

Algesic agents exciting muscle nociceptors

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Received: 1 August 2008 / Accepted: 27 November 2008 / Published online: 13 January 2009
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Abstract Morphologically, muscle nociceptors are free nerve endings connected to the CNS by thin myelinated (group III) or unmyelinated (group IV) afferent fibers. Not all of these endings are nociceptive; approximately 40% have a low mechanical threshold and likely fulfill non-nociceptive functions. Two chemical stimuli are particularly relevant as causes of muscle pain. The first is a drop in tissue pH, i.e. an increase in proton (H^+) concentration. A large number of painful patho(physio)logical alterations of muscle tissue are associated with an acidic interstitial pH (e.g. tonic contractions, spasm, inflammation). The second important cause of muscle pain is a release of adenosine triphosphate (ATP). ATP is present in all body cells, but in muscle its concentration is particularly high. Any damage of muscle cells (trauma, necrotic myositis) is accompanied by a release of ATP from the cells. Therefore, ATP is considered a general pain stimulus by some. ATP and protons are relatively specific stimuli for muscle pain; in cutaneous pain they play a less important role. The numerous agents that are released in pathologically altered muscle include substances that desensitize mechanosensitive group IV receptors. Capsaicin has a long-lasting desensitizing action, brain-derived neurotrophic factor, and tumor necrosis factor- α , a short-lasting one. Most of the agents exciting group IV units (e.g. low pH, ATP, capsaicin) activate not only nociceptive endings but also non-nociceptive ones. The only substance encountered that excites exclusively nociceptive group IV receptors is nerve growth factor (NGF). In rat muscle chronically inflamed with complete Freund's

adjuvant, most group IV endings are sensitized to mechanical (and to some) chemical stimuli. However, stimulants such as ATP, NGF, and solutions of low pH were found to be less effective in inflamed muscle. A possible explanation for this surprising finding is that in inflamed muscle the concentrations of ATP and NGF and H^+ are increased. Therefore, experimental administration of these agents is a less effective stimulus.

Keywords Muscle nociceptors · Algesic agents · Tissue acidity · ATP · NGF

Preface

I was a member of Robert Schmidt's department in Kiel for 14 years. Under his guidance I learned to work scientifically and do animal experiments. It was his idea to study the mechanisms of muscle pain—which was a new field at that time—and ever since I continued with this work. The subject is still of general interest, because the more we know about muscle pain, the more questions arise. Despite the research efforts by several groups in the world, deep somatic pain remains enigmatic, and there are still completely neglected aspects of musculoskeletal pain such as pain from fascia, ligaments, and periosteum.

The following article is a tribute to Robert Schmidt's scientific achievements and his continuous efforts to motivate people to enter novel research areas.

Introduction

Morphologically, muscle nociceptors are free nerve endings that are connected to the CNS via group III (thin myelinated)

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or group IV (unmyelinated afferent) fibers. The latter constitute the great majority of muscle nociceptors. However, not all free nerve endings with group IV fibers are nociceptors. Data from our group show that approximately 40% have a low mechanical threshold in the innocuous range and can be activated by non-painful deformation of the muscle tissue (low-threshold mechanosensitive (LTM) units, Hoheisel et al. 2005; Fig. 1d). These units are assumed to fulfill a non-nociceptive function, e.g. mediate pressure sensations from muscle (Graven-Nielsen et al. 2004; Light and Perl 2003). The remaining 60% have nociceptive properties in that they do not respond to weak, everyday stimuli but require tissue-threatening mechanical stimulation for activation. They are called high-threshold mechanosensitive (HTM) units in this article. The knowledge that unmyelinated muscle afferent units include non-nociceptive receptors, is more than 30 years old: the adjustment of circulation and respiration to the requirements of physical exercise is controlled by these units (McCloskey and Mitchell 1972). Nevertheless, in the literature there are still statements that all free nerve endings, all unmyelinated fibers, or even all small DRG neurons are nociceptive.

The high mechanical threshold of nociceptive muscle nociceptors is hard to explain considering the fragile structure of free nerve endings, because they consist of axoplasm surrounded by a semi-fluid cell membrane. Recent

data from invertebrates suggest that high-threshold mechanosensitive nerve endings possess membrane receptors of the TRPA (ankyrin-repeat transient receptor potential) family, whereas endings that are activated by gentle mechanical stimuli are thought to be equipped (among others) with degenerin/epithelial Na⁺ channels (for a review, see Goodman et al. 2004).

When tested with various combinations of inflammatory substances and mechanical stimuli, many group IV endings from muscle respond only to some of the stimulants (Kniffki et al. 1978). A possible reason for this specialized chemical sensitivity (or preferred susceptibility) is a particular combination of receptor molecules in the membrane of the free nerve ending.

Besides mechanical forces, two chemical stimuli are particularly relevant as causes of muscle pain. The first is a drop in tissue pH, i.e. an increase in proton concentration. Most of the patho(physio)logical alterations of muscle tissue are associated with an acidic interstitial pH. Possible reasons for a lowered pH are tonic contractions which lead to ischemia and increased proton concentration. The pain of fatiguing exercise is mainly due to an accumulation of lactic acid resulting in a decrease in pH, even if the arterial perfusion is normal. The pain during exercise is markedly increased if the muscle is ischemic. During ischemic contractions additional pain-producing and sensitizing

