Pronociceptive and Antinociceptive Neuromediators in Patellar Tendinopathy

Øystein Lian, Johan Dahl, Paul W. Ackermann, Frede Frihagen, Lars Engebretsen and Roald Bahr

Am. J. Sports Med. 2006; 34; 1801 originally published online Jun 30, 2006;
DOI: 10.1177/0363546506289169

Pronociceptive and Antinociceptive Neuromediators in Patellar Tendinopathy

Øystein Lian,*† MD, Johan Dahl,‡ MD, Paul W. Ackermann,‡ MD, PhD, Frede Frihagen,§ MD, Lars Engebretsen,*§ MD, PhD, and Roald Bahr,*‖ MD, PhD
*From the *Oslo Sports Trauma Research Center, Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, Norway, †Kristiansund Hospital, Kristiansund, Norway, ‡Orthopedic Laboratory, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, and §Orthopaedic Center, Ullevål University Hospital, Oslo, Norway*

Background: The occurrence of nerve ingrowth and its relation to chronic tendon pain (tendinopathy) are still largely unknown. In healthy tendons, the innervation is confined to the paratenon, whereas the tendon proper is devoid of nerve fibers. In this study on the pathogenesis of tendinopathy, the authors examined sensory and sympathetic nerve fiber occurrence in the patellar tendon.

Hypothesis: Nerve ingrowth and altered expression of sensory and sympathetic neuromediators play a major role in the pathophysiology of pain in patellar tendinopathy.

Study Design: Case control study; Level of evidence, 3.

Methods: Biopsies from the patellar tendon in patients with patellar tendinopathy (n = 10) were compared with biopsies from a control group (n = 10) without any previous or current knee symptoms compatible with patellar tendinopathy. The biopsies were stained immunohistochemically for sensory and autonomic nerve markers. The biopsies from the 2 groups were compared using subjective and semiquantitative methods.

Results: Chronic painful patellar tendons exhibited increased occurrence of sprouting nonvascular sensory, substance P–positive nerve fibers and a decreased occurrence of vascular sympathetic nerve fibers, positive to tyroxin hydroxylase, a marker for noradrenaline.

Conclusion: The altered sensory-sympathetic innervation suggests a role in the pathophysiology of tendinopathy. Ingrowth of sprouting substance P fibers presumably reflects a nociceptive and maybe a proliferative role, possibly as reactions to repeated microtraumata, whereas the decreased occurrence of tyroxin hydroxylase may represent a reduced antinociceptive role. These findings could be used to develop targeted pharmacotherapy for the specific treatment of tendinopathy.

Keywords: tendon; pain; jumper's knee; substance P (SP); noradrenaline

Tendinopathy is a major cause of sick leave,[9] as well as morbidity related to athletic performance.[21] However, the basic pathogenesis of pain and degeneration in chronic tendon disease is generally poorly understood, which limits our ability to develop specific therapeutic interventions. In the absence of inflammation, the chemical and morphologic substrate for the experienced pain is also mostly unknown. However, neurogenic inflammation was recently implicated in the origin of achillodynia,[13] suggesting new therapeutic targets to mitigate symptoms.

Patellar tendinopathy is believed to be a tendon overload injury caused by a combination of internal and external risk factors.[29] Studies using Doppler flow technique[6,7,28] and histologic evaluations[25,26] have demonstrated increased blood vessel density in patients with degenerative tendon disease. Blood vessels cannot explain the pain suffered by these patients, and thus other factors related to pain pathophysiology, among them neuromediators, have been suggested[3,5,19,33] but are yet unidentified in patellar tendinopathy.

Recent observations have established that normal innervation of the tendon envelope (paratenon and surrounding loose connective tissue) consists of sensory and autonomic nerve fibers,[2,3,5] which are suggested to play an

‖Address correspondence to Roald Bahr, MD, PhD, Oslo Sports Trauma Research Center, Norwegian School of Sport Sciences, PO Box 4014, Ullevål Stadion, 0806 Oslo, Norway (e-mail: roald@nih.no).
No potential conflict of interest declared.

The American Journal of Sports Medicine, Vol. 34, No. 11
DOI: 10.1177/0363546506289169

important role in the regulation of pain, inflammation, and tissue repair.[12,13,24,26] However, the healthy tendon proper is practically devoid of nerve fibers.[2,3,5] From other pain conditions, for example, low back pain, a relationship between sensory nerve ingrowth with expression of substance P (SP) in the disc and pathogenesis of pain has been shown.[16] Recently, SP was also demonstrated in Achilles tendinopathy.[5,10,33] In rheumatoid arthritis, a connection has been demonstrated between decreased sympathetic input (ie, low levels of noradrenaline) and decreased anti-inflammatory capability.[34]

However, in tendinopathy, the relative ratio of sensory and autonomic nerve fibers and their clinical relationship to pain have not been studied. Thus, in this study, we compared the occurrence of sensory and sympathetic nerve fibers between chronic painful patellar tendons and controls.

