BOOK CHAPTER FOR
THE BOOK ON TENDONS

Title:
TENDON INNERVATION AND
NEURONAL RESPONSE AFTER INJURY

Paul W. Ackermann, Daniel K-I Bring and Per A. F. H. Renström

Department of Molecular Medicine and Surgery,
Section for Sports Medicine and Orthopedics,
Karolinska Institutet
Stockholm, Sweden

Correspondence should be sent to:
Paul Ackermann, MD, PhD
Dept. of Orthopedics
Karolinska University Hospital
S-171 76, Stockholm
Sweden
E-mail: Paul.Ackermann@karolinska.se
Introduction

Tendon pain and injuries are frequent complications of repetitive motion and overuse syndromes and pose a huge burden on the health care system mainly due to the lack of specific treatment and long recovery time until return to work. The knowledge of the pathomechanisms underlying tendon disorders is still limited. Although some mechanical risk factors have been identified, the relationship to pain and inflammation is debatable [54] and still requires a biological explanation. Accumulating data support the hypothesis that the peripheral nervous system plays a major role in the regulation of pain and inflammation, maybe also in tissue repair.

In inflammatory conditions of the locomotor apparatus pain is almost a constant feature. However, in degenerative conditions pain is highly variable. Whether inflammation is a prerequisite to pain in degenerative disease is unclear. Notably, spontaneous ruptures of tendons, presumed to be caused by degeneration, are often not preceded by pain. On the whole, the relationship between pain, inflammation and degeneration of the locomotor system and specifically tendons remains obscure, a consequence of limited knowledge of neuroanatomy and pathophysiology.

Over the last decade, however, knowledge about specific neuronal signal substances, so called neuropeptides, involved in the regulation of pain, inflammation and also tissue repair has evolved. This chapter discusses tendon innervation focusing on neuropeptides including opioids, in tendon, proposing an endogenous peripheral system of pain inhibition. Moreover, recent findings on neuronal response to injury are presented, suggesting new pathways for tendon repair.

The novel data presented in this chapter considering tendon innervation and neuronal response to injury, may suggest new targets for pain treatment and specific pharmacotherapy in tendon disorders that could shorten recovery time.
Tendon innervation

Tendon innervation originates from three neighboring sources: cutaneous, muscular and peritendinous nerve trunks. From the myotendinous junction the nerve fibers cross and enter the endotenon septa. In the paratenon, nerve fibers form rich plexuses and send branches that penetrate the epitenon. Most nerve fibers do not enter the tendon proper, but terminate as nerve endings on the surface.

The nerves of tendons are composed of myelinated, fast-transmitting $A\alpha$- and $A\beta$-fibers and unmyelinated, slow-transmitting $A\gamma$, $A\delta$, B- and C-fibers. The nerve endings of myelinated fibers ($A\alpha$, $A\beta$) are of type I-III specialized mechanoreceptors, whose main function is to mediate physical energy (pressure, tension) into afferent nerve signals. The unmyelinated nerve endings of $A\gamma$, $A\delta$, and C-fibers are type IVa fibers, so called nociceptors. These nerve endings mediate deep tissue pain and hyperalgesia that are characteristic features of tendon pain. The nerve endings of B-fibers, which are autonomic, consist of type IVb fibers mainly located in the walls of small arteries, arterioles, capillaries, and postcapillary veins and exert vasomotor actions.

Over the last decade, the peripheral nervous system in addition to classical functions such as nociception and vasoactivity, has been found to also participates in the regulation of a wide variety of efferent actions on cell proliferation, cytokine expression, inflammation, immune responses and hormone release [12, 27, 33, 57, 61, 64, 67]. The paradoxical “efferent” role of afferent nociceptive fibers as suggested by Bayliss [11] more than one century ago is now widely recognized. So far, however, the innervation and neuronal regulation of periarticular tissues and especially tendons have received little attention. In particular, this applies to the specific neuronal mediators, i.e. the signal substances.

The mediators of the nervous system act through two principally different ways:
1) With fast transmitters, i.e. classical neurotransmitters (monoamines, acetylcholine, amino acids), which directly effectuate muscle contractions or afferently relay information on painful stimuli.

2) With a family of slow transmitters, neuropeptides, which slowly regulate physiological functions in the central as well as peripheral nervous system.

