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Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat

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Abstract

In inflamed tissue – including skeletal muscle – the concentrations of cytokines and neurotrophins are known to increase. However, nothing is known about a possible contribution of these agents to muscle pain and hyperalgesia. The present study investigated acute effects of cytokines and neurotrophins on response properties of slowly conducting muscle afferents. In anaesthetised rats, the impulse activity of single mechanosensitive group IV fibres innervating the gastrocnemius–soleus muscle was recorded and tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), nerve growth factor (NGF), or brain-derived neurotrophic factor (BDNF) were injected into the muscle. Changes in the mechanosensibility of the endings following administration of the agents were tested with repeated pressure stimuli of defined forces. A low mechanical threshold in the innocuous range was found in 44.4% of the units tested, 55.6% required strong, potentially tissue-damaging pressure stimuli for activation. NGF excited only units that had a high mechanical threshold, while IL-6 was a stimulant for low-threshold mechanosensitive units only. TNF-α and BDNF did not excite group IV units but had a desensitising action: after TNF-α or BDNF, the response magnitudes to pressure stimuli decreased significantly. The data indicate that cytokines and neurotrophins influence the impulse activity and mechanosensitivity of group IV muscle afferent units. These effects could be of functional significance when the agents are released from muscle cells under pathophysiological circumstances.
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Keywords: Group IV afferent units; Skeletal muscle; Cytokines; Neurotrophins; Nociception

1. Introduction

Recently, evidence has accumulated that not only the classic inflammatory substances like bradykinin, serotonin, and prostaglandins but also cytokines and neurotrophins contribute to pain and alterations of neuronal sensibility during tissue injury or inflammation (Apfel, 2000; De Jongh et al., 2003).

Tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are considered to be such inflammatory cytokines. TNF-α is a major product of activated macrophages and a mediator of diverse functions in damaged and inflamed tissue (Warren et al., 2002). IL-6 is involved in the initial phase of inflammation (Kishimoto et al., 1995) and is reported to have both pro- and anti-inflammatory actions (Gadient and Patterson, 1999). There is evidence that TNF-α and IL-6 are involved in peripheral nociception and inflammatory hyperalgesia, but the literature is controversial in this regard. In the rat, intraplantar and intramuscular injections of TNF-α and IL-6 have been shown to induce hyperalgesia (Cunha et al., 1992; Schäfer et al., 2003; Woolf et al., 1997). In another study, local administrations of TNF-α and IL-6 led to anti-nociception suggesting that both cytokines are mediators of peripheral analgesic actions (Czlonkowski et al., 1993). TNF-α and IL-6 also play an important role in skeletal muscle metabolism (Pedersen et al., 2001; Reid and Li, 2001). It has been shown that IL-6 is released locally in contracting muscle (Jonsdottir et al., 2000; Steensberg et al., 2000). TNF-α is

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synthesised in skeletal muscle myocytes, causes muscle weakness (Li and Reid, 2001) and is involved in recovery of muscle function after injury (Warren et al., 2002).

The neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) participate in structural and functional plasticity of nociceptive pathways (Merighi et al., 2004). NGF plays an important role in the development of nociceptive neurones (Lewin and Mendell, 1993) but is also involved in sensory modulation of the adult nervous system (Lewin et al., 1993; Merighi et al., 2004). NGF is synthesised in skeletal muscle (Amano et al., 1991; Stuerenburg and Kunze, 1998) and contributes to muscle regeneration following injury (Menetrey et al., 2000). In rats, local administration of NGF resulted in hyperalgesia (Lewin et al., 1993). In healthy humans, systemically administered NGF caused diffuse myalgias (Petty et al., 1994), while intramuscularly injected NGF evoked long-lasting mechanical allodynia and hyperalgesia (Svensson et al., 2003). BDNF meets many criteria of a neurotransmitter/neuromodulator (Merighi et al., 2004; Pezet et al., 2002). In C fibres innervating the rat skin, intracutaneous injection of BDNF led to sensitisation to noxious heat stimuli (Shu et al., 1999).