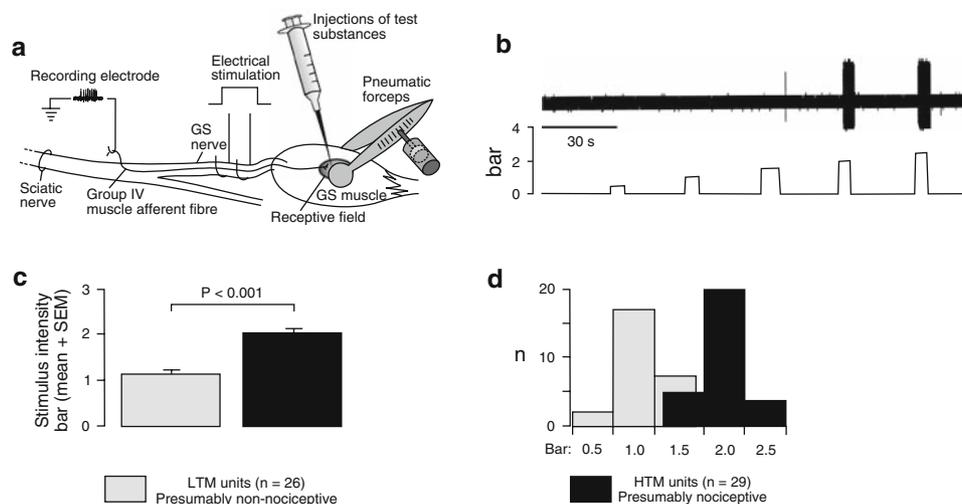


Fig. 1 Identification of high-threshold mechanosensitive (HTM) versus low-threshold mechanosensitive (LTM) muscle receptors with unmyelinated (group IV) afferent fibers. **a** Set-up for recording of the impulse activity of single group IV fibers supplying a receptive ending in the gastrocnemius-soleus (GS) muscle of the rat. Fiber bundles containing a few nerve fibers were split by hand from the sciatic nerve and put over a recording electrode. The GS nerve was electrically stimulated for measuring the conduction velocity of the fiber under study. Only fibers conducting at less than 2.5 m/s were accepted for study. The receptive ending was localized in the muscle with pressure stimuli delivered by a pneumatic forceps that could be closed with a defined force. The test substances were injected into that area of the muscle

from which the ending could be excited by mechanical stimulation (the receptive field). **b** Determination of the mechanical threshold of a group IV unit. The upper trace is an original recording of the impulse activity; the lower trace shows the pressure stimuli delivered by the forceps. The amplitude of the stimuli is given in the physical unit “bar”. Note that there was no resting activity in the beginning of the recording. The first clear response occurred at a pressure of 2 bar. **c** Pressure thresholds of 26 LTM (grey) and 26 HTM (black) units. **d** Distribution of the pressure threshold of the LTM and HTM units. There was little overlap between the two receptor populations; the dividing line between LTM and HTM units was a threshold of 1.5 bar

substances—such as bradykinin (BKN)—are released in muscle tissue. In inflamed tissue a decreased pH of 5–6 has been measured.

The second important cause of muscle pain is a release of adenosine triphosphate (ATP). ATP is an energy-rich molecule that is present in all body cells, but in muscle its concentration is particularly high. Any damage of muscle cells or increase in the permeability of the muscle cells' membrane is accompanied by a release of ATP from the cells (Burnstock 2007). Therefore, ATP is considered a general pain stimulus by some. Situations in which such an ATP release occurs range from muscle tear to a necrotic myositis. These two stimuli (ATP and protons) are relatively specific for muscle pain; in cutaneous pain they play a less important role.

The relative effectiveness of substances exciting muscle nociceptors is hard to compare, because the various groups who have tested these stimulants (Kellgren 1937–38; Kumazawa and Mizumura 1977; Mense 1977; Kaufman et al. 1982; Makowska et al. 2005; Cairns et al. 2007) used different animal models or different methods of administration (close intra-arterial or intramuscular injections, superfusion in vitro).

Under patho(physio)logical circumstances, not a single substance, but an unknown combination of substances (the inflammatory “cocktail” or “soup”) acts on muscle nociceptors. Experiments on human subjects (Babenko et al. 1999) and animals (Mense 1981) have shown that for instance BKN and serotonin injected together have a more than additive action with regard to evoking pain or exciting muscle nociceptors, respectively. In this article, the substances known so far are addressed one after the other, but this surely does not reflect the real situation. Moreover, to date only some of the entire spectrum of relevant substances are known, and in the future many more likely will be found.

Recently, a microdialysis technique has been used to measure the concentration of inflammatory substances in myofascial trigger points of patients (Shah et al. 2005). In this study, increased levels of the classic inflammatory agents and cytokines such as BKN, serotonin, substance P, and tumor necrosis factor (TNF)- α were found that may explain the tenderness of a trigger point. However, not all of these substances are probably responsible for the trigger point pain, because, e.g. TNF- α desensitizes muscle nociceptors to mechanical stimulation for 6–8 min (Hoheisel et al. 2005; Fig. 2a). In the concentration used, TNF- α (0.6 μ M) did not excite group IV units. The main acute effect of TNF- α appears to be a transient desensitization of the muscle receptors to mechanical stimuli. As other substances, too, desensitize muscle nociceptors (e.g. brain-derived neurotrophic factor, BDNF, Fig. 2b), it is possible that in the very body periphery—at the level of the receptive ending—there is an interaction between sensitizing and

desensitizing influences, similar to the CNS where pro- and anti-nociceptive processes are simultaneously active. Therefore, if an increased level of biologically active substances is found in a pathologically altered tissue, one cannot conclude that these agents contribute to the pain associated with the pathological condition. Another problem for the interpretation of elevated levels of biologically active substances is the time-course of many effects, i.e. acute effects likely differ from chronic ones.

