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Innervation of nociceptors in the menisci of the knee joint: an immunohistochemical study

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Abstract Using histology, we studied the innervation of nociceptors in the medial and lateral menisci of the knee joint. Specimens examined were taken from 16 patients during arthroplasty. The patients were 6 men and 10 women, with ages ranging from 14 to 76 years (mean 56 years). Immunohistochemistry with the unlabeled antibody biotin-streptavidin method was employed to detect protein gene product 9.5 (PGP 9.5) or substance P (SP) in the specimen. The antibody for PGP 9.5 detected nerve tissues in the menisci. Most but not all of the nerve fibers were associated with blood vessels. Nerve fibers and sensory receptors were found mainly in the peripheral, vascular zone, representing the outer one-third of the meniscus, and the innervated area was wider in the anterior and posterior horns. Pacinian and Ruffini corpuscles as well as free nerve endings were identified in these areas. Larger fibers coursed circumferentially in the peripheral zone, with smaller branches of nerve fibers running radially into the meniscus. Nerve fibers positive for SP were also detected in the menisci, but were fewer in number. Their branches also were fewer, oriented radially and paralleling blood vessels. This study showed that some of the pain in cases of meniscal tear could originate in the meniscus itself, especially with peripheral tears that may be accompanied by bleeding.

Introduction

Pain in a meniscal injury has been thought to originate either from distortion of the capsule by the torn menisci or from secondary synovitis, because of the absence of nerve fibers in the articular cartilage. The menisci function as stabilizers of the joint and shock absorbers [1, 5, 12]. Mechanoreceptors are important to their function, but innervation of the meniscus has received little attention. Day et al. [3] reported that nerves were present in the outer one-third of the meniscus and in the anterior and posterior horns. Zimny et al. [16] demonstrated neural elements in the perimeniscal tissue and in the outer and middle thirds of the meniscus by light and transmission electron microscopy. This study set out to identify mechanoreceptors and nociceptors.

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Materials and methods

Medial and lateral menisci were obtained from 16 patients undergoing total knee arthroplasty (6 men and 10 women with ages ranging from 14 to 76 years, mean 56 years). The menisci were fixed with Zamboni's fixative [15] containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4) at 4 °C. Then they were demineralized in 0.1 M ethylenediaminotetra-acetic acid (EDTA), pH 7.3, for 3 or 4 weeks at 4 °C [2]. The specimens were rinsed for at least 2 days in 0.1 M phosphate buffer containing 30% sucrose.Sections were mounted on separate gelatin-coated glass slides. Then an unlabeled-antibody biotin-streptavidin method (Stravigen, BioGenix Laboratories, Dublin, CAl.) was used [4]. Briefly,the sections were incubated with antisera to substance P (SP) (1 : 3000) and protein gene product 9.5 (PGP9.5) (1 : 5000) separately diluted in a solution of 1% normal goat serum in phosphate-buffered saline containing 0.3% Triton X-100, for 72–96 h in a humid chamber at 4 °C. Sections were then incubated with biotinylated anti-rabbit IgG antibodies for 24 h and streptavidin-horseradish peroxidase complex for 12 h at 4 °C. The immunohistochemical reaction product was developed by incubation of the sections in a solution of 0.05% diaminobenzidine hydrochloride (Sigma, St. Louis) and 0.01% hydrogen peroxide in 0.1 M phosphate buffer for 15–20 min. This reaction was completely blocked by preabsorption of the antibody with 10 nM/ml SP (Peptide Institute, Japan) and PGP9.5 (Cambridge Research Biochemicals, UK). After washing, tissue sections were dehydrated in a graded series of alcohol, and mounted under coverslips with Permount.

