Anat Embryol (1989) 179:415–421

Anatomy
and Embryology

The ultrastructure of sensory nerve endings in human anterior cruciate ligament*

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Summary. The sensory innervation of the anterior cruciate ligament (ligamentum cruciatum anterius) of the human knee joint was studied by light- and electron microscopy. The connective tissue between the synovial membrane and the cruciate ligament contains small Ruffini corpuscles and lamellar corpuscles with several inner cores. The connective tissue septa between the individual fascicles of the cruciate ligament contain Ruffini corpuscles and free nerve endings. The free nerve endings are innervated by C-fibres and myelinated A-delta fibres. The afferent axons of Ruffini corpuscles are myelinated and measure 4–6 µm in diameter, those of the lamellar corpuscles with several inner cores measure about 6 µm in diameter.

It is discussed, whether these receptors of the anterior cruciate ligament may influence the muscle tone via polysynaptic reflexes.

Key words: Human knee joint – Cruciate ligament – Mechanoreceptors – Sensory innervation pattern

Introduction

The development of prosthetic material to replace ligaments and articular surfaces and the development of advanced surgical techniques in orthopedics gave rise to the demand for a new approach to joint anatomy. Today joints are considered as functional units (Müller 1982). Apart from articular surfaces, capsules and ligaments, studies of joints now include also the muscles with their fascia, aponeuroses and insertions (van Mameren 1983; van Mameren and Drukker 1984; van der Wal et al. 1987), moreover the innervation (Grüber et al. 1986) and blood supply. Special attention is given to the sophisticated knee joint of primates and man. While walking and running, the total body weight alternatingly is shifted from one knee joint to the other. The ligaments, particularly the cruciate ligaments play an important role to secure the stability of the knee joint. According to Huson (1974) and Menschik (1974, 1975) the cruciate ligaments have a gearbox-like function (Getriebefunktion). This function is lost even after cutting one cruciate ligament.

The structure of the cruciate ligaments is explained in detail in textbooks as well as in research papers (for review see Barnett et al. 1961; Müller 1982; Haus and Refior 1987). The ligaments of the knee joint are referred to as passive elements of the locomotive system. The active elements, i.e. the muscles, are activated by impulses via monosynaptic or polysynaptic reflexes (Baxendale and Ferrell 1981; Ferrell 1980, 1985, 1988; Grüber et al. 1986). During an operation Grüber et al. (1986) were able to initiate a polysynaptic reflex of the semimembraneous and biceps femoris muscle by irritating the anterior cruciate ligament. The final histological proof of mechanoreceptors was not

Fig. 1. Free nerve endings adjacent to a blood vessel from the subsynovial connective tissue of the anterior cruciate ligament. *1*, Detail from a smooth muscle cell of the blood vessel; *2*, thickened nerve terminal only in part covered by a basal lamina. *3*, Schwann cell with axons; *4*, Fibrocyte. ×9660

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* Supported by Verein zur Förderung der Erforschung und Bekämpfung rheumatischer Krankheiten e.V., Bad Bramstedt and DFG (Ha 1194/3-1).

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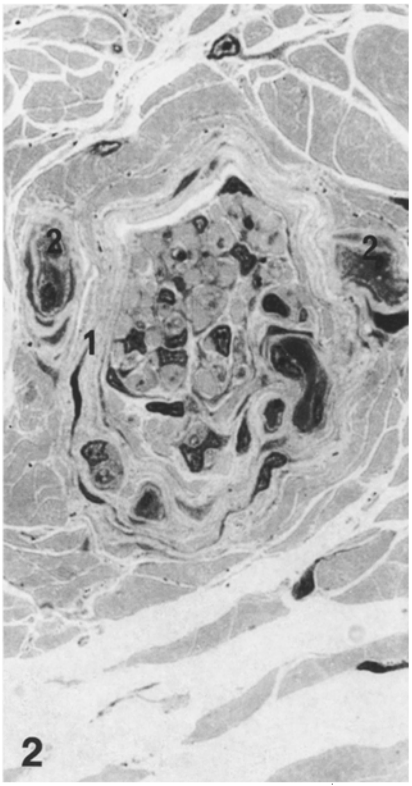