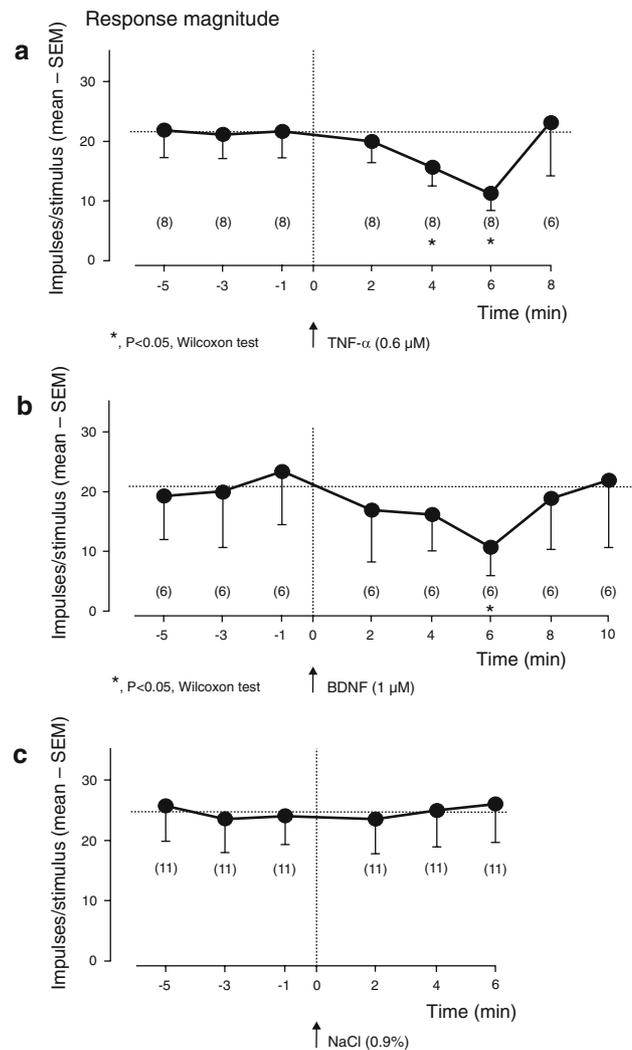


Fig. 2 Mechanical desensitization of group IV HTM receptors by TNF- α (a) and BDNF (b). Eight or six, respectively, group IV endings were tested with repeated noxious pressure stimuli at 2 min interval and their response magnitude measured (ordinate). After three stimulations, TNF- α or BDNF, respectively, were injected into the mechanical sensitive receptive fields of the endings and the mechanical stimulations continued. The mean of the first three responses was taken as baseline. Both test substances did not excite the group IV units at the concentration used. c Control injection of phosphate buffered saline to show that the injection procedure itself did not change the responsiveness of the units. Both TNF- α and BDNF caused a significant transient reduction in response magnitude 4–6 min after i.m. injection. * $P < 0.05$ in comparison to baseline, Wilcoxon test, two-tailed

This article addresses the key substances and their membrane receptors that are assumed to be involved in acute and chronic muscle pain. It does not attempt to give a comprehensive overview of all known algescic or sensitizing substances but focuses on those stimulants that have been studied systematically.

Stimulants for muscle nociceptors and their membrane receptors

Inflammatory substances

Protons (H^+ -ions)

Protons do not belong to the inflammatory substances in the strict sense, but are dealt with under this heading, because a decrease of pH is one of the key signs of tissue inflammation. It also occurs in hematoma, ischemia, cancer, myofascial trigger points, and exhausting exercise (Revic et al. 1949; Hood et al. 1988; Pan et al. 1988; Issberner et al. 1996; Reeh and Steen 1996; Shah et al. 2005). All these conditions are likely to activate the proton-sensitive receptors. A decrease in pH (an increased proton concentration) is probably the cause of pain in many cases of work-related musculoskeletal disorders, because when the ergonomics of the work place is poor, the workers are likely to perform tonic muscle contractions, which lead to ischemia and lowered pH. Contractions with little force appear to be sufficient to become painful if the same muscle (or part of a muscle) is used in a monotonous way. In this context the so-called cinderella concept is of interest (Hostens and Ramon 2005). It states that during monotonous work at low force level, just a few—and always the same—type I motor units are activated, which have the lowest activation threshold and therefore are the first to be recruited and the last to be derecruited. These muscle fibers do most of the work and are likely to be overloaded. The pain during tooth clenching and bruxism as well as tension-type headache may also be mediated by proton-sensitive membrane receptors, because under these conditions head muscles are overloaded and likely have a low pH.

In rats, intramuscular injection of buffer solutions at pH 6.0 excites more than 50% of the mechanosensitive group IV afferents (Hoheisel et al. 2004). When rats are given two unilateral intramuscular injections of pH 4 saline at an interval of 5 days, the animals develop chronic bilateral pain (Sluka et al. 2001), not only in the injected muscle but also in the paw. In humans, intramuscular injection of a buffer solution at pH 5.2 results in localized muscle pain (Steen et al. 2001). Intramuscular infusion of a solution at pH 5.2 produces moderate pain without signs of adaptation during the infusion (Issberner et al. 1996). The infusion

evokes all symptoms that are typical of muscle pain, namely pain and mechanical hyperalgesia at the infusion site and referred pain (Frey Law et al. 2007).

The relevance of a decreased tissue pH for muscle pain is evident from the great number of membrane receptors that occur on muscle nociceptors. They include a family of non-selective cation membrane channels, namely the acid sensing ion channels (ASICs), of which ASIC1 and ASIC3 are found on muscle free nerve endings (Sluka et al. 2003; Hoheisel et al. 2004). ASIC 3 receptors colocalize with substance P (SP) in dorsal root ganglion (DRG) neurons (Price et al. 2001). ASIC3 knock-out mice do not exhibit the mechanical hyperalgesia that occurs after repeated intramuscular injection of acid solutions in wild-type mice (Sluka et al. 2003) or after muscle inflammation (Sluka et al. 2007). Therefore, activation of ASIC3 receptors on muscle receptors appears to be essential for the central sensitization after repeated injections of acidic solutions and probably also after other muscle lesions. ASIC1 channels open at a pH of less than 6.9, while the ASIC3 subtype is activated at a pH of approximately 6.0 (Wemmie et al. 2006). A higher proportion of DRG cells with afferent fibers from muscle express ASIC3 receptors (approx. 50%) than neurons with afferents from the skin (10–20%; Molliver et al. 2005).

Another membrane receptor that is sensitive to a lowered tissue pH is the transient receptor potential receptor, subtype1 (TRPV1). This receptor is also sensitive to heat and capsaicin. The combined sensitivity to heat and lowered pH may explain the pain and deep tenderness felt during a general infection with high fever. The deep tenderness may be due to the fact that the raised body temperature together with a lowered tissue pH activate the TRPV1 receptors.