So far, nothing is known about a possible excitatory or sensitising action of cytokines and neurotrophins on unmyelinated muscle afferent fibres many of which are nociceptive. The present study was performed to investigate acute effects of intramuscularly injected TNF-α, IL-6, NGF, and BDNF on the response properties of group IV muscle afferent fibres. The experiments aimed at answering the question if cytokines and neurotrophins (which are known to be synthesised in normal and pathophysiological altered muscle tissue) may contribute to pain and hyperalgesia of an inflamed or injured muscle.

2. Methods

All experiments were carried out in accordance with the German law on the protection of animals and with the ethical proposals of the International Association for the Study of Pain (IASP, Pain 16, 1983); the experimental design was approved by the local ethics authority responsible for animal experimentation.

2.1. Surgical preparation

The experiments were performed on adult male Sprague–Dawley rats (320–450 g) deeply anaesthetised with thiopental sodium (Trapanal®, Byk Gulden/Altana Pharma) 100 mg/kg i.p. initially, followed by 10–20 mg/kg h of the same anaesthetic i.v. using an infusion pump to maintain a deep and constant level of anaesthesia. The anaesthesia was deep enough to abolish flexor reflexes and marked blood pressure reactions (exceeding 10 mmHg) to noxious stimuli or electrical stimulation of the GS nerves at intensities that activate group IV afferent fibres. Muscular relaxation was induced with pancuronium bromide (0.6 mg/kg h, Inresa GmbH). Mean arterial blood pressure and body core temperature were continuously monitored and kept at physiological levels (>80 mmHg, 37–38 °C). The animals were artificially ventilated with a gas mixture of 47.5% O_2, 2.5% CO_2, and 50% N_2. The left sciatic nerve and the left gastrocnemius–soleus (GS) muscle with both GS nerves were surgically exposed. To cover the wound, a pool was formed by sewing skin flaps to a metal ring and filling it with warm paraffin or silicone oil (38 °C).

2.2. Recording of neuronal impulse activity

The impulse activity of single group IV afferent units was recorded from thin nerve filaments separated from the proximal sciatic nerve (Diehl et al., 1993; Reinöhl et al., 2003). The action potentials of the nerve fibre under study could be clearly identified following electrical stimulation of the GS nerves (search stimulus: square pulses of 0.3 ms duration and 50 V amplitude, 0.2 Hz). Units were accepted as afferent if they were sensitive to mechanical stimulation of the GS muscle. Nerve fibres were identified as group IV according to their conduction velocity. The conduction velocity was calculated by dividing the distance between recording and stimulating electrode by action potential latency at 1.5 times electrical threshold. Fibres conducting at less than 1.5 m/s were classified as group IV (Diehl et al., 1993; Reinöhl et al., 2003). Impulses (action potentials) were counted using a template criterion (SPIKE 2 software, Cambridge Electronic Disign Limited). Background (ongoing) activity of the units was determined in a period of 30 s before testing the muscle with mechanical stimuli.

2.3. Determination of receptive properties

Mechanosensitive properties of the afferent units were determined with graded mechanical stimuli applied by hand to the exposed GS muscle. The stimuli were applied with an artist's brush (touch) and a forceps with broadened tips (diameter 5 mm, area 19.63 mm^2, moderate and noxious pressure, see below). Group IV muscle afferent units exhibited marked differences in mechanical threshold (Iggo, 1961; Mense and Meyer, 1985). Units responding to touch or moderate pressure (moderate pressure led to a weak deformation of the muscle) but not responding to stretching the muscle by bending the paw were classified as low-threshold mechanosensitive (LTM) units, units that required strong, potentially tissue-damaging, mechanical stimuli for activation as high-threshold mechanosensitive (HTM) units (Diehl et al., 1993; Mense and Meyer, 1985).