Fig. 2. Ruffini corpuscle from the subsynovial connective tissue of the anterior cruciate ligament. Perineural capsule (*1*), blood vessels (*2*) adjacent to the corpuscle. Semithin section. × 840

Fig. 3. Same Ruffini corpuscle as in Fig. 2 in ultrathin section. The incomplete perineural capsule (*1*) divides the corpuscle into compartments. *2*, blood vessels; *3*, Schwann cell. × 2280

provided by these authors nor by Schultz et al. (1984), Zimny et al. (1986) nor by Schutte et al. (1987) and Zimny (1988) in their light microscopic studies.

Our study shows for the first time electron microscopic figures of nerve endings in the human anterior cruciate ligament.

Material and methods

A number of 21 anterior cruciate ligaments (LCA) was studied. 19 specimens were obtained from knee joints with no pathological findings of people who died between 4 and 79 years of age. The specimens were taken 4 to 85 h after death occurred. 2 specimens were taken during operation in patients undergoing prosthetic surgery. Each specimen was divided into three parts (the tibial, medial and femoral portion) and subsequently fixed for 48 h in Karnovsky solution (Karnovsky 1965). During fixation each third of a ligament was again divided into three parts. The major part of the specimens was embedded in glycolmetacrylate after Rosenberg et al. (1960), the rest was postfixed in a 1% OsO_4 solution in 1% saccharose and embedded in Epon 812 after Luft (1961).

For electron microscopy we used only specimens embedded in Epon. Semithin sections (1–2 μm thick) were stained according to the Lazcko and Levai method (1975), ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963). Macrotome: Polycut S (Cambridge Inst.), ultramicrotome: Ultracut (Reichert), light microscope: Axiomat (Zeiss), electron microscope: Philips 300.

Results

The anterior cruciate ligament is covered on its ventral aspect by synovial membrane, the dorsal aspect merges into the connective tissue of the fibrous layer of the capsule. The nerves (branches of the tibial nerve) run together with blood vessels (branches of the arteria genus media) within the connective tissue of the fibrous layer and subsynovially between the synovial layer and the ligament. The nerves are surrounded by a perineurium and consist of myelinated and non-myelinated nerve fibres. Their diameter varies between 2 to 10 μm in myelinated and around 1 μm in non-myelinated fibres.

Three types of nerve endings were observed: free nerve endings, Ruffini corpuscles and Pacini corpuscles. All three types of nerve endings are present in all three portions of a ligament. They are located subsynovially and in the fibrous layer, some free nerve endings even occur in the periligamentum externum (comp. peritendineum externum). No nerve endings were found within the dense connective tissue of the ligaments.