**Neuropeptides**

Neuropeptides act as chemical messengers and regulators in the central as well as peripheral nervous system. They differ from classical neurotransmitters in several respects. Peripheral neuropeptides are synthesized in the cell bodies, i.e. the dorsal root ganglia (sensory) and the sympathetic chain (autonomic), and transported distally. In contrast classical neurotransmitters are synthesized in the axon terminals, thus exerting their effects more locally. Synthesis and turnover of classical transmitters are more rapid than those of neuropeptides leading to more long-lasting regulatory effects of the neuropeptides. Moreover, several neuropeptides are co-released, also together with classical transmitters [34], which offer a variety of functional interactions. The effects of neuropeptides and classical transmitters are elicited by different receptor mechanisms resulting in direct effects of classical transmitters, while neuropeptides act regulatory, “supervising”.

Several neuropeptides have been identified in both the central and peripheral nervous system. So far, however, research on the occurrence and functions of neuropeptides in tendons has been very limited. Neuropeptides that hypothetically are important for nociception and tissue homeostasis in tendon can be classified in three groups; sensory, opioid and autonomic, according to their function and original nerve fiber type finding.

*Sensory neuropeptides*
Sensory primary afferents, C- and Aδ-fibers, contain substance P (SP) claimed to transmit nociceptive signals [35, 40, 65]. Calcitonin gene-related peptide (CGRP) co-exists with and potentiates the effect of SP [56, 74, 77]. Both SP and CGRP have also been shown to exert pro-inflammatory effects such as vasodilation and protein extravasation [13, 47, 53]. In the periphery, release of SP leads to sensitization of surrounding primary afferents by enhancing cellular release of prostaglandins, histamines and cytokines [63, 72]. Recently, however, it has been demonstrated that SP also directly stimulates nociceptor endings [71]. CGRP, often co-localized with SP in unmyelinated C fibers, facilitates the release of SP and delays SP degradation in the spinal cord, thereby potentiating the nociceptive effect [38, 62, 77]. Sensory nerve fibers, interestingly, also seem to contain anti-nociceptive and anti-inflammatory peptides counteracting the effects of SP and CGRP. Thus, galanin (GAL), somatostatin (SOM) as well as opioids, all of which occurring in primary afferents, have been shown to inhibit inflammation and nociception [17, 20, 28, 68, 78]. These anti-nociceptive peptides probably exert their modulatory effect through inhibition of SP release [15, 25, 78, 79].

**Opioid neuropeptides**

The opioid neuropeptides and their receptors are, in similarity with sensory neuropeptides, produced by primary sensory neurons (dorsal root ganglia) and transported centrally and peripherally. The endogenous opioid peptides consist of three families (enkephalins, dynorphins and endorphins), which contain several biologically active products, e.g. Met-enkephalin-Arg-Pro (MEAP) and dynorphin B (DYN B), that exert physiological actions by interacting with opioid receptors. Enkephalins are thought to be the ligands for the δ-opioid receptor and dynorphins for the κ-receptor. Recent studies identified endomorphins as endogenous ligands.
for \( \mu \)-opioid receptors in addition to endorphins and the enkephalins. A fourth opioid receptor was also demonstrated, which is genetically closely related to the other receptors and responds to the endogenous agonist Nociceptin.

The endogenous opioids seem to provide a peripheral anti-nociceptive system. It was recently reported that intra-articular morphine in conjunction with arthroscopy had a significant analgesic effect in a dose-response manner (Likar et al. 1999). Notably, morphine inhibits SP release from peripheral sensory nerve endings [15, 79]. Thus, opioid receptors are therefore not only confined to the CNS, but also occur in the periphery, implicating that pain can be inhibited at a peripheral level [29]. Indeed, opioid receptors have been demonstrated on peripheral sensory nerve terminals of the skin [19, 66, 73]. As for tendons, the neuronal occurrence of endogenous opioids and receptors, has just recently been explored [2].

*Autonomic neuropeptides*

Autonomic B-fibers, in the periphery contain several mediators with sympathetic and parasympathetic effects. Neuropeptide Y (NPY) found in sympathetic fibers has been shown to be a potent vasoconstrictor. It often co-exists with the classical transmitter noradrenaline (NA) potentiating the vasoconstrictive action of NA [45]. In parasympathetic nerve fibers, vasoactive intestinal polypeptide (VIP) has been shown to be a potent vasodilator [44]. In addition to the well established vasoactive role of VIP, other important effects have been reported over the last years. Thus, VIP has been implicated in the regulation of immune cells and the expression of cytokines and growth factors.