Bradykinin (BKN)

BKN is cleaved from plasma proteins by the enzyme kallikrein. Its precursors can be found in blood plasma, and therefore are ubiquitously present in the body. In ischemic tissue, or when plasma extravasation occurs in damaged tissue, BKN is formed. The excitatory action of BKN is restricted to group III- and IV-endings in muscle (Kaufman et al. 1982; Mense and Meyer 1988), i.e. it does not excite muscle spindles or Golgi tendon organs. BKN is only mildly painful when injected into human skeletal muscle (Jensen et al. 1990; Babenko et al. 1999). In intact muscle, BKN acts mainly by binding to the G-protein coupled B2 receptor, a metabotropic receptor that activates an intracellular cascade of second messengers and eventually also protein kinase C. Through action on the B2 receptor, BKN can enhance the responsiveness to other algescic agents such as serotonin (5-HT; Hu et al. 2005). When a muscle is inflamed, another BKN receptor—the B1 receptor—is

synthesized in the dorsal root ganglion (DRG) cell, transported to the free nerve ending, and built in its membrane (Perkins and Kelly 1993; Kumazawa 1996). This *de novo* synthesis of a membrane receptor in response to an inflammatory stimulus is a good example of neuroplasticity in the peripheral nervous system.

Serotonin (5-hydroxytryptamine, 5-HT)

5-HT excites receptors with group III- and IV-fibers in muscle (gastrocnemius-soleus (GS) and masseter; Fock and Mense 1976; Mense 1977; Sung et al. 2007). Injection into human muscles results in short and modest pain (Babenko et al. 1999). It may contribute to the pain of fibromyalgia syndrome (FMS), because its metabolism is impaired in women with FMS and its concentration increased (Wolfe et al. 1997; Ernberg et al. 1999, 2006). 5-HT exerts its action through a large family of 5-HT receptors, but in the body periphery the 5-HT₃ receptor prevails. More than half of the ganglion neurons that supply the masseter and temporalis muscle express the 5-HT₃ receptor, a non-selective cation channel (Sung et al. 2007). Female rats exhibit a significantly higher expression of the 5-HT₃ receptor than males. However, the response magnitude to 5-HT of masticatory muscle afferent units does not differ between female and male rats.

Prostaglandins (PGs)

PGs of the E2 type have little excitatory action on group IV muscle afferent units, but they increase the response magnitude of these units to BKN (Mense 1981). For both PGE2 and 5-HT, this sensitizing action may occur without excitation of the unit under study. Thus, the sensitizing concentration of PGE2 and 5-HT is below that required for activation. This fits with the general observation that in the course of an inflammation, tenderness occurs first (due to nociceptor sensitization) followed by spontaneous pain (due to nociceptor excitation). The membrane receptor of PGE2 is the prostanoid receptor EP2. In delayed onset muscle soreness (DOMS), increased levels of PGs have been reported to be present (Tegeader et al. 2002).

Interleukins (ILs)

Among the many ILs, IL-1 and IL-6 are assumed to have a pronociceptive action, whereas IL-10 is thought to be antinociceptive. In our group, the action of intramuscularly injected IL-6 on muscle group IV afferent units was tested. At the doses used (25 μ l at a concentration of 1 μ M), the cytokine excited only LTM receptors, i.e. the non-nociceptive subtype of group IV afferent units (Hoheisel et al. 2005). The tissue levels of ILs are increased in DOMS

(Tegeader et al. 2002) and in myofascial trigger points (IL-1b, Shah 2005). However, if our own data from the rat GS muscle can be generalized, IL-6 is not an algesic agent, because it excites LTM (presumably non-nociceptive) units, only.

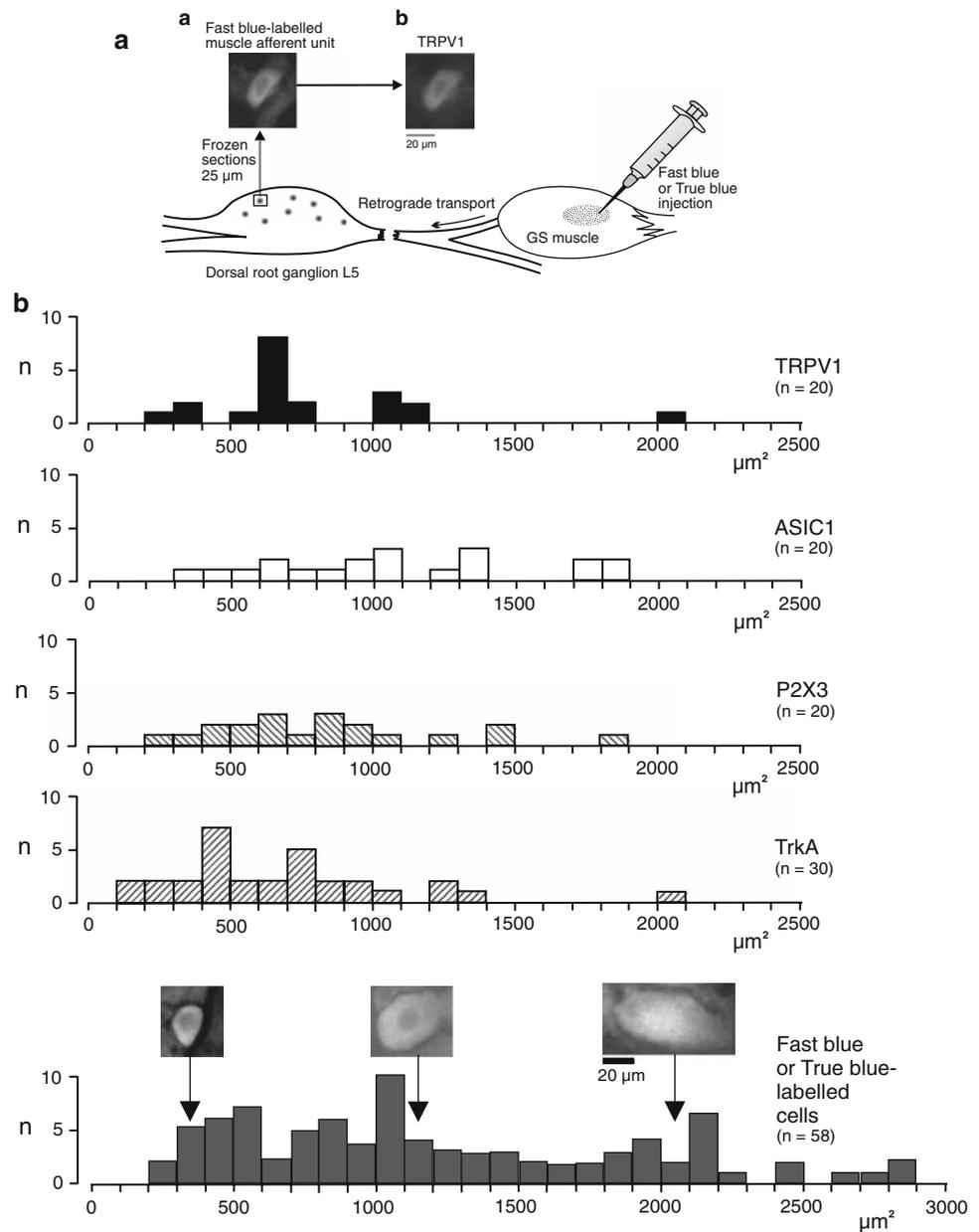
Nerve growth factor (NGF)