METHODS

Patient Groups

The patient group included athletes from different sports who were included in a prospective randomized trial comparing surgery with eccentric training.[8] The following diagnostic criteria were used for patellar tendinopathy: history of exercise-related pain in the proximal patellar tendon or the patellar insertion and distinct tenderness to palpation corresponding to the painful area.[11] To be included in the study, the patients had to have a clinical diagnosis of jumper's knee grade IIIB; that is, the patient had pain during and after activity and was unable to participate in sports at the same level as before pain.[22,32] In addition, the patient had to have thickening and signal changes on the MRI corresponding to the painful area to ensure that the biopsies were taken from the tendinopathic area. Patients had to have symptoms for a minimum of 3 months and be willing to undergo surgery. Subjects were excluded if they had a history of knee or patellar tendon surgery, inflammatory joint conditions, or degenerative conditions. Both knees were included if the patient had bilateral problems.

Each patient went through a standardized interview, and the information requested from each athlete included age, height, weight, and number of years participating in organized athletic training. Patients were asked to report the number of training hours per week during the competition season (sport-specific training, weight training, jump training, and other types of training). To assess the severity of the condition, the athletes with current patellar tendinopathy also self-recorded their symptoms and level of sports function using the Victorian Institute of Sport Assessment (VISA) questionnaire.[36] This brief questionnaire assesses symptoms, function, and the ability to play sport.[36] The maximal VISA score for an asymptomatic, fully performing individual is 100 points, and the theoretical minimum is 0.[36] The VISA questionnaire has shown excellent short-term test-retest reliability and has been shown to be a valid measure of symptoms in patients with patellar tendinopathy.[36]

The control group was selected from patients with tibia fractures from low-energy trauma treated with marrow nailing. These patients could have no current or previous knee symptoms compatible with patellar tendinopathy. Subjects in both groups had to be at least 18 years old (to ensure that the epiphyses were closed) and able to understand oral and written Norwegian.

Exclusion criteria in both groups were previous surgical treatment in or around the same knee, corticosteroid injections in or around the same knee, serious traumatic injury affecting the same knee, any rheumatic disease, and degenerative knee disorders. The study was approved by the regional committee for research ethics, participation was voluntary, and consent was obtained.

Surgical Technique

The surgical exposure was identical in the 2 groups, with a 5-cm longitudinal midline or lateral parapatellar incision, splitting of the paratenon, and exposure of the patellar ligament. The paratenon was split longitudinally, any pathologic paratenon tissue was removed, and the tendon was fully exposed. In both groups, the biopsies were taken from the proximal bone-ligament junction. The tendon tissue was excised using a full-thickness wedge-shaped incision, wide from the patellar pole and narrowing distally. In the patient group, all abnormal tissue was removed. If clearly abnormal tissue was not seen macroscopically, the excision was based on the MRI signal changes. Typically, a wedge with a proximal base 1 cm wide and extending to an apex 20 to 30 mm distal from the patellar pole was removed. In the control group, the biopsies were taken with a width of at least 5 mm and a length of at least 20 mm from the middle portion of the ligament starting at the bone-ligament junction.

Biopsy Procedure

The biopsy handling was identical in the 2 groups. Immediately after the surgical procedure, the biopsies were transferred to Zamboni solvent.[38] The biopsies were stored in this solution for 4 to 24 hours and then washed in 0.1-M phosphate-buffered saline (PBS), pH 7.2, with 15% sucrose (weight/volume) and 0.1% natriumazide. This washing was done until the yellow color from the Zamboni solution could no longer be seen in the PBS solvent. The biopsies were then stored in PBS at 4°C for a minimum of 48 hours.

The samples were sectioned at 154 μm on a Leitz cryostat, and frozen sections were mounted directly on SuperFrost/Plus glass slides, 3 sections per slide, and stained using the avidin-biotin or the hematoxylin and eosin systems, for immunohistochemistry and light microscopy, respectively.

Morphologic Study. The hematoxylin- and eosin-stained slides were subjectively assessed by a single blinded observer and graded according to the Bonar scale[15] with regard to tenocyte morphologic characteristics and vascularity (Table 1).

TABLE 1
Modified Bonar Scale[a]

	Grade			
	0	1	2	3
Tenocytes	Inconspicuous, elongated, spindle-shaped nuclei with no obvious cytoplasm at light microscopy	Increased roundness; nuclei becomes ovoid to round in shape without conspicuous cytoplasm	Increased roundness and size; the nucleus is round and slightly enlarged; a small amount of cytoplasm is visible	Nucleus is round and large, with abundant cytoplasm and lacuna formation (chondroid change)
Blood vessels	Inconspicuous blood vessels coursing in between bundles	Occasional cluster of capillaries; less than 1 per 10 high-power fields	1-2 clusters per 10 high-power fields	More than 2 clusters per 10 high-power fields

[a]See Cook et al.[15] The scale is a semiquantitative tendon score based on tenocyte and blood vessel morphologic characteristics (grading of ground substance and collagen is not included in this reproduction).