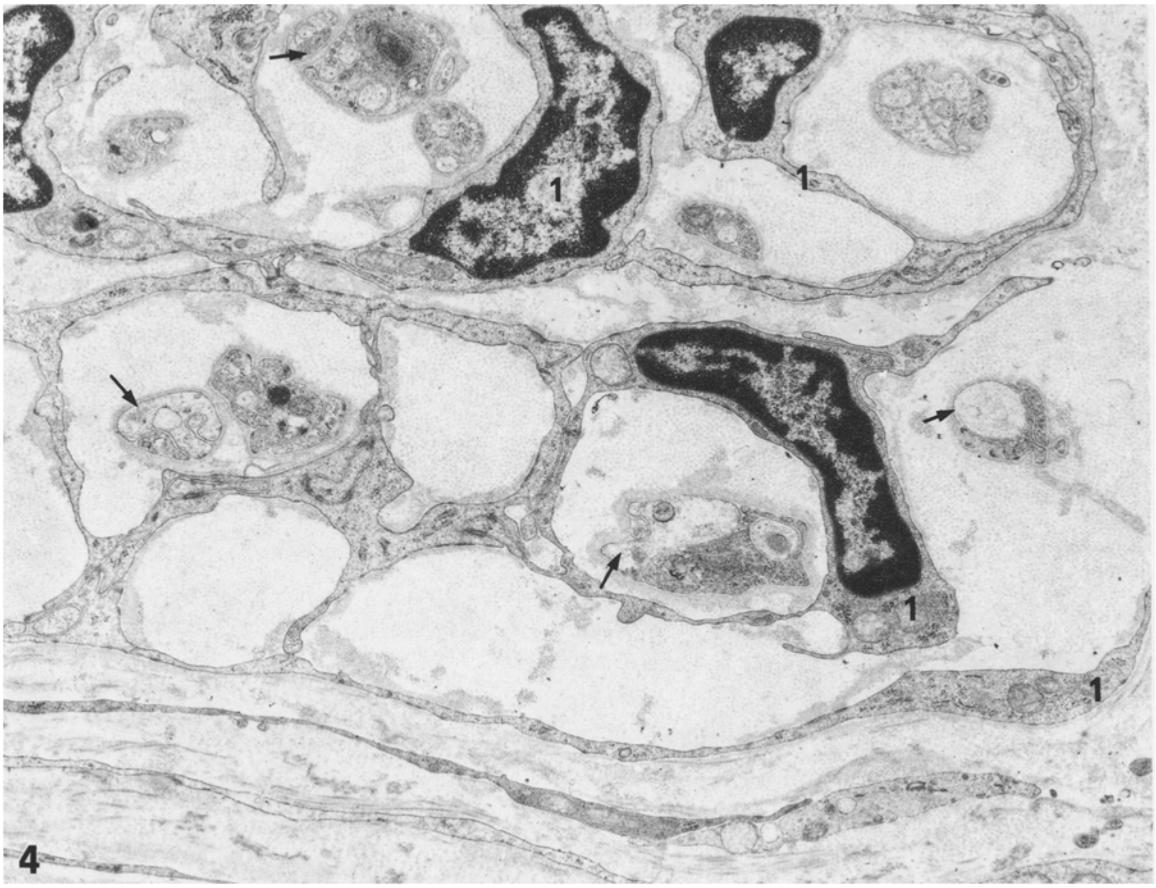


Fig. 4. Detail of a Ruffini corpuscle. *1*, septal and perineural cells. The nerve endings (*arrows*) are only partially surrounded by terminal Schwann cells. ×11730

Free nerve endings (Fig. 1) are formed at the end of non-myelinated or myelinated axons (diameter 2 μm). The axon terminal is slightly thickened and incompletely covered by a Schwann cell. Larger parts of the axolemma are only surrounded by a basal lamina. The terminal is filled with mitochondria, neurotubuli and neurofilaments and also empty vesicles of 20 nm diameter. Free nerve endings were mainly observed adjacent to blood and lymphatic vessels.

Ruffini corpuscles (Figs. 2, 3) are located within the subsynovial connective tissue of the cruciate ligament. The afferent axons are myelinated and measure 4–6 μm. The corpuscle is composed of nerve terminals, endoneural connective tissue and an incomplete perineural capsule.

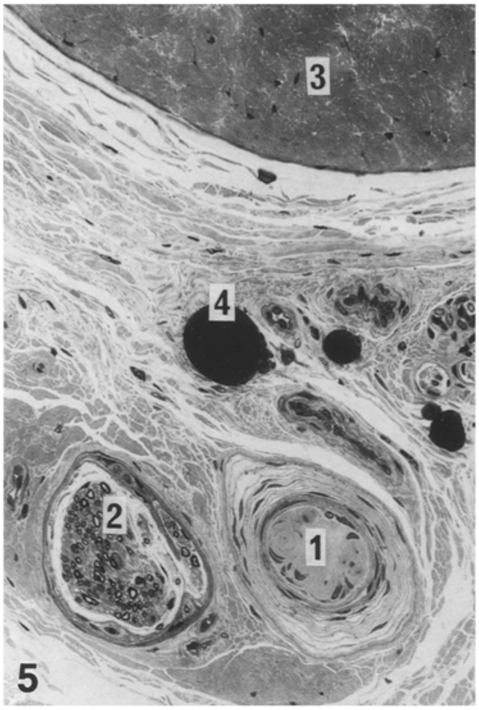
The nerve terminals contain mitochondria and vesicles and sometimes they acquire the form of small paintbrush-like protrusions (Figs. 3, 4). The axolemma is incompletely surrounded by a cytoplasmic lamella of a Schwann cell and thus the "naked" parts of the axolemma come directly into contact with the collagenous fibrils of the endoneurium of the corpuscle. The Schwann cells possess numerous thin cytoplasmic protrusions which often form several lamellae (Figs. 3, 4) and anchor between the collagenous fibrils of the endoneurium. These cytoplasmic lamellae of the Schwann cells often divide the endoneurium into cylindric compartments which on cross section appear in round or oval shape. The cytoplasmic membrane of Schwann cells is always covered by a basal lamina.

