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Y. Hirasawa · S. Okajima · M. Ohta · T. Tokioka

Nerve distribution to the human knee joint: anatomical and immunohistochemical study

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Summary The nerve distribution to the knee joints was analyzed in 5 cadavers and 10 joint capsules specimens were resected during total knee arthroplasty. We found nerve fibers immunoreactive for anti-substance P antibody in the articular capsule. By confocal laser scanning microscopy, we evaluated the three-dimensional structures of the Ruffini's corpuscles and the free nerve endings, both of which were immunoreactive for anti-protein gene product 9.5.

Résumé Nous avons analysé la distribution des nerfs de l'articulation du genou en utilisant des cadavres (n=5) et des capsules d'articulation (n=10) réséquées pendant une arthroplastie totale du genou. Nous avons découvert des fibres de nerfs immunoréactives pour l'anticorps P de l'antisubstance dans la capsule d'articulation. Au moyen d'une microscopie scanographique par laser confocal, nous avons évalué les structures tridimensionnelles des corpuscules de Ruffini et les terminaisons des nerfs libres, lesquelles étaient immunoréactives pour le produit 9.5 du gëne antiprotéine.

Introduction

It is well known that there are three types of nerve endings in the articular capsule such as Ruffini's corpuscle, free nerve ending and Pacinian corpuscle [2]. The Pacinian corpuscle is the receptor for the high frequency and quickly adapting group; on the other hand, Ruffini's corpuscle is the slowly adapting mechanoreceptor [2]. Free nerve ending is thought to be the receptor for pain and temperature [2].

Y. Hirasawa (✉) · S. Okajima · M. Ohta
Department of Orthopaedic Surgery,
Kyoto Prefectural University of Medicine,
Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto City,
Japan, 602–0841
Tel.: + 81-75-251-5549, Fax: + 81-75-251-5841

T. Tokioka
2nd Department of Anatomy,
Meikai University School of Dentistry,
1–1 Keyakidai, Sakado, Saitama, Japan 350–0283

Materials and methods

Five cadavers were used for macroscopic examinations. The medial joint capsules from 10 knees of 10 patients were partially resected during total knee arthroplasty performed between 1996 and 1997, and were used as immunohistochemical materials. Median age of the 10 patients was 60 (58–65) years.

Tissues were immersed in a fixative containing 2% para-formaldehyde in phosphate buffered saline (PBS) (0.15 mol/l NaCl in 20 mmol/l sodium phosphate buffer (pH 7.5) supplemented with 8% sucrose for 4 h at 40 C. Specimens were cryoprotected through a series of increasing concentrations of sucrose (10, 15, 20, and 25%) in PBS, embedded in OCT compound, quick-frozen, and cut into 10-µm sections on a cryostat. Frozen sections were mounted on slides, washed with 50 mmol/l Tris buffer (pH 7.5), and blocked with PBS containing 5% bovine serum albumin (BSA) for 30 min. Slides were incubated for 48 h with anti-substance P subtype polyclonal antibody (Chemicon) at concentrations of 1 µg/ml in 50 mmol/l and anti-PGP 9.5 polyclonal antibody (Ultra Clone, UK). Sections were washed three times with PBS, and incubated for 16 h with horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG at a final dilution of 1:20. After three times washing with PBS, sections were incubated for 30 min in DAB solution (50 mmol/l Tris buffer (pH 7.5), 0.05% 3,3'-diaminobenzidine tetrahydro-chloride), and then for 10 min in DAB solution containing 0.01% H_2O_2. Specimens were observed by light microscopy.

After incubation with anti-PGP 9.5 polyclonal antibody, sections were incubated for 12 h with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG antibody (Amersham, Japan), at a final dilution of 1:20. These sections were viewed on a confocal laser scanning microscopy (CSLM) system (MRC-600, Bio-Rad Laboratories/Axiovert Zeiss).