2.4. Quantitative mechanical stimulation

Repeated mechanical stimuli were applied to the mechanosensitive receptive field (RF, GS muscle exposed) of the unit using a forceps that could be closed pneumatically with defined forces (expressed in bar in text and figures). The forceps had broadened circular tips with a diameter of 5 mm (area 19.63 mm^2). The mechanical threshold of the RF was determined with graded stimulus intensities in steps of 0.5, 1.0, 1.5, 2.0, 2.5 and in a few cases 3.0 bar (stimulus duration 5 s, Fig. 1A). The mechanical threshold was defined as the lowest stimulus intensity that elicited action potentials (e.g. the third stimulus in Fig. 1A). Stimulus intensities of 0.5–1.5 bar corresponded to touch (0.5 bar) or moderate pressure stimuli (1–1.5 bar, the forceps caused a weak

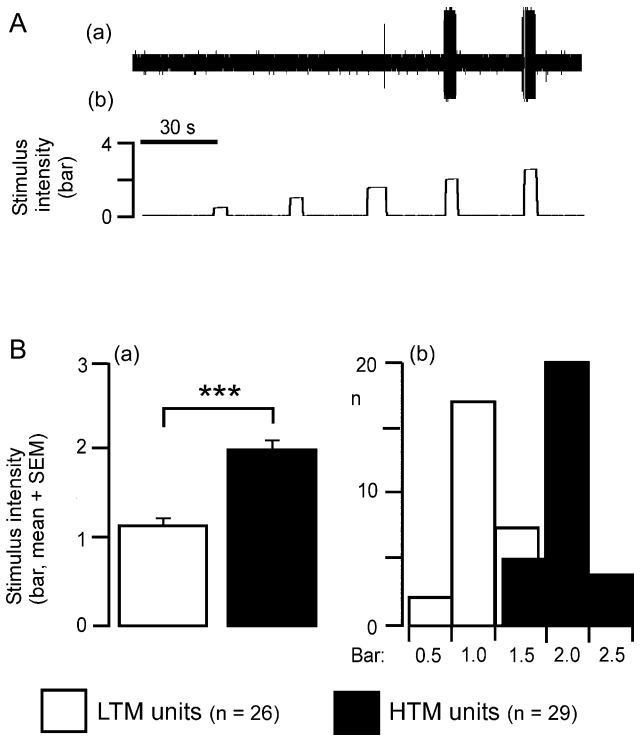

Fig. 1. Threshold of mechanosensitive group IV muscle afferent units. (A) Graded pressure stimuli applied to the receptive field of a high-threshold mechanosensitive (HTM) unit with the use of a pneumatic forceps. (a) Original registration of action potentials elicited by stimulus intensities shown in b. (b) Intensity and duration of mechanical stimuli. (B) Objective threshold of low-threshold mechanosensitive (LTM, open bars) and high-threshold mechanosensitive units (HTM, black bars) classified with stimuli applied by hand. (a) Mean mechanical thresholds of LTM and HTM neurones, respectively; ***$P<0.001$, *U*-test of Mann and Whitney. (b) Distribution of individual thresholds; effect size correlation $r=0.845$.

deformation of the muscle), while intensities of 2.0 bar or higher squeezed the muscle tissue and were termed 'noxious pressure'. In contrast to 0.5, 1.0 and 1.5 bar, awake rats reacted with withdrawal movements when intensities of 2 bar or higher were applied to the GS muscle through the intact skin. When applied to the exposed muscle in anaesthetised animals, these strong stimuli caused an increase in blood pressure of 3–10 mmHg (sciatic nerve intact). To determine sensitising or desensitising effects, the intensity of mechanical stimulation was adjusted to that stimulus intensity which elicited the first clear response of the unit (>10 action potentials per stimulus, e.g. the fourth stimulus in Fig. 1A). Mechanical stimulation of the receptive field was repeated every 2 min (Fig. 2). Each stimulus lasted for 5 s. After three stimuli—which served as a control—the test solution was injected into the receptive field of the unit without removing the stimulation forceps (Fig. 2B). Magnitudes of the neuronal response to mechanical stimulation were determined by summing up all impulses elicited during the stimulation period of 5 s (impulses per stimulus, imp/stim).