*Neuropeptides in tendons*

As information on neuropeptide prevalence in connective tissue was largely unexplored, we studied the neuropeptide occurrence in tendons compared to ligaments [1].
With a combined approach based on radioimmunoassay (RIA), immunohistochemistry (IHC) and high performance liquid chromatography (HPLC), three main classes of neuropeptides: autonomic, sensory and opioid in normal rat Achilles tendon and medial collateral ligament have been detected. The combined quantitative and morphologic analyses have demonstrated 12 neuropeptides; sensory (SP, NKA, CGRP, GAL, SOM), opioid (LE, ME, MEAP, MEAGL, nociceptin), and autonomic (NPY, VIP) in tendons [1].

**Occurrence and levels of neuropeptides**

*Sensory peptides*

Overall, several studies have disclosed the occurrence of sensory neuropeptides in both animal and human tendons [4, 24, 43]. In tendons, sensory neuropeptides with nociceptive and pro-inflammatory effects (SP and CGRP) are present, as well as sensory modulatory neuropeptides (GAL, SOM), which are known to act anti-inflammatory. Thus, the relative concentrations of sensory mediators and modulators could suggest physiological nociceptive thresholds.

The concentrations of (SP, CGRP) vs. (GAL) were demonstrated about 4 times higher in tendons compared to ligaments [4], which might reflect a tissue specific vulnerability to pain (Fig. 1A). The levels of sensory peptides can be changed in response to outer or inner stress [33], in addition possibly explaining why pain syndromes in tendons are more common than in ligaments (Fig. 1A).

In fact, increased levels of sensory neuropeptides have been shown to be involved in painful and inflammatory conditions. Several studies have demonstrated increased levels of sensory peptides in patients with rheumatoid arthritis [50] and in tendons of rats with experimentally induced arthritis [14]. One report also showed increased levels of SP to correlate with the pain caused by rotator cuff disease [24]. Other studies demonstrated increased levels of the neurotransmitter glutamate in painful patellar and Achilles tendons of patients with tendinosis.
[8, 9], indicating increased neuronal activity. Glutamate is known to exist peripherally and to interact with SP in nociception [18]. SP has in fact been shown to potentiate the effect of glutamate [18, 42] and was also demonstrated in Achilles tendons of patients with tendinosis [7].

The occurrence of sensory neuropeptides in tendons complies with nociceptive effects, possibly in combination with traditional neurotransmitters, and the neuropeptide levels indicate a vulnerability to develop painful disorders.

**Opioid peptides**

A peripheral neuronal source of anti-nociception to modulate the sensory system could be the endogenous opioid neuropeptides. Notably, data on opioids in the peripheral nervous system and specifically tendons are quite scarce. However, an opioid system in the Achilles tendon of rats was recently detected (Fig. 1B) [2]. This was the first observation on the occurrence of opioids including nociceptin in periarticular tissues. The results clearly demonstrated the existence of opioid peptides (enkephalin and nociceptin) in the tendon.

The physiological role of enkephalins like MEAP can be assumed to be anti-nociceptive [17, 46], anti-inflammatory [31, 39], vasodilatory [23, 52], immunosuppressive [16] and trophic [75, 80]. Notably, the release of SP from afferents in the cat knee joint is inhibited by intra-articular enkephalin-analogue injections [79]. Also nociceptin has been reported to reduce mechanosensitivity in the rat knee joint through inhibition of SP release [48, 49].

The lower concentrations of MEAP and nociceptin in tendons compared to ligaments (Fig.1B) could reflect a greater susceptibility to pain and inflammation, in particular when considering also the high levels of SP found in tendons. These combined data suggest that there is a balance between nociceptive and anti-nociceptive peptides under normal conditions. This balance may be altered under pathological conditions.
**Autonomic peptides**

The presence of autonomic peptides has been demonstrated in tendons of both animals [5] and humans [43]. The autonomic peptides, which appear in sympathetic (NPY) and parasympathetic (VIP) nerve fibers, are known to exert opposite effects in the regulation of vasoactivity.

One study showed that the concentration of NPY compared to VIP was 15-times higher in ligaments and twice as high in tendons (Fig. 1C). The observation would seem to reflect that the sympathetic tone is higher than the parasympathetic, and specifically higher in ligaments than in tendons. Such a difference between tissues may be assumed to be of importance for blood-flow, and hypothetically for healing capacity, possibly also for susceptibility to degeneration and injury. Notably, ligaments are more prone to rupture than tendons [76].