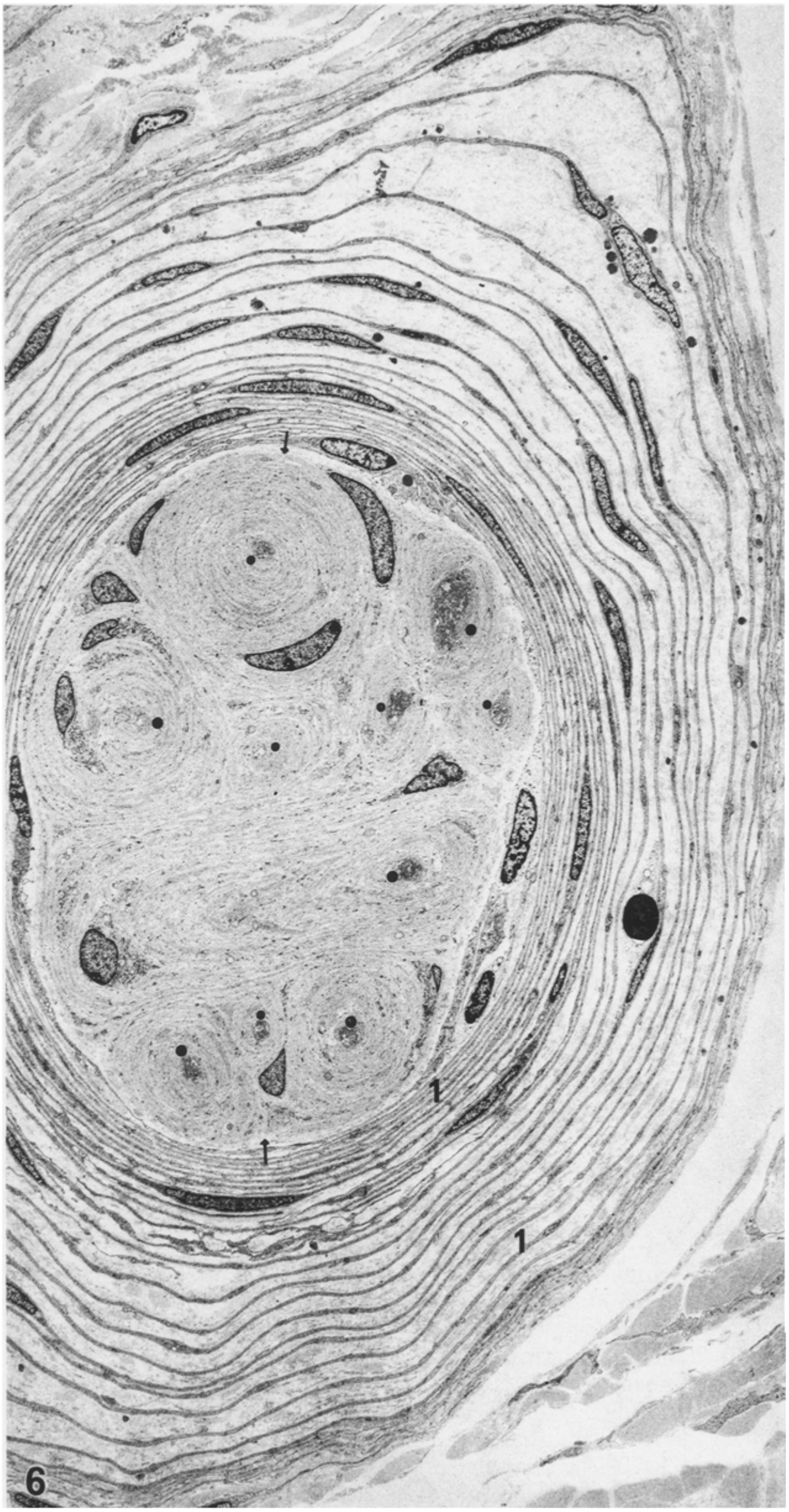
The endoneurial connective tissue consists of collagenous fibrils and fibroblasts. The collagenous fibrils are grouped in bundles and they leave the corpuscle through gaps of the perineural capsule to merge into the surrounding connective tissue.