Immunohistochemistry. The slides were rinsed for 10 minutes in PBS. Incubation with 10% normal goat serum in PBS for 30 minutes blocked nonspecific binding. Subsequently, the sections were incubated overnight in a humid atmosphere at +8°C with primary antisera for protein gene product 9.5 (PGP, 1:10 000, UltraClone, Wellow, United Kingdom), SP (1:10 000, Peninsula Laboratories, San Carlos, Calif), and tyrosine hydroxylase (TH, 1:5000, Peninsula Laboratories), a rate-limiting enzyme reflecting the occurrence of noradrenaline. The PGP is the carboxyl-terminal hydrolase to the ubiquitin protein, which is an important protein component in the axonal neurolemma and is used as a general nerve marker, which makes it possible to identify the total number of nerve fibers.[17,24,37] After incubation with the primary antisera, the sections were rinsed in PBS (3-5 minutes) and then incubated with biotinylated goat antirabbit antibodies (1:250, Vector Laboratories, Burlingame, Calif) for 40 minutes at room temperature. Finally, the sections were incubated for 40 minutes with Cy3-conjugated avidin (1:5000, Amersham International, Stafford, United Kingdom). Control staining was performed by omitting the primary antiserum. A Nikon epifluorescence microscope (Eclipse E800, Nikon, Yokohama, Japan) was used for the analyses. The slides were examined by 2 independent observers, who were blinded with regard to the group to which the slides belonged. The occurrence and neuromorphology of PGP, SP, and TH were subjectively assessed, and pictures were taken for subsequent semiquantitative analyses.

Semiquantitative Analysis. After the subjective assessment, the following steps identified in an earlier study[1] were applied to optimize the semiquantitative analysis: The patellar tendons were longitudinally sectioned, and the sections were numbered consecutively from the dorsal to the ventral aspect. Three sections from different levels (ie, ventral, middle, and dorsal parts of the tendon) were chosen to represent the full thickness of the tendon. Staining was performed simultaneously for all sections to be compared. For microscopic analysis, a video camera system (DXM 1200, Nikon) was attached to the epifluorescence microscope and connected to a computer. From each section, 3 images from the microscopic fields (× 20 objective) exhibiting the strongest immunofluorescence were stored in the computer. Thereafter, the images were analyzed using Easy Analysis software (Technooptik, Skarholmen, Sweden). The software denotes and considers all positively stained nerve fibers beyond a defined threshold of fluorescent intensity. The results were expressed as the fractional area occupied by positive fibers in relation to the total area. The fluorescent/total area was determined in 9 images in each biopsy of the patient and control groups, respectively. In the microscopic analysis, the mean interobserver coefficient of variation was 9.8% and the intraobserver variation 9.6%. For statistical analysis, the mean fluorescent/total area was calculated for each of the 10 biopsies from both the patient and the control groups.

Data Analysis

For continuous variables, the results are given as means with range, unless otherwise noted. Comparisons between groups were done using unpaired *t* tests, as noted in the "Results" section. An α level of .05 was considered significant.

A sample size analysis based on the results of earlier immunohistochemical semiquantification studies, using an estimated average of 0.65 and 1 and an SD of 0.3 and 0.4 in the 2 groups, respectively, as well as an α level of .05 and a β level of .30, resulted in a sample size of 10.

RESULTS

The mean age was 30 years (range, 24-34 years; n = 10) in the patient group and 29 years (range, 19-43 years; n = 10) in the control group. In the patient group, the mean number of years participating in organized training was 17 (range, 10-28 years; n = 10), and the mean number of total training hours per week was 14 (range, 6-24 hours; n = 10). The mean VISA score was 42 (range, 15-65; n = 10), and the

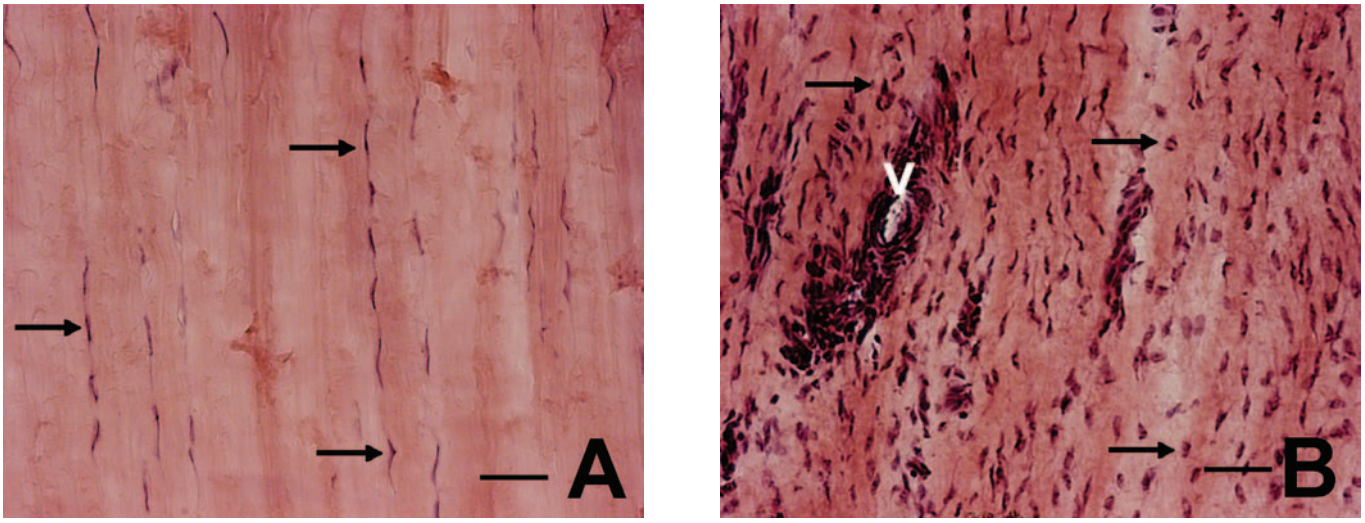

Figure 1. Hematoxylin and eosin micrographs of longitudinal sections through the patellar tendon of healthy control (A) and painful tendinopathy (B). Arrows denote tenocytes. The healthy tendon is homogeneous, with organized parallel collagen structure and thin, elongated tenocytes (A). The tendinopathy, on the other hand, is marked by collagen disorganization, increased cell count, activated tenocytes, and vascular ingrowth (V) in the tendon proper (B). Bar, 50 μm.

mean duration of symptoms was 36 months (range, 5-120 months; n = 10).