Results

The antibody for PGP9.5 detected nerve tissue in the meniscus. Nerve fibers and sensory receptors were found mainly in the vascular outer third of the menisci, with the

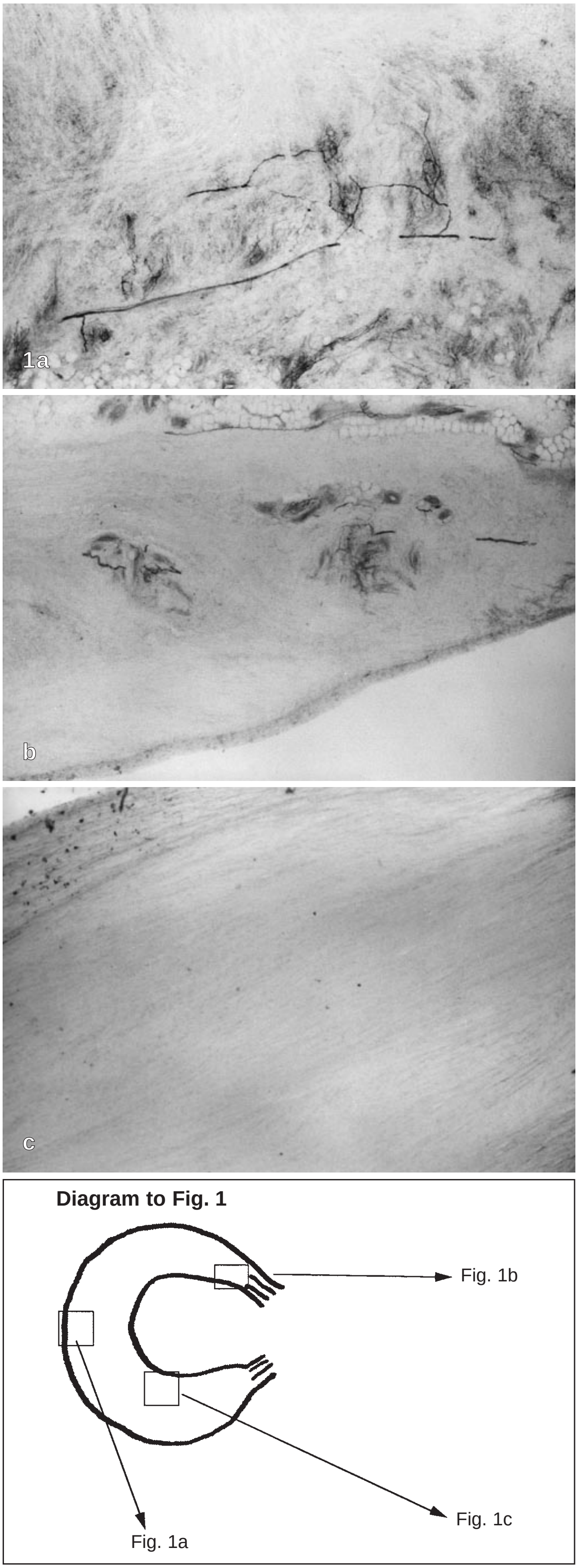
1a
b
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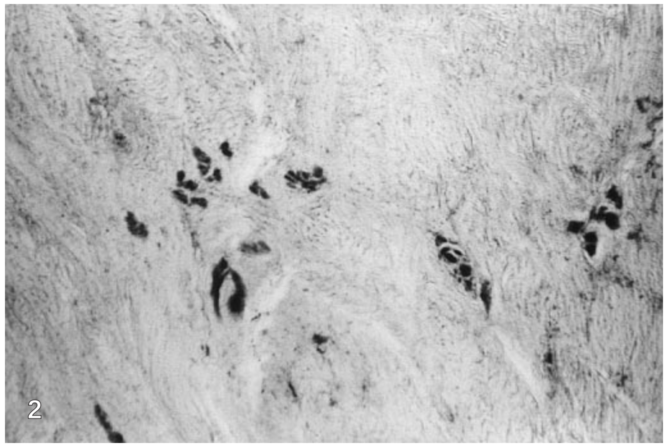
Diagram to Fig. 1
Fig. 1b
Fig. 1a
Fig. 1c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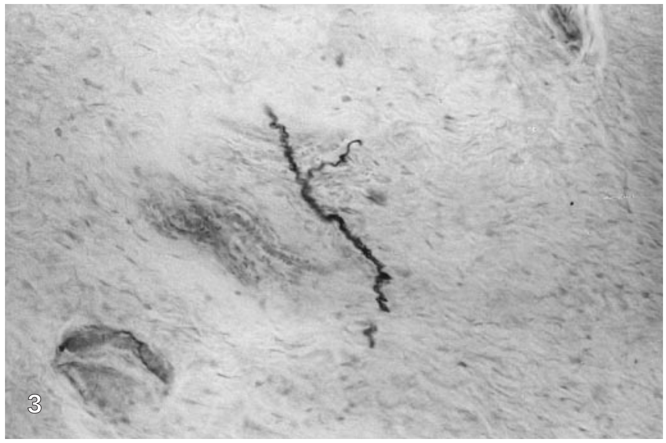
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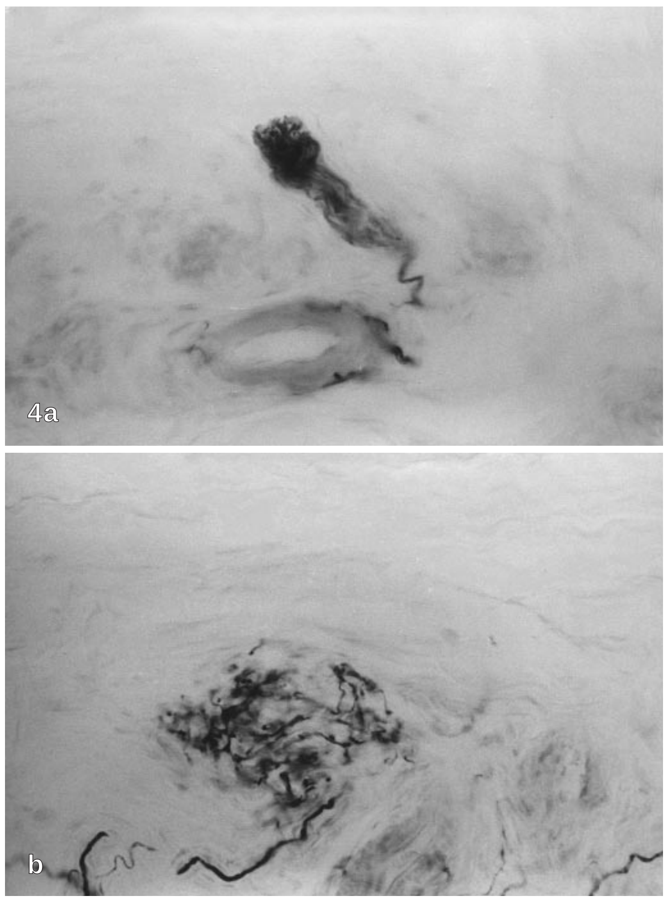
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◀ **Fig. 1** **a** Nerve fibers and sensory receptors were found mainly in the vascular outer third of the meniscus. Nerve fibers were usually associated with blood vessels, especially the larger fibers. Larger fibers coursed circumferentially in the peripheral zone, with smaller branches running radially and sometimes convolutedly toward the center of the meniscus (× 40; for topography, see diagram). **b** The positive area was wider in the anterior horns (× 40). **c** No nerve fibers or receptors were seen in the avascular intermediate or inner third of the menisci (× 100)