NGF, a neurotrophin, is known to control the development of the nociceptive and sympathetic nervous system. In the adult, NGF has a sensitizing action on peripheral nociceptors and on central neurons. It has a close relationship to skeletal muscle, because it is synthesized in muscle tissue. Its synthesis is increased when the muscle is inflamed (Men trety et al. 2000; Pezet and McMahon 2006). NGF is the first and only substance we encountered in many years of muscle pain research that excites exclusively HTM (presumably nociceptive) receptors in muscle (Hoheisel et al. 2005). Despite its strong excitatory action on HTM muscle receptors, intramuscular injection of NGF in humans is not painful (Svensson et al. 2003), and behaving rats likewise do not show pain-related behavior when NGF is injected into the gastrocnemius-soleus muscle (Hoheisel et al. 2007). The lack of pain during and directly after intramuscular NGF injection can be explained by the finding that at the spinal level, the NGF-induced afferent activity elicits mainly subthreshold potentials (Hoheisel et al. 2007) and not action potentials. Subthreshold potentials are not transmitted to higher centers, and therefore do not evoke subjective sensations. Nevertheless, these potentials have a strong sensitizing action on dorsal horn neurons (Hoheisel et al. 2007).

Possibly, the sensitizing action of subthreshold potentials in dorsal horn neurons—and probably also in higher nociceptive centers—is of general importance for the occurrence of chronic muscle pain. In some cases of painful work-related muscle disorders where employees perform tonic contractions with the same muscle over prolonged periods of time, microlesions are likely to occur that evoke subthreshold potentials in dorsal horn neurons. These potentials do not elicit subjective sensations, but sensitize the central neurons. Eventually, the sensitization may cause a chronic muscle pain syndrome. Professions that appear to be particularly prone to have work-related disorders are musicians and other workers who tonically contract their muscles at relatively little forces (see above, Cinderella hypothesis).

The membrane receptor for NGF is the tyrosine kinase receptor, subtype A (TrkA). These receptors have been identified on DRG cells supplying the rat GS muscle (Fig. 3). With regard to the algesic property of i.m. injection of NGF, differences between muscles were found: in behavioral experiments in rats, NGF at a concentration of

Fig. 3 Visualization of receptor molecules in the membrane of dorsal root ganglion cells supplying the rat gastrocnemius-soleus (GS) muscle. **A** Data acquisition. The retrogradely transported marker fast blue or true blue was injected into the GS muscle to visualize the cells in the dorsal root ganglion (DRG) L5 which had receptive endings in the GS muscle. Sections of the DRG were incubated with antibodies to the receptor molecules listed in **(B)**. Cells showing both true blue fluorescence and staining for receptor molecules were evaluated. The insets in **(A)** show a true blue-stained DRG cell that also exhibited immunofluorescence for the receptor molecule TRPV1. **B** size distribution of cells in the DRG L5 labelled retrogradely from the GS muscle with true blue. The lowermost panel shows all cells stained, the other panels show cells that were additionally immunoreactive for the receptor molecules TRPV1, ASIC 1, P2X3, and TrkA. Note that the cells showing IR for the receptor molecules were among the smallest of the size distribution. The numbers in brackets indicate the cells evaluated



0.8 μM did not evoke pain when injected into the GS muscle, but when injected into the multifidus muscle—one of the genuine low back muscles—it elicited a medium-level of pain-related behavior (Hoheisel and Mense, unpublished).

It would be interesting to know to what extent this finding can be extended to other muscles. It is intriguing to imagine that different muscles may have a different sensitivity to pain. When considering the genuine postural low back muscles versus muscles of the extremities, one may argue that low back muscles are mainly composed of red muscle fibers and fulfill postural functions, whereas extremity muscles contain mainly white fibers and have a locomotor function. However, also muscles in the same

body region fulfilling similar functions have been shown to differ in pain sensitivity: in a study by Svensson et al. (2003) on the sensitizing action of NGF in human masticatory muscles, the temporalis muscle was much less sensitized to mechanical stimuli than was the masseter muscle.

Figure 3 presents immunohistochemical evidence that in the DRG L5, somata supplying the GS muscle are equipped with TRPV1, ASIC1, P2X3, and TrkA membrane receptors. The receptor molecules were found particularly on the small cell bodies that likely have slowly conducting afferent fibers. Generally, DRG cells are assumed to possess the same receptor molecules as peripheral receptive endings; therefore the stimulating action of protons, ATP, and NGF on nociceptors in muscle may be due to a direct effect

mediated through binding of the stimulants to their specific membrane receptors.

Other substances

Adenosine triphosphate (ATP)

ATP activates purinergic P2X cation channels, particularly the P2X2 and P2X3 membrane receptors, which are expressed on slowly conducting muscle afferent units (Ambalavanar et al. 2005). The ATP concentration in muscle cells is particularly high, in the millimolar range. A recent study of our group showed that this ATP concentration is sufficient for activating group IV muscle units; approximately 60% of all mechanosensitive units were excited (Reinöhl et al. 2003). Intramuscular or close intra-arterial injections of ATP or analogues activate group IV fibers more effectively than A δ -fibers (Hanna and Kaufman 2004). Infusion of ATP into the trapezius muscle in humans evokes muscle pain (Mörk et al. 2003). In intracellular recordings from DRG cells in vitro, about two-thirds of the cells were depolarized by ATP (Connor et al. 2005).