2.5. Intramuscular injections

The injection volume was 25 μl throughout, the injections were performed using a syringe mounted in a micromanipulator. We injected the solutions into that area of the muscle where the unit showed the strongest response to mechanical stimuli. The figures (insets in Figs. 4 and 5) show projections onto the muscle surface of that muscle region from which the neurones could be excited with mechanical stimuli. To make sure that the injected solution had reached the receptive ending of the unit, unresponsive units were tested with an injection of hypertonic saline (5% NaCl, Fig. 4B) at the end of the experimental protocol. In our experiments, hypertonic saline stimulated all group IV units tested. To avoid recordings from fibres desensitised or sensitised by earlier drug injections, in each experiment only two fibres were tested, one in the medial head of the GS muscle, a second one in the lateral head.

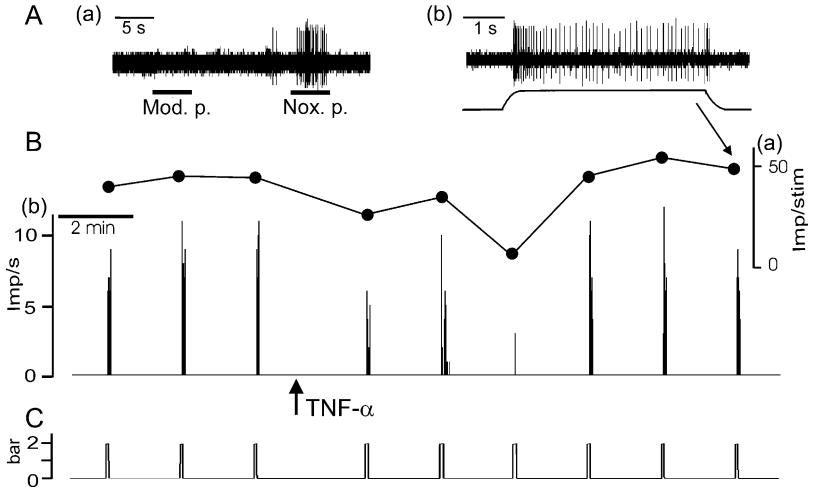

Fig. 2. Effect of intramuscular TNF-α injection on the response magnitude of a single group IV unit to mechanical stimulation. (A) Original registrations. (a) Response to mechanical stimuli applied by hand, the unit responded to noxious but not to innocuous pressure stimuli (Mod. p., moderate, innocuous pressure; Nox. p., noxious pressure). Bars underneath the recording indicate the duration of the stimuli. (b) Response to stimulation with the pneumatic forceps (last stimulus of the histogram shown in B). (Ba) Response curve to repeated stimulation of the receptive field (impulses per stimulus). (Bb) Peristimulus time histogram of the unit's activity from which the data in Ba were obtained (bin width 1 s). (C) Intensity and duration of mechanical stimuli: intensity 2 bar, duration 5 s, the stimuli were repeated every 2 min. The arrow underneath the abscissa indicates the time of intramuscular TNF-α injection. There was no direct activation of the unit by TNF-α.

2.6. Test substances

(1) Tumour necrosis factor-α (TNF-α, human recombinant; Sigma) 0.6 μM in phosphate buffered saline pH 7.4 (PBS, 0.25 μg in 25 μl), (2) interleukin-6 (IL-6, human recombinant; Sigma) 1 μM in PBS (0.65 μg in 25 μl), (3) nerve growth factor (NGF, human recombinant, Calbiochem®) 0.8 μM in PBS (0.52 μg in 25 μl) and (4) brain-derived neurotrophic factor (BDNF, human recombinant; Calbiochem®) 1 μM in PBS (0.7 μg in 25 μl). The concentrations were adjusted to values that are known to cause hyperalgesia when injected intramuscularly in animals or humans (Schäfers et al., 2003; Svensson et al., 2003). The pH of the test solutions was: TNF-α, pH 7.4 ($n=3$); IL-6, pH 7.3–7.4 ($n=3$); NGF, pH 7.2–7.3 ($n=3$); BDNF, pH 7.3–7.4 ($n=3$). Injections of vehicle (PBS, pH 7.4) served as controls.

