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NGF-evoked sensitization of muscle fascia nociceptors in humans

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ABSTRACT

Nerve growth factor (NGF) induces local hyperalgesia for a few days after intramuscular injection, but longer-lasting muscle pain upon systemic administration. As the muscle fascia is densely innervated by free nerve endings, we hypothesized a lasting sensitization of fascia nociceptors by NGF. We administered 1 μg NGF (dissolved in 100 μL saline) ultrasound-guided to the fascia of the *Musculus erector spinae* muscle at the lumbar level of 14 male volunteers and assessed hypersensitivity after 6 hours, and 1, 3, 7, 14, and 21 days. Pain upon mechanical stimuli (constant pressure and dynamic impact), upon exercise and electrically induced *M. erector spinae* contraction, and upon injection of 100 μL phosphate buffer pH 4 (at day 7 and 14 only) to the fascia of both NGF- and saline-treated muscles, was investigated. Injections into the muscle fascia did not cause acute pain. Local heat pain thresholds were unchanged following NGF and saline (control) administration. NGF evoked a lasting (days 1-7) and significant reduction of pressure pain, pressure thresholds, exercise-evoked muscle pain, and hyperalgesia to impact stimuli (12 m/s). Pain upon injected protons was significantly elevated ($P < 0.04$) for 2 weeks. NGF induced a sensitization of the muscle fascia to mechanical and chemical stimuli lasting for up to 2 weeks. As nociceptors in the fascia appear to be particularly prone to sensitization, they may contribute to acute or chronic muscle pain.

1. Introduction

Mechanical hypersensitivity is a cardinal symptom of acute and chronic muscle pain. Thin unmyelinated nociceptors of the muscles have been investigated intensely for their contribution to muscle pain [10,11,16]. Muscle nociceptors and low-threshold mechanosensitive muscle afferents can be excited by inflammatory mediators such as adenosine triphosphate or tissue acidification [10]. Only nerve growth factor (NGF) exclusively activated the nociceptors [11]. Interestingly, intramuscular NGF injection caused sensitization also at spinal level, with increased responses of second-order neurons to electrical stimulation of the peripheral nerve [12,34]. The injection of tumor necrosis factor into the gastrocnemius muscle increased intramuscular NGF levels and reduced muscle pressure thresholds for at least 1 day [24], further indicating the putative role of NGF in muscle pain. Noteworthy, muscle soreness is dependent on the availability of TrkA, the high-affinity receptor for NGF, and hyperalgesic behavior is increased by enhanced NGF expression [9].

The clinical implications of NGF have been convincingly shown by profound analgesic effects of NGF-capturing antibodies (Tanezumab; Pfizer Inc, New York, NY, USA) in the treatment of patients with low back pain [13]. Intramuscular injection of NGF in humans does not elicit acute pain, but causes mechanical hyperalgesia lasting several days [1,19,31,33]. Following systemic application of NGF, generalized muscle pain was reported [2]. The time course of sensitization contrasts that of mechanical hyperalgesia following intracutaneous NGF injections, which lasts several weeks [5,22]. Apart from tissue-specific sensitization patterns including central and peripheral mechanisms, the different time courses between dermal and muscular NGF responses might also be explained by sensitization of nociceptors innervating structures other than skeletal muscle. Of the different muscle tissue structures, particularly the fascia – having been under-investigated in muscle pain research during the last decades – may be a source for muscle nociception and chronic back pain. The muscle fascia has a dense neuronal (PGP9.5-positive) innervation with nonpeptidergic nerve fiber endings [35] and encapsulated mechanoreceptors [29,38]; it determines a "viscoelasticity" that modifies the activation of these receptors inside the fascia [30], and it is functionally integrated within the muscle tissue providing smooth gliding properties [15], but has less blood supply than the muscle and therefore, slower healing properties [25], all of which had been postulated to be relevant for clinical chronic back pain [25,27,30]. Experimentally induced muscle soreness by eccentric exercise

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elicits more pain upon hypertonic saline injected into the fascia overlaying the over-exercised muscle as compared to intramuscular injections [7]. Eccentric muscle exercise might cause elevated NGF levels that finally contribute to an enhanced sensitivity of the fascia.