Results

Nerves innervating the knee joint were divided into two types according to their pattern of distribution: *articular branches* innervated the joint directly and *minor articular branches supplying muscles* that passed through the knee joint.

The saphenous nerve projected a main branch at the medial aspect of the knee as it ran down the anterolateral edge

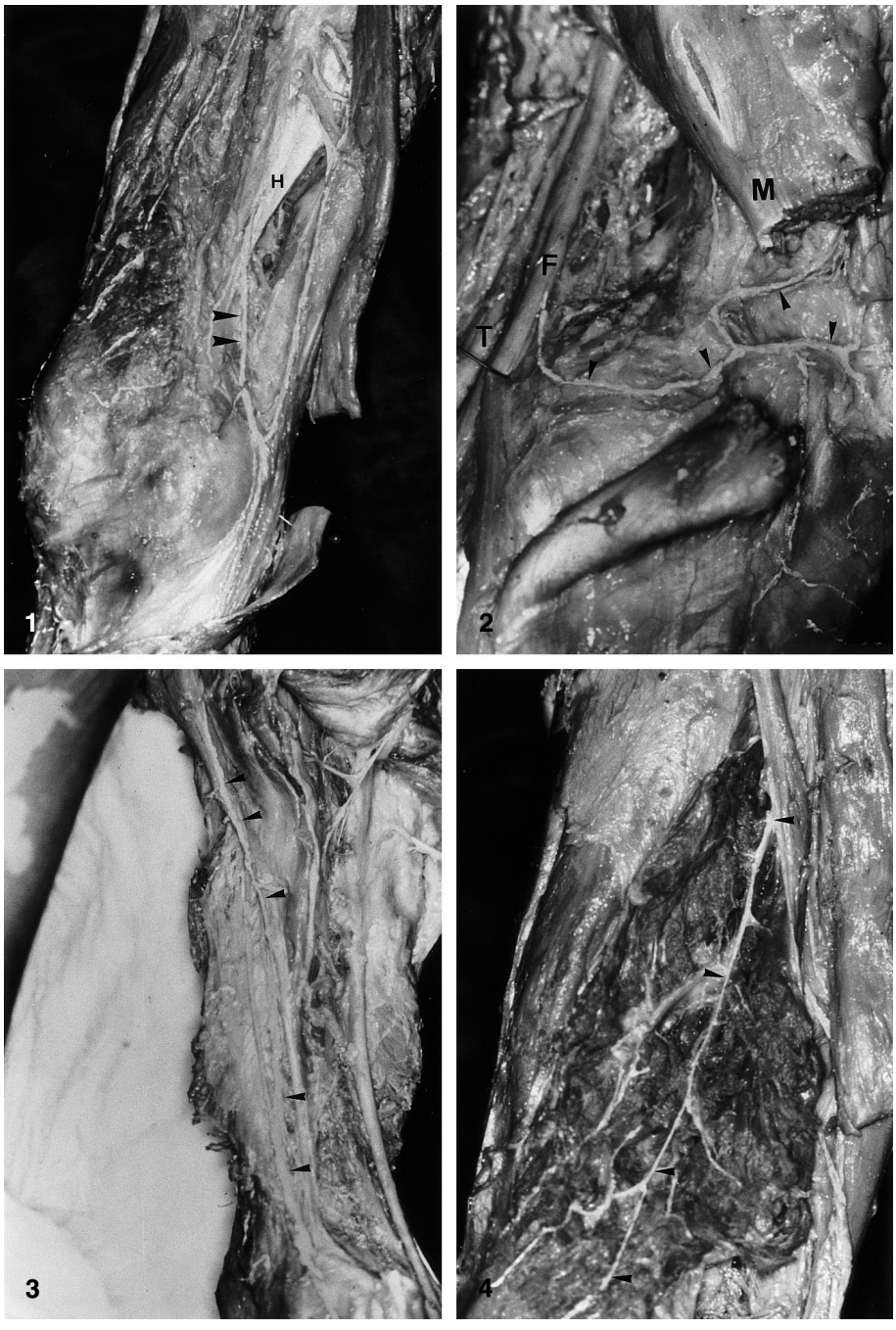


Fig. 1 Medial-upper superior view of the knee joint. The saphenous nerve (*arrowheads*) runs down the anterolateral edge of the sartorius projecting a main branch at the medial aspect of the knee. *H*, Hunter's canal