2.7. Response criteria

The majority of units were silent or exhibited only single action potentials of low frequency before i.m. injection. In these units, the occurrence of impulse activity was taken as a response (Fig. 4B, injection of 5% NaCl; Fig. 5A, NGF injection). In the (few) spontaneously active units, a response was defined as an increase in mean impulse activity by a factor of at least two within 30 s (Fig. 4A, IL-6 injection).

2.8. Statistical evaluation

Statistical comparisons were made using Fisher's exact test, the *U*-test of Mann and Whitney, and the Wilcoxon test for paired data. A probability level of less than 5% (two-tailed) was regarded significant. Responses to mechanical stimulation before and after intramuscular injection of test solutions were compared with the Wilcoxon test for paired data. As control value, the mean response magnitude of the three mechanical stimuli applied directly before substance injection was adopted (indicated by the dotted line in the figures).

3. Results

3.1. General findings

Recordings were made from 72 group IV units. High mechanical thresholds (HTM units) was found in 55.6% (40 of 72) had in that they required noxious mechanical stimuli for activation. A low mechanical threshold was found in 44.4% (32 of 72) and responded to weak deformation of the muscle (LTM units). As can be seen in Fig. 1, quantitative mechanical stimulation of the receptive field with the pneumatic forceps showed that both types of unit differed significantly in their mechanical threshold (HTM units: 1.98 bar ± 0.05 SEM, range 1.5–2.5, $n=29$; LTM units: 1.10 bar ± 0.06 SEM, range 0.5–1.5, $n=26$; $P<0.001$). The mechanosensitive receptive fields were distributed over the entire muscle.

Conduction velocities of the recorded group IV muscle afferent fibres varied between 0.65 and 1.46 m/s (HTM units: range 0.65–1.46 m/s, mean 0.89 m/s ± 0.03 SEM, $n=39$; LTM units: range 0.67–1.18 m/s, mean 0.88 ± 0.02 SEM, $n=31$).

Background activity of the units was low; most units were silent or exhibited only single action potentials of low frequency (HTM units: mean 1.84 imp/30 s ± 0.80 SEM, range 0–20 imp/30 s, $n=33$; LTM units: mean 1.74 imp/30 s ± 1.12 SEM, range 0–30 imp/30 s, $n=27$).

3.2. Intramuscular injections

3.2.1. Tumour necrosis factor-α (TNF-α)

Sixteen group IV muscle receptors (8 HTM, 8 LTM) were tested with intramuscular injections of TNF-α. None of these neurones was excited by TNF-α. However, TNF-α had an acute desensitising action in that the mean response magnitudes to mechanical stimulation declined significantly 4 and 6 min after TNF-α injection ($n=16$, Fig. 3A; control value 23.31 imp/stim ± 2.89 SEM, dotted line in Fig. 3A; 4 min post TNF-α 15.87 imp/stim ± 1.73 SEM; 6 min post TNF-α 12.34 imp/stim ± 1.89 SEM; $P<0.01$). As can be seen from Fig. 3, the TNF-α effect took several minutes to develop (the responses 4 and 6 min after TNF-α injection exhibited a stronger reduction than the first response 2 min after injection). In units with a high mechanical threshold,