Hypothesizing a particular sensitivity of the muscle fascia, we examined NGF sensitization of this tissue, which might possibly be linked to chronic muscle pain. We studied the time course of local hyperalgesia following an ultrasonic-controlled injection of NGF into the *Musculus erector spinae* fascia at lumbar level (L4-L5) in human subjects. Additionally, we explored whether NGF also had sensitized acid-sensing ion channels (ASICs) or transient receptor potentials (TRPV1) by injection of phosphate buffer (pH 4) into the pretreated fascia sites.

2. Methods

The local Ethics Committee of the Medical Faculty Mannheim, University of Heidelberg, approved the experimental protocol on human volunteers according to the Declaration of Helsinki. Fourteen healthy Caucasian male volunteers (mean age 24 ± 3 years) without history of low back pain (body mass index 22.5 ± 2 kg/m^2) participated in the study after having signed the written informed consent form. Prior to the NGF and saline administration, all volunteers underwent a training session to become familiarized with the test procedures and to acquire a "baseline quantitative sensory test." None of the volunteers withdrew from the study prematurely.

2.1. NGF administration

Injections of 1 μg human recombinant lyophilized NGF (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), dissolved in 100 μL isotonic 0.9% saline (Braun, Melsungen, Germany), were administered into the fascia of the *M. erector spinae* at lumbar level (L4-L5) and about 5-6 cm lateral to the midline. Injections of 100 μL 0.9% saline into the fascia at the contralateral site served as reference control. The fascia was precisely identified by ultrasound (Acuson X150; Siemens, Munich, Germany) (Fig. 1A) and solutions were administered under ultrasound control using a 27-g cannula and a 1-mL tuberculin syringe (Becton-Dickinson, Heidelberg, Germany) (Fig. 1B).

Volunteers were blinded to the substances injected into the fascia. The injection sites were marked with a felt-tip pen. Both labels were transferred to a translucent acetate sheet for future identification, particularly for the ultrasound-guided injections of the phosphate buffer pH 4 at day 7 and 14 (Fig. 1C).

Noteworthy, within day 1 to 3 of the investigation, the researchers and volunteers were no longer blinded due to the site differences of pain perception.

2.2. Experimental protocol

The test series described below were performed in randomized order at the saline and NGF sites at 6 hours and 1, 3, 7, 14, and 21 days after injections into the fascia. These tests included a variety of mechanical stimulation protocols, which were newly developed in order to specifically assess sensitization processes of the muscle fascia upon mechanical load and upon muscle contraction. Following the mechanical tests, 100 μL phosphate buffer (pH 4) was injected under ultrasound control into the fascia of the *M. erector spinae* at the NGF- and saline-pretreated sites at day 7 and 21 in order to estimate for TRPV1 sensitization. Thermal threshold tests of fascia nociceptors could not be performed for technical reasons.