Fig. 2 The common peroneal nerve (*F*), a branch of the sciatic nerve, projects articular branches (*arrowheads*) as it runs down the inner edge of the long head of the biceps femoris (*M*). *T*, tibial nerve

Fig. 3 The cutaneous branch of the obturator nerve (*arrowheads*) runs down the posterior and inner aspects of the adductor longus and emerges at the anterior edge of the gracilis to innervate the skin from the thigh to the inner aspect of the knee

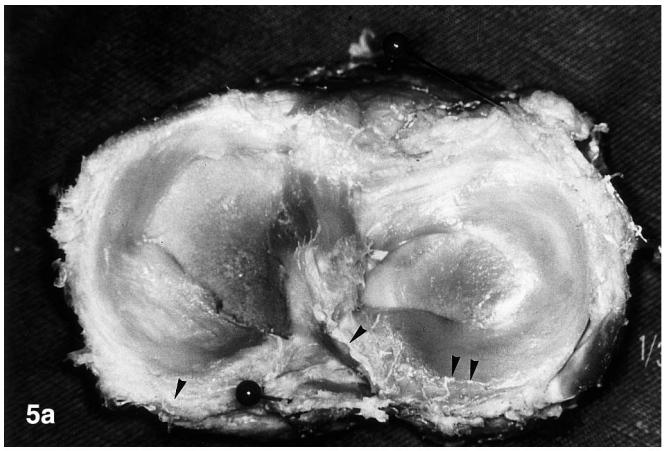
Fig. 4 The small branches of the femoral nerve (*arrowheads*) that pierce the vastus medialis and the vastus lateralis are distributed to the superoanterior aspect of the articular capsule

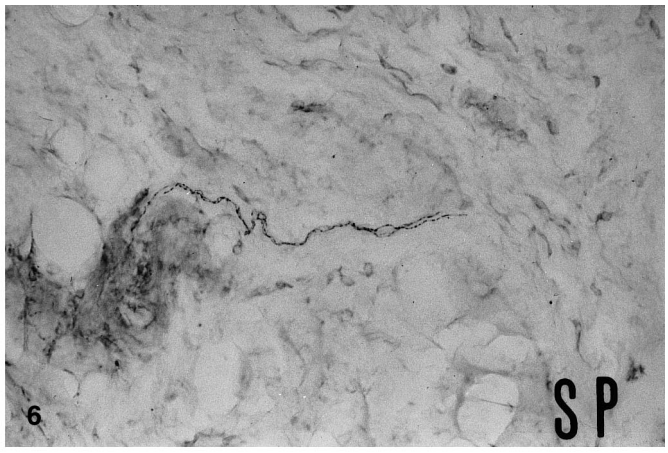
of the sartorius (Fig. 1). The main branch innervated the skin, that extended from medial to the anteroinferior side of the knee and a wide area covering the articular capsule.