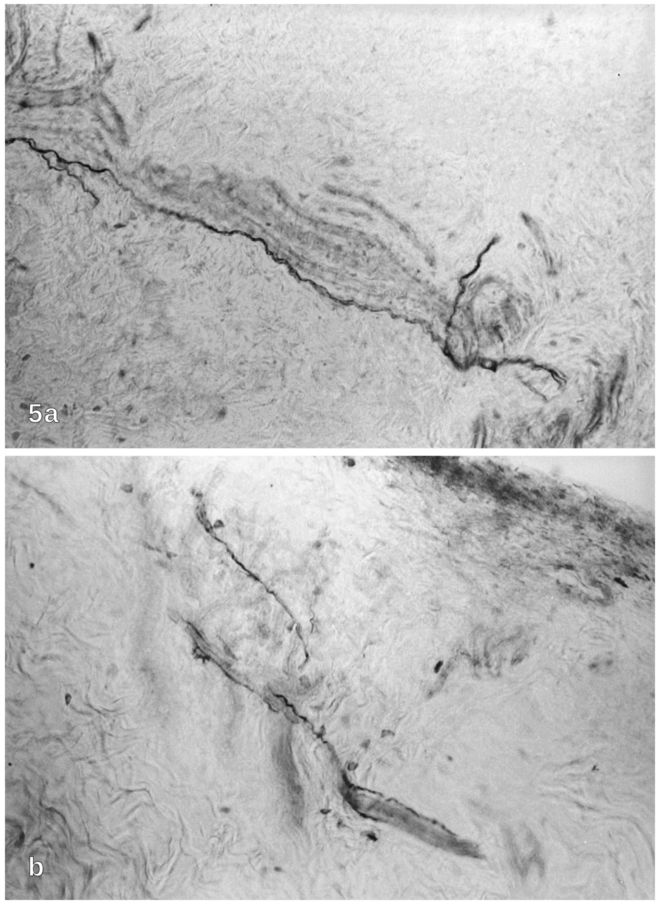
Fig. 2 Most nerve fibers were seen in the interstitial tissue (× 100)