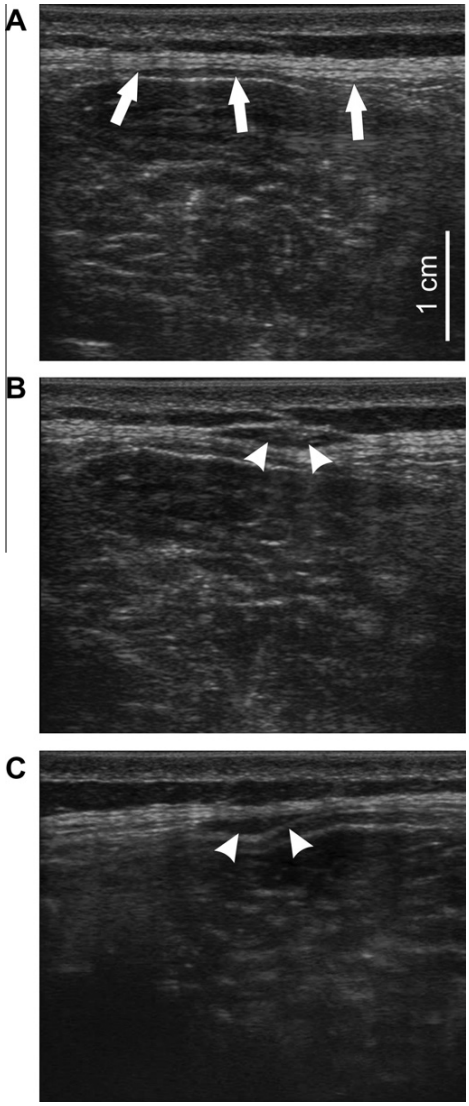

Fig. 1. Ultrasound images of a specimen depicting the fascia of the *Musculus erector spinae* (white arrows) before (A) and immediately after the injection of 100 μL nerve growth factor (B) and 100 μL phosphate buffer pH 4 (C). The edges of the resulting "blebs" located in the fascia are marked by arrowheads.

2.3. Exercise of paravertebral lumbar muscles

Prior to the test series, the volunteers were interviewed about movement-related pain at the injection sites. They were instructed to extend the lumbar spine actively by bending forward (touching toes with fingertips), rotate their upper body maximally left and right, and perform lateral flexion to stretch and activate paravertebral muscles. Any signs of discomfort or pain occurring during these movements were documented. Thereafter, volunteers lay on a bench (face down) for the remaining tests.

2.4. Mechanical impact pain

In order to assess mechanical impact pain, a cylindrical plastic bullet (12-mm height, 5-mm diameter, 0.5-g weight) was accelerated computer-controlled in a guiding barrel (length 8 cm) to 12 m/s and directed perpendicularly towards the skin surface at the injection sites. For a detailed description of this stimulation

method, see [14]. Volunteers were asked to rate the magnitude of impact pain on a visual analogue scale (VAS; Somedic, Hörby, Sweden) with the endpoints 0 (no pain) and 100 (worst pain imaginable). Impact stimuli were repeated 3 times for each site in 10-second intervals, and averaged VAS values were calculated for statistical analysis.

2.5. Mechanical pressure pain

A series of mechanical pressure sensitivity tests were performed to specifically assess sensitivity of muscle and fascia.

First, a handheld algometer (Somedic) equipped with a 9-mm rounded probe (cylindrical with a spherical tip, similar to [6]) was employed to deliver a constant force of 150 kPa for 10 seconds to the NGF- and saline-pretreated sites. The rate of application and static pressure was manually controlled, keeping the displayed values in a range between 145 and 155 kPa. Maximum perceived pain was recorded on a VAS (Somedic).

Secondly, pressure pain thresholds were assessed by delivering increasing forces (approximately 30 kPa/s) with the algometer (Somedic; 9-mm rounded probe) until the volunteers indicated the perception of pain. Assessments were repeated 3 times at each pretreated fascia site in 15-second intervals, and mean pressure pain thresholds were recorded for statistics.

Thirdly, tonic pressure pain was also tested using a larger stimulation surface (convex arc of a circle, radius 42.5 mm, length 67 mm, and width 50 mm) attached to an algometer (Wagner Instruments, Greenwich, CT, USA). A force of 1.79 N/cm^2, or 17.9 kPa, respectively, was applied for 10 seconds to the convex arc circle attached to the skin surface overlaying the muscle fascia by delivering a constant pressure of 60 N (Wagner Instruments). Volunteers rated the pressure-induced pain on a VAS (Somedic), and tests were repeated 3 times in 15-second intervals per site.

