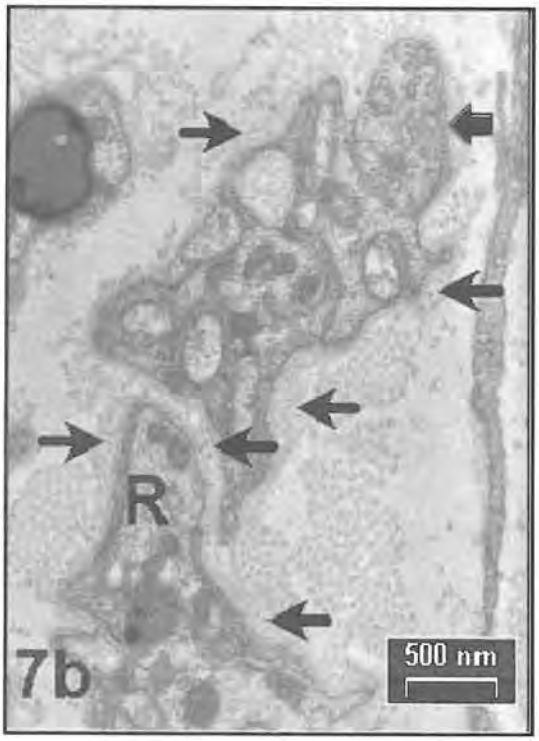


Fig. 7a,b. Higher magnification of the outlined areas within the receptive complex of the 1st type of ending shown in Figure 3, demonstrating axon terminals, partly or completely enveloped by Schwann cells. Small diameter collagen fibrils (large arrows) are intimately related to the receptor site (R), and additional loops of basal lamina are present (small arrows). Note the undulating transducer membrane of high contrast and underlying 'receptor matrix' with filamentous substructure at the receptive site. Thick arrow, vesiculated nerve profile containing numerous round and flattened clear vesicles.

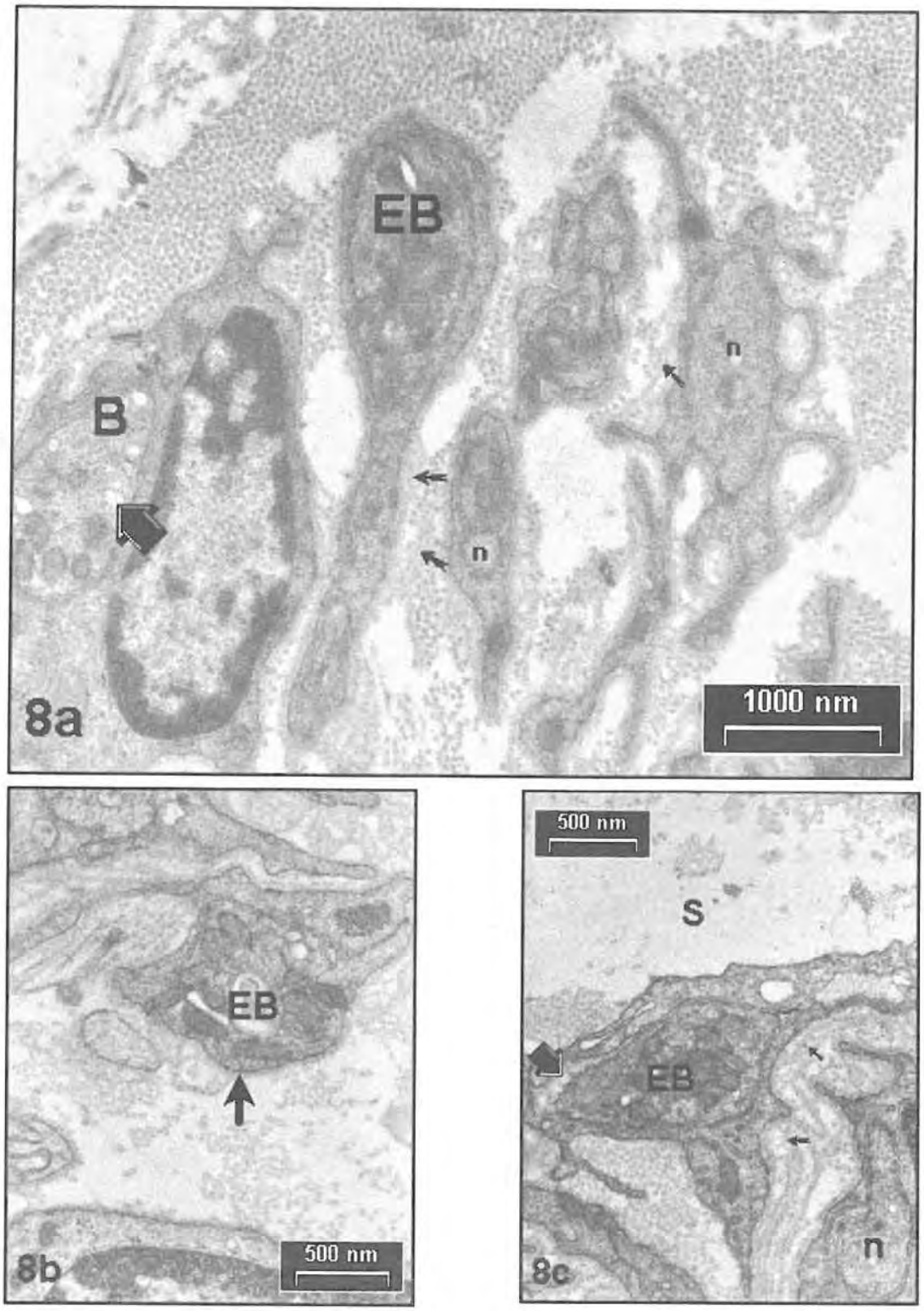


Fig. 8a,b,c. Electron micrographs of group II (Adelta) afferent nerve fibres in the receptive complex of the 2nd type of ending showing axonal beads (B) with peripherally distributed mitochondria, a central 'neurofilament core' (thick arrow, Fig. 8a), few neurotubules and scarce cell organelles. Axonal end bulbs (EB) containing randomly oriented mitochondria and variable numbers of vesicles, but in contrast to beads no neurofilament core and scarce neurotubules. S, subcapsular space; small arrows, multilayered basal laminae; n, thin axonal segments with neurotubules and neurofilaments. Note the undulating transducer membranes of high contrast (thin arrow in Fig.8b; thick arrow in Fig. 8c) with underlying 'receptor matrix' and accumulated vesicles.

The receptive complex sensu stricto was composed of axon terminals and associated Schwann cell coverings, both intimately related to parallel bundles of collagen fibrils measuring about 30 - 45 nm in diameter. The receptive complex of both types of Ruffini endings showed similar ultrastructural specialisations. Nerve fibres branched frequently within the receptive complex and exhibited focal axonal enlargements (varicosities or beads). Considerable variation in the vesicular content, the proportion of vesicles to mitochondria, and the mean axonal diameter was observed in these varicosities. Generally, the varicosities were only partly enveloped by associated Schwann cell processes (Fig. 7 *a, b*). According to Heppelmann et al. (1990), the 'bare' area of axolemma lacking Schwann cell covering, was referred to as the 'receptive site'. The axolemma at the receptive site usually showed a varying degree of undulation. Collagen fibrils often closely approached this receptive site, but direct contact was seldom observed.

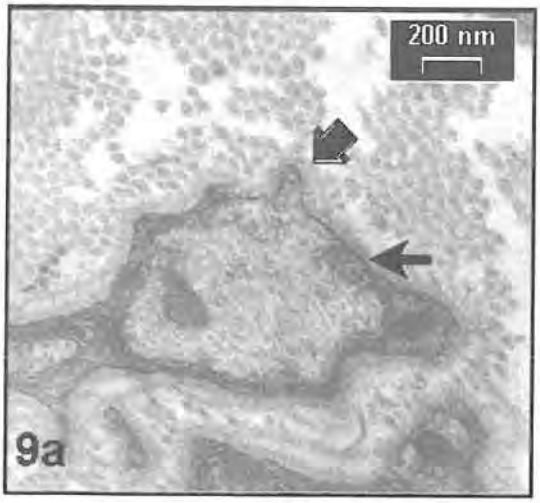
Large varicosities (measuring 1 - 1.5 µm), with 'neurofilament core' (Heppelmann et al. 1990), and a large number of peripheral and parallel mitochondria, were regularly found in the type 2 ending (Fig. 8*a*). Other varicosities had no neurofilament core, but were filled with randomly oriented mitochondria (Fig. 8*a, b*). They also contained varying numbers of clear vesicles and some dense cored vesicles.

Vesicles were scattered between the mitochondria or clumped within a distinct 'receptor matrix' (Andres and v. Düring, 1973; Andres et al., 1985). This matrix (Fig. 8*b*) was characterised by a filamentous substructure and lay under the bare axolemma at the receptive site. The receptor matrix was sometimes present in small axonal extensions known as axonal 'herniations' or 'fingers' (Byers, 1985), pointing to nearby endoneural collagen fibrils (Fig. 9*a*). The larger varicosities were commonly accompanied by smaller varicosities (mean average diameter 0.2 - 0.5 µm) containing scarce mitochondria and a larger number of small agranular vesicles 30 - 60 nm in diameter (Fig. 8 *b*). The smaller varicosities were most numerous in type 1 endings (Fig. 7*b*). Some of these smaller varicosities in the receptor complex of our type 2 ending contained a variable number of small granular vesicles, 30 - 60 nm in diameter (Fig. 10). These varicosities were completely or only partly ensheated by Schwann cells.

A striking structural specialization of the corpuscles presently described was the presence of multilayered basal laminae around the axon terminals and associated Schwann cells (Figs 7*a, b*, 8*a, b*). Particularly, the type 2 ending appeared focally well endowed with multilayered basal lamina (Fig *4*).

Both types of ending contained small diameter collagen fibrils (diameter on average 20 nm). These collagen fibrils were single or grouped and were commonly oriented tangential to the nerve terminals (Fig. 11). When sectioned transversally they were seen as electron-dense 'dots' around nerve terminals and associated Schwann cells. Comparable electron-dense dots were found along longitudinally sectioned nerve terminals and recognised as transversely sectioned small diameter collagen fibrils (Fig. 11). The small diameter collagen fibrils were always enclosed by one or several loops of multilayered basal laminae, and intimately related to the transducer membrane at the receptive sites (Figs 7*a, b*, 8*a, b*). They were regularly anchored in the basal laminae.

Schwann cells in both types of endings commonly exhibited long cytoplasmic extensions projecting far into the surrounding endoneural connective tissue, embracing collagen bundles. In some instances axon terminals in the Ruffini-like endings described here contained very electron -dense bodies, vacuoles, lamellar formations and multivesicular bodies (Fig. 9*b*).



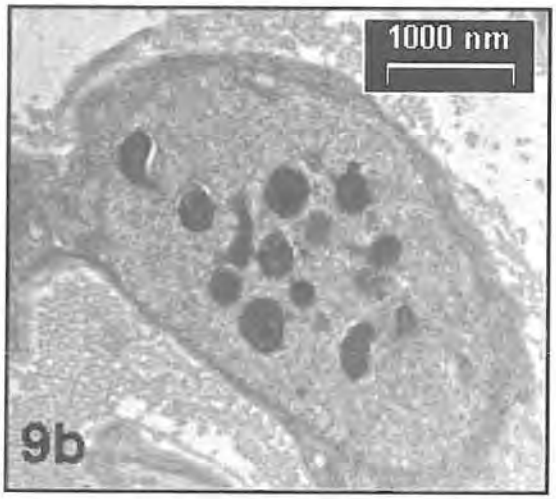


Fig. 9. Electron micrographs of axonal profiles in the receptive complex of the 2nd type of ending. (a) Fingerlike axonal herniation (thick arrow) in intimate relationship with the surrounding endoneurial connective tissue. Note the high contrast of the adjacent transducer membrane, and the underlying electron-dense axoplasm with accumulated small agranular vesicles (thin arrow). (b) Axonal profile with filamentous substructure containing multiple homogeneous densely osmiophilic bodies, recognised as end products of mitochondrial degeneration.

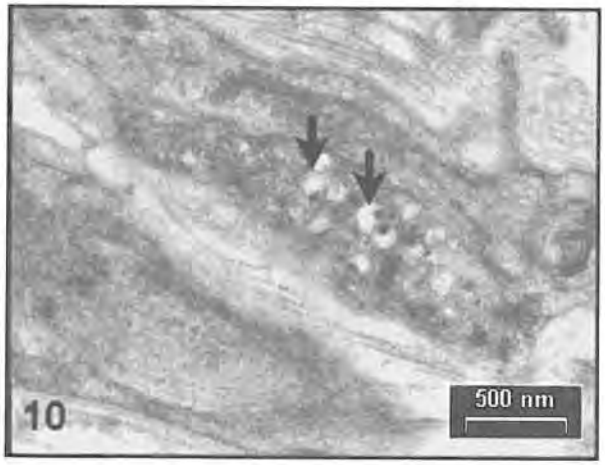


Fig. 10. Electron micrograph of a vesiculated nerve profile in the receptive complex of the 2nd type of ending, containing predominantly granular vesicles. Large granular vesicles with eccentric core and elaborate electron-lucent halo (arrows).

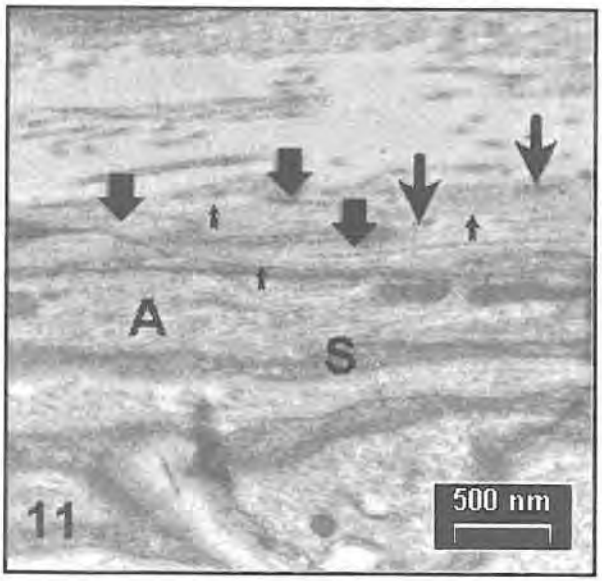


Fig. 11. Electron micrograph illustrating the intimate relationship between small diameter collagen fibrils and the axon terminal (A) with associated Schwann cell (S). Longitudinally sectioned axon terminal. Parallel small diameter collagen fibrils (thick arrows) enclosed within additional loops of basal lamina (small arrows). Note multiple electron-dense spots (thin arrows) within successive layers of basal laminae recognized as transversely sectioned small diameter collagen fibrils.

DISCUSSION

The morphofunctional classification of articular receptors as type I through IV, established by Freeman & Wyke (1967), has been widely used in the field of articular neurology, and includes Ruffini endings (type I), pacinian corpuscles (type II), Golgi endings (type III) and free nerve endings (type IV). Types I through III are corpuscular endings with mechanoreceptive properties, while noncorpuscular endings (type IV) mainly serve as nociceptors. Unfortunately the literature on the subject of nerve endings is confusing. It is often dificult to be certain with precision that different investigators are refering to the same ending. This may be due to a misinterpretation of nerve endings by the investigator, to a physiological variety of nerve endings in one individual, or to interspecies variation. Specific ultrastructural features of articular receptors may be important in order to speculate about their possible physiological function and specific functional importance for a particular joint.

The observation of small numbers of mechanoreceptive endings in the facet joint capsule of the human lumbar spine agrees with the observations of McLain (1994) on cervical facets and suggests that each mechanoreceptor has a relatively large receptive field, which emphasises the clinical and neurosurgical importance of the facet joint. Structurally both types of corpuscular endings found in the facet joint capsule of the human lumbar spine are comparable to the 2 types of Ruffini corpuscles described by Halata et al. (1984) in the knee joint capsule of monkey.

Ruffini corpuscles with an extensive subcapsular space, comparable to our type 2 endings, have also been found in the hairy skin of cats (Chambers et al. 1972), and in knee joint capsule of the dog (Schenk et al. 1996). The presence of a 'central core' (the receptive complex) situated within an extensive subcapsular space, has been implicated as an important criterion for the differentiation between large encapsulated Ruffini corpuscles and the well-defined Golgi tendon organ (Sklenska, 1972; Andres & von Düring, 1973; Andres, 1974). Golgi tendon organs and Ruffini endings are generally described as stretch receptors registrating altered tension of the surrounding collagen, as collagen bundles pass through these corpuscles (Schoultz & Swett, 1972 and 1974; Nitatori, 1988; Zimny, 1988; Backenköhler et al. 1996; Schenk et al. 1996).

Halata and Munger (1986) reported finding a specific corpuscle with a varying degree of encapsulation involved in the protopathic sensibility of the human glans penis. These corpuscles (referred to as 'genital end bulb) were characterised by a central meshwork of densely arborising nerve terminals ultrastructurally identical in appearance to noncorpuscular nerve endings. In this respect the ultrastructural resemblance with our type 2 Ruffini-like endings is remarkable. Indeed, the afferent terminals in our Ruffini-type endings share comparable structural specialisations with noncorpuscular endings of fine afferent nerve terminals (Andres et al. 1985; Heppelmann et al. 1990; von Düring & Andres, 1990).

In agreement with recent electrophysiological studies demonstrating group III and group IV mechanosensitive units in the lumbar facet joint (see Introduction), we did find our corpuscular endings supplied by thinly myelinated group III (A delta) and unmyelinated group IV (C) nerve fibres. Apparently the nerve supply of our Ruffini-like endings differs from the well-known corpuscular endings in tendons or other articular capsules. Indeed, as generally accepted corpuscular endings are generally supplied by a myelinated medium-sized axon measuring 2 - 4 µm in diameter classified as a group II fibre, dividing into several unmyelinated nerve fibres after entering the corpuscle (Halata, 1977; Halata & Munger, 1980,1981; Halata et al. 1984, 1985; Strasmann et al. 1987; Zimny, 1988; Halata & Haus, 1989; Haus & Halata, 1990; Heppelmann et al., 1990; Backenköhler et al. 1996).

According to Heppelmann et al. (1990), the largest axonal profiles are afferent terminals from thinly myelinated group III (A delta) nerve fibres. The presence of a 'neurofilament core' within the terminal part of the group III afferent axon is known to be a conclusive criterion in the distinction between beads along the course and the end bulb or the last axonal bead at the tip of the sensory ending.

The smaller axonal profiles, lacking a neurofilament core and containing a variable number of vesicles, probably represent axonal beads and/or end bulbs of nerve terminals from unmyelinated Group IV (C) afferent nerve fibres or autonomic efferent terminals. However, a conclusive morphofunctional classification of vesiculated nerve profiles using conventional EM is impossible.

Vesiculated nerve profiles characterised by numerous granular vesicles, 30 to 60 nm in diameter, are accepted to be adrenergic in function and probably derived from vasomotor nerve fibres.

It is generally believed that the 'bare' areas of axolemma intimately related to the surrounding endoneural connective tissue, are involved in mechano-electric transduction.
Stretching of the fibrous capsule is assumed to cause a physical distortion of the transducer membranes at the receptive sites (Schoultz & Swett, 1972). To our knowledge, direct contacts with collagen fibrils have never been demonstrated. In addition, the literature concerning the significance of the finger-like axonal projections in mechanoreception is contradictory (Byers, 1985; Maeda et al. 1989; Sato et al. ,1992). However, the presence of the small diameter collagen fibrils in intimate relation to multilayered basal laminae at the receptive sites, as described here, is suggestive. Comparable multilayered basal laminae have been observed in periodontal Ruffini endings (Byers, 1985; Byers & Dong, 1989; Maeda et al., 1989, 1991; Kannari, 1990; Kannari et al. 1991). Multiple layering of basal laminae has been assumed to represent a morphological sign of axonal regeneration (Ide, 1983; Sandoz & Zenker, 1986; Sasamura, 1986). To our knowledge a possible functional significance of the multilayered basal laminae remains to be elucidated.

Kannari et al.(1991) also observed periaxonal small diameter collagen fibrils intimately related to multilayered basal laminae. They assumed that the multilayered basal laminae thereby act as an 'adhesive device' or linkage between the afferent terminal and the small diameter collagen fibrils. Indeed, such structrural specialisations at the receptive sites probably facilitate the perception of the acquired mechanical stimulus. Stimulation of the receptive complex would narrow the extracellular spaces between successive layers of basal laminae, causing a deformation and displacement of small diameter collagen fibrils and a physical distortion of the transducer membrane. Small diameter collagen fibrils are thought to be highly elastic, recoil after deformation, and to be resistant to stretch (Harkness, 1968; Schoultz & Swett, 1972).