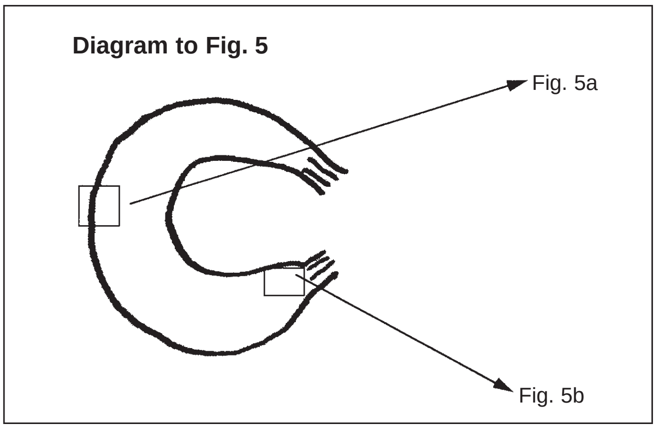
Fig. 3 Many free nerve endings were seen in these peripheral areas (× 100)

Fig. 4 **a** Pacinian corpuscles were identified in these peripheral areas (× 200). **b** Golgi-like tension receptor was identified in these peripheral areas (× 200)

positive area being wider in the anterior and posterior horns (Fig. 1a,b). No nerve fibers or receptors were seen in the avascular intermediate or inner third of the menisci (Fig. 1c). Similar patterns of innervation were seen in both the medial and lateral meniscus. Nerve fibers were usually associated with blood vessels, especially larger fibers. Larger fibers coursed circumferentially in the peripheral zone,with smaller branches running radially and sometimes convolutedly toward the center of the meniscus (Fig. 1a). Most nerve fibers were seen in the interstitial tissue (Fig. 2), where many free nerve endings also were seen (Fig. 3). Pacinian and Ruffini corpuscleswere also identified in these peripheral areas (Fig. 4a, b).

Nerve fiber immunoreceptors for SP,which is specific for nociceptive fibers, were also detected in the menisci, again mainly in the vascular outer third,with the positive area being relatively wider in the anterior and posterior horns. None of the nerve fibers in the avascular intermediate or inner third was unstained. The SP-positive zone was narrower than the PGP9.5-positive area. Again, most fibers were associated with blood vessels in the interstitial tissue, especially larger fibers, which tended to course circumferentially, as was the case for PGP9.5. SP-immunoreactive fibers were fewer in number than the PGP9.5-positive nerves and receptors. Their diameters were also smaller than the PGP9.5-positive nerve fibers. Their branches were also fewer and ran radially, parallelling blood vessels. Many free nerve endings were seen in the peripheral areas, but Pacinian and Ruffini corpuscles were not positive for SP (Figs. 5a,b, 6).