2.6. Pain upon muscle contraction during tonic pressure

Since pilot experiments revealed that tonic pressure was more painful when the underlying muscle was contracted, we combined tonic pressure stimulation with lumbar muscle exertion. A force of 150 kPa (algometer with 9-mm rounded probe, Somedic) was delivered to the skin above the injection sites and volunteers were asked to move their ipsilateral leg up and down by lifting it 30 cm from the surface and to lower it back subsequently. This exercise was repeated 3 times in 10-second intervals while the leg was kept relaxed in between. Volunteers estimated their muscle pain perceived during the 3 leg lifts on a VAS (Somedic). Possible increases of the tonic pressure stimulus during the muscle contraction were recorded.

2.7. Pain upon electrically induced muscle contraction

Two electrocardiogram electrodes of 15 mm diameter (Tyco Healthcare, Bayern, Germany) were attached to the skin surface of the *M. erector spinae* equidistantly at 2.5 cm cranial and caudal from the NGF and saline injection sites, respectively. Electrocardiogram electrodes were connected to a constant current stimulator (Digitimer DS7, Hertfordshire, UK) attached to a pulse generator (PG1; Rimkus, Parsdorf, Germany). Electrical pulses (width 0.5 ms) were delivered at 2 Hz with increasing intensity. Volunteers were instructed to indicate when the electrical pulses were perceived and when they induced pain. Electrical pulses were further increased (~5 mA per second) until muscle twitches were observed. Following a resting period of 15 seconds, this current intensity that individually induced the muscle twitch was delivered continuously for 10 seconds (2 Hz, pulse width 0.5 ms) and the corresponding volunteers' pain sensation recorded on the VAS (Somedic). For each day and each site of investigation, the individual stimulus intensity that was required to induce a muscle twitch was determined.

2.8. Heat pain thresholds

To control for sensitization of skin nociceptors by NGF, heat pain thresholds were recorded by a 25 × 50 mm Peltier thermode (Somedic, Sweden) attached to the skin surface of both injection sites. The surface temperature was increased from 32°C at a rate of 1°C per second (SenseLab, Somedic) until the volunteer indicated heat pain by pressing a handheld switch. At that time, the temperature value was stored on a computer and the temperature was immediately decreased to 32°C. Thermal thresholds were assessed 3 times with 10-second intervals in between, and average heat pain temperature values were calculated for statistical analysis.

2.9. Pain upon phosphate buffer pH 4

An approved pharmacist provided sterile phosphate buffer pH 4 solutions, of which 100 μL was administered under ultrasound control to the fascia of the *M. erector spinae* at both NGF- and saline-pretreated sites at days 7 and 14 of the study (Fig. 1C). The NGF and saline sites were exactly identified by means of the labels transferred to the acetate sheet. Volunteers rated pain intensity using an 11-point numerical rating scale with 2 anchor points, 0 (no pain) and 10 (worst pain imaginable). The subjects were asked to rate pain intensity at 2-second intervals within the first 10 seconds, and thereafter in 5-second intervals for another 50 seconds. This narrow time investigation of proton-induced pain required the use of a numerical rating scale instead of the previously mentioned VAS.

The phosphate buffer was injected at the end of the investigation in order to rule out that possible hyperalgesic responses may confound the experimental protocol.

2.10. Statistics

Data were compiled in Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA) and analyzed using Statistica 7.0 software package (StatSoft, Tulsa, OK, USA). Significant differences (P-level 0.05) were evaluated by analysis of variance (ANOVA) with the factorial groups "substance" (NGF vs saline) and "time" of investigation, followed by Fisher's least significant difference post hoc test. All values are depicted as means ± SEM.

3. Results

Injection of saline or NGF into the fascia did not evoke acute pain. Rotation and flexion of the lower back caused a sensation of discomfort at the NGF application sites at 6 hours and at days 1 and 3, but volunteers reported this sensation as not disturbing or irritating during activities of daily life.