Glutamate

Glutamate is the transmitter of nociceptive afferent fibers from muscle in the spinal cord. Since the spinal terminations and the nociceptive ending in muscle are parts of the same neuron, glutamate occurs also in the membrane of the nociceptor. Every time a nociceptor is excited, it releases glutamate in the tissue through the axon reflex (Rees et al. 1994; Lawand et al. 2000). Glutamate is also a stimulant for muscle nociceptors, because their membrane is equipped with NMDA receptors. NMDA glutamate receptors were also found on masseter ganglion cells (Cairns et al. 2003; Dong et al. 2007). In the rat, glutamate excites slowly conducting muscle afferents from masticatory muscles (Cairns et al. 2002; Svensson et al. 2005) and evokes pain-related behavior in animals after injection into these muscles (Ro et al. 2003). In humans, intramuscular glutamate injection elicits pain (Ge et al. 2005; Svensson et al. 2005).

With regard to glutamate as an analgesic agent, a clear gender difference was found: it evokes greater pain responses in women than in men (Svensson et al. 2003), and muscle receptors with slowly conducting afferent fibers in female rats show larger responses to glutamate than those in male rats (Cairns et al. 2001, 2002). If this finding reflects a generally higher sensitivity of female muscle nociceptors, it could explain the higher prevalence of some chronic muscle pain syndromes in women (e.g. temporomandibular pain (TMD); Rollman and Lautenbacher 2001; Huang et al. 2002; Nekora-Azak et al. 2006). One of the hormones influ-

encing the nociceptive system in females is estrogen. In rats, an increase in estrogen is associated with an elevated mechanical threshold of putative mechanical nociceptors in masticatory muscles (Mann et al. 2006). In female TMD patients, higher levels of pain are found during hormonal cycle times when estrogen levels are low (LeResche et al. 2003).

The NMDA receptor is an ionotropic glutamate receptor, i.e. the molecule has the form of a channel through the membrane of the receptive ending through which ions can pass (predominantly Ca⁺⁺). DRG cells of masticatory muscles can be depolarized by glutamate through activation of NMDA receptors (Pelkey and Marshall 1998). Glutamate-evoked discharges can be attenuated by ketamine, an uncompetitive NMDA receptor blocker (Cairns et al. 2003; Dong et al. 2006). In pathologically altered muscle tissue, glutamate levels are elevated (Rosendal et al. 2004), this holds true also for DOMS (Tegeer et al. 2002). Muscle inflammation induced by complete Freund's adjuvant apparently activates the peripheral NMDA receptors, because treatment of the myositis rats with AP5—a competitive NMDA antagonist—attenuates the inflammation-induced reduction of bite force (Ro et al. 2005). This effect is likely due to a peripheral site of action of AP5, since the antagonist does not readily cross the blood brain barrier to penetrate into the CNS (Wong and Kemp 1991).

Estrogen appears to act on the NMDA receptor by phosphorylating the receptor protein, i.e. it couples a phosphate residue to the NMDA protein molecule. The phosphorylation increases opening probability and open time of the NMDA channel. Through this mechanism the ion currents through the NMDA receptor channel are enhanced (Tang et al. 2008).

Collectively, the data on glutamate suggest that the neurotransmitter is an effective stimulus for the activation of muscle nociceptors and also for the sensitization of dorsal horn neurons.

Capsaicin

Capsaicin is the active ingredient of chilli pepper; it is the specific ligand of the TRPV1 receptor. As mentioned above, the TRPV1 receptor is also sensitive to H⁺ ions and heat. Capsaicin stimulates group III and IV muscle afferent units (Kaufman et al. 1982; Hoheisel et al. 2004). In the latter study, approximately 50% of the group IV units were activated by capsaicin. About half of the DRG cells supplying the rat GS muscle express the TRPV1 receptor, and approximately the same proportion of trigeminal ganglion cells innervating the masseter muscle are depolarized by capsaicin. (Hoheisel et al. 2004; Connor et al. 2005). When injected i.m. in relatively high concentrations (close to 1 mM), capsaicin desensitizes the group IV muscle receptors

to subsequent mechanical and chemical stimuli, i.e. the receptors can no longer be stimulated by experimental stimuli (Hoheisel et al. 2004). Microneurographic recordings from muscle nerves in humans showed that also muscle nociceptors in humans respond to injections of capsaicin into the muscle close to the receptive ending (Marchettini et al. 1996). The capsaicin injections elicited strong muscle pain. Since capsaicin is assumed to be a specific stimulant for the TRPV1 receptor, these data demonstrate that human muscle nociceptors are equipped with this receptor molecule.

TRPV1 receptors have been found in nociceptors close to taste buds and in the gustatory receptors themselves (Simon and de Araujo 2005). Therefore, the burning sensation a hot meal causes in the mouth could be mediated by the excitation of nociceptors of the tongue mucosa or by taste buds.

Stimulants of muscle nociceptors that probably do not act through membrane receptors

Hypertonic saline (sodium chloride (NaCl) 5–6%)

A large increase in extracellular Na^+ does not occur under (patho)physiological conditions but it can be easily induced experimentally by injecting or infusing hypertonic saline i.m. (Graven-Nielsen 2006). In these experiments, the high Na^+ concentration—and not the hypertonicity of the solution—appears to be the effective stimulus (Mense 2007). Hypertonic saline may also excite muscle nociceptors indirectly by releasing glutamate (Tegeer et al. 2002).

Hypertonic saline has long been known to excite muscle receptors with groups III and IV afferent fibers (Paintal 1960; Kumazawa and Mizumura 1977, Mense 1977, Cairns et al. 2003). Recently, it has been shown to activate dorsal horn neurons at a high frequency (Hoheisel et al. 2007). Specific membrane receptors for NaCl are not known, several mechanisms are being discussed for its action on sensory receptors: one is shrinkage of the free nerve endings with ensuing opening of mechanosensitive cation channels. Another possibility is release of other excitants such as glutamate (Hamill and Martinac 2001; Ro et al. 2007). The high extracellular concentration of sodium ions induced by administration of hypertonic saline could result in a depolarization of the ending via an influx of Na^+ ions through unspecific sodium channels (Mense 2007). Kellgren (1937–38) was one of the first to inject hypertonic saline as a pain stimulus in clinical studies, and since then it has been widely used because it causes well reproducible pain in humans and aversive behavior in rats (Svensson et al. 1995; Ro et al. 2003; Graven-Nielsen 2006; Hoheisel et al. 2007).