It may be noted that Pickar & McLain (1995) demonstrated electrophysiologically a directional sensitivity to spinal loading of slowly adapting Ruffini-type mechanosensitive afferents in the lumbar facet joint. In this context, the apparent variable orientation of periaxonal small diameter collagen fibrils in our encapsulated Ruffini-like endings on studying serial sections may be of interest and needs further investigation. It is tempting to suggest that the required mechanical stimulus of our type 2 endings is transferred to their receptive complex throughout the extensive fluid-filled spaces between successive inner capsular cells, thereby acting as a 'pressoreceptor'. It might be of interest to mention in this context the close resemblance between some of their afferent terminals and worn out pressoreceptor endings within the carotid sinus (Knoche et al. 1974; Abdel-Magied et al. 1982; Shin et al. 1987).

Tight junctions are known to provide specific permeability barrier characteristics. The intercellular features of the perineural cells and inner capsular cells create a blood-nerve barrier that provides a constant microenvironment for the axon terminals within the receptive complex (Vandenabeele et al. 1997). The perineural sleeve accompanying the capillaries in the periphery of the subcapsular space probably helps to maintain the integrity of the perineural barrier. Our observations on the intercellular junctions between capsular cells in the Ruffini corpuscles are in agreement with the results of Ovalle & Dow (1983). The gap junctions are involved in intercellular communication and cytofunctional synchronism of inner capsular cells (Ovalle & Dow, 1983).

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Chapter 4.2

Immunohistochemical Studies of Facet Joint Innervation

The joint capsule of the facet joint is richly innervated by a variety of noncorpuscular 'free' nerve endings, as demonstrated by electron microscopy. With the use of conventional histological staining methods a conclusive identification of nerve endings is impossible. Immunohistochemistry however, using specific neuronal markers allows identification of the innervation pattern and permits speculations on possible functions.

SP - and CGRP - immunoreactivity has been demonstrated in the lumbar facet joint of rat (Cavanaugh et al. 1989; Ahmed et al. 1993; Suseki et al. 1997), rabbit (El-Bohy et al. 1988), and man (Giles & Harvey, 1987; Grönblad et al. 1991; Ashton et al. 1992; Beamen et al. 1993). Immunoreactivity to SP generally appeared sparsely distributed. Reports on the relationship with local blood vessels are contradictory. Grönblad et al. (1991) reported SP-immunoreactive varicosal nerve endings in close apposition to local blood vessels. Others noted that they were exclusively nonvascular in position (Ashton et al. 1992; Ahmed et al. 1993; Suseki et al. 1997). Some authors did not mention the relationship of SP-immunoreactive nerves with local blood vessels (El-Bohy et al. 1988; Giles & Harvey, 1987).

SP, an 11 amino acid neuropeptide and a well-known peptide marker of sensory nerves, is generally believed to have a primary role in pain transmission and assumed to be susceptible to stimulation by direct pressure or by chemical irritation. SP is also known to participate in inflammation, it has an important effect on blood vessel permeability (Lembeck & Holzer, 1979), and is assumed to have a co-regulatory vasomotor function with CGRP. CGRP often colocalizes with SP. CGRP is also a well-known peptide marker of sensory nerves and a potent vasodilator neuropeptide (Brain et al. 1985; Ashton et al. 1992).

Grönblad et al.(1991) suggested that the perivascular SP-immunoreactive nerves in the joint capsules of the facet joint are mainly involved in local vasoregulation (Grönblad et al. 1991). However, their perivascular location does not necessary exclude a nociceptive function (Beamen et al. 1993). The predominantly nonvascular appearance of the SP-immunoreactive nerve fibres, running freely in the stroma of the capsule, as noticed in most studies, almost certainly indicates that the main action of SP in lumbar spine is not vasoregulatory. SP-immunoreactive nerves are assumed to be susceptible to stimulation by direct pressure or by chemical irritation, and thus contribute to nociceptive transmission (Giles & Harvey, 1987; El-Bohy et al. 1988; Ashton et al. 1992; Ahmed et al. 1993; Beamen et al. 1993; Suseki et al. 1997). Gilette et al. (1993) demonstrated that spinal somatosensory neurons in cats have receptive fields in lumbar facet joints.

Immunohistochemistry, using specific neuronal markers allowed the identification of sympathetic nerves in the facet joint. The sympathetic nerve supply of the facet joint in the rat lumbar spine has been identified as being primarily or exclusively vascular in nature, using antisera against neuropeptide Y (NPY), as well as tyrosine hydroxylase (TH), and dopamine beta-hydroxylase (DBH) (Ahmed et al. 1993; Suseki et al. 1997). Ashton et al. (1992) demonstrated the profuse sympathetic innervation of the human lumbar facet joint in close apposition to blood vessels, using antisera against the C-flanking peptide of neuropeptide Y (CPON).

NPY, TH, DBH, and CPON are generally considered to be noradrenergic sympathetic postganglionic nerve fibre markers in the peripheral nervous system. NPY is known to be a potent vasoconstrictor. CPON is a noradrenergic sympathetic postganglionic nerve fibre marker in the peripheral nervous system, and has an identical distribution and coexists with NPY. TH is the rate-limiting enzyme in the catecholamine synthesis. DBH is an enzyme that converts dopamine to noradrenaline in the catecholamine synthesis. NPY and TH were co-localized in the facet joint of rat lumbar spine (Ahmed et al.1993a). It is generally accepted that the main function of the sympathetic nerves in the facet joint, is regulation of local blood flow.

Sympathetic nerves have been reported to be involved in the neural mechanism of joint pain (Wong, 1993) and joint inflammation (Levine et al. 1984,1985, 1986; Fitzgerald, 1989). Morphologically, Suseki et al. (1997) recently demonstrated the key role of sympathetic nerves of the lumbar facet joint in spinal pain transmission, using neuronal tracing techniques and immunohistochemistry. They demonstrated that sensory innervation of lumbar facet joints occurs not only ipsilateral and segmental by spinal (somatic) nerves, but also bilateral and nonsegmental through the paravertebral sympathetic trunk and its rami communicantes, similar to the transmission of visceral pain.

Further attempts were made in the present account in studying the immunohistochemical characteristics of nerve endings in the facet joint capsules of the human lumbar spine, by both light and electron microscopy. Special attention is hereby given to the distribution pattern of sensory and sympathetic nerves and the immunohistochemical characteristics of corpuscular nerve endings.

Our samples are all taken from patients with severe chronic and disabling back pain. Abnormalities due to the cause(s) of the chronic pain condition should be expected. Studies from 'normal' tissues from persons without spinal disorders are necessary to decide unequivocally whether our observations are normal phenomenons.

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Immunohistochemistry of the Facet Joint Capsule, with Special Reference to the Co-localization of Sensory and Sympathetic Neuronal Markers

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Based on: Vandenabeele F, Creemers J, Lambrichts I (1998) Localisation of substance P, neuropeptide Y, dopamine-beta-hydroxylase, and neurofilament immunoreactivity in the facet capsule of the human lumbar spine. *Cell Biology International* **21** (in press).

ABSTRACT

The pathways and functions of the nerve fibres supplying the lumbar facet joint was determined immunohistochemically. We localised neurofilament (NF) and S100 as general neuro-glial markers. The distribution pattern of nerve endings was studied using antiserum against synaptophysin (SYN). The functions of these nerve endings were determined using SP as sensory marker related to pain, and NPY together with DBH as noradrenergic sympathetic postganglionic nerve fibre markers.

Immunoreactivity to NF,S100 and SYN revealed a panoramic view of the distribution and termination pattern of the nerve fibres supplying the lumbar facet joint. Immmunoreactivity to NPY and DBH was often co-localized and commonly close to blood vessels. SP-immunoreactive fibres were found vascular and nonvascular in location. A subpopulation of SP-positive fibres reacted also with NPY and DBH, both vascular and nonvascular in location.

Nerves containing SP have been implicated in nociception, inflammation, and vasoregulation. A paravascular localization of SP, in particular when colocalized with DBH together with NPY, probably reflects a vasoregulatory role. Nonvascular SP-immunoreactive nerve fibres are likely involved in pain transmission. The co-localization of SP and sympathetic neuronal markers in nonvascular noncorpuscular 'free' nerve endings of the facet joint capsules, supports the hypothesis that facet pain is transmitted by sympathetic afferent fibres.

INTRODUCTION

It has been reported that the lumbar facet joint receives nerve fibres from both dorsal root ganglia, and sympathetic ganglia (Suseki et al. 1997). Sensory information projecting from deep spinal tissues has been demonstrated to follow apparent sympathetic routes, as mentioned above. Therefore we decided to take another look at the pathways and functions of the nerve fibres supplying the lumbar facet joint using immunohistochemistry, and sought to determine wether a co-localization of sensory and sympathetic neuronal markers is present in facet joint capsules. Previous immunohistochemical studies on the innervation of the lumbar facet joint did not mention on the co-localization of sensory and sympathetic neuronal markers in the facet joint capsules. To date, the concept of co-existence and co-transmission of neurotransmitters introduced by Burnstock (1976) and demonstrated in different tissues, is widely accepted.

MATERIALS AND METHODS

Tissue Preparation.

Tissue samples of facet joint capsules from 14 recurrent lumbar disc herniation operations were subjected to immunohistochemical analysis. Commonly, samples were obtained from both facet joints at one or more operative levels. Only tissue that has to be removed for surgical reasons was available – that is when the exposures required for the surgical decompression reached the facet joint. Part of tissue samples was processed for parallel electron microcopic studies on facet innervation as reported elsewhere (Vandenabeele et al., 1995 and 1997).

Antisera.

Monoclonal anti-human antibodies to NF, Synaptophysin (SYN) and polyclonal anti-human antisera to S100,SP,NPY and DBH were used. The NF antiserum (Biogenex) was raised in mouse against human brain neurofilament, and labels the 200 kD polypeptide subunit of neurofilament. S-100 antiserum (Dako) was raised in rabbit against epitopes on the a and b chains of bovine S-100 protein. SP antiserum (Biogenex) was raised in rabbit against a synthetic substance P bound to a carrier protein (keyhole limpet hemocyanin). Synaptophysin antiserum (Dako) was raised in rabbit against synthetic human 38 kDa SYN peptide coupled to keyhole limpet haemocyanin. NPY antiserum (Affiniti) was raised in rabbit against synthetic porcine NPY without conjugation to a carrier protein. DBH antiserum (Affiniti) was raised in rabbit against bovine adrenal DBH.
S100 is a protein present in the supporting glial cells of the CNS and the Schwann cells of the PNS. Neurofilament is a neuron-specific intermediate cytoplasmic filament. Neurofilament is built up of a triplet of polypeptides of approximately 70, 150, and 200 kDaltons. S100 and NF are considered to be general nerve markers. SYN is a transmembrane protein of the presynaptic vesicles of neurons that participates in the release of neurotransmitters. The neuropeptide is involved in mediating sensory modalities, particularly nociception (Hökfelt T et al. 1975, Henry 1982). NPY co-exists and co-operates with NOR in the large granular vesicles of postganglionic sympathetic neurons (Ekblad et al. 1984) and is a potent vasoconstrictor (Hakanson et al. 1986). DBH is an enzyme localized in the granular vesicles of these fibres and is involved in catecholamine synthesis.

Immunocytochemistry.

Specimens were prepared using the following protocol: tissue was taken directly from surgery and fixed for 12-24 hours in 4% zinc formalin (Unifix, Klinipath) at room temperature. Materials were automatically embedded in paraffin, and sectioned serially at 0.5 µm thickness on a Reichert Ultracut microtome. Sections were mounted on slideglasses coated with poly-L-lysin.
The antisera were used at the following concentrations: NF and S-100 (ready to use solutions), SP (1: 3000), SYN (1/50), NPY (1:2000), and DBH (1:750). NF, S100, SP, NPY and DBH were detected using the labelled streptavidin-biotin (LSAB) method, whereas SYN was detected using the labelled avidin-biotin (LAB) method.

(1) LSAB method.
Sections were incubated with (1) the primary antibody for 30 minutes at room temperature, (2) universal biotinylated goat IgG secondary antibody (Supersensitive Multilink, Biogenex) for 20 minutes at room temperature, (3) alkaline phosphatase-conjugated streptavidin (Biogenex) for 20 minutes at room temperature, and (3) Fast red as alkaline phosphatase substrate. 10% human AB serum was added to the link antibody, as blocking solution, just before incubation.

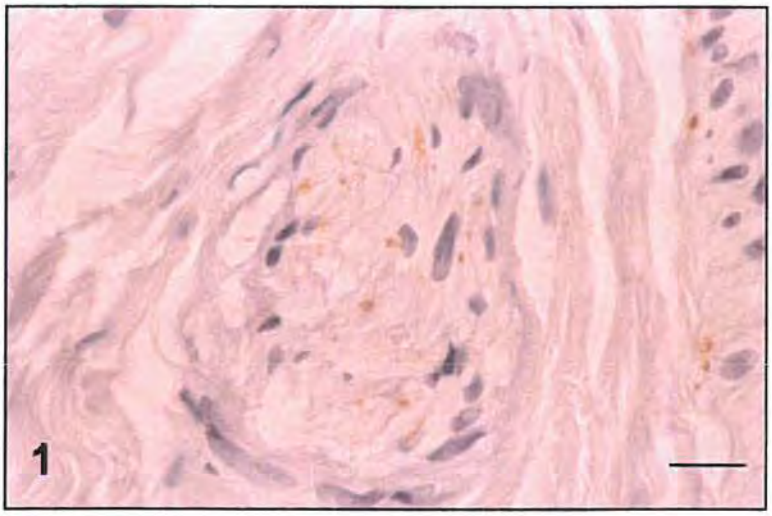
(2) LAB method.
Sections were incubated with (1) the primary antibody for 30 minutes at room temperature, (2) biotinylated anti-rabbit Ig G secondary antibody (Dako) for 20 minutes at room temperature, (3) peroxidase (HRP)- conjugated avidin (Dako) for 20 minutes at room temperature (Dako), and (3) diaminobenzidine (Dako) as peroxidase substrate.

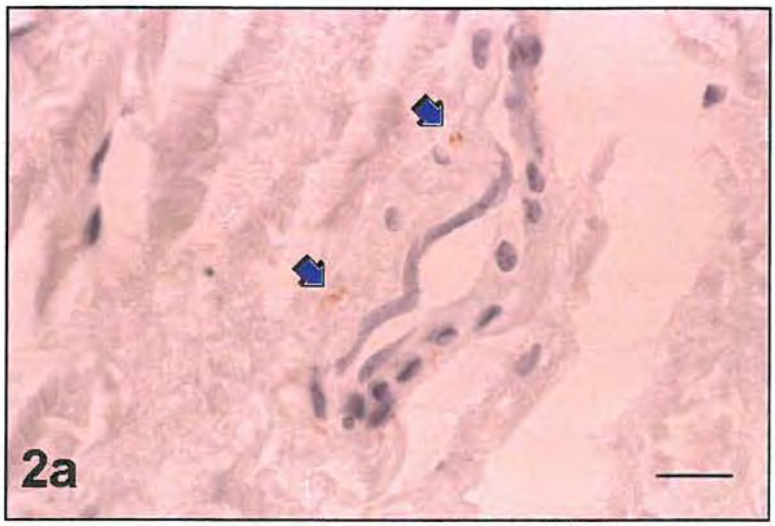
After each step, the slides were washed in 0.1 M PBS. Finally, sections were counterstained with Mayer's hematoxylin, air-dried at room temperature, and coverslipped with Aquatex (Merck). Observation for immunoreactivity was made using a Zeiss photobinocular microscope. Control sections in which the primary antisera were omitted were included. They were further processed similar to the processing of the experimental sections.

RESULTS

Immunohistochemical Findings

NF immunoreactive, S100- ir, SYN- ir, SP- ir, NPY- ir and DBH- ir nerve fibres were found in the dense fibrous layer and synovial plical tissue of the facet joint capsule. No nerve fibres were found in the synovial lining membrane. NF- ir, S100- ir,and SYN- ir fibres were more abundant than the others. The innervation pattern appeared more extensive in the vicinity of blood vessels (Figs 2*a, b;* 3 *a, b; 5 c, d).* NF- ir fibres generally appeared of larger calibre. Immunoreactivity to NF and S100 co-localized in the largest fibres. NF- ir and S100- ir glomerular and lamellated paciniform corpuscular endings were regularly found (Figs 4 *g-n*). Some corpuscular endings showed SP-immunoreactivity (Fig. 5*a*). SYN- ir axon terminals were found on consecutive sections within these corpuscular neural elements (Fig. 1). SYN- ir, SP- ir, NPY- ir and DBH- ir fibres were of smaller diameter and varicosal, and commonly showed scarce or no immunoreactivity to NF (Fig. 3*a*). Most of them were observed in the vicinity of blood vessels or in the tunica adventitia. Part of these fibres were found freely in the stroma, without relation to bood vessels. Immunoreactivity to NPY and DBH was often co-localized in the same neural elements on consecutive sections (Figs 5*d, e*). Notably, part of SP- ir fibres co-localized with immunoreactivity to NPY and DBH, on studying consecutive tissue sections (Figs 6*a-c*).





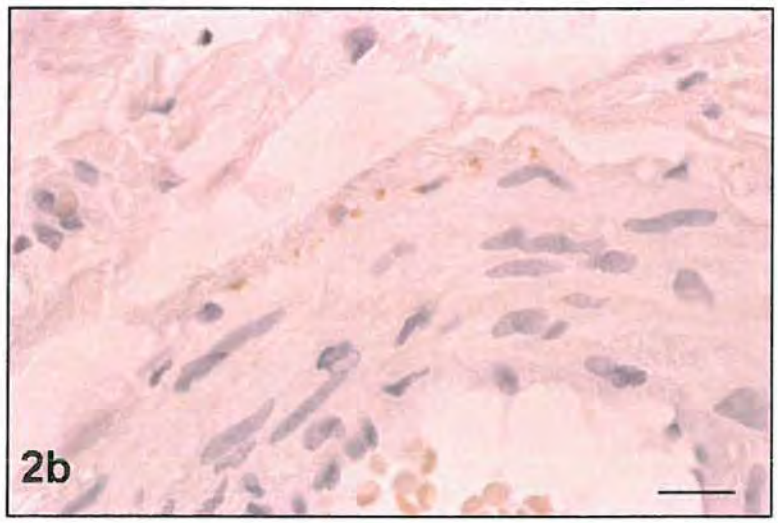
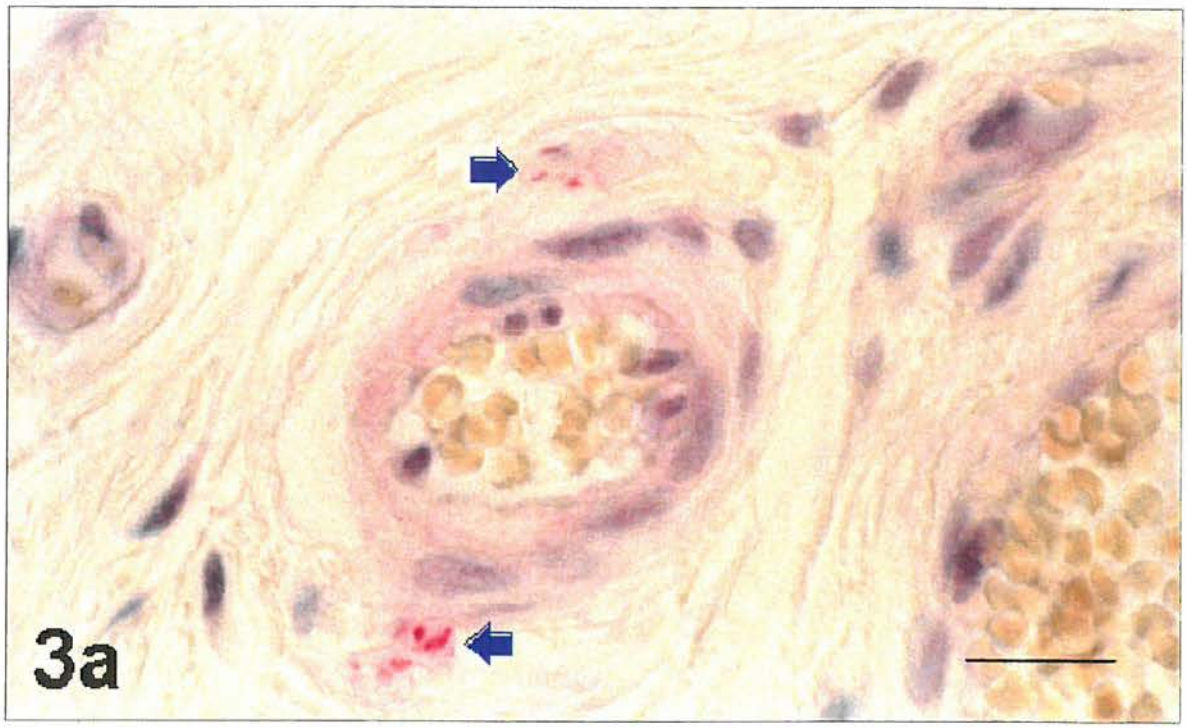


Fig. 1 and 2. Human lumbar facet joint. Light micrographs of Mayer's hematoxylin-stained paraffin sections of the dense fibrous capsule. (1) Synaptophysin - immunoreactivity in a corpuscular nerve ending. (2a) Synaptophysin - immunoreactive 'free' nerve endings in the tunica adventitia of a venule. (2b) Synaptophysin - immunoreactive 'free' nerve endings in the tunica adventitia of an arteriole.
BAR = 2.5 µm.