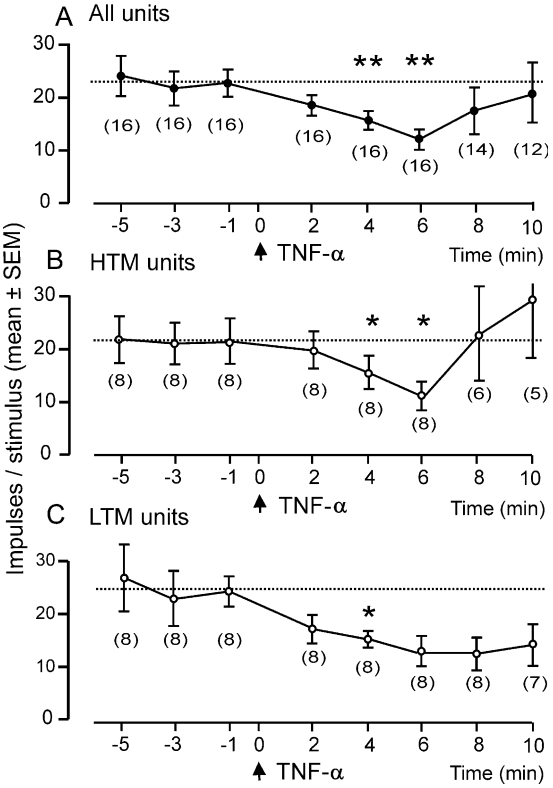

Fig. 3. Mean response magnitudes of group IV units to repeated pressure stimuli applied before and after intramuscular TNF-α injection. (A) All units, (B) high-threshold mechanosensitive (HTM) units, (C) low-threshold mechanosensitive (LTM) units. Dotted lines in A–C indicate the control value (=mean of the first three stimuli; −5, −3, and −1 min). The asterisks show the level of significance in comparison with the control value (Wilcoxon test of paired data). *$P<0.05$; **$P<0.01$. The figures in parentheses are the number of units tested. Arrows mark the time of intramuscular TNF-α injection (0 min).

the decrease in response magnitude lasted for a few minutes only, 8 min after TNF-α injection, the mean response magnitude had returned to control values (Figs. 2 and 3B). Units with a low threshold showed a trend towards a longer-lasting desensitisation (Fig. 3C).

3.2.2. Interleukin-6 (IL-6)

Two neurones out of 17 (10 HTM, 7 LTM) tested with IL-6 showed a response to intramuscular injection of IL-6 (11.8%). Both responsive neurones had a low mechanical threshold (Fig. 4A). Units with a high mechanical threshold ($n=10$) were not excited by this cytokine (Fig. 4B and C). Twelve group IV units (7 HTM, 5 LTM) were tested with repeated mechanical stimuli before and after IL-6. Compared with the control values, the mean response magnitudes were largely unchanged by IL-6 (Fig. 4D; all units: control value 35.30 imp/stim ± 8.01 SEM (dotted line in Fig. 4D), 6 min post IL-6 31.33 imp/stim ± 5.64 SEM, $n=12$; HTM units: control 25.09 imp/stim ± 6.64 SEM, 6 min post IL-6 23.14 imp/stim ± 3.12 SEM, $n=7$; LTM units: control 49.60 imp/stim ± 15.61 SEM, 6 min post IL-6 42.80 imp/stim ± 11.52 SEM, $n=5$).