Microscopy

The morphologic appearance of the painful tendons in the tendon proper differed significantly compared with the appearance of the controls. The proper tendinous tissue exhibited signs of tendinosis (collagen degeneration, fiber disorientation, hypercellularity, angiogenesis, and absence of inflammatory cells) in all but 1 of the patients, whereas only a few of the controls exhibited early signs of tendinosis (Figure 1).

Semiquantitative assessment of tenocyte morphologic characteristics and of angiogenesis according to the Bonar scale, as signs of early and later stages of tendinosis, respectively, was performed.[15] Tenocyte changes occurred in all but 1 of the painful tendons, whereas only 3 of 10 controls exhibited these changes ($P = .006$). Angiogenesis, considered to be the last histologic sign of tendinopathy,[15] was found in 4 of 10 painful tendons but in none of the controls ($P = .038$).

Immunohistochemistry

Overall, the subjective immunohistochemical assessment confirmed the morphologic appearance. However, it also provided more detailed information about sensory (SP) and sympathetic (TH) nerve fiber occurrence in the patellar tendon. Thus, the majority (7/10) of the painful tendons exhibited an increased number of nerve fibers positive to SP and notably decreased levels of TH.

Sensory Nerves

Closer subjective analysis showed that the increased number of SP-positive fibers in the painful tendons occurred mainly as thin, varicose, sprouting nonvascular nerve terminals within the tendon proper (Figure 2B). Notably, these SP-positive nerves in the painful tendons were found over a larger area, more spread out within the tendon than in the controls. The SP-positive nerve fibers, seen as free nerve endings interspersed between the proper collagen fibers, often accompany the loose connective tissue ingrowth within the tendon proper of the painful tendons. Contrary to what one might have expected, no differences were noted between the groups regarding the small subpopulation of vascular SP-positive fibers. In both groups, SP was regularly seen in larger nerve bundles (Figure 2A).

Sympathetic Nerves

Subjective analysis demonstrated a great difference not only in the occurrence of TH-expressing nerve fibers between patients and controls but also in their morphologic distribution. In both groups, TH-positive nerves were present as free nerve endings throughout the tendon proper, but unlike the sensory nerves, the majority of the TH-positive nerves were distinctively related to the blood vessels (Figure 3A). In the patients, there was a distinct decrease in the occurrence of TH-positive nerves. Some TH-positive free nerve endings were still seen, but the vessel-related TH nerves in the patients were significantly diminished (Figure 3B).

General Nerve Occurrence

The neuronal localization of SP and TH staining was confirmed by positive immunoreactivity for PGP, a general nerve marker. The subjective analysis of PGP showed a distinctively higher nerve fiber occurrence in the chronic pain group compared with the controls. Nerves existed as