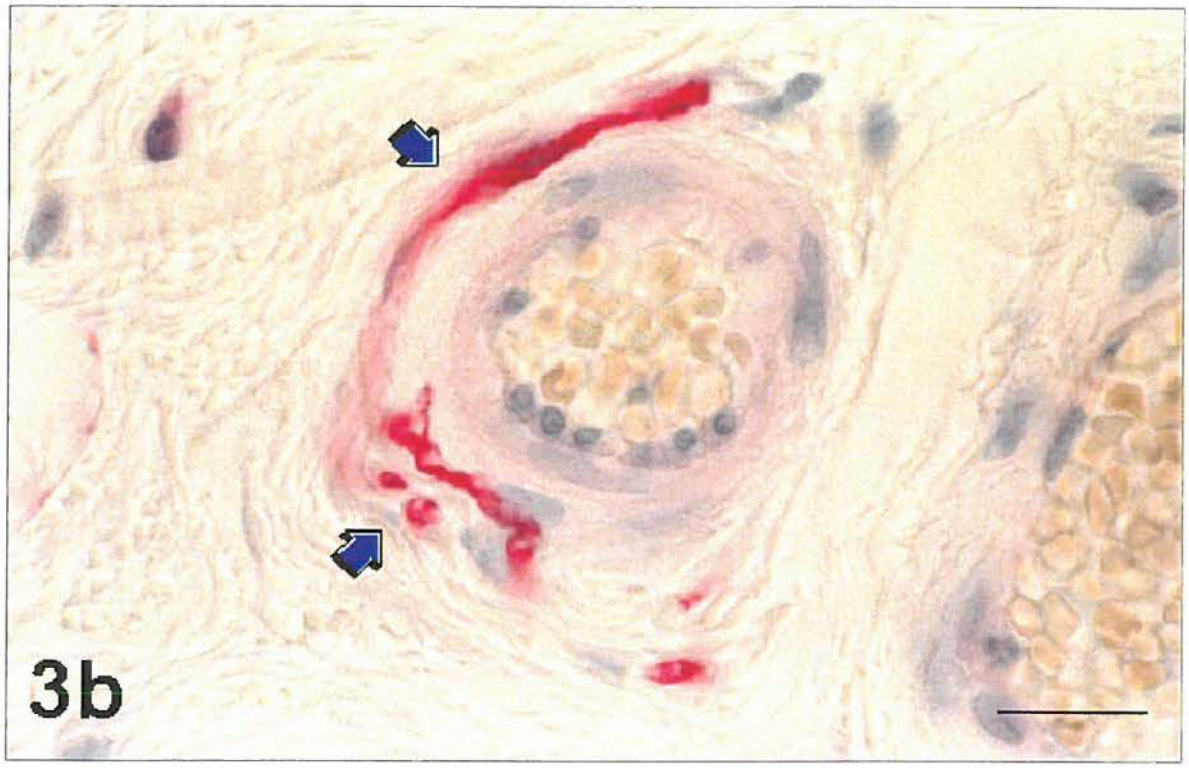


Fig. 3. Human lumbar facet joint. Dense fibrous layer of the facet joint capsule. Light micrographs of Mayer's hematoxylin-stained paraffin sections. (a) Scarce immunoreactivity to NF (arrows) in the tunica adventitia of an arteriole. (b) Consecutive section, showing a varicosal S100 - immunoreactive nerve ending (arrow). BAR = 25 µm.

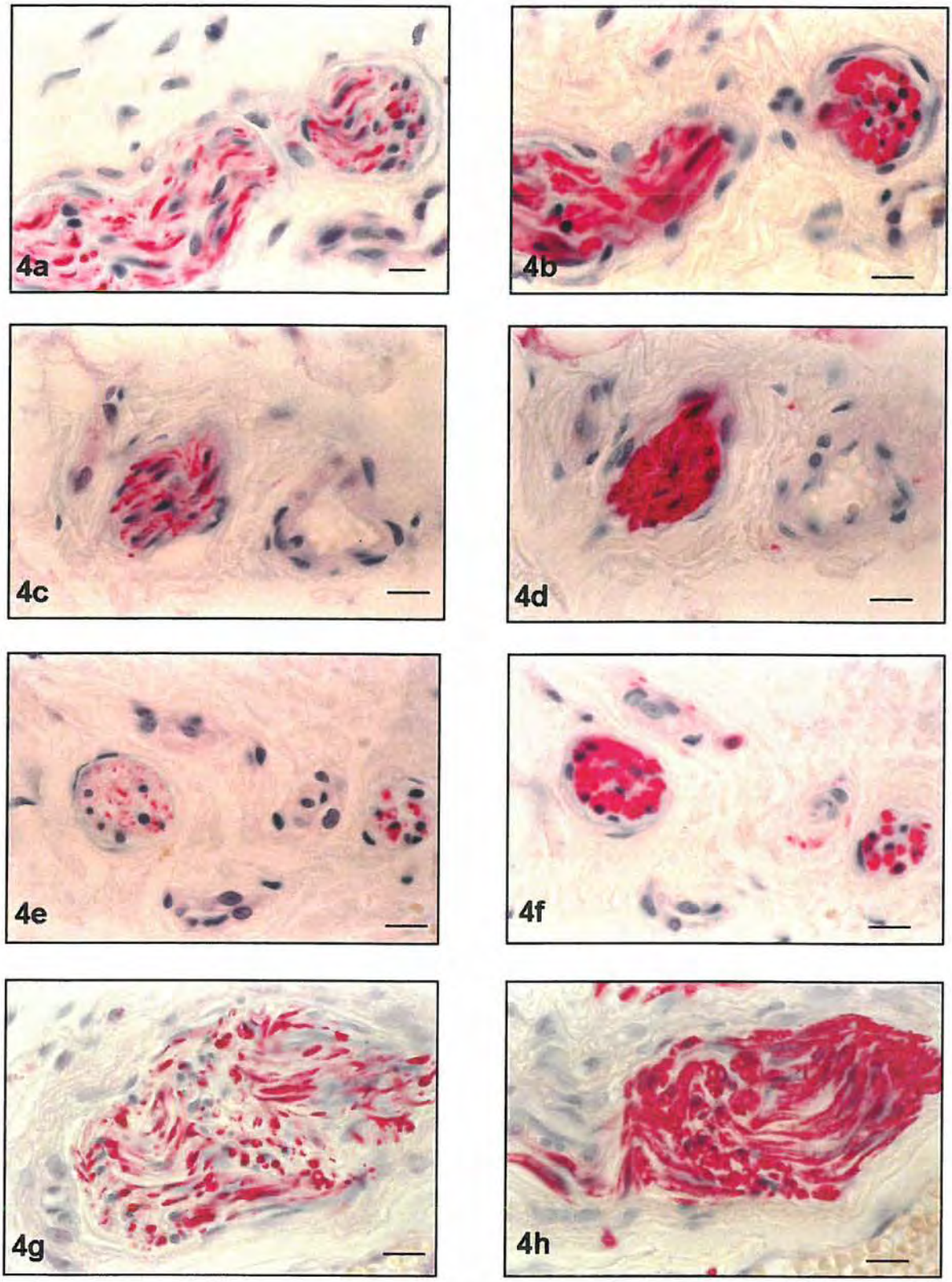


Fig. 4a-h. Human lumbar facet joint. Light micrographs of consecutive Mayer's hematoxylin-stained paraffin sections, demonstrating NF-immunoreactivity (a,c,e,g) and S100-immunoreactivity (b,d,f,h) in neural structures. (a,b) Transversally and longitudinally sectioned small nerve bundle. (c,d,e,f) Paravascular nerve endings and small nerve bundles. (g,h) Large corpuscular-like neural structure. BAR = 15 µm.

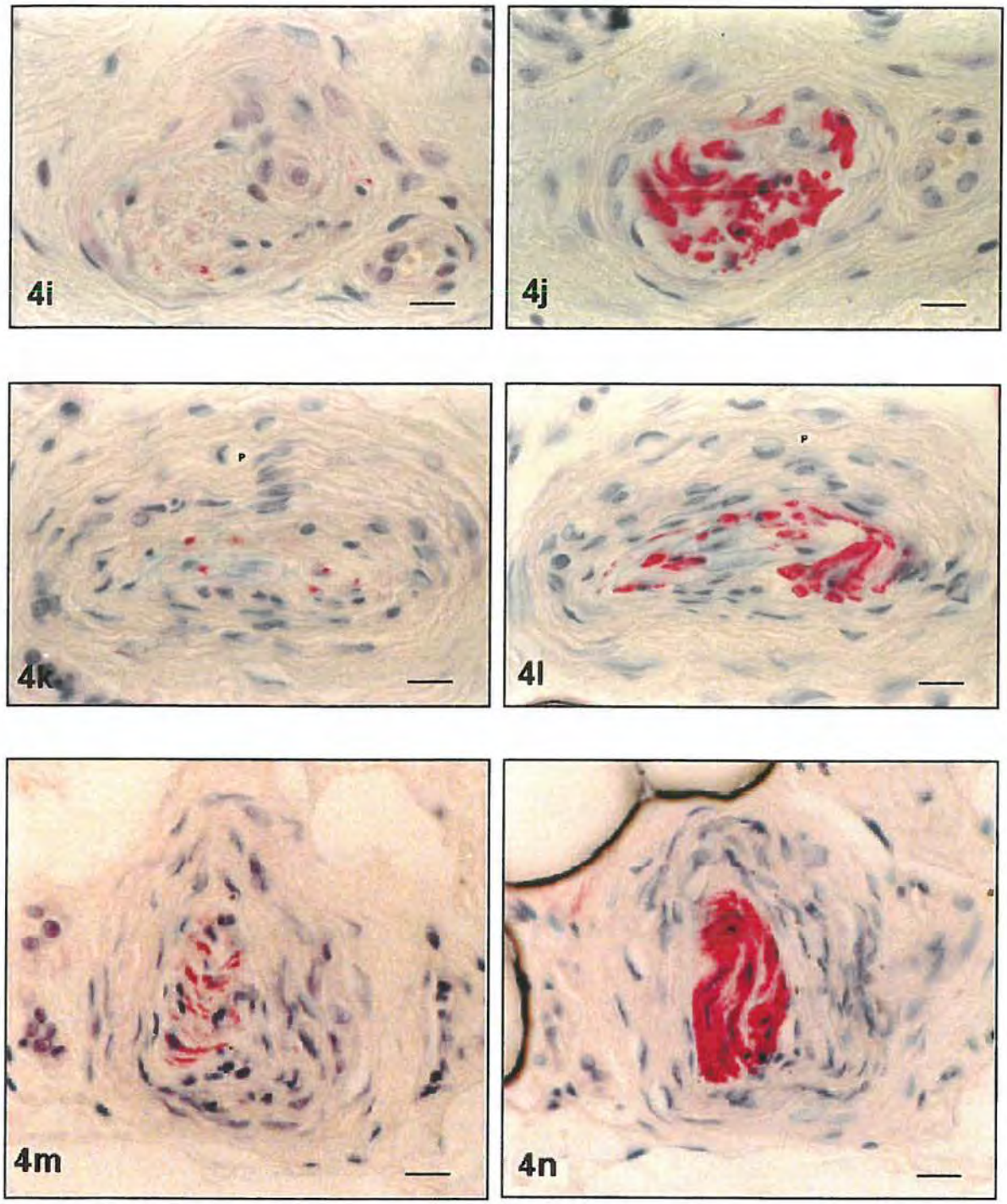


Fig. 4 i-n. Human lumbar facet joint. Light micrographs of consecutive Mayer's hematoxylin-stained paraffin sections, showing NF - immunoreactivity (i,k,m) and S100 - immunoreactivity (j,l,n) in corpuscular neural structures. (i, j) Paravascular glomerular corpuscular (encapsulated) nerve ending. The receptive complex is loosely arranged and surrounded by a narrow subcapsular space. (k-n) Lamellated corpuscles, characterised by a well-developed perineurial capsule. BAR = 15 µm.

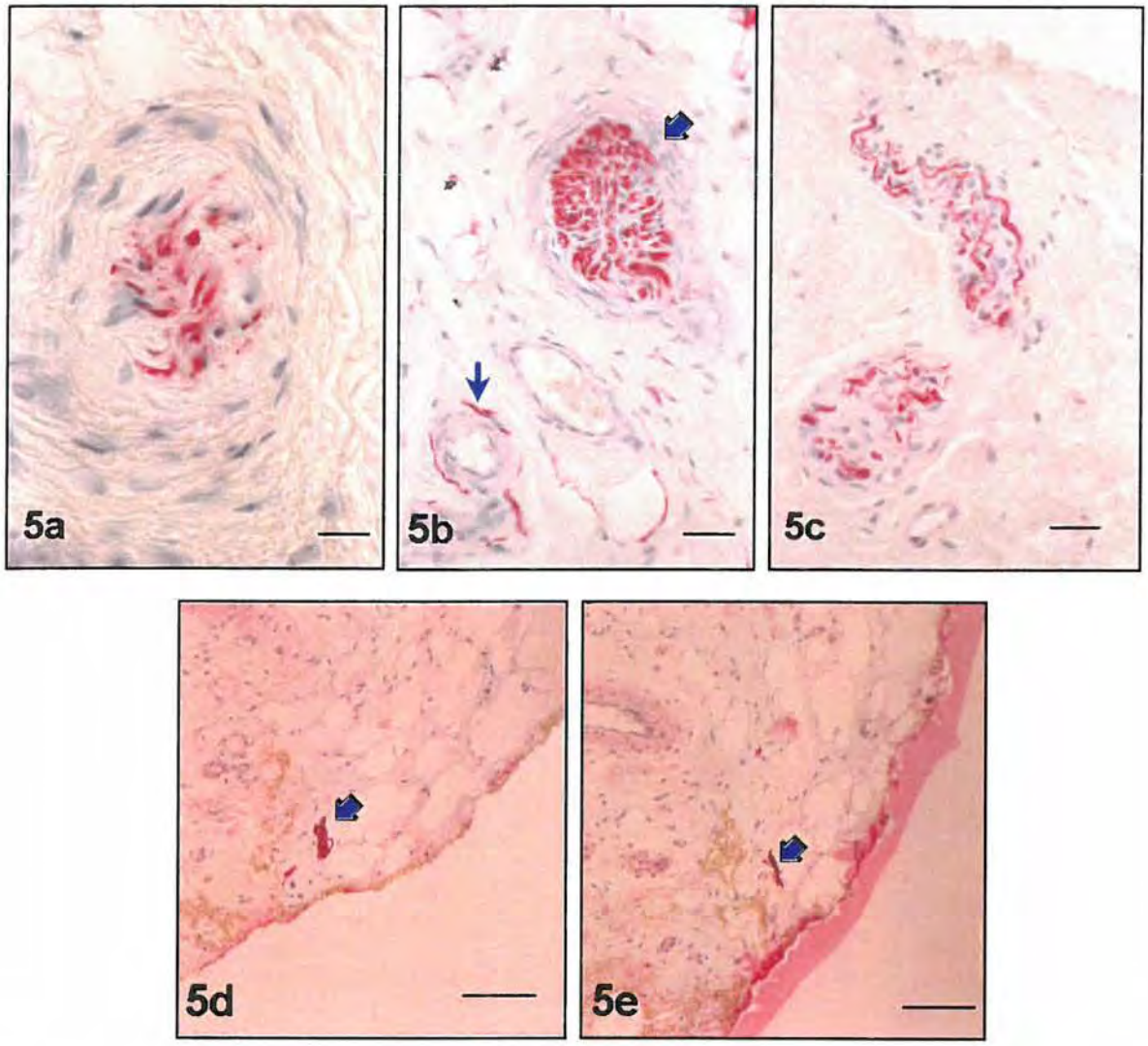


Fig. 5. Human lumbar facet joint. Light micrographs of Mayer's hematoxylin-stained paraffin sections.

(a-c) *Dense fibrous layer*: (a) SP - immunoreactive corpuscular nerve ending. BAR = 15 µm.
(b) Paravascular S100 - immunoreactive large nerve bundle (thick arrow) and 'free' nerve endings (thin arrow); BAR = 25 µm. (c) NF - immunoreactive nerve bundles. BAR = 25 µm.
(d,e) *Synovial plical tissue*: (d) DBH - immunoreactive paravascular nerve ending (arrow), (e) NPY - immunoreactive paravascular nerve ending (arrow). BAR = 200 µm.

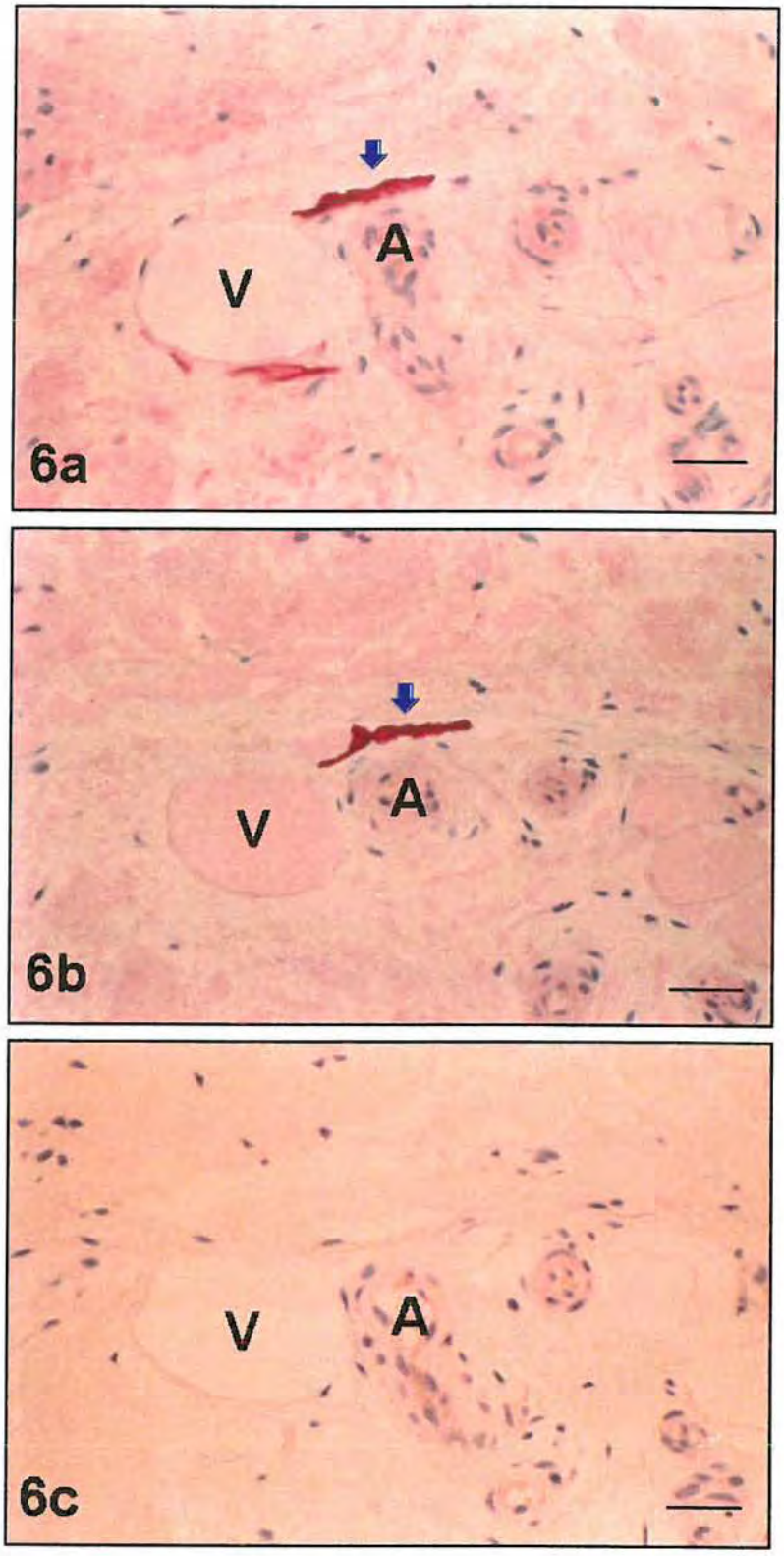


Fig. 6. Human lumbar facet joint. Light micrographs of Mayer's hematoxylin-stained paraffin sections. Dense fibrous layer of the facet joint capsule, showing immunoreactivity to SP (arrow; Fig. 6a) and NPY (arrow; Fig. 6b) in the same paravascular nerve ending on consecutive sections. Note the absence of immunoreactivity to the 200 kD polypeptide of NF (Fig. 6c). Venule (V). Arteriole (A). BAR = 70 µm.