3.1. Sensitization to mechanical stimuli

Baseline quantitative sensory tests were performed during the training session, and these did not differ significantly from those recorded at the saline injection site, at which the impact stimulation evoked a sensation of VAS 9.2 ± 0.8 at 6 hours to VAS 5.8 ± 0.6 at day 21 ($P > 0.05$, ANOVA). The pain sensation had a pounding quality. At the NGF site, impact pain was significantly elevated as compared to saline control ($P < 0.03$, ANOVA; Fisher's least significant difference post hoc test) with maximum responses of VAS 13.8 ± 1.4 at day 1 and VAS 13.4 ± 1.8 at day 3 (Fig. 2A).

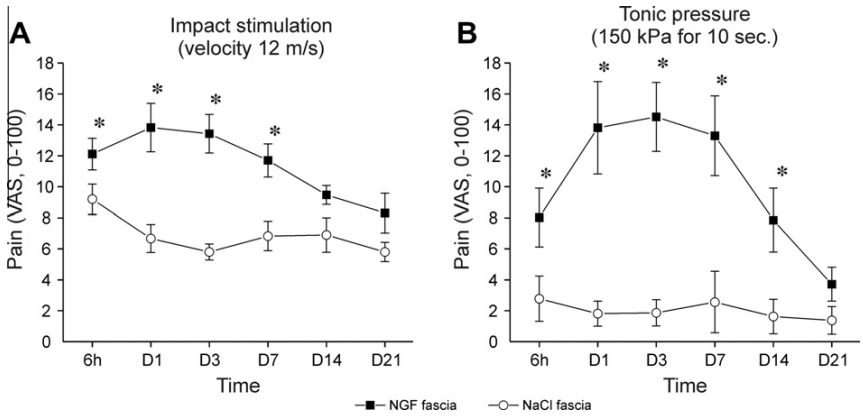

Fig. 2. Pain induced by mechanical impact stimulation (velocity 12 m/s; (A) and tonic pressure (150 kPa for 10 seconds); (B) recorded from muscle fascia at 6 hours, and 1, 3, 7, 14, and 21 days after injection of 1 μg nerve growth factor (solid symbols) and 0.9% NaCl (open symbols) into the contralateral fascia of the *Musculus erector spinae*. Significant differences between the pretreated sites are indicated by asterisks (analysis of variance, Fisher's least significant difference post hoc test; $P < 0.05$).

The pressure pain sensation thresholds assessed with the rounded 9-mm algometer probe decreased significantly at day 1 from 881 ± 147 kPa at the saline-treated site to 392 ± 56 kPa after NGF administration ($P < 0.03$, ANOVA). Accordingly, mild pressure of 150 kPa caused virtually no pain at the saline and NGF fascia. However, the magnitude of pressure-induced pain was perceived significantly stronger at the NGF sites ($P < 0.007$, ANOVA) at day 1-7 with a VAS of 13.9 ± 2.6 on average (Fig 2B). Delivering a constant pressure of 17.9 kPa via a convex arc covering the pretreated muscle fascia also evoked an altered pain response. On average, at the saline-treated sites, perception was recorded at VAS 1.2 ± 0.8 throughout the 21-day observation period, whereas at the NGF sites, sensation was elevated significantly ($P < 0.02$, ANOVA) at day 1-3 (average VAS 8.2 ± 2, data not shown).

3.2. Pain upon muscle contraction

The tonic pressure of 150 kPa directed to the injection sites was slightly increased when the subjects were lifting their ipsilateral leg by 30 cm, thereby contracting their *M. erector spinae*. The peak force measured during lifting the ipsilateral leg was 169 ± 3 kPa at the saline site and 183 kPa ± 9 kPa at the NGF site ($P > 0.05$; ANOVA). The pain during leg lifting was significantly enhanced at the NGF injection sites (VAS 22 ± 3 up to day 7) as compared to the saline-treated fascia, at which the leg lifting was perceived as basically pain-free (VAS 2 ± 1.3, $P < 0.002$, ANOVA) (Fig. 3A).