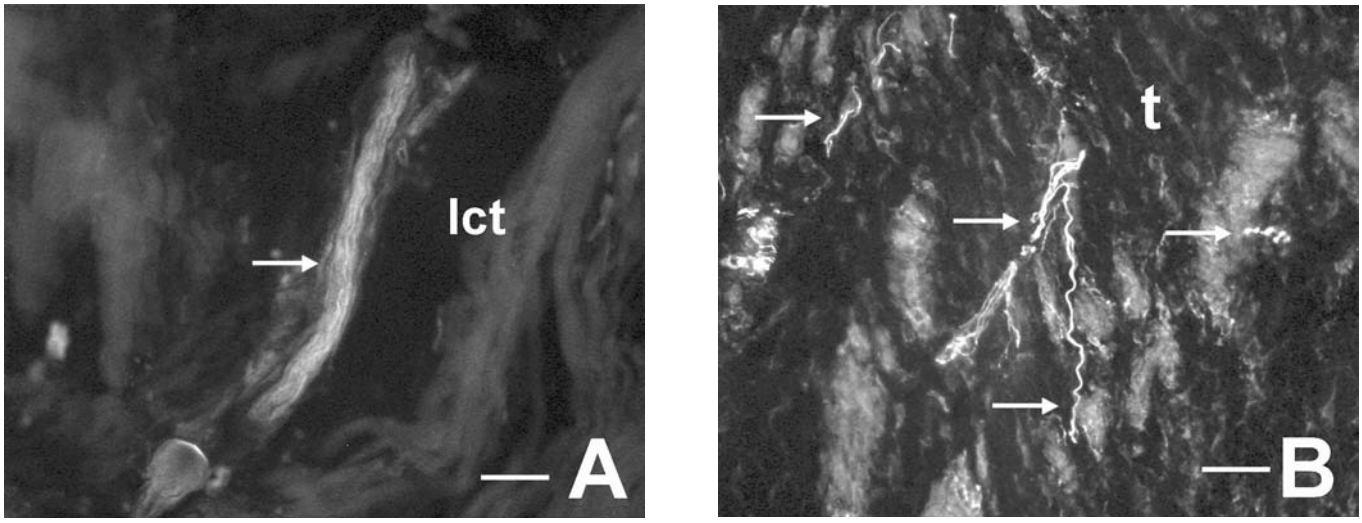

Figure 2. Immunofluorescence micrographs of longitudinal sections through the patellar tendon of healthy control (A) and painful tendinopathy (B) after incubation with antisera to substance P (SP). In the control tendon, SP-positive nerve fibers are mainly present as vascular nerve fibers and as large bundles (arrow) in the loose connective tissue (lct; A). In painful tendinopathy, increased spread and sprouting of SP-positive nerve fibers (arrows) are seen (B). These sprouting nerves even invade the tendon proper (t). Bar, 50 μm.

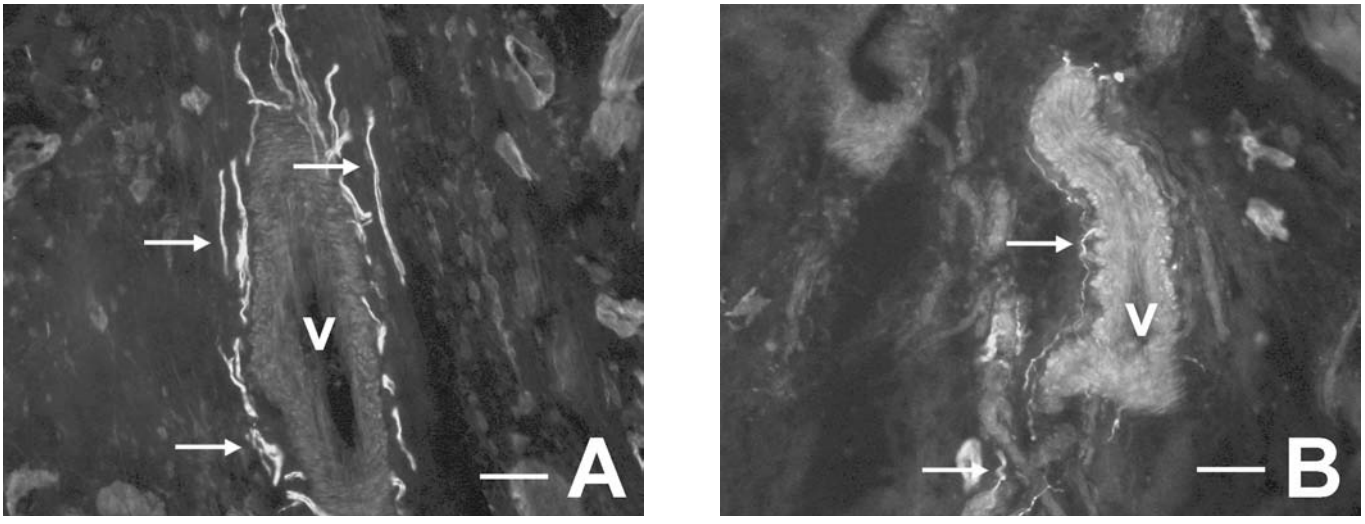

Figure 3. Immunofluorescence micrographs of longitudinal sections through the patellar tendon of healthy control (A) and painful tendinopathy (B) stained for tyrosine hydroxylase (TH, a marker for noradrenaline). Arrows denote nerve fibers. In the healthy tendon, a strong relation is seen between blood vessels and TH-positive nerves (A). In painful tendinopathy, a decreased number of TH-positive nerves, which are blood vessel related, are seen. V, blood vessel. Bar, 50 μm.

both vascular and nonvascular free nerve endings and in larger bundles.

Semiquantitative Immunohistochemistry

Computerized image analysis of SP, TH, and PGP expression showed similar differences in occurrence as assessed subjectively, although not all results were significant. The occurrence of SP was 22% ($P = .567$) and that of PGP 54% ($P = .098$) higher in the chronic painful tendons than in the controls. The occurrence of TH in the chronic painful tendons was 53% lower than in the controls ($P = .018$) (Figure 4).

DISCUSSION

This study demonstrates that the composition of nerve fibers expressing sensory (SP) and sympathetic neuromediators (TH) appears to differ between patients with painful