The perineural capsule is incomplete and reveals gaps. It consists of flat perineural cells. The number of capsular layers varies between 2 and 5. Unlike fibroblasts the perineural cells are covered by a basal lamina. Capillaries may be included in a perineural capsule (Figs. 2, 3).

Pacini corpuscles with up to 10 inner cores were found on the ventral aspect of the LCA located between the synovial layer and the ligament (Fig. 5). A corpuscle consists of nerve terminals, inner cores and a perineural capsule (Fig. 6). The afferent axon is myelinated and measures 4 to 8 μm in diameter; inside the corpuscle it looses its myelin sheath and divides into several branches (up to 10). The individual branches acquire a spiral course inside the inner cores and they contain accumulations of mitochondria and clear vesicles (diameter 20 nm) (Figs. 7, 8). In some areas the terminal axon reveals spike-like protrusions (Figs. 7, 8) which protrude into the lamellar system of the inner core. These protrusions are only covered by a basal lamina, their axoplasm contains microfilaments and they abut the collagenous fibrils which are located in the space between the inner core cells.

The inner core consists of terminal Schwann cells which are arranged on the terminal axon like beads on a string. A cross section at the level of the Schwann cell nucleus (in the middle of an inner core-internodium) reveals a thin





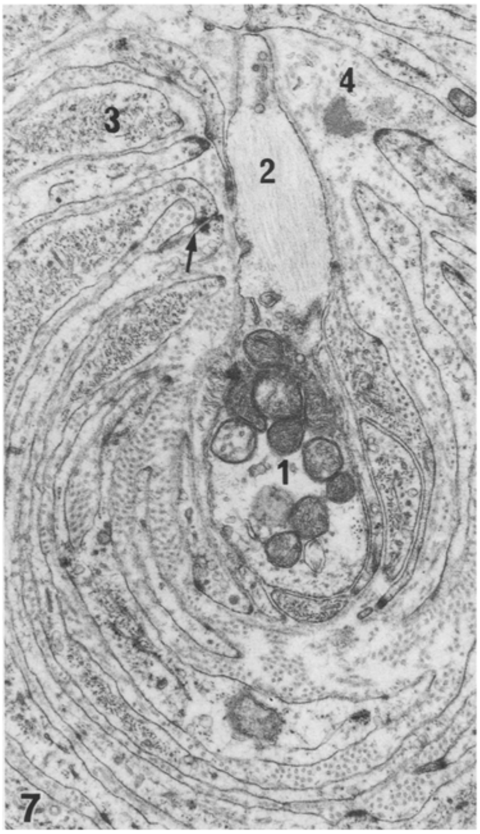
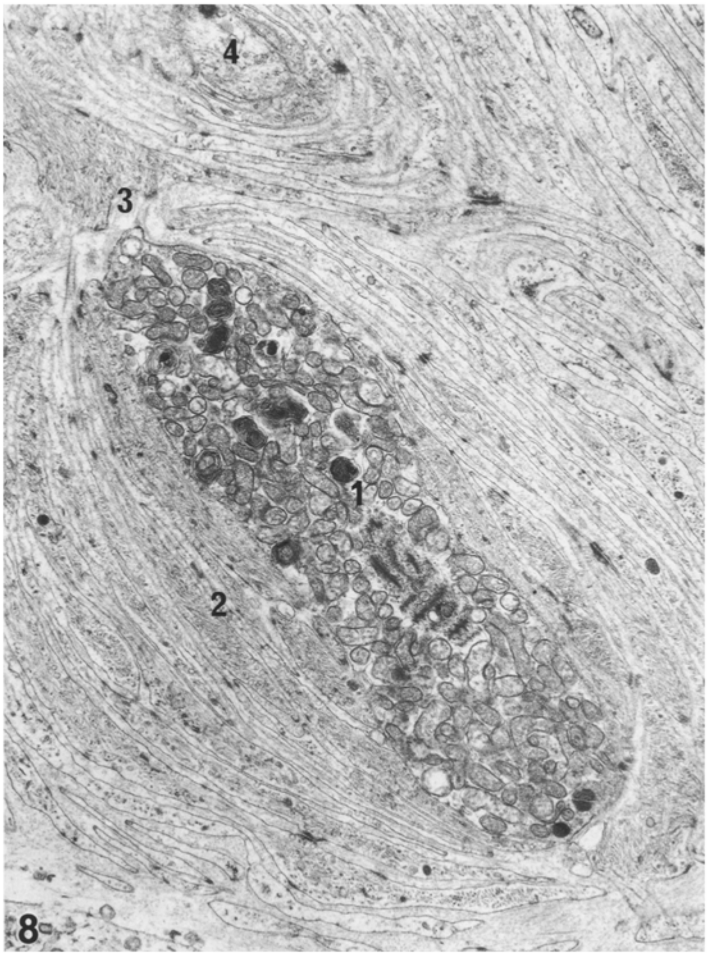