DISCUSSION

Several studies have described the sensory (SP and CGRP) and sympathetic (NPY, CPON, TH, DBH) in the facet joint of different animals, including man (vide supra).

Immunoreactivity to NF, S100 and SYN has not previously been described in the human lumbar facet joint, but were readily identified in the present study. The poor immunoreactivity to NF in the smallest neural elements of the facet joint capsules in our material, that are likely nerve endings, agrees with the observations of El-Bohy et al. (1988). The conclusions of El-Bohy et al. (1988) can be summarized as follows: (1) the lack of immunoreactivity to NF in the smallest diameter axons relates to their paucity of neurofilaments, (2) SP-ir fibres that were not NF-ir represent C fibres and probably the smallest Aδ fibres, (3) nerve fibres that were SP- ir and NF- ir represent larger diameter C and/or Aδ fibres, and (4) large calibre NF-ir nerves that were not SP-ir are α- and γ-motorneurons. SP is generally known to be present in C and Aδ fibres, but not in larger-diameter fibres (Nicoll, 1980; Pernow, 1983).

Interestingly, we reported a subpopulation of varicosal SP-immunoreactive nerves in the facet capsule that co-localized with the well-known sympathetic markers NPY and DBH, but lacked NF immunoreactivity. Uptill now, a similar colocalization with SP was not demonstrated in the human facet joint. Clearly, these varicosal nerve endings belong to the C fibres.

The co-localization of SP-, DBH- and NPY- immunoreactivity in perivascular endings probably reflect a vasoactive role, and represent noradrenergic postganglionic sympathetic fibres. SP is known to have a potent vasodilatory effect. NPY mediates autonomic modalities, thereby acting as a potent vasoconstrictor. The co-localization of SP-, DBH- and NPY- immunoreactivity in other fibres, found distant from blood vessels, is consistent with current concepts that sensory innervation of the facet joint follows sympathetic routes (vide supra), and attributes to the now widely accepted concept of co-transmission and co-existence of different neurotransmitters and/or neuromodulators to occur in the same nerve profiles, introduced by Burnstock (1976) and demonstrated in different tissues. SP is generally known as a modulator in nociception and 'neurogenic inflammation' by a direct vascular effect and by mast cell degranulation (Mapp et al., 1990; Grönblad et al. 1991; Zimmerman, 1992).

In general, literature on the innervation of the synovial membrane is contradictory. Immunohistochemically, synovial tissues were shown to be innervated by both sensory and sympathetic nerve fibres (Mapp et al. 1990), including the facet joints (Ahmed et al. 1993). Others localized immunohistochemically the neural elements of the lumbar facet joint capsules in the dense fibrous layer and in the adipose tissue of the synovial plical tissue, but none in the synovial lamina intima (Giles & Harvey, 1987;El-Bohy et al. 1988; Grönblad et al. 1991, Ashton et al. 1992), in agreement with our observations (unpublished data). Notably, to our knowlegde, all electron microscopic studies suggested the synovial lamina intima of synovial joints lacks any type of innervation, including the facet joints (Vandenabeele et al. 1995). Different reports on innervation of the synovial lining membrane, may be due to (1) interspecies variation, (2) topographical misinterpretation (misinterpretation of perivascular subintimal nerves as intimal), (3) immunohistochemically non-detectable low levels of epitopes, and (4) use of unspecific neuronal markers.

Interestingly, Mapp et al. (1990) reported finding immunohistochemically neural elements in normal synovial tissues, but confirmed the absence of any type of innervation in the superficial layers of inflammed joints. Mapp et al. (1990) suggested that low and/or non-detectible levels of epitopes in the synovial membrane are secondary to synovial inflammation. They considered that in inflammed synovial joints: (1) synovial growth outflanks neural growth in the superficial layers of the synovial membrane, restricting synovial nerves to more deeper layers, and (2) the release of neuropeptides is increased, reducing the stores in the nerves. Likely, facet joints were affected in our patients. This might explain the absence of immunohistochemically detectable nerve endings in the synovial lamina intima.

Notably, structural characteristics of neural elements that are studied immunohistochemically may explain non-detectable low levels of epitopes and hence scarce or absent immunoreactivity. NF-immunoreactivity is poor or not detectable in the smallest diameter axons and free nerve endings because of their paucity of neurofilaments (Friede & Samorajski, 1970). We assume that differences in immunoreactivity to NF may be secondary to possible interneuronal differences in the expression of dominant polypeptide subunits (Vandenabeele et al., 1998). Scarce or absent immunoreactivity to S-100 might relate to a thin or absent Schwann cell covering (Mapp et al., 1990).

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S-100, NF and SP Immunoreactivity in Corpuscular Nerve Endings of the Human Lumbar Facet Joint: a Light- and Electronmicroscopic Study

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ABSTRACT

Corpuscular endings are generally known to be involved in mechanoreception. Recent studies suggested their nociceptive role. Mechanoreceptors of the posterior longitudinal ligament and intervertebral disc of human lumbar spine were assumed to be involved in the mediation of spinal pain. The objective of the present study is to determine whether the corpuscular endings of the human lumbar facet joint might have a similar nociceptive function.

We localized neurofilament (NF) and S-100 as general neuro-glial markers and substance P (SP) as marker for sensory neurons in Ruffini-like endings and Golgi tendon-like organs of the facet capsule by light- and electron microscopic immunocytochemistry on lowicryl resin sections.

Light microscopically, all mechanoreceptors demonstrated intense immunoreactivity to NF and S-100, but reacted poorly to SP.

Ultrastructurally, the receptive complex of both types of corpuscular endings exhibited varicose group III and group IV axon terminals, partly enveloped by associated Schwann cells. S-100 gold particle labeling was associated with the nuclear region and with thin cytoplasmic extensions of Schwann cells, whereas NF labeling was localised on the axon terminals. Labeling for S-100 and NF was intense and uniformly distributed. SP gold particle labeling was observed over the receptive sites, related to vesicles, 45 - 80 nm in diameter. Vesicles were often in close proximity to microtubules.

The demonstration of the nociceptive neuropeptide SP in the Ruffini-like endings and in Golgi tendon-like organs of the human lumbar facet joint indicates that these mechanoreceptors may mediate pain reception in the lumbar spine.

INTRODUCTION

The lumbar facet joints are known to be richly innervated. Noncorpuscular nerve endings have been demonstrated in the facet capsule of human lumbar spine by electron microscopy (Vandenabeele et al. 1995). Noncorpuscular nerve endings are assumed to be involved in nociception and local vasoregulation. Ruffini-like endings have been demonstrated in the facet capsule of human lumbar spine by light microscopy (Ozaktay et al.,1991), and recently by electron microscopy (Vandenabeele et al. 1997). Electrophysiological studies identified mechanosensitive afferent units in the facet joint capsule of the lumbar spine (Cavanaugh et al. 1989; Yamashita et al. 1990; Avramov et al. 1992; Pickar & McLain, 1995).

It is generally accepted that corpuscular endings mainly serve as mechanoreceptors, collecting information on joint motion during routine daily activities. Particular attention was given by McLain (1994) to the crucial role of mechanoreceptors of the lumbar facet joint, in proprioception and protective muscular reflexes of the lumbar spine.

Light microscopical immunocytochemical studies suggest that corpuscular nerve endings might be pain sensitive: immunoreactivity to the nociceptive neuronal marker substance P (SP) has been demonstrated in corpuscular nerve endings of the posterior longitudinal ligament in human lumbar spine (Liesi et al., 1983), and in human bone periosteum (Grönblad et al. 1984). Roberts et al. (1995), demonstrated substantial immunoreactivity to calcitonin gene-related peptide (CGRP), but scarce staining for SP in corpuscular nerve endings of the outermost regions of the human intervertebral disc and the human longitudinal ligament. CGRP is also a neuronal marker for sensory nerve endings, including nociception.

McLain (1994) tentatively suggested that mechanoreceptors of the facet joint might be responsible for pain reception in the spine. He proposed that they respond to potentially damaging capsular stress during extreme or excessive motion of the joint. Yamashita et al. (1990) demonstrated mechanosensitive units with high-thresholds that respond to mechanical strain, and are likely pain-sensitive under certain conditions. In the present study, attempts are made to investigate the putative role of corpuscular endings of the human lumbar facet joint, in pain transmission. Therefore, a neuroimmunohistochemical analysis of corpuscular nerve endings in human lumbar facet joint capsule is undertaken. The occurence and distribution of immunoreactivity to the nociceptive neuropeptide substance P (SP) in these receptors are determined. Antisera to neurofilament (NF) and S-100 are used as general neuronal markers.

MATERIALS AND METHODS

Operative tissue samples of the facet joint capsule at lumbosacral spinal levels were obtained from 12 patients with chronic low back pain and sciatica, undergoing spinal surgery for recurrent lumbar disc herniation. The tissue samples were fragments of tissue that needed to be removed for posterior fusion operations. Part of these tissue samples were used to study the ultrastructural characteristics of encapsulated Ruffini-like endings, on routine epoxy resin sections, as reported elsewhere (Vandenabeele et al., 1997). Tissue samples for light- and electron microscopical immunocytochemistry were processed as follows:

Fixation and tissue processing

Tissue samples were cut free and immediately fixed by immersion in a solution of 0.25% glutaraldehyde in 0.1M phosphate buffer plus 4 % paraformaldehyde and 0.15 mM CaCl2, for 1 h at room temperature. The specimens were additionally fixed by immersion in 4 % paraformaldehyde in 0.1 M PBS plus 0.15 mM CaCl2, for another 12 h at 2 °C. After dehydratation the samples were embedded in lowicryl resin (Unicryl). The resin polymerized in ultraviolet light and at -20°C.

Serial semithin sections (0.5 µm) were cut with glass knives for light microcopy on a Reichert microtome. The sections were stained with a solution of thionin and methylene blue (0.1% aqueous solution) and studied in a Zeiss photobinocular microscope. Semithin sections containing corpuscular nerve endings were selected and subsequent sections were mounted on slideglasses coated with biobound, and processed for light microscopical immunocytochemistry.

For electron microscopy, serial ultrathin sections (60 nm) were cut with diamond knives, mounted on Formvar-coated nickel grids, and processed for immunocytochemistry as described below. Ultrathin sections were examined by transmission electron microscopy with a Phillips EM 208 operating at 80 kV. The quantification of gold particle labeling was carried out using the video-image analysis system 'Analysis' (Sis).

Immunocytochemistry

The following antisera were used:

1) mouse monoclonal antibody to neurofilament protein (2F11 clone; ready to use solution; Dako) reacting with the 70 kD and 200 kD polypeptide subunits,

1) rabbit polyclonal antibody to the a and b chains of S-100 protein (ready to use solution; Dako), and

1) rabbit polyclonal antibody to SP (ready to use solution; Biogenex) bound to a carrier protein (keyhole limpet hemocyanin).

Light Microscopy. Semithin unicryl resin sections of tissue samples containing corpuscular nerve endings, were processed for immunocytochemistry using following procedures:

A) Labelled streptavidin-biotin (LSAB) method. Sections were incubated in 1) phosphate buffered saline (PBS) and the primary antibody, overnight, at room temperature, in a moist chamber, 2) universal biotinylated goat IgG secondary antibody (Supersensitive Multilink, Biogenex) for 20 minutes at room temperature, 3) streptavidine, labelled with alkaline phosphatase (Biogenex) for 20 minutes at room temperature, and 4) Fast red as alkaline phosphatase substrate. 10% human AB serum was added to the secondary antibody, as blocking solution, prior to incubation. After each step, the slides were washed in 0.1 M PBS. Finally, sections were counterstained with an 0.1% aqueous solution of thionin and methylene blue, coverslipped with immersion oil and immediately photographed in a bright-field microscope.

A) Silver enhanced post-embedding colloidal-gold immunocytochemistry. Sections were incubated in 1) blocking buffer (PBS + 0.8% BSA + 0.1% IGSS gelatin + 5% FCS), 2) primary antibody overnight at room temperature in moist chamber, 3) gold conjugated secondary antibody (goat anti-mouse for NF; goat anti-rabbit for SP) diluted in blocking buffer for 90 min at room temperature. After each step, the slides were washed in buffer (PBS + 0.1% IGSS + 0.8% BSA). Finally, sections were silver enhanced, air-dried and stained with with an 0.1% aqueous solution of thionin and methylene blue, coverslipped with immersion oil and immediately photographed using a epi-polarisation microscope.

Electron Microscopy. For ultrastructural analysis, post-embedding colloidal-gold immunocytochemistry was applied to thin sections, using the following protocols:

A) for immunocytochemistry with monoclonal antibody to NF, sections were: 1) incubated overnight at room temperature on a drop of primary antibody, 2) rinsed with 0.1 M PBS, 2) incubated for 20 minutes at room temperature with universal biotinylated goat IgG secondary (link) (Supersensitive Multilink, Biogenex), 4) rinsed with 0.1 M PBS, 4) incubated with goldparticles of 5 nm conjugated to streptavidine (1:25; Orion) for 1 h at room temperature, 5) washed with optimax wash buffer (Biogenex), 6) postfixed with 2% glutaraldehyde in 0.1 M phosphate buffer, and 7) washed with distilled water. 10% human AB serum was added to the secondary antibody, as blocking solution, prior to incubation.

B) for immunocytochemistry with the polyclonal antibodies to S100 and SP, sections were: 1) incubated overnight at room temperature on a drop of primary antibody, 2) rinsed with 0.1 M PBS, 3) incubated for 1 h at room temperature with goat anti-rabbit IgG antibody conjugated to gold particles of 10 nm (1:25; Orion), 4) washed with optimax wash buffer (Biogenex), 5) fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, 6) washed with distilled water.

Sections were air-dried at room temperature, routinely stained using uranyl acetate and lead citrate, and examined using a Phillips EM 208 electron microscope. Quantification of immunogold labelling was carried out, using a video-image analysis system which included a Kodak camera attached to the electron microscope, and the software package 'Analysis' (Sis). According to Liem et al. (1997), a terminal axon in the receptor was considered labelled and used for further analysis when the density of gold particle labelling (expressed as the number of gold particles per µm2) covering the terminal was 5 times higher as the background level (endoneurium, perineurium, capsular stroma).

For light and electron microscopical immunocytochemical control sections, the primary antisera were omitted, but further processed in an identical way as the experimental sections.

RESULTS

Light microscopy

Histologically, clustered corpuscular nerve endings with a varying degree of encapsulation and compartmentalisation, were clearly identifiable on consecutive sections. The final verification was made by electron microscopy (vide infra). The endings were referred to as encapsulated Ruffini-like endings, as reported elsewhere (Vandenabeele et al., 1997). Additionally, we observed a few larger corpuscular endings, that were classified as Golgi tendon-like organs (Andres and von Düring, 1973; Andres, 1974; Sklenska, 1972). Freeman and Wyke (1967) classified Ruffini endings as articular receptors of type I, and Golgi tendon organs as type III.

Light microscopical immunoreactivity to S-100 (Fig. 1*a,b*) and NF (Figs 2*a*, 3*a*) was intense in both types of endings (Biotine-Streptavidin technique, Silver enhanced post-embedding colloidal-gold immunocytochemistry). Immunostaining for SP using the Biotine-Streptavidin detection method was weak (Fig. 2*b*), but more convincing using Silver enhanced post-embedding colloidal gold immunocytochemistry & epi-polarisation microscopy (Fig. 3b).

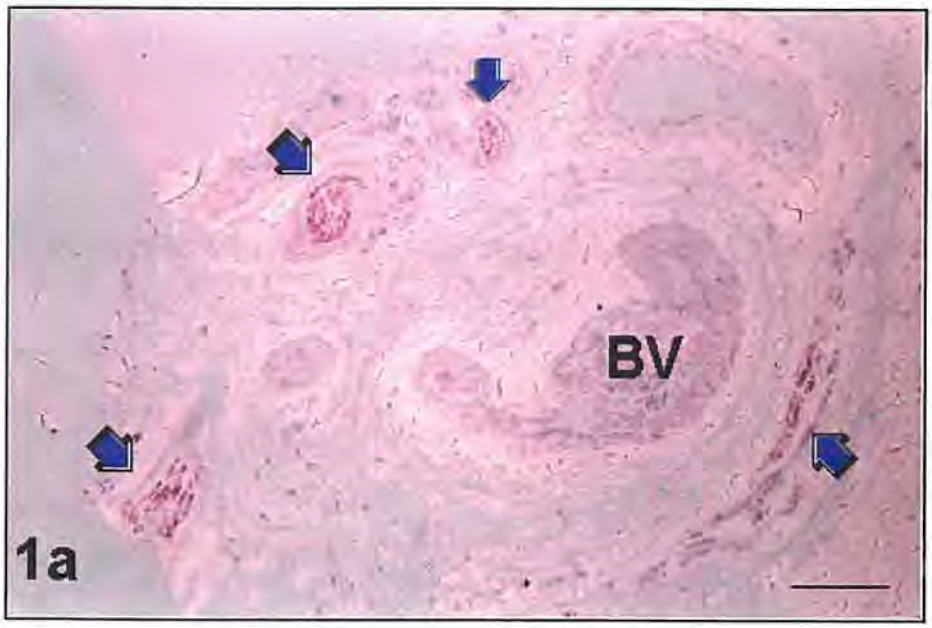

Fig. 1a Light micrograph of thionin-methylene blue-stained lowicryl resin semithin sections of the dense fibrous capsule, showing immunoreactivity to S100 in paravascular corpuscular nerve endings (arrows). Blood vessel (BV). BAR = 500 µm.

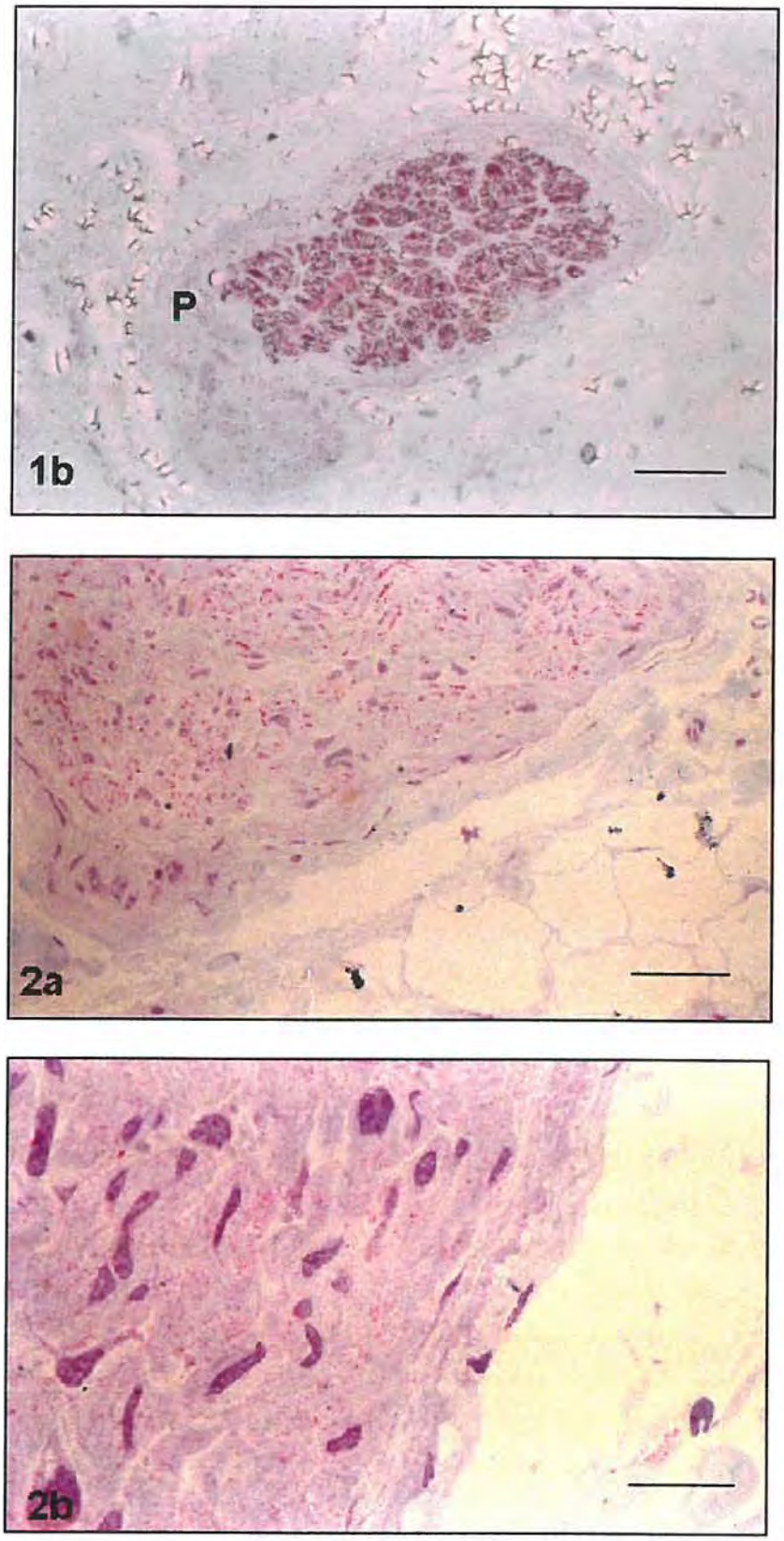

Fig. 1b; 2a, b Light micrographs of thionin-methylene blue-stained lowicryl resin semithin sections of the dense fibrous capsule, showing a Golgi tendon-like corpuscular nerve ending. The receptive complex shows intense immunoreactivity to S100 (1b). Immunoreactivity to NF (2a) and to SP (2b) in the axons of the receptive complex. Note the absence of immunoreactivity in the perineurial capsule (P). 1b, BAR = 105 µm; 2a, BAR = 70 µm; 2b, BAR = 25 µm.