Electrical stimuli of 22 ± 2 mA were applied to provoke twitches of the *M. erector spinae* at the injection sites. The perceived pain recorded during the 10 seconds of electrically induced muscle contractions did not differ significantly between the fascia pretreatments ($P > 0.3$, ANOVA) and was, on average, recorded at VAS 27 ± 10 for saline and VAS 38 ± 6 for the NGF sites (for time-course, see Fig. 3B).

3.3. Sensitization to chemical stimuli

Phosphate buffer solutions (100 μL, pH 4) were administered to the fascia pretreated with saline and NGF at day 7 and 14. Pain intensity was maximal during the proton injection and gradually declined throughout the 1-minute recording period (Fig. 4). Pretreatment with NGF enhanced proton-induced pain at 7 and 14 days after the injection ($P < 0.04$, ANOVA), particularly within the initial 30 seconds after administration of the low pH solution (Fig. 4).

3.4. Heat pain thresholds

We additionally assessed the heat pain thresholds of skin nociceptors to control for possible sensitization by NGF diffusing from

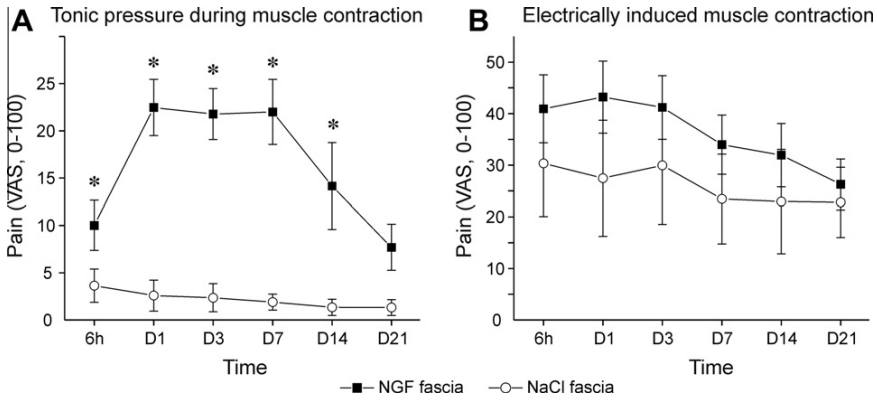

Fig. 3. Pain induced by tonic pressure during active muscle contraction (leg lift) (A) and upon electrically induced contraction of the muscle (B) at 6 hours, and 1, 3, 7, 14, and 21 days after injection of 1 μg nerve growth factor (solid symbols) and 0.9% NaCl (open symbols) into the contralateral fascia of the *Musculus erector spinae*. Significant differences between the pretreated sites are indicated by asterisks (analysis of variance, Fisher's least significant difference post hoc test; $P < 0.05$).