Altogether, quantitative analysis of tendons has clearly demonstrated the occurrence of autonomic, sensory and opioid peptides presumed to be involved in nociception, inflammation and vasoactivity. The relative levels of counteracting neuropeptides may be assumed to represent the tissue homeostasis of different functions and, possibly the susceptibility to external stress. In fact, increased levels of pro-nociceptive sensory mediators have been shown in painful tendon conditions.

**Morphological distribution of neuropeptides**

Morphological studies by immunohistochemistry have verified the neuronal occurrence of 11 neuropeptides.

**Sensory fibers**

Nerve fibers immunoreactive to SP, CGRP, NKA, GAL and SOM were consistently identified in the Achilles tendon of the rat [4]. The highest fiber density was observed in the
surrounding tissues, i.e. the paratenon and loose connective tissue (Fig. 2). The ratio of vascular vs. non-vascular fibers was higher in the surrounding loose connective tissue than in the paratenon, which exhibited a large number of non-vascular free nerve endings (Fig. 2C). Thus, the abundance of immunopositive vascular fibers in the surrounding loose connective tissues may reflect an important role in the regulation of blood flow to the proper structures. Both SP and CGRP, in particular the latter, are potent vasodilators [13]. In addition, they have pro-inflammatory effects, e.g. by enhancing protein extravasation and leukocyte chemotaxis. The occurrence of sensory free nerve endings unrelated to vessels predominantly seen in the paratenon suggests a nociceptive role.

**Opioid peptides and receptors**

Morphological studies demonstrated the neuronal presence of several enkephalins, i.e. LE, ME, MEAP and MEAGL, in peripheral nerve fibers of the rat Achilles tendon (Fig. 3D) [2]. All four enkephalins predominantly occurred in the loose connective tissue, the paratenon and musculo-tendinous junction, and no enkephalins were found in the tendon proper. Hypothetically, this difference in anatomical distribution might suggest that regulation of painful disorders of the Achilles tendon mainly occurs in the surrounding tissues, which during normal condition also harbor the sensory and autonomic neuropeptides. The existence of an opioid system in connective tissues, as demonstrated by neuronal immunoreactivity to enkephalins, was supported by opioid receptor studies based on binding assays and immunohistochemistry [2]. Of the three opioid receptors (δ-, κ-, μ-) studied, however only the δ-opioid receptor (DOR) could be detected by immunohistochemistry (Fig. 3D). Double staining disclosed co-existence of each of the enkephalins with DOR in the nerve fibers (Fig. 3D), which is in accordance with other studies suggesting that enkephalins are the main ligands for DOR [21]. DOR exerts a potent inhibitory effect on SP-release [30, 79].
There are several reports demonstrating that treatment with delta opioid agonists in the periphery elicit both anti-inflammatory and anti-nociceptive effects in models of inflammation [55, 69, 81]. Presumably, the co-existence of enkephalins and DOR in vascular nerve fibers reflects inhibition of neurogenic pro-inflammatory actions, whereas the co-existence in free nerve endings reflects inhibition of nociception.

The present findings suggesting a neuronal source of opioids in the periphery probably reflect an anti-nociceptive system in tendons, which may be exploited in the therapeutical setting by drugs acting selectively in the periphery.

*Autonomic fibers*

Both sympathetic (NPY, NA) and para-sympathetic (VIP) fibers were identified in the tendon by immunohistochemistry [5]. The autonomic fibers were mostly observed as networks around blood vessels located in the loose connective tissue around the tendon proper (Fig. 2). Over all, the observations seem to reflect that the regulation of blood flow to tendons predominantly occurs in the adjacent loose connective tissue.

Altogether, there seems to exist a complex neuropeptidergic network in tendons with a specific ratio of potentiating and inhibitory mediators. The observations may well reflect unique tissue requirements under normal conditions including the susceptibility to stress. The most conspicuous findings pertained to the abundance of sensory fibers in the surrounding tissues of the tendon, whereas the tendon proper, notably, was almost devoid of nerve fibers (Fig. 2). This would seem to reflect that the neuronal regulation of tendons highly depends on the innervation of the surrounding tissues.
Neuronal response after tendon injury

Having established the normal occurrence of nerve fibers and their expression of different neuropeptides mainly localized in the surrounding structures of the tendon, i.e. the paratenon, this section aims to describe the distribution of nerve fibers and their expression of neuropeptides after injury and during healing of tendinous tissue. In addition to cytokines and growth factors, neuropeptides in the periphery may participate in tissue repair [12, 27, 57, 61].