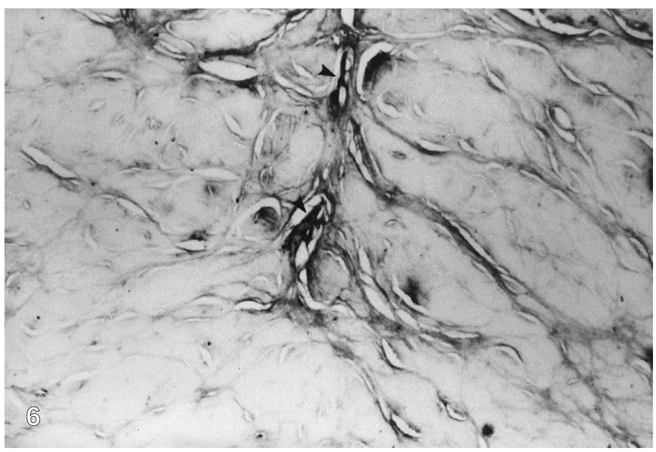


Fig. 5 **a** Substance P (SP)-positive zone was narrower than the protein gene product (PGP) 9.5-positive area. Most fibers were associated with blood vessels in the interstitial tissue, especially larger fibers, which tended to course circumferentially, as was the case for PGP9.5. Their branches were also fewer and ran radially, parallelling blood vessels (× 100; for topography see diagram). **b** The positive area was wider in the posterior horns (× 100)

Fig. 6 SP-immunoreactive fibers (*arrowheads*) were fewer in number than the PGP9.5-positive nerves and receptors. Their diameters were also smaller than PGP9.5-positive nerve fibers (× 100)

Discussion

The medial and lateral menisci have an important function in load transmission, gliding movements, nutrition of the articular cartilage,and stability of the knee joint, as do the cruciate and other ligaments [1, 5, 12]. Progressive osteoarthritis has been observed after meniscectomy. To assure smooth gliding movement and avoid excessive motion, the innervation in the meniscus is very important. Zimny et al. [16] noted nerve fibers penetrating from the perimeniscal tissue into the outer third of the meniscus, with a heavier concentration at the horns, and identified Ruffini endings, Golgi tendon organs and Pacinian corpuscles. Our study demonstrated nerve fibers and receptors mainly in the vascular outer third of the meniscus. Nerve fibers probably do not exist in the avascular area.

In this study we found nerve fibers and receptors possessing the neuropeptides SP and PGP9.5. SP belongs to a family of closely related peptides known as tachykinins and is one of the neurotransmitters in nociceptive fibers [6, 9, 10, 11]. Pain in a meniscal injury has been thought to arise from traction on the capsule from torn menisci or from secondary synovitis. As we have demonstrated that SP-positive nerve fibers were peripherally located and typically associated with blood vessels, we suggest that some of the pain in cases of meniscal tear might arise from the meniscus itself, especially for peripheral tears accompanied by bleeding.

Based on the results of our investigations, the mechanism of pain sensation at the time of a fresh meniscal tear can be explained as follows: When a meniscal tear occurs in the avascular (non-innervated) area, pain derives from stimulation, resulting from deterioration of the micromilius around the torn meniscus, to nociceptors in the synovia and joint capsule. On the other hand, when a meniscal tear occurs in the vascular (innervated) area, some of the pain derives from a direct injury to nerve tissues in the meniscus as well as the aforementioned mechanism [13].

As sequelae to the meniscal tear, the following event can be speculated. When a torn meniscus is stable and maintains normal function, pain may not arise. However, pain can arise from stimulation to nociceptors of the meniscus itself, if the tear progresses over to the vascular area. Furthermore, when the meniscus become unstable, much of the pain may originate from traction on the capsule, caused by the torn meniscus, or from secondary synovitis, caused by associated injury of the articular cartilage [3,5, 14].

The population of nerve fibers and receptors within a meniscus provides the central nervous system with information related to mechanical stimulation including motion and excessive loading, and may be related to protective reflexes.This is supported by our observation of Pacinian corpuscles, which are rapidly adapting receptors.

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