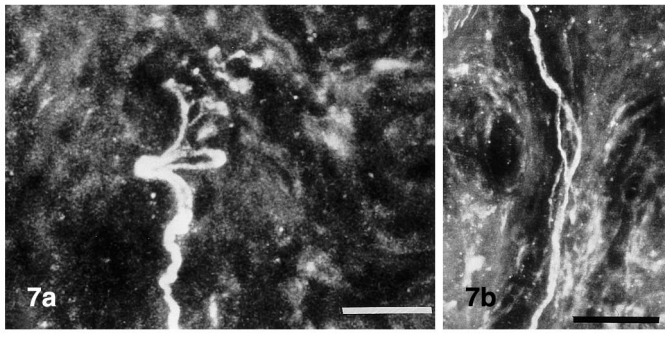
The sciatic nerve divided into the tibial and common peroneal nerves at the popliteal area. Subsequently, the tibial nerve projected articular branches at the popliteal fossa (Fig. 2). These articular branches pierced the adipose tissue of the popliteal fossa proceeding to the deeper layers in order to innervate the articular capsule following the superomedial popliteal vessels and the superolateral vessels.

The common peroneal nerve projected articular branches as it ran down medially along the long head of the biceps femoris (Fig. 2). These branches ran towards the deep part of the long head of the biceps femoris and innervated the posterior and lateral side of the articular capsule. The common peroneal nerve also projected an articular branch as it ran down to the origin of the lateral head of the gastrocnemius and extended to the head of the fibula. This branch ran with the inferolateral popliteal vessels and innervated the anterolateral side of the articular capsule.

The cutaneous branch of the obturator nerve ran down the adductor longus and emerged at the gracilis to innervate the skin medially from the thigh to the knee (Fig. 3).







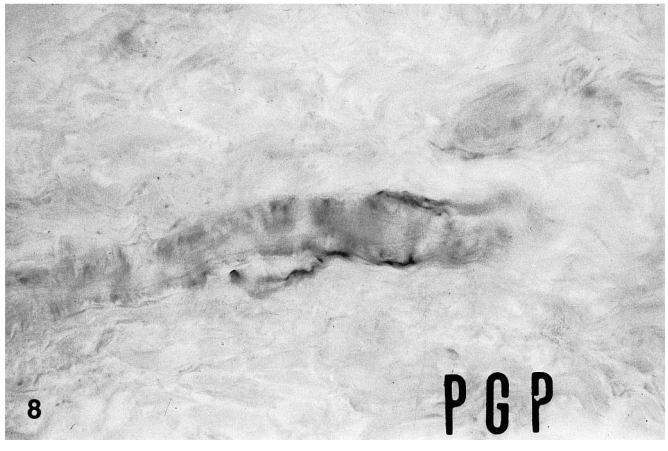


Fig. 5 **a** The branches, which are divided from the nerves innervated to the joint capsule, are distributed to anterior and posterior cruciate ligament. Many capsule branches (*arrowheads*) pierce the joint capsule and are distributed to the peripheral border of meniscus. **b** Innervation at tibial insertion of anterior cruciate ligament of the knee joint. Small branches (*arrowheads*) pierce anterior cruciate ligament

Fig. 6 Immunoreactive nerve bundles for anti-PGP 9.5 antibody. These bundles innervate the vessels. ×400

Fig. 7. **a** Ruffini's corpuscle immunoreactive for anti-PGP 9.5 antibody by CSLM. This structure exhibits the spray formations like rape blossoms. *Bar*=25 µm. **b** Free nerve endings immunoreactive for anti-PGP 9.5 antibody by CSLM. *Bar*=25 µm

Fig. 8 Immunoreactive fibers for anti-substance P antibody in the synovial connective tissue. ×200

The minor branches of the femoral nerve that pierced the vastus medialis and the vastus lateralis were distributed to the articular capsule (Fig. 4). The branches, divided from the nerves innervated to the joint capsule, were distributed to the anterior and posterior cruciate ligaments. Many branches pierced the joint capsule and were distributed to the peripheral border of the menisci (Fig. 5).