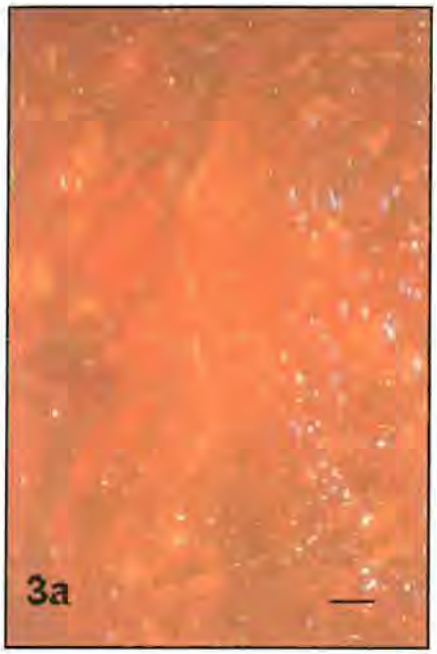

Fig. 3 Dense fibrous capsule. Corpuscular nerve ending. Light micrographs of lowicryl resin semithin sections, showing colloidal-gold immunoreactivity to NF (Fig. 3a) and to SP (Fig. 3b) in consecutive sections of the receptive complex, using epi-polarisation microscopy. BAR = 15 µm.

Electron microscopy

The presence of focal axonal enlargements (varicosities or beads), partly enveloped by associated Schwann cell processes and containing a variable number of mitochondria and vesicles with a rather dark appearance, verified the function as corpuscular nerve ending. The plasma membranes of the varicosities were poorly visualized, due to the use of unosmicated specimens embedded in lowicryl resin for immunocytochemistry. The 'bare' axolemma of the axon terminals, devoided of Schwann cell covering, has been referred to as the 'receptive site'.

The receptive complex in both types of corpuscular endings appeared compartimentalized by thin cytoplasmic extensions of fibroblast-like cells. Ruffini endings were characterized by a loosely arranged receptive complex and a more-or-less extensive subcapsular space. The receptive complex of the Golgi-tendon-like organs was more compact and almost completely filled the corpuscle, leaving a narrow subcapsular space. Small myelinated axons were regularly seen in the receptive complex of Golgi tendon-like organs, but exceptionally present in Ruffini-like endings.

Colloidal-gold immunocytochemistry for S-100, demonstrated strong and specific intracellular gold particle labeling over the thin cytoplasmic extensions and nuclear region of the Schwann cells (Fig. 4a). Axon terminals were devoid of labeling for S-100, whereas they exhibited specific gold particle labeling for NF (Fig. 4b).

Most group III and group IV axonal varicosities exhibited weak, but significant, gold particle labeling for SP. Axon terminals were considered labeled as the density of gold particle labeling to SP was more than 5 times higher as the backgroundlabeling of the capsular tissue. Labeling for SP was more intense over the receptive sites and commonly associated with small vesicles, 45 - 80 nm in diameter, in the vicinity of microtubules (Fig. 4c).

No reference is made in the present study to unencapsulated 'free nerve endings' (type IV, according to Freeman and Wyke, 1967), since they could not routinely be localized. A conclusive identification of labelled noncorpucular nerve endings on unicryl resin sections, was even more problematic.

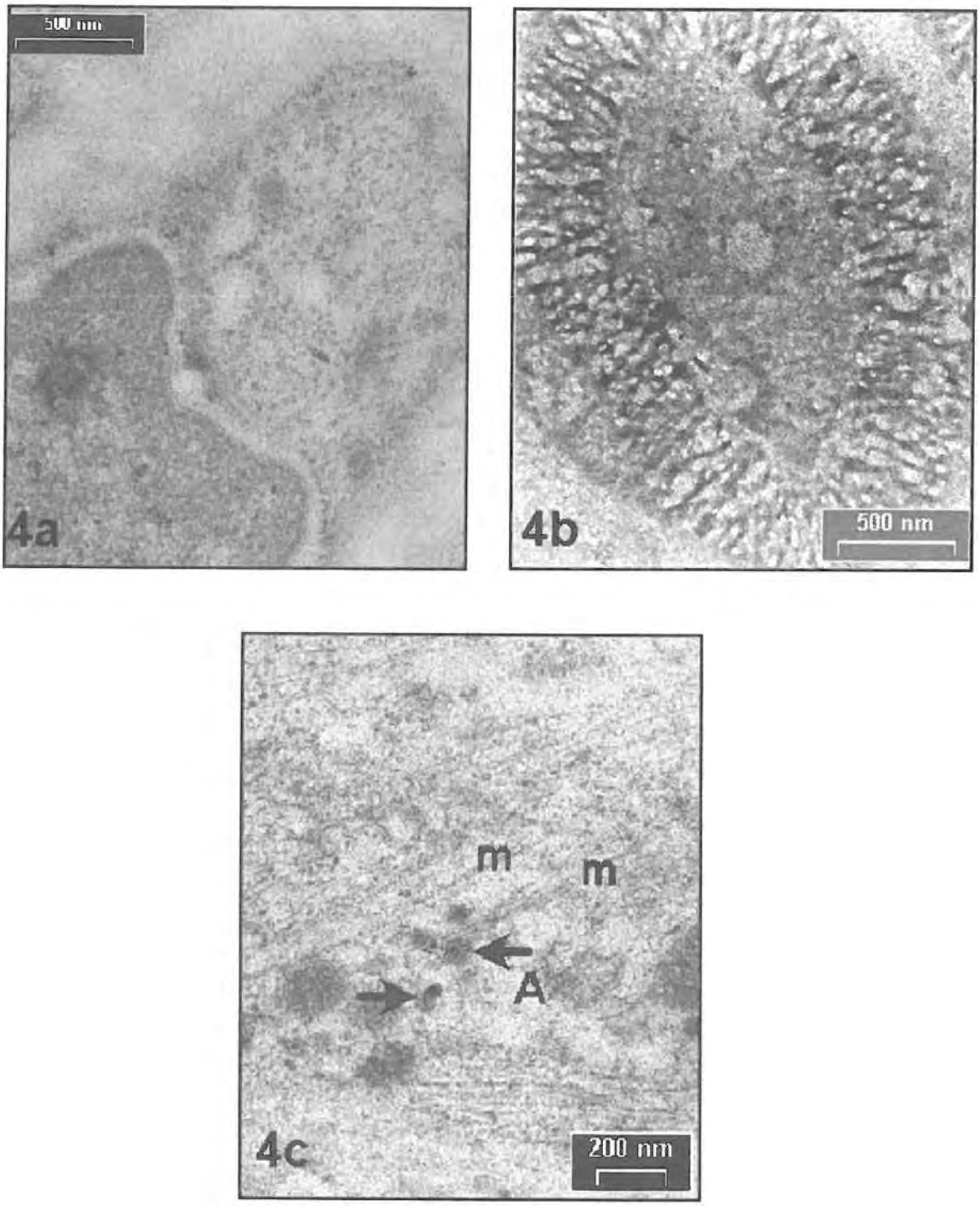

Fig. 4. Electron micrographs. a) Colloidal-gold immunocytochemistry for S-100, demonstrating strong and specific intracellular gold particle labeling over the thin cytoplasmic extensions and nuclear region of the Schwann cell. (b) Colloidal-gold labeling for NF in the axon of a small myelinated nerve fibre. (c) The receptive site of an terminal axon (A), showing gold -particle labeling for SP in small vesicles, 45 - 80 nm in diameter (arrows). Microtubules(m). Axon (A).

DISCUSSION

Speculations on functional properties of sensory receptors based on their morphology, location and relationship to the surrounding tissue, remains mostly speculative (Lambrichts et al. 1992). Axons of sensory neurons are classified according to conduction velocities, functional nature of fibres, total fibre diameter, and type of sheath. The terminology of Lloyd & Chang (1948) is commonly used for joint nerves. Myelinated afferent fibres are classsified into groups I to III. The unmyelinated afferent fibres are group IV fibres.

Group III and group IV afferent fibres may have both mechanosensitive and nociceptive properties (Andres et al. 1985; Willis & Coggeshall, 1991).

Noncorpuscular 'free' nerve endings of group III and group IV afferent nerve terminals respond best or only to noxious movements of the joint (Willis & Coggeshall, 1991), and are thought to be primarly involved in joint nociception, especially during inflammation (Schaible & Schmidt, 1985).

Based on the morphological criteria of Heppelmann et al. (1990), nerves supplying Ruffini-like endings of the human lumbar facet joint, are likely to be thinly myelinated group III (A?) and unmyelinated group IV (C) fibres (Vandenabeele et al. 1997).

Electrophysiological recordings of conduction velocities demonstrated group III and group IV mechanosensitive capsule units in the lumbar facet joint, based on conduction velocities (Yamashita et al.,1990; Avramov et al.1992). Some of these units showed high thresholds and were assumed to mediate nociceptive properties.

The current study demonstrates NF-, S-100- and SP- immunoreactivity in corpuscular endings of the facet joint in human lumbar spine, by light and electron microscopy. The problematic localization of noncorpuscular 'free' nerve endings in the capsular tissue is inherent to poor visualization of membrane structures due to fixation and tissue processing. Staining of lowicryl resins was generally poor. Corpuscles have been referred to as encapsulated Ruffini-like endings and Golgi tendon-like organs. S-100 and NF are well-known general glia-neuronal markers. S-100 is a soluble calcium-modulated protein specific for the supporting glia cells of the CNS and the Schwann cells of the PNS, whereas neurofilament is a neuron-specific intermediate cytoplasmic filament, built up of a triplet of polypeptides of approximately 70, 150, and 200 kDalton. SP is an 11 amino acid neuropeptide believed to be involved in mediating sensory modalities, particularly nociception.

As expected, SP immunoreactivity was mainly localised on small synaptic vesicles at the receptive sites, supporting Tay & Wong (1992).

The immunocytochemical results presented here, showing the well-known nociceptive neuropeptide SP in Ruffini-like endings, as well as in Golgi tendon-like organs of the facet joint capsule in human lumbar spine, is of particular interest. It suggests that corpuscular nerve endings might be pain-sensitive, corroborating the electrophysiological recordings of Yamashita et al. (1990) and Avramov et al. (1992). They might be involved in the mediating back pain, supporting the morphological studies of Liesi et al. (1983), McLain (1994), and Roberts et al. (1995).

Ruffini endings and Golgi tendon organs are generally known as slowly adapting joint receptors responding to stretch. Ruffini endings are thought to be very sensitive, whereas Golgi tendon organs are relatively insensitive. Reports on the range of action of Ruffini endings in joint motion, have been contradictory. Ruffini endings should only respond at the extremes of joint motion, whereas Golgi tendon organs respond in different positions of the joint, but maximally at the extremes of joint motion (Willis & Coggeshall, 1991).

Likely, the role of SP as pain transmitter in the corpuscular endings could be restricted to mechanical strain during extensive capsular stretch at extremes of joint movement, as tentatively suggested by McLain (1994). Notably, mechanoreceptors are known to be more sensitive to mechanical stimuli in inflammed joints, causing abnormal painful responses to normal stimuli (Roberts et al. 1995).

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Chapter 4.3

Innervation of the Interspinous and Supraspinous Ligaments in the Human Lumbar Spine: an Electron-microscopic and Immunohistochemical Study

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I. Lambrichts

Based on: F. Vandenabeele, Creemers J, Lambrichts I (1996) Neurohistology of the human interspinous ligament: a transmission electron microscopic and immunohistochemical study. *Jaarboek van de Nederlandse Vereniging voor Microscopie*, 173 – 175.

ABSTRACT

The distribution pattern, structure and function of neural elements in the interspinous and supraspinous ligaments of the human lumbar spine were investigated by means of transmission electron microscopy and immunohistochemistry for neurofilament (NF), substance P (SP) and neuropeptide Y (NPY).

Electron microscopy revealed small myelinated and unmyelinated nerve fibres, and their, mainly noncorpuscular, nerve endings.

The immunohistochemical staining revealed no recognizable corpuscular nerve endings. It confirmed the presence of nonvascular and vascular noncorpuscular 'free' nerve endings, located both superficially and deep in the ligaments. The nonvascular SP- ir varicosal nerve endings might be involved in nociception.

INTRODUCTION

It is generally accepted that the interspinal and supraspinal ligaments are mainly designed to limit flexion in the vertebral column (Heylings, 1978), providing stability and protection for the vertebral joints during flexion of the lumbar spine (Yahia & Newman, 1989). Yahia & Newman (1993) suggested their role in spinal proprioception and pain transmission.

Human supraspinous and interspinous ligaments have been reported to be well innervated both by unencapsulated and encapsulated nerve endings, as demonstrated by light microscopy (Yahia et al. 1988; Yahia & Newman, 1989), scanning electron microscopy (Yahia & Newman, 1989, 1993), and transmission electron microscopy (Yahia & Newman, 1989). Yahia & Newman (1989) demonstrated noncorpuscular 'free' nerve endings and Ruffini endings. There have been few immunohistochemical studies on the innervation of the human supraspinous and interspinous ligaments, using antibodies against the general neuro-glial markers NF and S100 (Yahia et al. 1992; Rhalmi et al. 1993; Yahia & Newman, 1993; Jiang et al. 1995).
More detailed information on nerve function is available for the spinal ligaments of rat and rabbit, demonstrating a sensory and sympathetic innervation (El-Bohy et al. 1988; Ahmed et al. 1991,1993). Untill now immunohistochemical data on the nerve function in human supraspinous and interspinous ligaments are lacking.

Therefore we studied the nerve structure and function in human spinal ligaments by means of transmission electron microscopy and immunohistochemistry, using the general neural marker NF, the sensory marker SP as well as the sympathetic marker NPY.

MATERIALS AND METHODS

Surgical specimens of the interspinous ligaments and the supraspinous ligament were obtained at lumbar levels. Samples were obtained from 12 patients during routine neurochirurgical procedures for recurrent disc herniations with therapy-resistent low back pain and sciatica. Simultaneously tissue samples were obtained of the paired facet joints at the operative level for light- and electronmicroscopy and immunohistochemistry, as described above.

The fresh surgical specimens for transmission electron microscopy were immediately immersed in a solution of 2 % glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3). Specimens were postfixed in 2 % osmium tetroxide for 1 h, stained with 2 % uranyl acetate in 10 % aceton for 20 min, dehydrated in an ascending aceton series and embedded in Araldite. Semithin sections were cut on a Reichert Ultracut E microtome and stained with thionin-methylene blue (0.1% aqueous solution) for light microscopy. Serial ultrathin sections (0.06 µm) were prepared from selected tissue blocks, mounted on 0.7% formvar-coated grids, contrasted with uranyl acetate and lead citrate, and finally examined in a Philips EM 400 electron microscope.

Immediately after excision, the tissue samples for immunostaining were fixed in zinc formalin 4% (Unifix, Klinipath) for 24 hours. All specimens were processed routinely in a tissue processor for embedding in paraffin. Frozen paraffined tissue blocks were sectioned at 3 µm thickness, placed on slides precoated with biobond (British Biocell International) to increase section adhesion, and air-dried. Sections were processed for immunohistochemistry using the labelled streptavidin-biotin method. Sections were incubated with antibodies to NFP, NPY, and SP (as specified elsewhere) for 20 minutes at room temperature. After rinsing in PBS, sections were incubated with biotinylated goat anti-immunoglobulins (Multilink, Biogenex Laboratories USA; with 10 per cent normal human AB serum to inhibit nonspecific staining) for 20 minutes at room temperature. The slides were washed again with PBS, incubated with alkaline phosphatase-conjugated streptavidin for 20 minutes at room temperature.

Finally, sections were washed, stained with fast red chromogen (Biogenex Laboratories, USA) for 10 minutes, counterstained with Mayer's hematoxylin, and coverslipped. Immunohistochemical controls were prepared by incubating sections in PBS instead of the primary antibody and then processed in a manner identical to that previously described for the experimental sections. The slides were examined and photographed using bright-field light microscopy.

RESULTS

Electron microscopy

Spinal ligaments were found to be richly innervated. Neural structures were mainly localized in the superficial areolar layer of the ligaments and within fibro-elastic septa in the deeper ligamentous substance. Neural structures were usually found in the vicinity of blood vessels.

We identified single or bundled small myelinated (0.8 - 2.9 µm) and unmyelinated nerve fibres (0.2 - 0.8 µm), and mixed nerves (Figs 3 *a,b,f*). Noncorpuscular 'free' nerve endings were regularly observed. Their axons contained variable numbers of accumulated mitochondria and various types of vesicles, including small granular vesicles, large granular vesicles and small agranular vesicles (Fig. 3*g*).

Morphological characteristics of these nerve endings were identical to those described in the facet joint capsule (Vandenabeele et al., 1995). Notably, we regularly observed small globular endings (10 - 20 µm) related to neurovascular bundles (Figs 3 *c,d,e*). Vesiculated nerve profiles were embedded within the elaborated endoneural space of these corpuscular endings.

Occasionally, noncorpuscular 'free' nerve endings were observed in the deeper stroma of the ligament, mainly nonvascular. Despite an extensive investigation of the specimens studied, the deeper substance of the fibro-elastic ligamentum flavum lacked any type of innervation.

Immunohistochemistry

NF- ir nerves and varicose SP- ir and NPY- ir nerve endings were found in the superficial sheaths of the supraspinous and interspinous ligaments, mainly close to blood vessels, but also freely distributed in the deeper ligamentous substance as well as in fibroadipose septa (Figs 1 *a-c*; 2 *a-b*). No recognizable sensory corpuscles were found immunohistochemically.