Fig. 5. Lamellar corpuscle from the subsynovial connective tissue between the anterior and posterior cruciate ligament. *1*, lamellar corpuscle; *2*, nerve fascicle with myelinated and non-myelinated axons; *3*, fascicle of the anterior cruciate ligament; *4*, fat cell. Semithin section. ×210

Fig. 6. Lamellar corpuscle from the subsynovial connective tissue between the anterior and posterior cruciate ligament. 10 inner cores (*dots*), subcapsular space (*arrows*) and multilayered perineural capsule (*1*). ×1680

Fig. 7. Detail of the inner core of a lamellar corpuscle. Terminal axon (*1*) filled with mitochondria and vesicles. Finger-like protrusion (*2*) reaching into the longitudinal cleft of the inner core (*4*). Inner core consisting of cytoplasmic lamellae of the terminal Schwann cell (*3*). Desmosome-like contacts between lamellae (*arrows*). ×17000



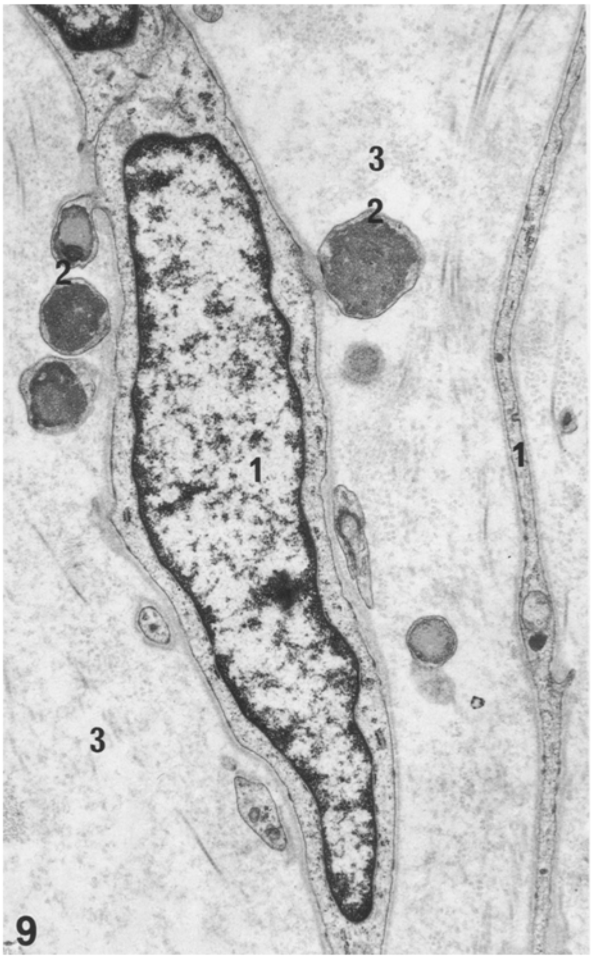


Fig. 8. Detail of the inner core of a lamellar corpuscle. *1*, terminal axon with mitochondria and opaque inclusions consisting of microfilaments; *2*, lamellar system of the terminal Schwann cell of an inner core; *3*, longitudinal cleft between the cytoplasmic lamellae of the terminal Schwann cell; *4* passing axon of a neighbouring inner core. ×10350