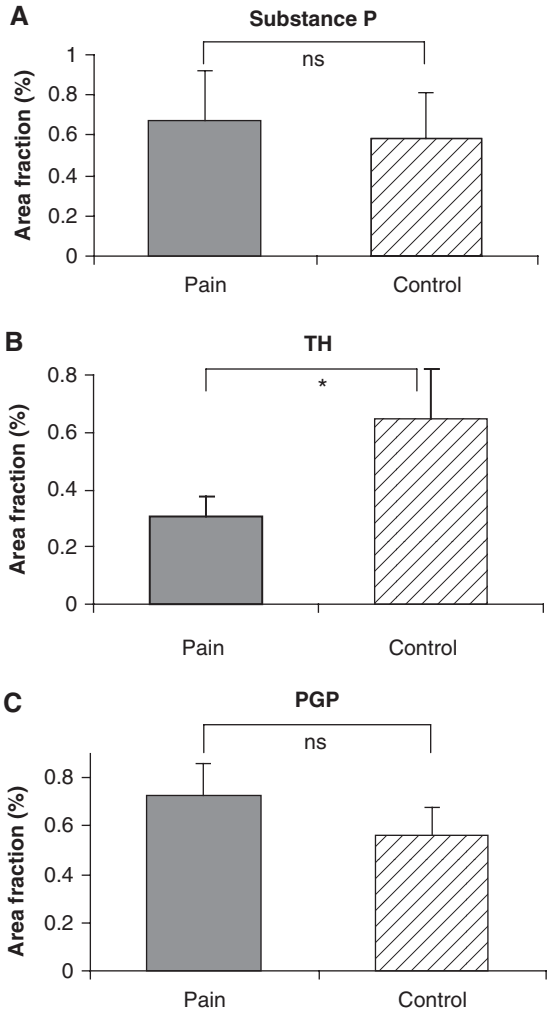

Figure 4. Mean area fraction occupied by nerve fibers (%) immunoreactive to SP (A), TH (B), and PGP (C) in painful patellar tendons as compared with controls (± standard error of the mean). *$P < .05$. Not significant (ns), $P > .05$. SP, substance P; TH, tyrosine hydroxylase; PGP, protein gene product.

patellar tendinopathy and controls. Most notably, a painful tendon is characterized by an increased number of SP-positive nonvascular nerve endings and a vascularly related decrease in TH, a marker of noradrenaline.

Tendinopathy is defined as a chronic and painful tendon disorder. The patients of this study had a mean symptom duration of 3 years and a VISA score of 42, which indicates a high level of pain and significant disability.[36] All patients exhibited transformed tenocytes, in contrast to only 3 of 10 controls. Angiogenesis, considered to be an advanced histologic sign of tendinopathy,[15] was found in 4 of 10 painful tendons but in none of the controls. This means that the histopathologic findings in this study can be interpreted as characteristic of a patient group with chronic and severe complaints of patellar tendinopathy. We do not know the activity level in the control group, but because the described histopathologic changes are assumed to be typical of a chronic overload injury, we do not expect to find these changes in asymptomatic tendons. However, from a methodological point of view, this possibility cannot be completely excluded.

The altered peripheral sensory-sympathetic innervation in patients suggests a role in the pathophysiology of tendinopathy.[31] Increased ingrowth of sensory nerves into the painful tendon proper, seen as sprouting free nerve endings, may explain the pain by reflecting intensified nociceptive transmission as a response to, for example, repetitive mechanical stimuli. The sympathetic nerves that are believed to act antinociceptively exhibited a reduced occurrence in the patients studied, thus supporting the notion that the observed changes in peripheral innervation are involved in the regulation of tendon pain.

The peripheral nervous system is known to react to outer and inner stress. It has been demonstrated that sensory nerve ingrowth and decreased sympathetic innervation occur as a response to tendon injury,[4] indicating that repeated microtrauma might be an initiator of the neuronal response. Moreover, the same study established that nociception during early healing is related to increased sensory and decreased autonomic neuromediator occurrence.

In this study, we focused on SP and not on calcitonin gene-related peptide because the research on SP is further advanced compared with that on calcitonin gene-related peptide. Thus, the relationship between SP and chronic pain is more established, also with regard to tendinopathy and tendon healing.[14,23,33]

The increased occurrence of SP in tendinopathy may reflect a multitude of actions. Substance P has been found to participate in inflammatory actions like vasodilation, plasma extravasation, and release of cytokines, in addition to its role in nociception, where SP has been reported to directly stimulate nociceptor endings in an autocrine/paracrine manner.[35] Similar actions may be presumed to occur in tendinopathy because it has been demonstrated that SP receptors are present.[23] The existence of SP within the tendon proper of the painful patellar tendons was in fact observed mainly in free nerve endings, indicating that the main function of SP in tendinopathy is nociceptive rather than vasoactive.

The reduction in TH, that is, vasoregulatory noradrenaline, suggests a suppressed antinociceptive function. A recent report has demonstrated that noradrenaline release leads to secretion of opioids from leukocytes.[30] Notably, a similar pattern of decreased vascular TH and increased free SP-positive nerve fibers is seen in patients with painful rheumatoid arthritis.[34]

In addition, the up-regulation of SP seen within the pathogenic tendon proper might reflect a trophic role. Substance P has in fact been shown to stimulate proliferation of fibroblasts[14] and endothelial cells,[27] as well as the production of transforming growth factor β in fibroblasts.[20] It is therefore tempting to speculate that SP contributes to the morphologic changes observed in tendinopathic patients, that is, tenocyte transformation, hypercellularity, and presumably neovascularization. Whether neuronal and cellular alterations in tendinopathy can be correlated requires further studies. It remains to be seen if the pain level is related to the ratio of SP to TH or to the degree of neovascularization.