Neuropeptides and repair

Neuropeptides elicit and convey responses to stress and injury [33], responding very specifically both in the central- and peripheral nervous system to peripheral injury. After peripheral nerve lesion, a number of studies show that the expression of different neuropeptides follows a specific temporal pattern in accordance with the healing phases [36,59]. Moreover, administration of SP and CGRP to open wounds accelerated healing time. This would seem to reflect a regulatory role of neuropeptides in response to injury, which possibly also could play a role in tendon repair.

New nerve fiber ingrowth after injury

Both human [24, 60, 70] and animal studies [10, 22, 51, 58] indicate specific nerve fiber response to tendon injury, where nerve fiber ingrowth often is associated with increased experience of pain. Our studies of tendon injury specifically addressed the question of nerve regeneration during healing of experimental Achilles tendon rupture. Nerve regeneration at different time points (1-16 weeks) was investigated by immunohistochemistry including semi-quantification of neuronal markers for new and mature fibers [3].

In the first week post rupture, corresponding to the inflammatory phase, there was increased nerve fiber activity in the surrounding loose connective tissue. From one to six weeks post rupture (regenerative phase), there was a striking shift in neuronal occurrence from the
surrounding loose connective tissue into the rupture site of the tendon proper (Fig. 4-5), in normal conditions notably devoid of nerve fibers. The peak expression of new nerve fibers in the rupture site occurred between weeks two and six, while mature nerve fibers were seen somewhat later, between weeks four and six. During weeks 8 to 16 post-rupture (remodeling phase), the nerve fibers decreased significantly at the rupture site (Fig. 4). They successively returned to normal in the paratenon and surrounding loose connective tissue - reflecting the plasticity of the peripheral nervous system.

In summary, tendon injury is characterized by nerve fiber ingrowth at the rupture site, a peak nerve fiber expression during the regenerative phase followed by nerve fiber withdrawal during the remodeling phase. The ingrowth and later withdrawal of nerve fibers in the tendon proper after rupture indicate that a neuronal supply is fundamental for tissue healing. Presumably, new nerve ingrowth is a prerequisite for delivery of neuronal mediators required for tissue repair.

**Temporally orchestrated neuropeptide occurrence during repair**

So far there are very limited data on the peripheral expression of neuropeptides after injury of musculo-skeletal tissues. Increased expression of SP and CGRP two weeks post injury has been observed in fracture- and skin healing [32, 37, 41]. As for ligament healing Grönblad et al. studied SP and CGRP expression 4 and 14 weeks after rupture and found an increase at week four and a decrease at week 14 [26]. Altogether, the temporal expression of neuropeptides indicates their involvement in not only the acute response to injury, but also in tissue regeneration.

Bearing this in mind, we studied the temporal expression of specific neuropeptides at different time points (1-16 weeks) post-rupture using the same experimental Achilles tendon rupture as mentioned above. The study entailed morphological and semi-quantitative analyses by
immunohistochemistry of sensory (SP, CGRP), sensory modulating (GAL), and autonomic (NPY, VIP) neuropeptides [1, 6].

One week post-rupture (inflammatory phase), SP and CGRP fibers were predominantly located in blood vessel walls surrounded by inflammatory cells in the loose connective tissue (Fig. 7A). This complies with a nociceptive and pro-inflammatory role. During week two to six (regenerative phase), the expression of SP and CGRP peaked (Fig. 6). Notably, this peak occurred at the rupture site of the tendon proper, seen as free sprouting nerve endings among fibroblasts and newly formed vessels (Fig. 7B), which may prove to reflect a role for SP/CGRP in cell proliferation. Up to week four the occurrence of GAL, NPY and VIP was sparse. Subsequently, the expression of GAL, NPY and VIP increased to reach a peak at the end of the regenerative phase, around week six, followed by their successive decrease, SP and CGRP included, till the end of the experiment (week 16) (Fig. 6). The early appearance of SP and CGRP in tissues surrounding the tendon would seem to comply with a nociceptive and pro-inflammatory role, whereas the later occurrence in the rupture site may prove to reflect a role in cell proliferation. The emergence of GAL, VIP and NPY, in the early remodeling phase, in addition to regulation of vasoactivity, represents modulatory effects on SP and CGRP. This modulation may be required to switch from the nociceptive, inflammatory and regenerative processes to the remodeling phase.