An overview of the effectivity of some of the above stimulants on mechanosensitive muscle group IV afferent units is shown in Fig. 4.

Potassium chloride (KCl)

In cats, close arterial injection excites group III- and IV-fibers of the GS muscle (Fock and Mense 1976; Kumazawa and Mizumura 1977, Mense 1977). In humans, KCl injection into craniofacial muscle has been reported to be as painful as hypertonic saline (Jensen and Norup 1992). The most likely mechanism of action is that high concentrations of KCl depolarize nerve endings and afferent fibers by reversing the K^+ equilibrium potential which leads to an inward K^+ current (O'Shaughnessy et al. 1993).

Interactions and modulatory influences

The following section deals mainly with sensitizing processes, because they are the hallmark of beginning pathological alterations in the body periphery and are responsible for pain symptoms such as allodynia (pain sensations evoked by innocuous stimuli) and hyperalgesia (enhanced pain sensations evoked by noxious stimuli). It should be kept in mind, though, that a sensitization of peripheral receptors with ensuing increased nociceptive input to the spinal cord is likely to lead to a central sensitization. Therefore, the decision whether allodynia or hyperalgesia is due to peripheral or central sensitization is often difficult to make.

BKN has long been known to sensitize group IV receptors to mechanical stimulation (Mense and Meyer 1988). It also induces sensitization to heat, and this sensitization is mediated by activation of protein kinase C (PKC) ϵ (Cesare et al. 1999). On the other hand, the excitatory actions of *BKN* on muscle group IV endings can be enhanced by other chemical stimuli: following injection of 5-HT or PGE2 the responses to *BKN* are increased (Mense 1981).

PG E2 sensitizes muscle receptors to *BKN* (Mense 1981). In the responses of group IV muscle receptors to *BKN*, a PG component appears to be included, because when the PG synthesis is inhibited by a cyclooxygenase blocker such as acetylsalicylic acid, the response magnitude of the muscle receptors to *BKN* is reduced (Mense 1982). Likewise, close arterial injections of arachidonic acid, the precursor of PGs, into the femoral artery have been reported to increase the responses of slowly conducting muscle afferents to static muscle contractions of the triceps surae muscle (Rotto et al. 1990). In this study, it is unclear if the muscle receptors were sensitized against the mechanical or chemical component of the static contractions.

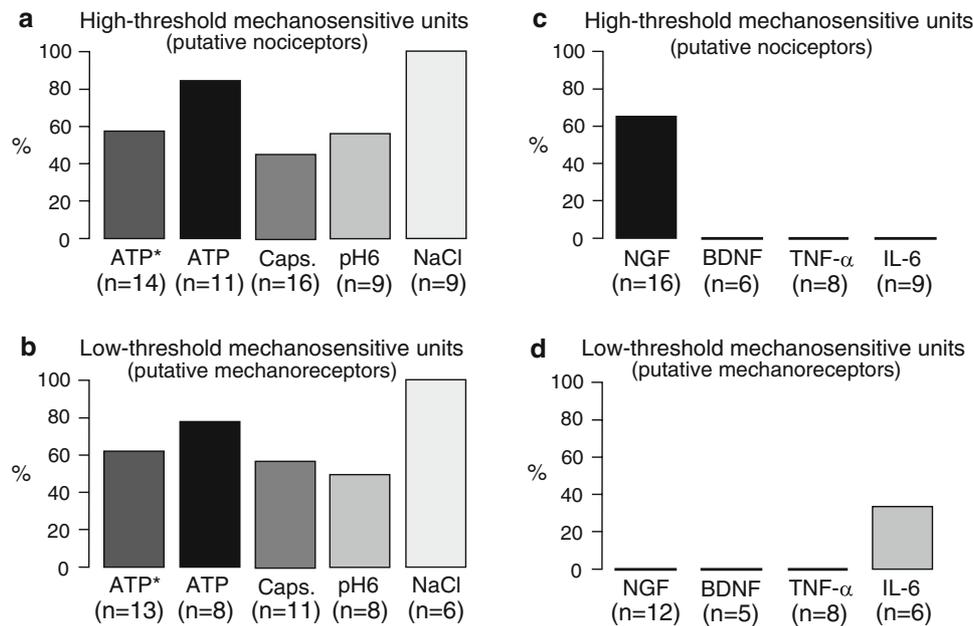


Fig. 4 Effectiveness of some endogenous agents as stimulants for muscle group IV units. **a** Proportion of high-threshold mechanosensitive endings responding to the agents. ATP*, native ATP solution, concentration 7.6 mM, pH 5.5; ATP, concentration 7.6 mM, pH of the solution adjusted to 7.4; Caps., capsaicin, concentration 0.67 mM; pH 6, phosphate buffer, pH 6.0; NaCl, hypertonic NaCl solution, concentration 5%. The injection volume was 25 μ l throughout. **b** Proportion of excited low-threshold mechanosensitive units, labelling as in (a).

Glutamate in high concentrations (1 M) excites and sensitizes slowly conducting afferent units from the rat masseter muscle to mechanical stimulation (Cairns et al. 2001; Cairns et al. 2007). Mechanical hypersensitivity was likewise observed following i.m. injections of an agonist of the metabotropic glutamate receptors (Lee and Ro 2007). Again, protein Kinase C ϵ appears to be involved in this effect.

Capsaicin first excites group IV muscle receptors and then desensitizes them to mechanical and chemical stimuli for at least 30 min after injection close to the receptive ending (Hoheisel et al. 2004). The desensitizing action has been used clinically for treatment of hyperalgesic states, for instance topical application of capsaicin in neuropathic pain patients (Dworkin et al. 2007).

TNF- α and *interleukin-6 (IL-6)* likewise have an acute desensitizing action on the mechanical responsiveness of nociceptive group IV muscle afferent units in the rat (but just for several min after injection close to the receptor (Hoheisel et al. 2005)).