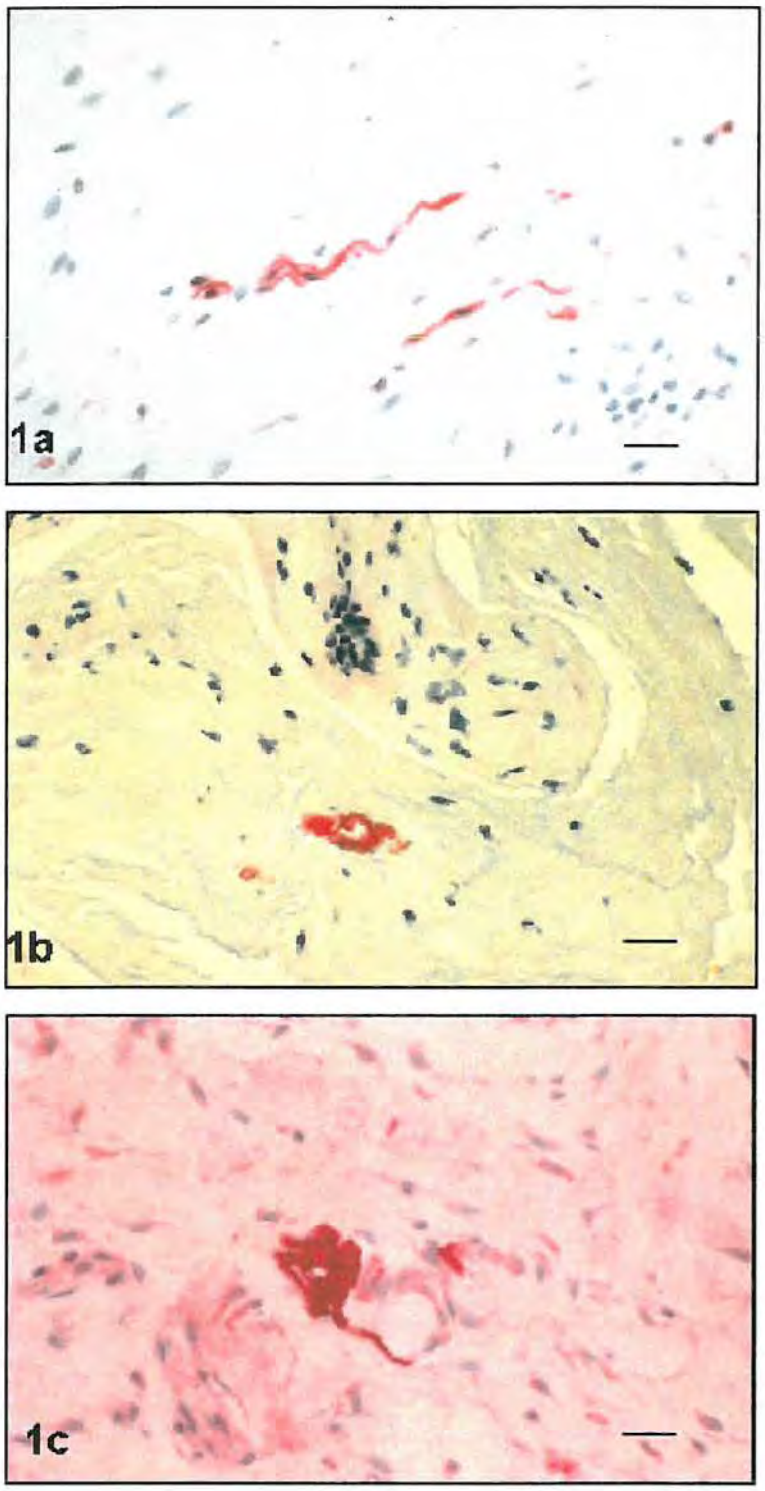
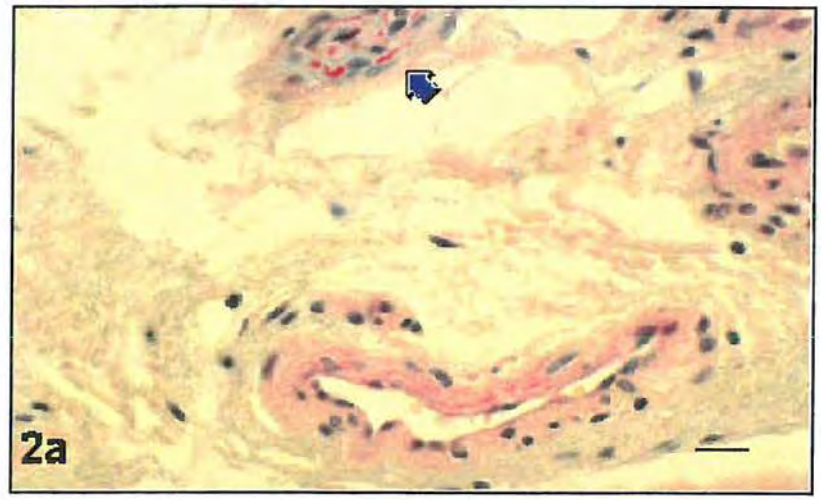
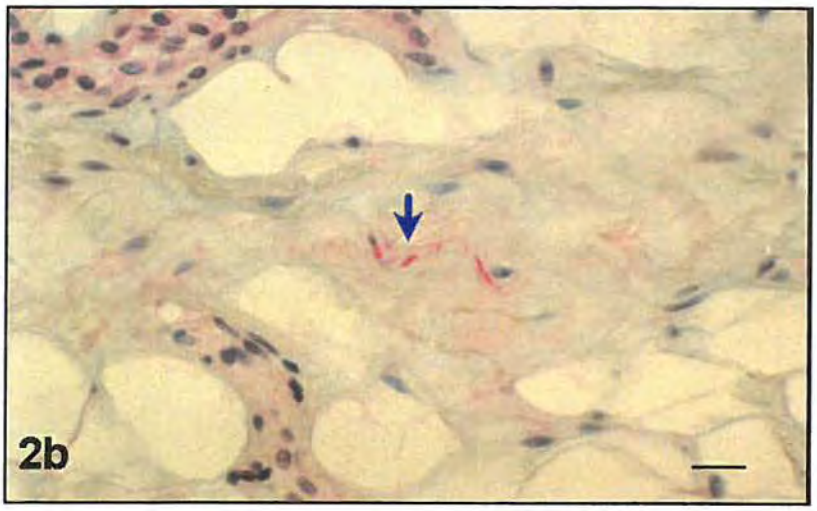


Fig. 1. Light micrographs of Mayer's hematoxylin-counterstained paraffin sections of the supraspinous ligament and interspinous ligament, showing immunoreactivity to NF, SP and DBH. BAR = 30 µm.

(a,c) Superficial layer: (a) NF- immunoreactive nerve, (c) DBH- immunoreactive nerve ending.
(b) Deep ligamentous substance: SP-immunoreactive nerve.





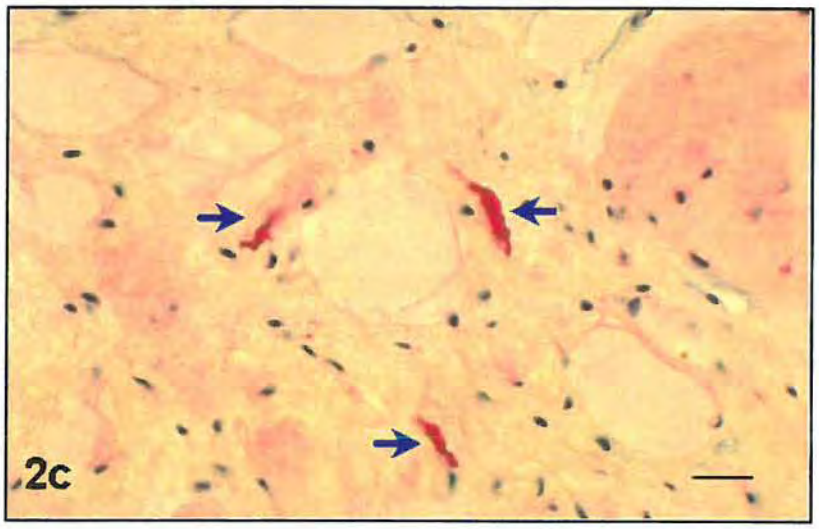


Fig. 2. Light micrographs of Mayer's hematoxylin-counterstained paraffin sections of the superficial layer of the supraspinous ligament, showing immunoreactivity to NF and NPY. (a) Paravascular NF-immunoreactive nerve bundle. (b) Paravascular NF-immunoreactive nerve fibre. (c) NPY-immunoreactive nerve endings. BAR = 28 µm.

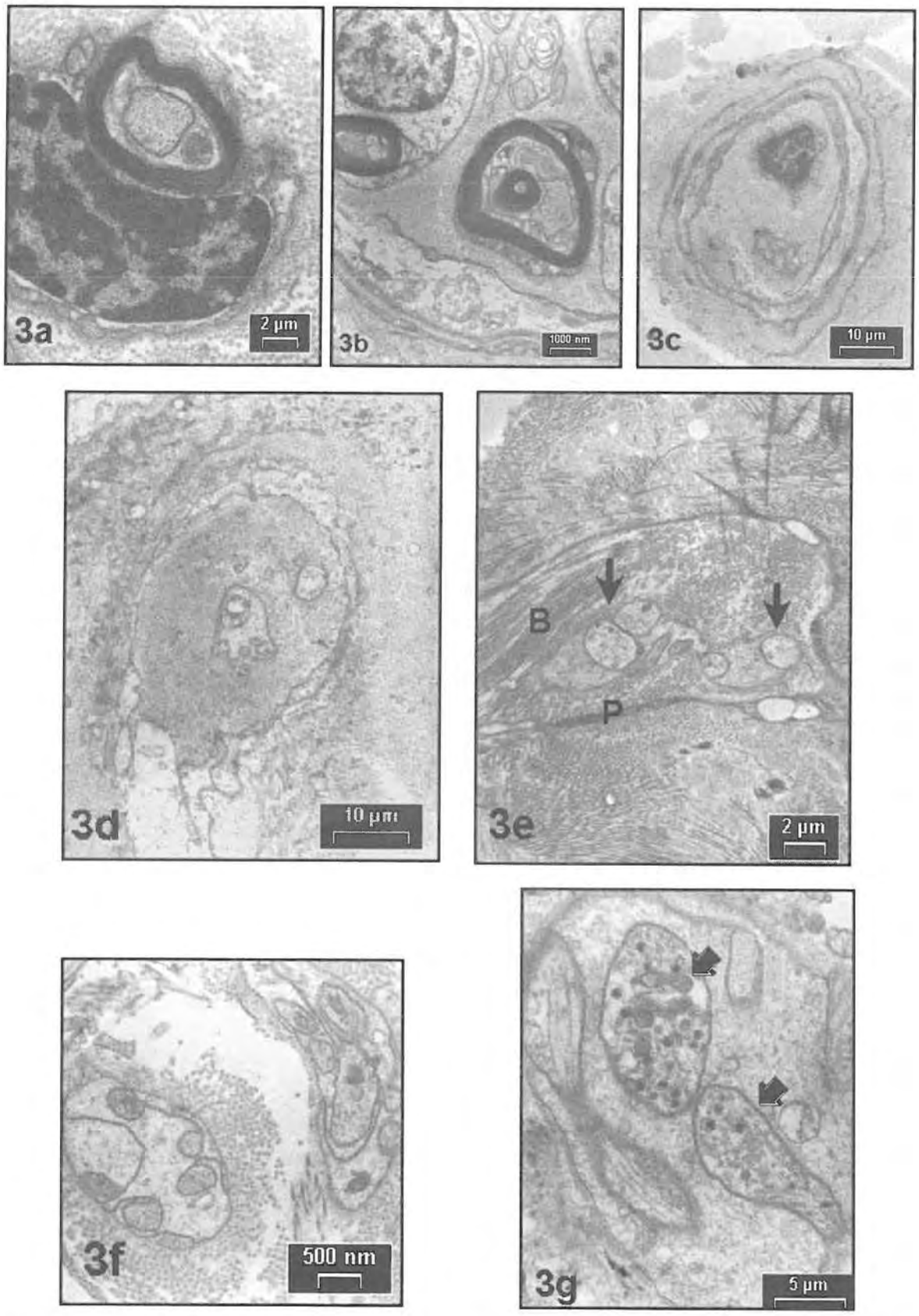


Fig. 3. Supraspinous ligament and interspinous ligaments. Electron micrographs. (a) Small myelinated nerve fibre. (b) Large mixed nerve. (c,d) Small lamellated corpuscles. (e) Unmyelinated nerve fibre. Continuous perineurial capsule and well-developed endoneurium. Note a collagen bundle entering the endoneurium. (f) Unmyelinated nerve fibres. (g) Vesiculated nerve profile with granular vesicles.

DISCUSSION

The results of this study have shown that the supraspinous and interspinous ligaments are richly innervated. Immunohistochemistry confirmed the presence of NF- ir, NPY- ir, and SP- ir nerves in the interspinous and supraspinous ligaments, often closely associated with blood vessels. The distribution pattern of NF-ir fibres is in accordance with the findings of El-Bohy et al. (1988), Rhalmi et al. (1993), Yahia and Newman (1993) The perivascular noncorpuscular 'free' varicose NPY- ir and SP- ir nerve endings are likely vasoregulatory. The nonvascular appearance of the other SP- ir and probably NPY- ir fibres, would seem to indicate that their action is not vasoregulatory. Likely they mediate sensory modalities. They may prove to play a significant role in the pathogenesis of low back pain (Grönblad et al., 1991).

The structure and distribution of the vesiculated nerve profiles probably represent different physiological conditions (Vandenabeele et al., 1995). It is generally accepted that profiles containing vesicles with an electron-dense granule or core, are adrenergic in function. Vesiculated nerve profiles characterized by an accumulation of mitochondria most probably represent sensory nerve endings of type III and IV fibres, thought nociceptive in function.

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Chapter 4.4

Immunohistochemical Study of Neurochemical Markers in the Lumbar Spine of the Human Fetus

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Based on: Vandenabeele F, Creemers J, Lambrichts I, Vanormelingen L (1998) Immunohistochemical study of neurochemical markers in the lumbar spine of the human fetus. *Cell Biology International* **21** (in press).

ABSTRACT

The pathways and functions of the nerves supplying the lumbar spine of the human fetus have been investigated immunohistochemically. The outer annulus fibrosus was richly innervated. The nucleus pulposus lacked any type of innervation. The dorsal dura was scarcely innervated.
The rami communicantes showed immunoreactivity to SP and suggests that somatosensory fibres originating in the deep structures of the lumbar spine follow 'sympathetic routes'.

INTRODUCTION

The lumbar vertebral column and its associated structures is known to be well innervated. Most studies focussed on the sensory and autonomic innervation of the human lumbar spine in the adult. The origin, pathways and termination pattern of the nerves and nerve plexuses supplying the vertebral column of the human fetus, have been reported by Groen et al. (1990). They demonstrated that the origin of nerves supplying the vertebral column is in the sympathetic trunks and their ramifications, and suggested that spinal pain is transmitted by sympathetic afferent fibres through the sympathetic trunks.

Recent clinical and experimental studies confirmed their hypothesis, as mentioned above. To our knowledge, immunohistochemical data on the neurohistology of the human fetal spine are absent. Therefore we decided to examine the pathways and functions of the nerve fibres supplying the lumbar spine in the human fetus, using immunocytochemistry.

MATERIALS AND METHODS

The entire lumbar spinal column with the epaxial musculature was removed as a unit from a fetus of 30 weeks gestational age, and immersed in zinc formalin 4% (Unifix, Klinipath) for 24 hours. Then the specimen was decalcified in De-cal (National Diagnostics), embedded in paraffin, and sectioned serially in a transversal plane at 7 µm thickness. The sections were subjected to immunohistochemical analysis using the labelled streptavidin-biotin method as describe elsewhere, and primary antisera against the 200 kD polypeptide of NF (Klinipath), the 70 and 200 kB polypeptide of NF (Dako) and S100 (Dako) as general neuro-glial markers, and against the sensory marker SP (Biogenex). Immunohistochemical controls were prepared by incubating sections in PBS buffer instead of the primary antibody.

RESULTS

Immunoreactivity to NF and S100 gives a panoramic view of the distribution, and termination pattern of nerves supplying the lumbar spine (Figs 4 *a-e*). In general, S100- ir fibres were more numerous than the NF- ir fibres. Immunoreactivity to S100 is found in nerve bundles, single axons and in varicosal nerve endings. Immunoreactivity to NF is found in larger neural elements.

Substantial immunoreactivity to S100, but none to NF was detected in the paravertebral sympathetic trunk and its rami communicantes, dural sleeves, ventral dura (Fig. 4*b*), while none or few could be detected in the dorsal dura. No immunoreactivity to NF was detected in the spinal cord, except for the anterior funiculus (Fig. 1*a*). Scarce or no immunoreactivity to S100 and NF was detected in the facet joints. NF- ir and S100- ir nerve fibres were found in the outer lamellae of the annulus fibrosus, while none could be found in the nucleus pulposus (Fig. 3*b*). Immunoreactivity to NF was detected in the ventral and dorsal roots of spinal nerves. Notably, immunoreactivity to NF was more substantial in the dorsal nerve roots.

Substantial immunoreactivity to SP was detected in the spinal cord, (Fig. 1*c*) dorsal root ganlion (Figs 2*a-c*), spinal nerves, epaxial muscles, dorsolumbar fascia, supraspinal ligament, and interestingly in the rami communicantes to the paravertebral sympathetic trunks (Fig. 3*a*). The immunoreactivity to SP was more substantial in the dorsal rami.

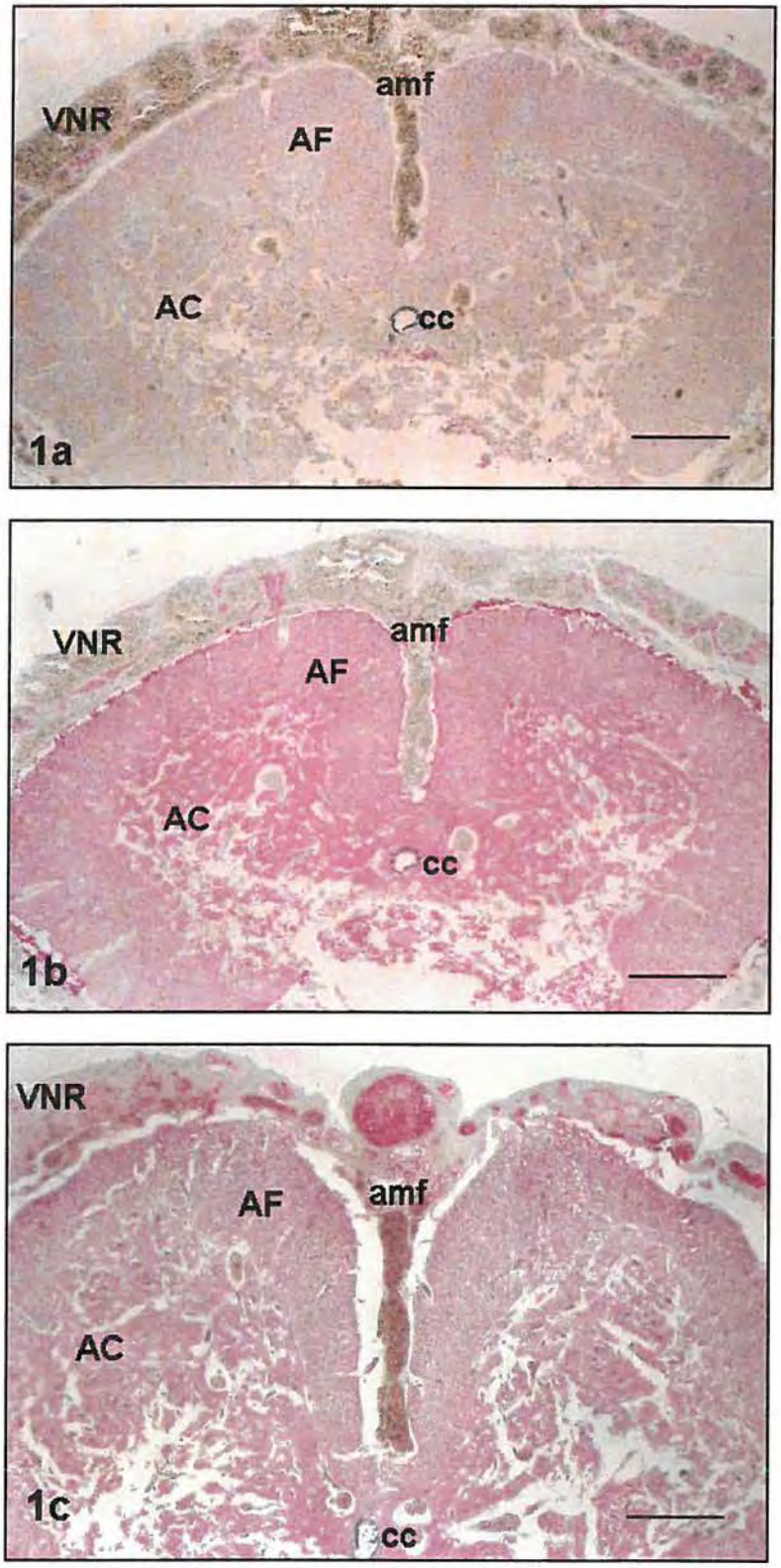


Fig. 1. Light micrographs of Mayer's hematoxylin-counterstained transverse paraffin sections through the spinal cord in the lumbar region of the human fetus, showing immunoreactivity to Neurofilamentprotein (Fig. 1a), S100 (Fig. 1b) and Substance P (Fig. 1c). Central Canal (cc). Anterior column (AC). Anterior median fissure (amf). Anterior funiculus (AF). Ventral nerve root (VNR). BAR = 350 µm.