Fig. 9. Detail of a perineural capsule of a lamellar corpuscle. Layers of perineural cells (*1*) covered by a basal lamina. Gaps (*3*) between the basal laminae of adjacent perineural cells filled with collagenous fibrils. Lysosome-like structures within the perineural cells (*2*). ×10350

terminal axon in the centre (less than 1 μm diameter) and two or three Schwann cell nuclei in the periphery of the inner core. The number of cytoplasmic lamellae varies between 20 and 30 (Fig. 6). At a level where two subsequent Schwann cells meet, the terminal axon becomes thicker (up to 8 μm) and contains accumulations of mitochondria. At this level the number of inner core lamellae reduces to 5. A cross section through a Pacini corpuscle displays inner cores with many Schwann cell lamellae as well as inner cores with few lamellae (Fig. 6). The space between lamellae is filled with collagenous fibrils oriented parallel to the long axis of the corpuscle. In some areas neighbouring cytoplasmic lamellae are joined through desmosome-like contacts (Fig. 7). The cytoplasmic membrane of terminal Schwann cells shows micropinocytotic vesicles.

The capsule of the corpuscle consists of 15 to 30 layers of flat perineural cells (Figs. 6, 9). The space between the inner cores and the innermost layer of the perineural capsule (subcapsular space) contains fibroblasts (Fig. 6). The space between the perineural cells is lined by the basal laminae of adjacent perineural cells and it contains collagenous fibrils oriented parallel to the long axis of the corpuscle. Some perineural cells display cytoplasmic protrusions (Fig. 9) containing lysosome-like structures. Unlike in Ruffini corpuscles the perineural capsule of Pacini corpuscle is complete and has no gaps. Moreover the endoneurium is scarce and consists of collagenous fibrils in the spaces between inner core lamellae. The subcapsular space shows fibroblasts in addition to collagenous fibrils.

Discussion

The light microscopic structure of sensory nerve endings in joint capsules has been known for many years (Gardner 1950; Barnett et al. 1961; Polacek 1966). Some electron microscopic studies (Halata and Groth 1976; Halata 1977; Halata and Munger 1980; Halata et al. 1984; Strasmann et al. 1987) revealed the ultrastructure of sensory nerve endings in the joint capsule of the shoulder and knee in different mammals. The ultrastructure of sensory nerve endings in human knee joint capsule was first demonstrated by Halata et al. (1985). The ultrastructure of sensory nerve endings in the cruciate ligaments had not been studied before. The light microscopic studies of Schutte et al. (1984), Zimny et al. (1986) and Grüber et al. (1986) present figures which due to the fixation and staining techniques used are not convincing.

In this study three types of nerve endings were observed: free nerve endings, Ruffini corpuscles and Pacini corpuscles. The free nerve endings resemble in structure those found in skin (Kruger et al. 1981). They are supplied by thin non-myelinated C-fibers or myelinated A-delta-fibers. They represent either primitive mechanoreceptors of high thresholds (cf. Andres et al. 1985), or nociceptors (Kruger et al. 1981), or thermoreceptors (Spray 1986), or are even multimodal (Kruger 1987).

The function of Ruffini corpuscles in the skin has been known since the publications by Chambers et al. (1972) and Biemesderfer et al. (1979). They act as slowly adapting stretch receptors. The function of Ruffini corpuscles was also studied in the joint capsule (Boyd and Roberts 1953; Boyd 1954; Skoglund 1956; Grigg 1975; Grigg and Hoffman 1984; Grigg et al. 1973, 1982; McClosky 1978) and proven to act as slowly-adapting stretch receptors. A comparison shows the resemblance of the ultrastructure of Ruffini corpuscles of the fibrous layer of the knee joint capsule and that of those of the cruciate ligament. This suggests a similar function to that found in Ruffini corpuscles in the skin (Chambers et al. 1972; Biemesderfer et al. 1978; Halata 1988).