NGF i.m. elicits large responses in high-threshold mechanosensitive group IV afferent units from rat GS muscle (Hoheisel et al. 2007), but does not induce mechanical sensitization of these units for up to 3 days after intramuscular injection (Hoheisel et al. 2005). The growth factor causes long-lasting allodynia (reduction in pressure-pain-threshold) in

The proportions in panel (a) and (b) were almost identical. **c** and **d** Responsiveness of high-threshold mechanosensitive and low-threshold mechanosensitive receptors to NGF, concentration 0.8 μ M; BDNF, concentration 1 μ M; TNF- α , concentration 0.6 μ M; and IL-6, concentration 1 μ M. Injection volume as in (a, b). Note that NGF excited exclusively high-threshold mechanosensitive (presumably nociceptive) units

humans and also in rats (Lewin et al. 1993; Svensson et al. 2003; Hoheisel et al. 2007). After systemic administration, NGF induces long-lasting myalgias with women exhibiting stronger reactions than men (Petty et al. 1994).

Hypertonic saline plays a questionable role as a sensitizing agent, because there are discrepancies in the literature. The stimulus has been found not to alter the mechanical threshold of slowly conducting muscle afferent units (Mok et al. 2005; Sung et al. 2007). On the other hand, in behaving rats hypertonic saline induced a clear hyperalgesia to pressure stimuli 1 day after i.m. injection (Hoheisel et al. 2007). Of course, the latter effect could be due to a central rather than a peripheral sensitization. The finding that an injection of hypertonic saline induces referred pain—a centrally mediated effect—speaks in favor of this assumption.

In many animal models of chronic pain, an experimental inflammation is induced to evoke allodynia and/or hyperalgesia. The two symptoms are often attributed to a sensitization of nociceptors in the inflamed tissue with the tacid assumption that in inflamed tissue nociceptors respond more strongly to algogenic stimuli. However, this does not hold for all stimulants, particularly not for those that are released from the inflamed tissue. An example is shown in Fig. 5. In this experiment, we injected phosphate buffers at different pH (7.4, 6, and 5) into a chronically inflamed

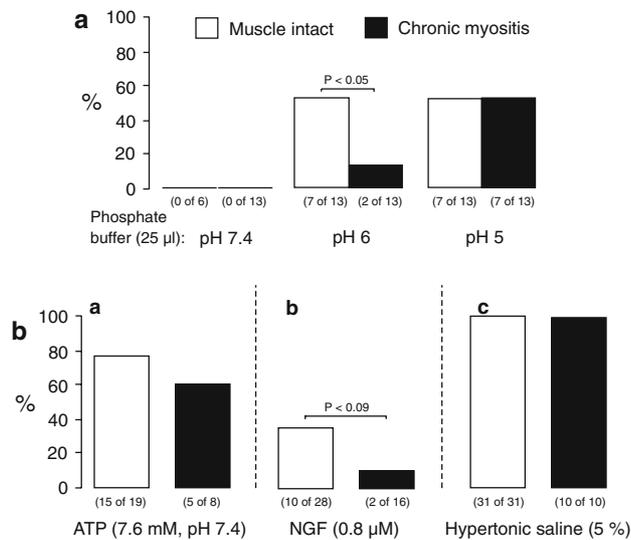


Fig. 5 Reduction of the excitatory effectivity of various endogenous stimulants in chronically inflamed muscle. **A** comparison of the effectivity of buffer solutions at pH 7.4, 6.0, and 5.0. Left panel, injections of a solution at pH 7.4 (neutral) had no excitatory action on group IV muscle receptors, either in intact or in inflamed muscle. A solution at pH 6.0 had a significantly reduced effect in inflamed muscle (middle panel), and a solution at pH 5.0 excited the same proportions in intact and inflamed muscle. **Ba, Bb**, both ATP and NGF had a reduced effectiveness in inflamed muscle, but the difference did not reach statistical significance. As stated in the text, these effects are assumed to reflect the release of the H^+ , ATP, and NGF in inflamed muscle. Hypertonic saline (right panel) excited all units tested in intact and inflamed muscle. This might indicate that the interstitial Na^+ concentration (and hypertonicity) is not markedly changed by the inflammation

muscle and compared the proportion of excited group IV units with that of an intact (non-inflamed) muscle. Buffer at a neutral pH was ineffective in both conditions, and a solution at pH 6 was significantly less effective in inflamed than in intact muscle. The pH 5 solution had the same excitatory action on endings in inflamed and intact muscle. At first thought, this was a surprising finding, because usually an algescic agent such as buffer at pH 6 is expected to be more effective in inflamed muscle. However, inflamed muscle tissue has a lowered pH, and from the data of Fig. 5A one may conclude that the pH in the inflamed muscle was close to 6. If in the muscle tissue the pH is close to 6, this pH is no longer a stimulus, because the proton concentration in the inflamed muscle and in the stimulating solution are approximately the same. A similar result was found with injections of ATP and NGF (Fig. 5Ba, c). Both stimuli were less effective in inflamed muscle, and this may be due an inflammation-induced release of ATP and NGF. When elevated levels of ATP and NGF are already present in the inflamed muscle, injections of additional ATP and NGF apparently are less effective stimuli.

Recent findings with NGF as a stimulant of muscle group IV units indicate that the data obtained from one

muscle cannot be simply transferred to other muscles. An injection of NGF (concentration of $0.8 \mu M$) into the gastrocnemius-soleus muscle in behaving rats did not cause acute pain reactions. However, the same NGF solution injected into the multifidus muscle evoked pain-related behavior (Hoheisel and Mense, unpublished). Other examples from the literature are that slowly conducting afferent units from the rat temporalis muscle differ from the masseter muscle regarding the activation of NMDA receptors (Dong et al. 2006). Svensson et al. (2005) likewise reported that intramuscular injection of glutamate into the splenius muscle in human subjects-induced pain referral, but no referral was observed when glutamate was injected into the masseter muscle.

In the future, differences in pain phenomena between various muscles may become an important field of research, considering the different composition and function of skeletal muscles (red versus white muscles, postural versus locomotor muscles, tonic versus phasic muscles). According to the data in the literature cited above, we conclude that muscle pain differs from cutaneous and visceral pain in many aspects, but there is also evidence that different muscles may differ with regard to their pain-evoking properties.

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