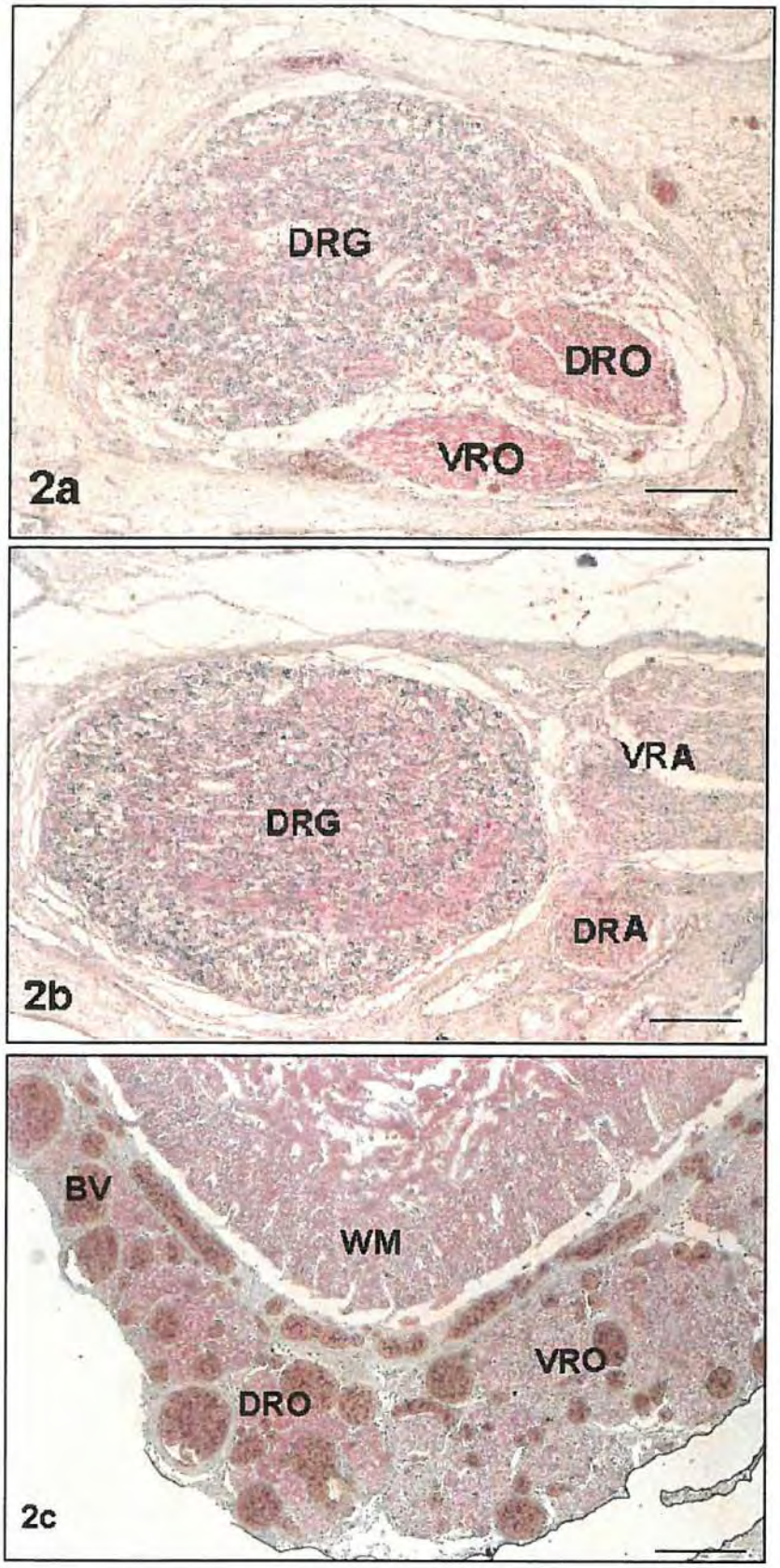
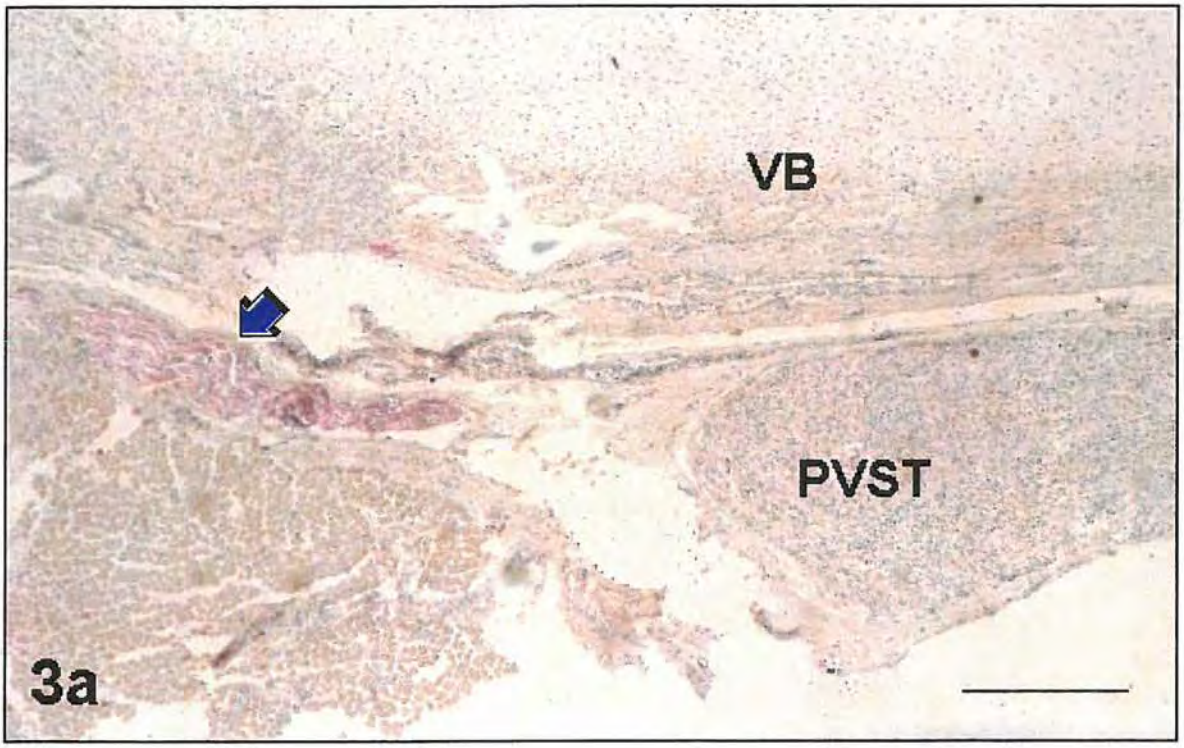


Fig. 2. Light micrographs of Mayer's hematoxylin-counterstained transverse paraffin sections of the human fetal spine at a lumbar level. Note significant SP-immunoreactivity of the dorsal ramus (DRA) in Figure 2b. Dorsal root ganglion (DRG). White matter (WM). Blood vessel (BV). Ventral nerve root (VRO). Dorsal nerve root (DRO). Ventral ramus (VRA). BAR = 350 µm.



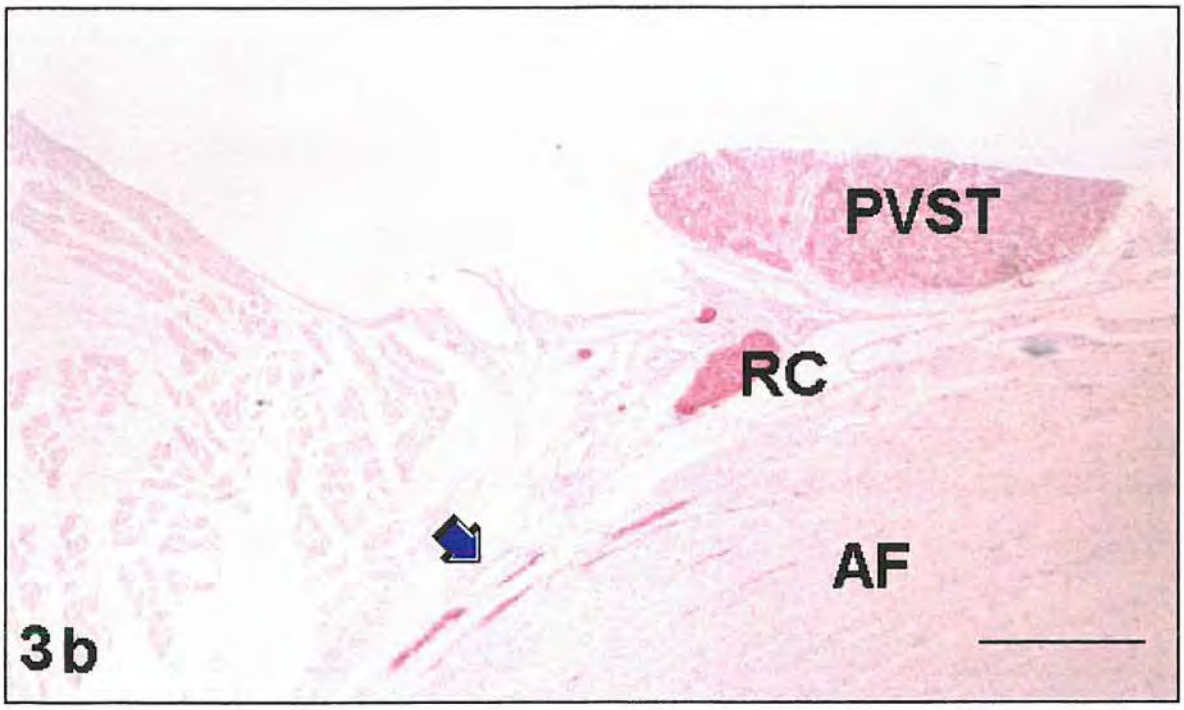


Fig. 3. Light micrographs of Mayer's hematoxylin-counterstained transverse paraffin sections of the human fetal spine at a lumbar level. (a) Note ramus communicans (blue arrow) to the paravertebral sympathetic trunk (PVST), showing immunoreactivity to SP. Vertebral body (VB). BAR = 100 µm. (b) S100 - immunoreactive nerves in the outer lamellae of the annulus fibrosus (AF). Ramus communicans (RC). Paravertebral sympathetic trunk (PVST). BAR = 200 µm.

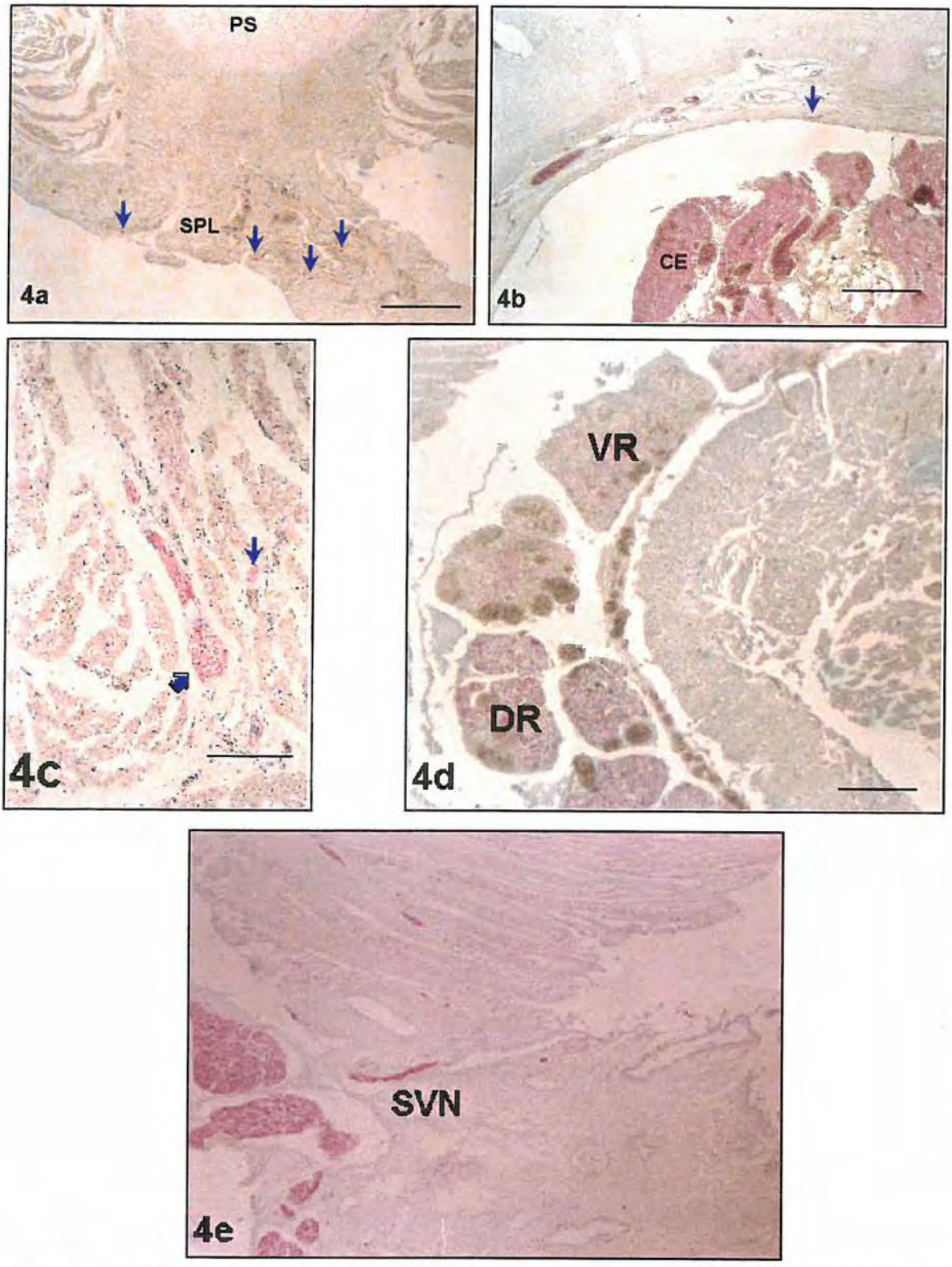


Fig. 4. Light micrographs of Mayer's hematoxylin-counterstained transverse paraffin sections in the lumbar region of the human fetal spine. (a) S100-immunoreactive nerves (arrows) in the supraspinal ligament (SPL). BAR = 300 µm. (b) S100-immunoreactive nerve (arrow) in the ventral spinal dura mater. Cauda equina (CE). BAR = 300 µm. (c) S100-immunoreactive nerves (arrows) in a sheath of the dorsolumbar fascia. BAR = 30 µm. (d) Immunoreactivity to NF (200 kD polypeptide subunit) spinal nerve roots. Note more substantial NF-immunoreactivity in the dorsal nerve root (DR). Ventral nerve root (VR). BAR = 350 µm. (e) Sinuvertebral nerve (SVN) entering the vertebral canal, showing S100- immunoreactivity. BAR = 300 µm.

DISCUSSION

The sparse immunoreactivity to NF in the smallest neural elements agrees with the observations of Sieger et al. (1984) and El-Bohy et al. (1988), and probably relates to their paucity of neurofilaments. Differences in NF immunoreactivity between the ventral and dorsal roots of the spinal nerves might be explained by a dominant expression of the 200 kD polypeptide subunit in the dorsal (sensory) root.

The demonstration of immunoreactivity to the nociceptive neuropeptide SP in rami communicantes, implicates the lumbar sympathetic trunk as a possible sensory (nociceptive) pathway, supporting the neuroanatomic studies of Groen et al. (1990) in the fetal spine, and recent clinical, electrophysiological and immunohistochemical observations in the adult (vide supra).

The paucity of NF- ir, S100- ir and SP- ir nerves in the dorsal dura correlates with neuroanatomic studies in the spinal dura mater of the adult (vide supra). The presence of SP- ir fibres in the outer annulus fibrosus and the lack of neural structures in the nucleus pulposus, is in agreement with previous studies on the innervation of the intervertebral disc (vide supra).

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CHAPTER 5

Summary and Conclusions

Summary

The purposes of our studies are set out in chapter 1. The knowledge of the distribution and of the structure and function of the nerves present in the lumbar spine, is of fundamental importance to understand the neurological mechanisms of low back pain. We reviewed the milestones in the study of the nerve supply to the lumbar spine. The different classifications of low back pain are given.

Our work concerns the neuromorphology of the lumbar vertebral column and associated structures in man. We attempt to elucidate light- and electronmicroscopical and immunohistochemical aspects of the nerve endings in the human lumbar spine. The study also elucidates the ultrastructural aspects of the human spinal meninges, with special reference to the spatial relationships of its different layers.

Chapter 2, focusses on the macroscopic and microscopic anatomy of the deep structures in the lumbar spine that are putative sources of pain transmission. The ad hoc literature data are reviewed. Special attention has been given to the microscopic structure of the facet joint. Our study on the ultrastructural organization of the human spinal arachnoid and dura mater is included in this chapter.

Chapter 3 deals with the extrinsic and intrinsic nerve supply of the lumbar spine.
Chapter 3.1 summarizes the origin and the distribution pattern of the extrinsic nerve supply of the lumbar spine, including the sinu-vertebral nerves, peridiscal nerve plexus and rami of the spinal nerves. Their termination pattern in the deep spinal tissues of both the ventral and dorsal compartment is described.
Chapter 3.2 concerns the light-, and electronmicroscopic and immunohistochemical observations on the intrinsic nerves in the ventral and dorsal compartment of the lumbar spine, except for our ultrastructural and immunocytochemical studies on the facet joints and supra- and interspinous ligaments, that are described in chapter 4.
Chapter 3.3 reviews the ad hoc literature on the sensory transmission in the lumbar spine, with special reference to the existence of afferent autonomic pathways and the 'sympathetic' modulation of spinal pain pathways.

Chapter 4 deals with our ultrastructural and immunocytochemical studies on the facet joints and supra- and interspinous ligaments.

In the facet joint, both nonmyelinated and thinly myelinated nerve fibres, as well as their 'free' nerve endings were regularly encountered in the joint capsule and adjacent synovial plical tissue, mainly perivascular in location. The 'free' nerve endings are characterized by an enlarged axonal profile containing vesicles, mitochondria, neurotubuli and neurofilaments. The axonal profiles are usually partly enclosed by thin sheaths of Schwann cell cytoplasm. The 'bare' or 'naked' area of the axolemma is defined as the receptive area. Two major types of vesiculated nerve profiles are observed at the electron microscopic level. Nerve profiles that contain mainly small granular vesicles predominate. The latter are considered adrenergic in function. A second type of nerve ending is encountered, containing varying numbers of small agranular vesicles and mitochondria.

Two variants of this type of nerve ending are described. One variant is predominantly characterized by accumulated mitochondria along with small agranular vesicles that are in close association with the receptive site. On morphological grounds, they represent noncorpuscular endings ('free nerve endings') of thinly myelinated group III (Ad) and nonmyelinated group IV (C) afferent nerve fibres.

The function of the other variant, containing predominantly small agranular vesicles and few or no mitochondria, is uncertain. The possible functions of 'free' nerve endings based on their ultramorphological aspects are discussed. The possibility of coexistence and/or cotransmission is suggested.

This chapter also deals with some immunohistochemical characteristics of the facet joint capsule and related synovial plical tissue. The ad hoc literature is reviewed. Our immunohistochemical studies confirmed that the facet joint capsule is richly innervated, by both sensory and autonomic nerves. The nerve fibres are found both vascular and nonvascular in location and might be involved in vasoregulation and pain transmission, respectively. We demonstrated the presence of a subpopulation of SP-immunoreactive nerve endings that is also immunoreactive for NPY and DBH, supporting (1) the concept of coexistence and/or cotransmission of neurotransmitters, as well as (2) the hypothesis that facet pain might be transmitted by sympathetic afferents.

By light microscopy, two types of corpuscular nerve endings could be observed: glomerular and lamellated corpuscles. Glomerular corpuscles predominate, whereas small lamellated (Paciniform) corpuscles are occasionally present. On ultramorphological grounds the glomerular corpuscles are classified as encapsulated Ruffini-like endings. Most glomerular corpuscles were small and spherical. Others are larger and fusiform, and classified as Golgi tendon-like endings, classically described at myotendineus junctions. Ultramorphologically, the axon terminals in the receptive complex of corpuscular endings, can be classified either as group III (Ad) or as group IV (C) fibres. Encapsulated corpuscular nerve endings function in mechanoreception for sense of movement. They are essential for spinal proprioception, ensure protective muscular reflexes and provide stability of the vertebral column.

This chapter focusses on some peculiar ultramorphological characteristics of the axon terminals in the encapsulated Ruffini-like endings of the lumbar facet joint. Periaxonal small diameter collagen fibrils situated within multilayered basal laminae are found in close association with the multiple receptive sites of their receptive complex. The functional relevance of these ultramorphological aspects in mechanoreception is discussed.