By immunohistochemistry, we demonstrated that many nerve bundles reacting for anti-PGP 9.5 antibody were present at the subsynovial connective tissue. Sometimes these bundles projected the small branches to the synovial membrane (Fig. 6). The branches in the synovial membrane often tapered (Fig. 7). Rarely, the branches made spray formations like rape blossoms (Fig. 7). Immunoreactive nerve fibers around the vessels in the synovial membrane and in the capsule were also observed. Immunoreactive fibers for anti-substance P antibody were also observed in the synovial connective tissue (Fig. 8). The number of the immunoreactive fibers for anti-substance P antibody was much less than that of the immunoreactive fibers for anti-PGP 9.5 antibody. There were no specific immunoreactive structures for anti-substance P antibody like PGP 9.5.

Discussion

The extrinsic afferent innervation of the joints follows Hilton's law: joints are innervated by articular branches

of the nerves supplying the muscles that cross the joint [9]. Kennedy stated that two groups of articular nerves distributed to the human knee: a posterior group and an anterior group [4]. The anterior group comprises the articular branches of the femoral, the common peroneal and the saphenous nerve. The posterior group consists of the posterior articular nerve as a branch of the tibial and the obturator nerves. We evaluated the saphenous nerve, which innervates a wide area covering the articular capsule. The tibial nerve projected several posterior articular branches at the popliteal fossa. The common peroneal nerve also projected articular branches to the anterior and lateral articular capsule. Interestingly, the branches to the anterior cruciate ligament (ACL) originate from the branches to the anterior capsule and the branches to the posterior cruciate ligament (PCL) come from the branches to the posterior capsule, reflecting that ACL is innervated by the anterior group and PCL by the posterior group.

The articular capsule of the knee joint is richly innervated and three types of nerve endings such as Ruffini's corpuscles, free nerve ending and Pacinian corpuscles are present in the knee joint [2]. Confocal laser scanning microscopy (CSLM) is a powerful tool for analyzing the three dimensional morphology of complex structures [5]. PGP 9.5 is an enzyme named *ubiquitin carboxyl-terminal hydrolase*, which is found in the cytoplasm of neurons at a high level [1,8]. The spray formation in this study corresponds to Ruffini corpuscles and the tapered axons are thought to be free nerve endings. Polacek described the details of the different types of Ruffini corpuscles by silver impregnation, such as simple spray, encapsulated spray and encapsulated spirals [7]. However, the silver impregnation stained not only the axoplasm but also the nuclei of Schwann cells and fibroblasts. Sometimes the silver impregnation makes it difficult to exhibit the complex neural structures. We evaluated the precise structure of Ruffini corpuscles immunohistochemically by using anti-PGP 9.5 antibody. The spiral formations in the present study have three floors and each floor has the small branche-like sprays. Probably, this structure is beneficial for the monitoring of the tension of collagen fibers. The tapered structure in this study corresponds to the free nerve ending. In the case of free nerve endings, it is difficult to identify these as the tapered nerve is blinded by the normal light microscopy. However, CSLM makes it possible to slice the immunoreactive structures and to reconstruct them. In Fig. 7, the free nerve ending corresponds to the four slices out of 20 slices by CLSM. No other specific structures were observed in the other 16 slices, reflecting that this structure ended in these 4 slices. In this way, we identified this structure as free nerve ending.

Over 20 kinds of physiologically active peptides have been discovered in the central and peripheral nervous system. Particularly well known are substance P and calcitonin gene related peptide (CGRP), neuropeptides involved in the detection of sensation by peripheral nerves. Substance P was discovered as a peptide that has the ability to contract the smooth muscle in the bowel, and found in sensory neurons [3]. About 20% of the dorsal root ganglion cells are known to contain substance P [6]. Substance P is thought to be one of the transmitter substances of sensation such as pain, pressure and temperature. In this study, the number of the immunoreactive fibers for anti-substance P antibody was less than that for anti-PGP 9.5 antibody. However, it was not possible to compare the number of immunoreactive fibers at the time of surgery with the number existing before arthritic changes in the same patient. Probably, the nerve fibers including substance P in the joint capsule are related to inflammation or pain in osteoarthritis patients, but a more detailed study will be needed to elucidate the functions of substance P in the joint capsule of such patients.

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