The protracted presence of SP in tendinopathy is pathologic, in contrast to its trophic role in normal tendon healing. For progression of normal tendon healing, it has been shown that a strict temporal orchestration of neuromediator occurrence is essential. Thus, an initial up-regulation in SP at the healing site during the inflammatory and regenerative phases is followed by disappearance of SP and emergence of TH, representing a progress of healing.[4] However, in tendinopathic patients, the normal healing process appears to be at a standstill, characterized by high levels of SP and low levels of TH. Considering the similarities to rheumatoid arthritis,[34] increased SP expression in tendinopathy might even be part of a neuroinflammatory process. The classic vessel-related proinflammatory actions of SP may occur in the tendon envelope, that is, the paratenon and loose connective tissue, as demonstrated by an experimental study, whereas in the tendon proper, no classic inflammation was seen.[13] These observations are similar to the mostly free SP nerve endings seen in the current study. The prolonged release of SP from free nerves in the painful tendons may, as a neuroinflammatory process, suppress the synthesis of growth factors and increase the levels of stromelysin (endopeptidase, metalloproteinase) in the tendon.[18] Hence, the demonstrated up-regulation of SP might lead to subsequent matrix destruction in the pathogenic tendon proper.

In this study, the variation between biopsies was high, and the semiquantitative analysis confirmed only 1 of the 3 subjective analyses of the neuromediators in question. However, the trends all pointed in the same direction. The semiquantitative method takes only the fields with highest density of immunofluorescence into account, thus overlooking histologic differences, such as extensive nerve sprouting. The semiquantitative analysis should therefore only be regarded as a complement to the subjective analysis. Although the up-regulated SP occurrence was demonstrated exclusively by subjective analysis, the observations were corroborated by a recent report on Achilles tendinosis demonstrating increased SP levels in tendinopathy.[33]

In conclusion, this study demonstrates a differentiation in the sensory and sympathetic neuromediator pattern in patients with painful tendinopathy. The dominance of nonvascular SP nerve endings as well as the decrease of the antinociceptive modulator noradrenaline suggest a pathophysiologic up-regulation of pain. Both these neuropeptides, known to be essential for normal healing, exhibit a disturbed balance that may contribute to the degenerative and painful processes of tendinopathy. The understanding of the neuronal pathomechanisms may suggest new therapeutic targets to mitigate the symptoms in patients with painful tendon disorders.

ACKNOWLEDGMENT

The Oslo Sports Trauma Research Center has been established at the Norwegian School of Sport Sciences through generous grants from the Norwegian Eastern Health Corporate, the Royal Norwegian Ministry of Culture, the Norwegian Olympic Committee & Confederation of Sport, Norsk Tipping AS, and Pfizer AS. The authors thank Sverre Løken, MD, for help with surgical procedures and taking biopsies.

REFERENCES

1. Ackermann PW, Ahmed M, Kreicbergs A. Early nerve regeneration after Achilles tendon rupture: a prerequisite for healing? A study in the rat. *J Orthop Res*. 2002;20:849-856.
2. Ackermann PW, Finn A, Ahmed M. Sensory neuropeptidergic pattern in tendon, ligament and joint capsule: a study in the rat. *Neuroreport.* 1999;13;2055-2060.
3. Ackermann PW, Li J, Finn A, Ahmed M, Kreicbergs A. Autonomic innervation of tendons, ligaments and joint capsules: a morphologic and quantitative study in the rat. *J Orthop Res*. 2001;19:372-378.
4. Ackermann PW, Li J, Lundeberg T, Kreicbergs A. Neuronal plasticity in relation to nociception and healing of rat Achilles tendon. *J Orthop Res*. 2003;21:432-441.
5. Ackermann PW, Renström P. Sensory neuropeptides in Achilles tendinosis: transactions of the Third Biennial Congress of the International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine. *Arthroscopy*. 2001;17(suppl):516.
6. Alfredsson H, Lorentzon M, Bäckman S, Bäckman A, Lerner U. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *J Orthop Res*. 2003;21:970-975.
7. Astrom M, Westlin N. Blood flow in the human Achilles tendon assessed by laser Doppler flowmetry. *J Orthop Res*. 1994;12:246-252.
8. Bahr R, Fossan B, Løken S, Engebretsen L. Surgical treatment versus eccentric training for patellar tendinopathy (jumper's knee): a randomized controlled trial. *J Bone Joint Surg Am.* In press.
9. Barnard P. *Musculoskeletal Disorders and Workplace Factors: A Critical Review of Epidemiologic Evidence For Work-Related Musculoskeletal Disorders Of The Neck, Upper Extremity, And Low Back*. Cincinnati, Ohio: US Department of Health and Human Services, National Institute of Occupational Safety and Health; 1997.
10. Bjur D, Alfredson H, Forsgren S. The innervation pattern of the human Achilles tendon: studies of the normal and tendinosis tendon with markers for general and sensory innervation. *Cell Tissue Res*. 2005; 320:201-206.
11. Blazina ME, Kerlan RK, Jobe FW, Carter VS, Carlson GJ. Jumper's knee. *Orthop Clin North Am*. 1973;4:665-673.
12. Brain SD. Sensory neuropeptides: their role in inflammation and wound healing. *Immunopharmacology*. 1997;37:133-150.
13. Bring DK, Heidgren ML, Kreicbergs A, Ackermann PW. Increase in sensory neuropeptides surrounding the Achilles tendon in rats with adjuvant arthritis. *J Orthop Res*. 2005;23:294-301.
14. Burssens P, Steyaert A, Forsyth R, van Ovost EJ, De Paepe Y, Verdonk R. Exogenously administered substance P and neutral endopeptidase inhibitors stimulate fibroblast proliferation, angiogenesis and collagen organization during Achilles tendon healing. *Foot Ankle Int*. 2005;26: 832-839.
15. Cook JL, Feller JA, Bonar SF, Khan KM. Abnormal tenocyte morphology is more prevalent than collagen disruption in asymptomatic athletes' patellar tendons. *J Orthop Res*. 2004;22:334-338.
16. Freemont AJ, Peacock TE, Goupille P, Hoyland JA, O'Brien J, Jayson MI. Nerve ingrowth into diseased intervertebral disc in chronic back pain. *Lancet*. 1997;19:178-181.
17. Gulbenkian S, Wharton J, Polak JM. The visualization of cardiovascular innervation in the guinea pig using a antiserum to protein gene product 9.5 (PGP 9.5). *J Auton Nerv Syst*. 1987;18:235-247.
18. Hart DA, Kydd A, Reno C. Gender and pregnancy affect neuropeptide responses of the rabbit Achilles tendon. *Clin Orthop Relat Res.* 1999;365:237-246.
19. Khan KM, Cook JL, Maffulli N, Kannus P. Where is the pain coming from in tendinopathy? It may be biochemical, not only structural, in origin. *Br J Sports Med*. 2000;34:81-83.
20. Lai XN, Wang ZG, Zhu JM, Wang LL. Effect of substance P on gene expression of transforming growth factor beta-1 and its receptors in rat's fibroblasts. *Chin J Traumatol.* 2003;6:350-354.