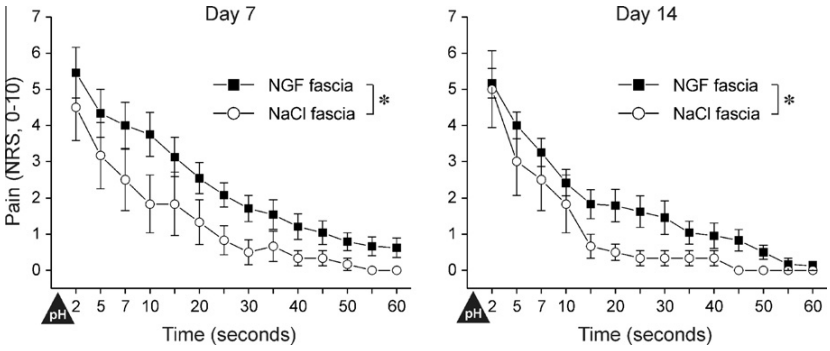

Fig. 4. Pain induced by the injection of 100 μL acidic solution (phosphate buffer pH 4) into the fascia of the *Musculus erector spinae* at day 7 (left panel) and day 14 (right panel) after administration of 1 μg nerve growth factor (solid symbols) and 0.9% NaCl (open symbols). Significant differences between the pretreated sites are indicated by asterisks (analysis of variance, Fisher's least significant difference post hoc test, $P < 0.05$). Note that pain upon proton injection was recorded at short intervals (2, 5, 7, and 10 seconds and thereafter, every 5 seconds), which demands a numeric rating scale (NRS) instead of a visual analogue scale.

the injection sites in the fascia toward the skin nerve fiber endings. At both injection sites, a reduced heat pain threshold of 42 ± 0.9°C (saline) and 42 ± 0.3°C (NGF) was recorded at 6 hours. Heat pain thresholds continuously increased up to 44 ± 0.5°C (saline) and 44 ± 0.6°C (NGF) at 2 weeks after the injections. Changes of thermal thresholds were virtually identical at the saline and NGF injection sites ($P > 0.6$, ANOVA) throughout the entire 21-day observation period (data not shown).

4. Discussion

We injected NGF into the fascia of the *M. erector spinae* at lumbar level (L4-L5) and observed a long-lasting sensitization to mechanical pressure and to chemical stimulation with acidic solution. Sensitization was confined to deeper tissues, as we did not observe heat sensitization of the skin. Sensitization to brush or light touch can be precluded due to our previous observations [22]. The present data suggest that NGF sensitizes nociceptors terminating in the muscle fascia. Their specific assessment required the development of mechanical sensitivity test protocols. Even though some of the test procedures were not used before, our data provide evidence that the sensitization of muscle fascia nociceptors to mechanical and chemical stimuli may contribute to the pathophysiology of chronic musculoskeletal pain.

4.1. NGF-evoked nociceptor sensitization of the muscle fascia

NGF has been reported to exert substantial sensitizing effects on skin and muscle nociceptors in response to mechanical, thermal, chemical, and electrical stimuli [20,22,31,32]. A dense network of nociceptors was also found in the fascia [35,38], which is a collagen- and elastin-containing structure separating the contractile elements of the muscle from neighboring tissue.

Upon injection into the fascia, NGF might also have reached the superficial skin layers. Sensory tests of hypersensitivity employing contact heat stimuli activate nociceptors of the uppermost skin layers [36]. Furthermore, mechanical impact stimuli cause an activation of superficially located skin nociceptors. After injection of NGF into the dermis of forearm skin, both mechanical pinprick and impact stimuli, as well as heat, cause an intense hyperalgesia peaking at about 1 week for heat and 3 weeks for mechanical responses [5,22]. While supra-threshold mechanical impact stimuli (12 m/s) delivered to the NGF-treated fascia evoked elevated pain, but supra-threshold heat stimuli applied to the overlaying skin of the NGF-injected fascia did not provoke heat hyperalgesia, we assume that the NGF did not diffuse into the superficial skin layers. Independent of NGF, we found a short-lived heat hyperalgesia at both investigation sites 6 hours after injection of the substances, which is most probably linked to the injury-induced release of inflammatory mediators, for instance, prostanoids from keratinocytes [4].

4.2. Hypersensitivity to constant pressure and contraction-related pain

Pressure pain can be evoked in absence of cutaneous sensory input and is mediated by group III and IV muscle-afferent fibers [8]. Here, pressure pain thresholds were reduced after NGF and pain ratings of static pressure significantly increased. Interestingly, when the tested muscle was contracted during the tonic pressure stimulus, pain increased. This indicates that the sensitized free nerve fiber endings within the muscle fascia are stimulated more effectively when the fascia is "pre-stretched" by the muscle contraction.