Summary

In summary, tendinous tissues are supplied with a complex network of neuronal mediators, which may be assumed to be involved in the regulation of nociception, vasoactivity and inflammation. In response to injury, the expression of neuropeptides is significantly altered in a temporally orchestrated manner suggesting a well-synchronized mechanism in nociception and tissue repair.
The relevance of the present findings pertains to the possibility of intervening in different efferent mechanisms of the peripheral nervous system, which presumably are implicated in a wide variety of functions such as vasoactivity, nociception, inflammatory responses and regeneration. Specific neuronal intervention, however, presupposes that the innervation of the musculoskeletal system according to specific mediators is clarified. Tendons are supplied with a complex peptidergic network, which presumably takes part in maintaining tissue homeostasis. Interestingly, ingrowth of new nerves seems to be a fundamental feature of tissue repair.

Whether neuronal mediators could be utilized in tissue engineering has so far not been explored. As for pharmacotherapy, it may prove that this can be targeted to specific neuronal mediators to enhance or inhibit their effects in painful conditions of the tendons.
References

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FIGURE LEGENDS

Figure 1A-C: Radioimmunoassay tissue concentrations (pmol/g) of sensory (SP, CGRP and GAL) (A), opioid (MEAP, N/OFQ and DYN B) (B) and autonomic (NPY and VIP) (C) neuropeptides in the Achilles tendon and collateral ligaments of rat knee (mean ± s.e.m.).

Figure 2A-C: Overview micrographs of longitudinal sections through the Achilles tendon built up by putting together computerized images of smaller micrographs. Incubation with antisera to a general nerve marker (PGP). Micrographs depict the proximal half of the Achilles tendon at increasing magnification in figures (A-C). Arrows denote varicosities and nerve terminals. The typical vascular localization of NPY is depicted in (B), whereas the free nerve endings are typical localization of SP (C). The immunoreactivity is seen in the paratenon and surrounding loose connective tissue, whereas the proper tendinous tissue, notably, is almost devoid of nerve fibers. Pt = paratenon.

Figure 3 A-D: Immunofluorescence micrographs of longitudinal sections through the Achilles tendon after incubation with antisera to NPY (A), VIP (B) and double staining (co-localization) of SP and CGRP (C), LE and DOR (D). The NPY-positive fibers are arranged as nerve terminals in the vessel walls. VIP-positive nerves are arranged as a “fence”, surrounding the proper tendon, of small varicosities in the paratenon. A co-existence of SP and CGRP is seen in the paratenon, indicating possible pro-inflammatory actions. The immunoreactivity displaying co-existence of LE and DOR is seen as free nerve endings in the paratenon, which indicates a potential peripheral anti-nociceptive system. t = tendon tissue; Pt = paratenon; Bar = 50 µm.

Figure 4: Area occupied by nerve fibers (%) immunoreactive to GAP (regenerating nerve fibers) and...
PGP (mature nerve fibers) in relation to total area, in the mid third of the tendon, over 16 weeks post rupture (mean±s.e.m.).

Figure 5A-B

Overview micrographs of longitudinal sections through the Achilles tendon two weeks post rupture built up by putting together computerized images of smaller micrographs. Incubation with antisera to a nerve growth marker, GAP-43. Micrographs depict the proximal half of the Achilles tendon at increasing magnification in figures (A-B). Arrows denote varicosities and nerve terminals. The GAP-positive fibers, indicating new nerve fiber ingrowth, are abundantly observed in the healing proper tendon tissue. Some GAP-fibers are also located in the loose connective tissue, however most are localized as free nerve endings within the healing proper tendon tissue.

Figure 6

Area occupied by nerve fibers (%) immunoreactive to SP, CGRP and GAL in relation to total area, in the mid third of the tendon, over 16 weeks post rupture (mean±s.e.m.).

Figure 7A-B

Immunofluorescence micrograph of longitudinal sections through healing Achilles tendon 1- (A) and 2- (B) weeks post rupture after incubation with antisera to CGRP. Nerve fibers immunoreactive to CGRP are seen as vascular and free nerve endings in the loose connective tissue (A). CGRP- immunoreactivity occurs mainly in the healing tendinous tissue as sprouting free nerve fibers. v = blood vessel; lct = loose connective tissue; t = proper tendon tissue; Bar = 50 µm.

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