21. Lian Ø, Engebretsen L, Bahr R. Prevalence of jumper's knee among elite athletes from different sports: a cross-sectional study. *Am J Sports Med.* 2005;33:561-567.
22. Lian Ø, Holen K, Engebretsen L, Bahr R. Relationship between symptoms of jumper's knee and the ultrasound characteristics of the patellar tendon among high level male volleyball players. *Scand J Med Sci Sports.* 1996;6:291-296.
23. Ljung BO, Alfredson H, Forsgren S. Neurokinin 1-receptors and sensory neuropeptides in tendon insertions at the medial and lateral epicondyles of the humerus: studies on tennis elbow and medial epicondylalgia. *J Orthop Res*. 2004;22:321-327.
24. Lundberg JM, Alm P, Wharton J, Polak JM. Protein gene product 9.5 (PGP 9.5). *Histochemistry.* 1988;90:9-17.
25. Maffulli N, Testa V, Capasso G, et al. Similar histopathological picture in males with Achilles and patellar tendinopathy. *Med Sci Sports Exerc*. 2004;36:1470-1475.
26. Movin T, Gad A, Reinholt FP, Rolf C. Tendon pathology in long-standing achillodynia: biopsy findings in 40 patients. *Acta Orthop Scand*. 1997; 68:170-175.
27. Nilsson J, von Euler AM, Dalsgaard CJ. Stimulation of connective tissue cell growth by substance P and substance K. *Nature.* 1985; 315:61-63.
28. Öhberg L, Lorentzon R, Alfredson H. Neovascularisation in Achilles tendons with painful tendinosis but not in normal tendons: an ultrasonographic investigation. *Knee Surg Sports Traumatol Arthrosc*. 2001;9:233-238.
29. Renstrom P, Johnson RJ. Overuse injuries in sports: a review. *Sports Med.* 1985;2:316-333.
30. Rittner HL. Leukocytes in the regulation of pain and analgesia. *J Leukoc Biol.* 2005;78:1215-1222.
31. Roberts WJ, Kramis RC. Sympathetic nervous system influence on acute and chronic pain. In: Fields HL, ed. *Pain Syndromes in Neurology*. Essex, England: Butterworth; 1990:85-106.
32. Roels J, Martens M, Mulier JC, Burssens A. Patellar tendinitis (jumper's knee). *Am J Sports Med.* 1978;6:362-368.
33. Schubert TE, Weidler C, Lerch K, Hofstadter F, Straub RH. Achilles tendinosis is associated with sprouting of substance P positive nerve fibres. *Ann Rheum Dis*. 2005;64:1083-1086.
34. Straub RH, Gunzler C, Miller LE, Cutolo M, Scholmerich J, Schill S. Anti-inflammatory cooperativity of corticosteroids and norepinephrine in rheumatoid arthritis synovial tissue in vivo and in vitro. *FASEB J*. 2002;16:993-1000.
35. Ueda H. In vivo molecular signal transduction of peripheral mechanisms of pain. *Jpn J Pharmacol.* 1999;79:263-268.
36. Visentini PJ, Khan K, Cook J, Kiss ZS, Harcourt PR, Wark JD. The VISA score: an index of severity of symptoms in patients with jumper's knee (patellar tendinosis). Victorian Institute of Sport Tendon Study Group. *J Sci Med Sport*. 1998;1:22-28.
37. Wilkinson KD, Lee KM, Desphande S, Duerksen-Hughes P, Boss JM, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science.* 1989;246:670-673.
38. Zamboni L, De Martino C. Buffered picric acid-formaldehyde: a new, rapid fixative for electron microscopy. *J Cell Biol*. 1967;35:148.