We did not find signs of muscle soreness or movement-related pain, as reported before in masseter or tibial muscle after intramuscular injection of 5 μg NGF [1], but volunteers still reported a sensation of discomfort. These differences between the studies might be due to the dose of administered NGF (1 vs 5 μg). In addition, local muscle contraction evoked by electrical stimulation was not perceived as more painful at the NGF site. Therefore, it can be suggested that 1 μg NGF had not sensitized the fascia sufficiently to cause pain upon bending/stretching or electrically induced muscle contraction.

4.3. Hypersensitivity to acidic solutions

Acidic pH activates and sensitizes muscle nociceptors [10,17,21] most likely via ASICs and TRPV1. Accordingly, increased responses to acidic pH might indicate sensitization of ASICs and TRPV1. Here, we recorded increased pain upon acidic phosphate buffer administered to muscle fascia at the NGF sites, as compared to the saline-treated fascia for about 2 weeks. In accordance with a recent study investigating enhanced NGF-mediated heat responses of trigeminal neurons [26], the present experimental data suggest that NGF might cause facilitated activation of muscle fascia ASIC3 and/or TRPV1 receptors [28,37]. Unfortunately, selective activation of muscle fascia TRPV1 by heat cannot be performed easily.

Interestingly, in the human muscle soreness model of eccentric exercise, the injection of hypertonic saline into muscle fascia was more painful as compared to the pain responses recorded upon intramuscular injections [7]. Moreover, pain in response to intramuscular saline injections was not different between the eccentric

exercised and normal muscle [7]. As NGF has been assumed to contribute to exercise-induced muscle soreness [18], our data would suggest a particular contribution of the muscle fascia nociceptors in muscular pain, possibly mediated by NGF-induced sensitization processes.

4.4. Time course of NGF-evoked hypersensitivity

NGF-evoked hypersensitivity of human muscle nociceptors lasted for up to 7 days [1,31,32], whereas human skin was hypersensitive for several weeks [5,20,22]. The time course of mechanical hyperalgesia from previous studies administering NGF intramuscularly revealed a peak of hyperalgesia at day 1, which returned to baseline after 4-7 days [1,19]. In our study regarding NGF administration to the muscle fascia, a slightly prolonged sensitization of at least 7 days was observed for mechanical stimulation, whereas upon injection of protons, elevated pain was recorded even after 2 weeks. Strikingly, higher doses of intramuscular NGF provoked a shorter-lasting hyperalgesia [19,33]. Distal application sites, such as the anterior tibial muscle, did not evoke a more protracted hyperalgesia [1], which would have been expected considering the concept of internalization of the NGF-trkA-receptor complex, its axonal transport to the dorsal root ganglia, and the consecutive induction of receptor protein expression changes involved in the hyperalgesic responses. Thus, the most probable explanation for the different time courses of NGF-evoked hypersensitivity might be a differential response to NGF between muscle and fascia. Such tissue specificity might be suggested when comparing the time difference of NGF-induced hyperalgesia in skin [22] vs muscle [1,33].

NGF-evoked sensitization processes may contribute to clinical musculoskeletal pain, as suggested by analgesic effects of anti-NGF antibodies in patients suffering from low back pain [3,13] or by the elevated cerebrospinal fluid levels of NGF in fibromyalgia patients [23]. Our results indicate that NGF provokes a distinct and long-lasting sensitization of nociceptors within the fascia of the *M. erector spinae*. Although a link to clinical musculoskeletal pain conditions is apparent, it should be noted that there are marked differences of this model as compared to chronic back pain patients; for instance, there is no pain upon spontaneous muscle use, but only upon stimulation under experimental conditions. Yet, the sensitization of acid-induced pain following NGF injection appears to be of particular importance for the translation from the experimental pain model presented herein to the clinical condition of muscular pain, as the increased sensitivity of muscle fascia nociceptors to inflammatory mediators (including tissue acidification) may contribute to the pathophysiology of clinical low back pain.

Conflict of interest statement

The authors declare no conflicts of interest.

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