Immunohistochemistry applied on the corpuscular nerve endings in the human lumbar facet joint capsules, at both light and electron microscopic level, demonstrated the presence of the sensory marker SP in vesicles situated in the receptive sites of their receptive complex. The presence of the nociceptive neuropeptide SP probably indicates the encapsulated corpuscular endings to function as pain generators in the lumbar facet joint, and thus mediate pain perception in the low back, supporting recent electrophysiological recordings.

It is generally accepted that only structures that are innervated by pain fibres (unmyelinated group IV or C fibres, and thinly myelinated group III or Ad fibres), are putative sources of pain generation. On morphological and electrophysiological grounds they both terminate in the lumbar facet joint capsule, as noncorpuscular 'free' as well as corpuscular encapsulated nerve endings, that share the function of pain generator. Under normal conditions nociceptive nerve endings have high mechanical thresholds, responding only to noxious stimuli. Under pathologic conditions such as inflammation and tissue damage, they become sensitized by various chemical mediators, responding spontaneously or at lower thresholds. The encapsulated corpuscular nerve endings of the lumbar facet joint are assumed to be activated at the extremes of motion during prolonged and pathological capsular stress.

This chapter also reports on our preliminary immunohistochemical analysis of neural structures in the lumbar spine of the human fetus. Immunoreactivity to NF and S100 gives a panoramic view of the distribution and termination pattern of nerves supplying the lumbar spine. Immunoreactivity to the nociceptive neuropeptide SP is demonstrated in rami communicantes, implicating the lumbar sympathetic trunk as a possible sensory (nociceptive) pathway.

Conclusions

The spinal dura and arachnoid closely adhere, without any naturally occurring 'subdural space'.

The innermost layer of the dura (dural border cell layer) is continuous with the outermost layer of the arachnoid (arachnoid border cell layer). The dural border cell layer is easily disrupted. It is characterized by multiple large extracellular spaces, the absence of extracellular collagen and few intercellular contacts, implicating structurally a leak of cohesion. Disruption of the dural border cell layer creates an artifactual 'subdural space', or actually an 'intradural space'.

The spinal dura mater mainly serves as a protective covering.

The spinal arachnoid and pia mater lack any type of innervation. The spinal dura mater is scarcely innervated. It mainly serves as a protective covering.

The human lumbar facet joint is richly innervated, by both noncorpuscular 'free' nerve endings and corpuscular endings.

Four major types of nerve endings are present in the dense fibrous layer and subintimal layer of the synovial membrane, mainly perivascular in location: noncorpuscular 'free' nerve endings and small encapsulated Ruffini-like endings predominate, along with occasional small lamellated (paciniform) corpuscles and golgi-tendon like corpuscles.

The human lumbar facet joint has both a sensory and an autonomic innervation.

NF and S100 immunoreactive nerve fibres of larger diameter and numerous S100 and SYN immunoreactive nerve endings are present both in the facet capsule (dense fibrous layer and synovial subintimal layer), and in the synovial plical tissue. A subpopulation of noncorpuscular 'free' nerve endings that is simultaneously immunoreactive for the sensory neuronal marker SP, and the autonomic markers NPY and DBH, is demonstrated in the facet joint capsule and adjacent synovial plical tissue. This indicates that the concept of co-transmission and co-existence of neurotransmitters can be applied for the lumbar facet joint. This is in agreement with the ad hoc literature about pain transmission in the lumbar spine following sympathetic routes.

Corpuscular nerve endings with mechanoreceptive fields in the facet joint capsule, are likely involved in the mechanisms of low back pain.

It is pointed out for the first time that immunoreactivity to the nociceptive neuropeptide SP is situated in the receptive complex of encapsulated Ruffini-like endings. Colloidal gold immunohistochemistry demonstrated SP in accumulated vesicles at the multiple receptive sites.

Sensory transmission in the lumbar spine also follows 'sympathetic' routes.

Clinical, electrophysiological, and neuroanatomical studies indicate that the lumbar spine has a dual intrinsic sensory innervation. Sensory (pain) transmission serving the lumbar spine may reach the dorsal root ganglia: (1) ipsilateral and segmental, by somatic (spinal) nerves, and (2) bilateral and polysegmental by sympathetic (visceral) nerves through the paravertebral sympathetic trunks and its ramifications. We demonstrated immunoreactivity to the nociceptive neuropeptide SP in rami communicantes of the lumbar spine in the human fetus. This suggests the existence of afferent (nociceptive) autonomic pathways, and agrees with the concept of co-existence and co-transmission of neurotransmitters in the lumbar spine.

The ligamentous structures of the lumbar spine are richly innervated.

On morphological grounds the ligamentous structures of the lumbar spine (facet joint capsules, outer annulus fibrosus, lumbodorsal fascia and spinal ligaments) are richly innervated, by both noncorpuscular 'free' nerve endings and corpuscular nerve endings.
Immunohistochemically, a sensory and an autonomic innervation is demonstrated. Likely, they play a crucial role in spinal proprioception and pain transmission.

Closing remarks

Exact knowledge of the innervation of the vertebral column and its associated structures is essential for better understanding of the mechanisms involved in low back pain. Clinical and electrophysiologically there is substantial evidence for disc pain, facet pain and sciatica.

We reviewed the ad hoc literature on the neurohistology of the ventral compartment and undertook microscopic studies as to the anatomic substrate of sensory transmission in the dorsal compartment. We demonstrated noncorpuscular 'free' nerve endings and corpuscular nerve endings in the facet joint capsules, in the lumbodorsal fascia and in the supraspinal as well as the interspinal ligaments, at both light and electron microscopic level and by immunohistochemistry. The concept of co-existence and co-transmission of neurotransmitters in a single neuron is discussed. The possible role of sympathetic routes and corpuscular endings in spinal pain pathways was explored.

Attemps were made in investigating the immunohistochemical characteristics of 'healthy' deep tissues of the human lumbar spine in the fetus.
Special attention was given to the ultrastructural characteristics and spatial relationships of the spinal meninges.

CHAPTER 6

Samenvatting en Conclusies

Samenvatting

Lage rugpijn is een belangrijk probleem in de medische praktijk. Meestal zijn de klinische bevindingen en radiologische onderzoeken aspecifiek, en blijft de oorzaak ('cause of pain') onduidelijk. Het vinden van de vermeende pijnbron ('source of pain') is in de meerderheid van de gevallen nog een grotere uitdaging. De pijn kan uitgaan van de wervelkolom met zijn geassocieerde structuren (primaire pijn). De pijn kan veroorzaakt worden door contractie van de paravertebrale spieren (secundaire pijn) of kan waargenomen worden op een andere plaats dan in de eigenlijke pijnbron (gerefereerde pijn). Primaire pijn kan ingedeeld worden volgens de vermeende pijnbron, in pijn uitgaande van (1) de bewegende structuren van de wervelkolom (tussenwervelschijven en/of facetgewrichten), (2) de weefsels rond de wervelkolom (paravertebrale spieren, ligamenten, ...), of van (3) een geknelde spinale zenuw.

De kennis van de structuur en functie van de neurale receptoren in de wervelkolom met zijn geassocieerde structuren is essentieel om inzicht te verwerven in de etiologie van lage rugpijn. Inderdaad, alleen structuren die bezenuwd worden door vrije zenuwuiteinden van pijn-gevoelige vezels (C en A delta vezels) vormen een mogelijke pijnbron.

De bezenuwing van de lumbale wervelzuil speelt bovendien een belangrijke rol in de proprioceptie van de lumbale wervelzuil. Proprioceptie wordt voornamelijk verzorgd door mechanoreceptoren. Mechanoreceptoren zijn meestal - al of niet omkapselde - sensorische lichaampjes. In de wervelzuil onderscheidt men verschillende types van omkapselde sensorische lichaampjes. De klassificatie van omgekapselde zenuwuiteinden is meestal niet eenvoudig.

Gedetailleerde morfologische gegevens over de ultrastructuur van de vliezen en de onderlinge verhoudingen van de vliezen in de wervelkolom ontbreken bij de mens en steunt in de klassieke anatomische handboeken grotendeels op waarnemingen bij het dier en op de structuur van de craniale vliezen bij de mens.

Het onderzoek beoogt enerzijds de neurohistologie van sommige weefsels in de humane lumbale wervelzuil te beschrijven, en anderzijds de ultrastructuur van de spinale dura mater en arachnoidea aan te geven.
Het proefschrift is ingedeeld in vijf hoofdstukken:

In hoofdstuk 1 wordt het doel van dit proefschrift en het nut van een gedetailleerde kennis omtrent structuur en functie van de neurale receptoren in de lage rug omschreven. Een kort historisch overzicht schetst de belangrijkste mijlpalen in de studie naar de bezenuwing van de lage rug. De verschillende classificaties van pijn uitgaande van de wervelkolom worden kort beschreven.

In hoofdstuk 2 wordt een overzicht gegeven van de macroscopische en microscopische anatomie van de verschillende structuren van de lumbale wervelkolom. De ultrastructurele morfologie en onderlinge verhoudingen van de spinale vliezen bij de mens - voor het eerst beschreven in deze studie - worden voorgesteld.

In hoofdstuk 3 wordt een overzicht gegeven van de oorsprong en het verloop van de zenuwen die de verschillende structuren van de lumbale wervelkolom bezenuwen en wordt een overzicht gegeven van de neurale receptoren in het ventrale en dorsale gedeelte van de lage rug (oa. de tussenwervelschijven, facetgewrichten, spinale vliezen en de meer oppervlakkig gelegen "diepe" paravertebrale structuren zoals ligamenten en spieren). Dit hoofdstuk is gebaseerd op 'ad hoc' literatuurgegevens aangevuld met eigen waarnemingen. Het belang van het orthosympatisch systeem voor de perceptie van prikkels afkomstig vanuit de lage rug wordt beschreven. De afferente informatiestroom bereikt waarschijnlijk via de viscerale sympatische plexus rond de wervelkolom en de grensstreng het CZS. mechanismen van beïnvloeding worden kort besproken, gebaseerd op ad hoc literatuurgegegevens.

De werking van het somatomotorisch systeem wordt voortdurend beïnvloed door het orthosympatisch zenuwstelsel, en vice versa. De verschillende mechanismen van beïnvloeding worden kort besproken, gebaseerd op ad hoc literatuurgegegevens.

Hoofdstuk 4 beschrijft de morfologie, distributie en functie van de neurale structuren in het facet gewricht, en het ligamentum supra- en interspinale. Hierbij wordt gebruik gemaakt van licht- en elektronenmicroscopische seriecoupes, en immunohistochemische technieken.
Wij konden geen neurale structuren in het cellulair gedeelte van de synoviale membraan in het facet gewricht aantonen. Enkel het fibreuze gedeelte van het gewrichtskapsel en het subintimale weefsel van de synoviale membraan en de intra-articulaire synoviale plooien blijken bezenuwd. Het lichtmicroscopisch onderzoek toont glomerulaire en gelamelleerde omkapselde lichaampjes.

De glomerulaire receptoren worden het meest frequent aangetroffen. De gelamelleerde zijn eerder zeldzaam. Het elektronenmicroscopisch onderzoek toont de structuur van gemyeliniseerde en niet-gemyeliniseerde zenuwvezels en van dikkere gemengde zenuwbundels. Er worden gedetailleerde gegevens verstrekt over de morfologie van niet-omkapselde 'vrije' zenuwuiteinden en omkapselde Ruffini-achtige lichaampjes.

Het aantal mechanoreceptoren in het facet gewricht is eerder gering, maar de aanwezige mechanoreceptoren hebben waarschijnlijk een vrij groot receptief veld.

De niet-omkapselde 'vrije' zenuwuiteinden in het facetgewricht bevinden zich meestal in de nabijheid van bloedvaten. Zij bevatten weinig mitochondriën, en worden gekenmerkt door de aanwezigheid van synaptische blaasjes. We onderscheiden kleine (30 - 60 nm) en grote (70 - 120 nm) blaasjes. De meerderheid van deze synaptische blaasjes bevat een centrale elektronendichte korrel of granulum omgeven door een klare halo. Vrije zenuwuiteinden die vooral kleine granulaire blaasjes bevatten, zijn vermoedelijk postganglionaire zenuweindigingen van het orthosympathische zenuwstelsel.

Op morfologische gronden zijn de vrije zenuwuiteinden in het kapsel van het lumbale facetgewricht, gekenmerkt door de aanwezigheid van mitochondriën en kleine doorzichtige synaptische blaasjes, afkomstig van ongemyeliniseerde (C of groep IV) en dunne gemyeliniseerde (A delta of groep III) zenuwvertakkingen. Zij zijn vermoedelijk afferent en pijngevoelig. De perceptie van pijn vergroot bij inflammatie en weefselbeschadiging. Hun topografische relatie met locale bloedvaten laat echter veronderstellen dat ze bovendien vasoregulerend zijn.

Twee types van omkapselde lichaampjes worden in het kapsel van het lumbale facetgewricht aangetroffen. Het eerste type komt het meest voor en is gegroepeerd. Het zijn kleine glomerulaire Ruffini-achtige omkapselde lichaampjes. Daarnaast bevat de kapsel een tweede type van omkapselde lichaampjes. Deze worden zelden aangetroffen. Het zijn grote spoelvormige lichaampjes van het Golgi-type, te herkennen aan hun grote subcapsulaire ruimte.

De ultrastructurele kenmerken van het sensoriële complex zijn in beide types van lichaampjes dezelfde. De zenuwuiteinden in het receptor complex van de omkapselde Ruffini-achtige lichaampjes zijn doorgaans omringd met opeenvolgende basale laminae. Het naakte axolemma van de zenuwuiteinden is het eigenlijke receptieve gedeelte. In de onmiddellijke nabijheid van de receptieve zone bevinden zich dunne periaxonale collageenvezels. Deze situeren zich tussen de opeenvolgende lagen van de basale lamina. De Ruffini-achtige lichaampjes dienen waarschijnlijk voor de perceptie van druk en spanning in de gewrichtskapsel. Zij reguleren bovendien de spierspanning in de paravertebrale musculatuur en zijn verantwoordelijk voor de proprioceptie in de lage rug. versterkt.

De aanwezigheid van dunne collageenvezeltjes ter hoogte van de receptieve delen van het omkapselde Ruffini-achtige lichaampje werd voor het eerst beschreven in deze studie en heeft geleid tot het voorstellen van de hypothese als zouden deze vezeltjes een belangrijke rol kunnen spelen in de overdracht van een mechanische prikkel. Hierbij veronderstellen we dat de opeenvolgende basale laminae waartussen deze vezeltjes liggen, de perceptie van de prikkel versterkt.

Immunohistochemische technieken werden toegepast op het gewrichtskapsel en de synoviale plooien van het facet gewricht. Op paraffine coupes werden NF en S100 (neuro-gliale merkers), SYN (merker voor zenuwuiteinden), NPY en DBH (merker van postganglionaire zenuweindigingen), en SP (sensorische merker, merker van pijnvezels) aangetoond in oa. gemyeliniseerde en niet-gemyeliniseerde zenuwvezels, gemengde zenuwvezels, en in 'vrije' zenuwuiteinden. De neuronale structuren bevinden zich meestal in de onmiddellijke nabijheid van bloedvaten.

Bovendien werden in opeenvolgende coupes sensorische en autonome merkers in hetzelfde zenuwuiteinde aangetoond. Dit zowel in paravasculaire als in vrije zenuweindingen. Er wordt dieper ingegaan op het concept van co-existentie en co-transmissie van neurotransmitters in een neurale receptor. Het aantonen van zowel sensorische als autonome neurotransmitters in dezelfde neurale receptor doet vermoeden dat de perceptie van een pijnprikkel in het kapsel van het facet gewricht eveneens via afferente (viscerale) sympathische vezels kan verlopen. Dit is naar alle waarschijnlijkheid ook van toepassing voor vrije zenuwuiteinden die zich niet in de onmiddellijke nabijheid van bloedvaten bevinden. Verschillende neuro-anatomische en klinische studies toonden reeds aan dat een pijnprikkel vanuit de lumbale wervelkolom of geassocieerde structuren, het CZS kan bereiken via de paravertebrale ganglia in de grensstreng van het orthosympatisch systeem. Vanzelfsprekend bevestigt het aantonen van SP in paravasculaire autonome zenuwuiteinden (NPY-ir, DBH-ir) de gekende vasoregulerende functie van SP.

Door gebruik te maken van licht- en elektronenmicroscopische coupes werden NF, S100 en SP aangetoond ter hoogte van Ruffini-achtige receptoren en Golgi -achtige lichaampjes in het gewrichtskapsel van het facet gewricht. SP wordt aangetoond ter hoogte van synaptische blaasjes in het receptieve deel van de zenuweindigingen. Perceptie van pijn in het facet gewricht zou dus eveneens kunnen gebeuren via omkapselde sensorische zenuweindigingen.

In dit hoofdstuk wordt ook de morfologie en structuur van de neurale receptoren in het ligamentum supraspinale en interspinale beschreven met behulp van licht- en elektronenmicroscopisch onderzoek, en immunohistochemische technieken. De oppervlakkige lagen van deze ligamenten worden sterk bezenuwd.

In dit hoofdstuk worden eveneens de eerste resultaten beschreven van de immunohistochemische technieken toegepast op ontkalkt materiaal van de menselijke foetale lumbale wervelzuil. De distributie van NF, S100 en SP wordt in kaart gebracht in de verschillende neurale structuren. SP wordt ondermeer aangetoond ter hoogte van het ruggenmerg, de spinale ganglia en de rami communicantes van het orthosympatisch systeem.

Het aantonen van SP in de ramus communicans en in de ganglia van de orthosympatische grensstreng, is waarschijnlijk een bevestiging van de hypothese dat het orthosympatisch systeem pijnprikkels doorgeeft. Behalve physiopathologische en klinische is verdere immunohistochemische exploratie hieromtrent noodzakelijk.

CONCLUSIES

De arachnoidea bestaat uit twee te onderscheiden lagen: een buitenste laag aan elkaar sluitende cellen die tegen de dura aanligt en een binnenste laag bestaande uit sterk vertakkende cellen en bindweefsel.

De dura mater bestaat uit drie lagen: een buitenste laag van afgeplatte cellen die grenst aan de epidurale ruimte, een middenste fibro-elastische laag en een binnenste laag van afgeplatte cellen.

In de wervelkolom van de mens is de dura mater niet gescheiden van de arachnoidea door een subdurale ruimte.

De binnenste laag van de dura bevat weinig celjuncties, geen collageen, en vertoont grote extracellulaire ruimten.

De vliezen van de wervelkolom bij de mens klieven gemakkelijk ter hoogte van de binnenste laag van de dura. De vermeende subdurale ruimte -indien aanwezig- is eigenlijk een intradurale ruimte.

De dura mater van de wervelkolom is in geringe mate bezenuwd en vormt voornamelijk een beschermende huls rond het CZS.

De arachnoidea bevat geen neuronale structuren.

Het facet gewricht (gewrichtskapsel, subintimale weefsel van de synoviale membraan) wordt rijkelijk bezenuwd door niet - omkapselde 'vrije' zenuweindigingen, merendeels in de onmiddellijke nabijheid van bloedvaten.

Er kunnen geen neuronale structuren worden aangetoond tussen de synoviale cellen.

Elektronenmicroscopisch bevat het facet gewricht drie types van niet-omkapselde 'vrije' zenuwuiteinden.

Het facet gewricht bevat weinig omkapselde sensorische zenuweindigingen. Omkapselde lichaampjes van het Ruffini-type komen het meest frequent voor, naast gelamelleerde (Paciniforme) lichaampjes. De grootste Ruffini-achtige receptoren zijn te vergelijken met Golgi-achtige lichaampjes.

Sommige niet-omkapselde 'vrije' zenuwuiteinden in het facet gewricht zijn tergelijkertijd immunoreactief voor NPY en DBH (autonome merkers), en voor SP (sensorische merker). Dit is in overeenstemming met het het concept van co-existentie en co-transmissie van neurotransmitters, en met de hypothese omtrent het bestaan van een eventuele afferente informatiestroom vanuit het facet gewricht via de grensstreng en de viscerale sympathische plexus rond de wervelkolom.

De perceptie van pijn gebeurt mogelijk ook via de omkapselde lichaampjes van het facet gewricht. Wij beschreven SP in synaptische blaasjes ter hoogte van het receptieve deel van de zenuwuiteinden in deze receptoren.

De ligamenteuze structuren van de lumbale wervelkolom worden eveneens uitgebreid bezenuwd zowel door 'vrije' zenuwuiteinden als omkapselde sensoriële zenuweindingen. De ligamenteuze structuren spelen aldus een voorname rol zowel in de proprioceptie van de wervelkolom als in de pijnperceptie. Het ligamentum flavum is niet bezenuwd.