The function of Vater-Pacini corpuscles is also well known (Loewenstein and Skalak 1966; Loewenstein 1971). Small lamellar corpuscles – often referred to as Golgi-Mazzoni corpuscles (Andres 1966; Grigg et al. 1982) – adapt rapidly (Schmidt 1971) and are a type of velocity receptor (Gottschaldt 1973). In the cruciate ligament large Vater-Pacini corpuscles were observed (Zimny 1988) as well as smaller lamellated corpuscles with several inner cores. Large Vater-Pacini corpuscles were described as found to be present in large numbers in the subcutis of the finger in primates and man. They are rapidly adapting receptors and respond to vibration (Iggo and Andres 1982). In joint capsules the large Vater-Pacini corpuscles were observed in periarticular connective tissue (Polacek 1966) and they were also observed in the connective tissue of the membrana interossea cruris of the rat (Zelena 1978). The later finding shows groups of corpuscles on the membrane and within the periost. Smaller corpuscles with one or several inner cores are mainly found in the dense connective tissue of the fibrous layer and in the (supportive) ligaments of the joint capsule (Halata et al. 1985; Strasmann et al. 1987). The afferent axon of a corpuscle with several inner cores measures up to 6 μm in diameter. All terminals inside one corpuscle (up to 10 terminals were found) originate from the same myelinated axon. The question is whether this describes a special variety of lamellar corpuscle with a special structure for better reception of stimuli or whether we are dealing with structural changes as a result of degenerative and regenerative processes. From the studies of Zelena (1981, 1984a, 1984b) and Zelena and Jirmanova (1988) it is known that regenerating Vater-Pacini corpuscles in the membrana interossea of the rat show several inner cores.

The function of mechanoreceptors in the joint capsule is still under debate. Initially they were considered to play an important role for joint position (kinaesthesia) (Gardner 1950; Boyd and Robertson 1953; Boyd 1954; Freeman and Wyke 1967). But studies and observations in patients with endoprosthesis (Grigg et al. 1973; McClosky 1978) showed that the sense of position is transmitted through the sensory innervation of the muscles and therefore remains intact even after total endoprosthesis, even if joint capsule and ligaments are radically removed. Moreover joint capsules without corpuscular nerve endings have been described (mandibular joint of the mouse – cf. Silbermann and Livne 1979; Dreessen et al. 1988).

Investigations by Ferrell (1980) on the knee joint of the cat have proven that mechanoreceptors of joint capsules and ligaments provide information on the angle of the joint during the whole movement. Lundberg et al. (1978) observed the influence of joint receptors on the regulation of muscle tone. They were found to reduce the tension of the muscle during the terminal phase of a movement through an interneural connection with motor neurons on the same segmental level. Grüber et al. (1986) observed a polysynaptic reflex of the semimembranous muscle and the biceps muscle initiated through an irritation of the anterior cruciate ligament. All muscles acting on one joint probably have a feedback connection through reflex cycles with the respective connective tissue structures to assure optimum control during movement. This view is supported by the topographic arrangement of receptors and by the fact that apart from joint capsules, muscle fasciae and insertions also contain small lamellar corpuscles (van der Wal et al. 1987).

Reflexes initiated by the stretching of the ligaments probably influence the muscle tone (Lundberg et al. 1978; Ferrell 1980; Grüber et al. 1986) and guarantee the appropriate joint position for optimum movement. This suggests that during necessary operations the ligaments must be treated with maximum care and that especially the dorsal aspect of the knee joint capsule and the cruciate ligaments have to remain intact.

Acknowledgements. The authors wish to thank Ms Birgit Knutz and Mr Stefan Schillemeit for their valuable technical assistance. We wish also to thank Dr. med. Theresa Rettig for translating the manuscript.

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Accepted January 19, 1989