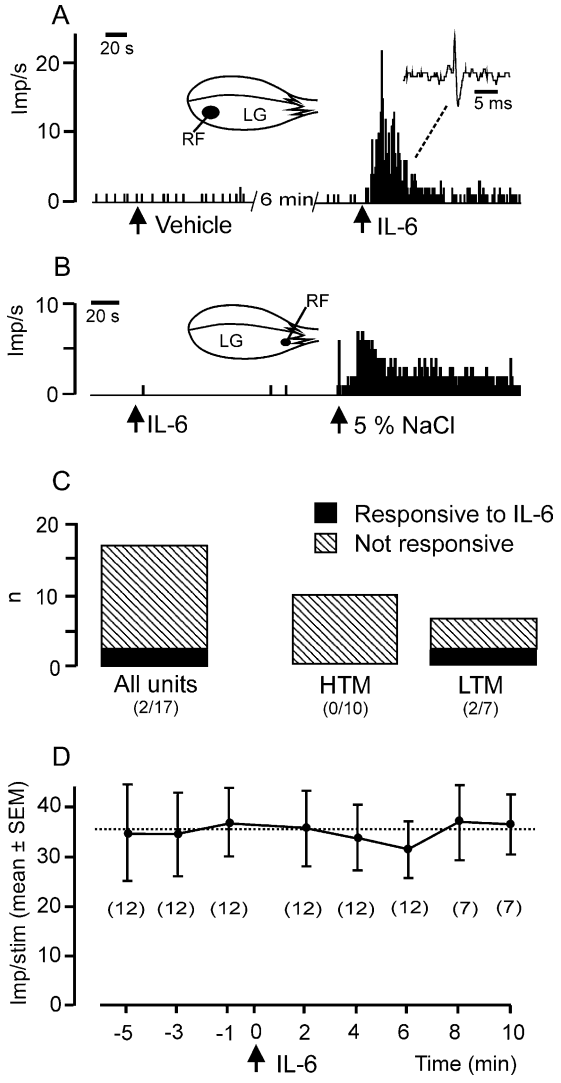

Fig. 4. Effects of intramuscular interleukin-6 (IL-6) injection. (A) Low-threshold mechanosensitive (LTM) unit responding to IL-6 injection. Lack of a response to the vehicle and marked response to IL-6. Arrows mark the time of injection. Inset: individual action potential of the IL-6-induced response. RF, receptive field; LG, lateral head of the gastrocnemius muscle. (B) High-threshold mechanosensitive (HTM) unit responding to hypertonic saline (5% NaCl) but not to IL-6. (C) Number of group IV units tested with IL-6. All units, all units investigated; HTM, high-threshold mechanosensitive units; LTM, low-threshold mechanosensitive units. Black areas, number of units responding to IL-6; hatched areas, unresponsive neurones. (D) Mean response magnitudes of group IV units to mechanical stimulation of the receptive field before and after IL-6 injection. The figures in parentheses are the number of units tested.

3.2.3. Nerve growth factor (NGF)

From 28 units (16 HTM, 12 LTM) tested, 10 responded to the NGF injection (35.7%). An example of a responsive

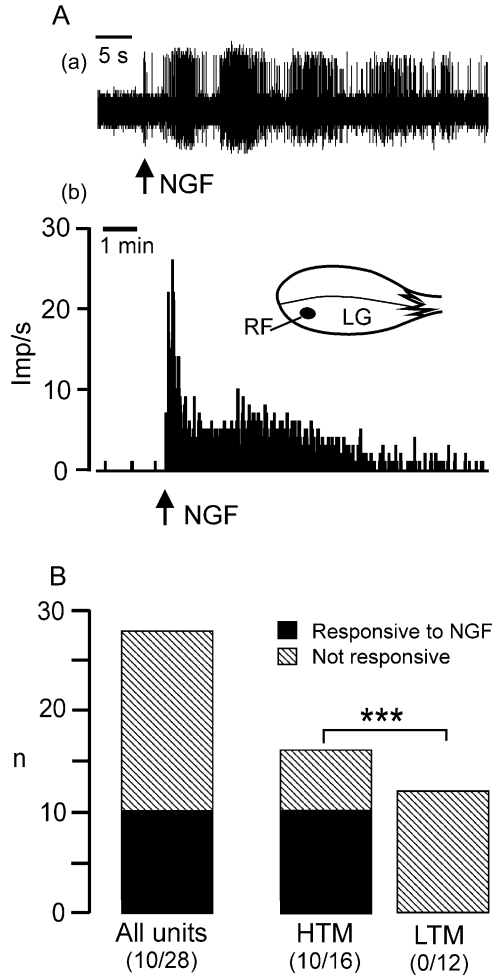

Fig. 5. NGF-induced excitation of group IV units. (A) Impulse activity of a high-threshold mechanosensitive (HTM) group IV afferent unit. (a) Original registration during the first minute of the NGF-induced response shown in b. Arrow, time of NGF injection. (b) Time histogram of the response to NGF; LG, lateral head of the gastrocnemius muscle. (B) Number of group IV units tested with NGF. All units, all units tested; HTM, high-threshold mechanosensitive units; LTM, low-threshold mechanosensitive units. Black areas, number of units responding to NGF; hatched areas, unresponsive neurones. The figures in parentheses are the number of units tested. ***$P<0.001$, Fisher's exact test.

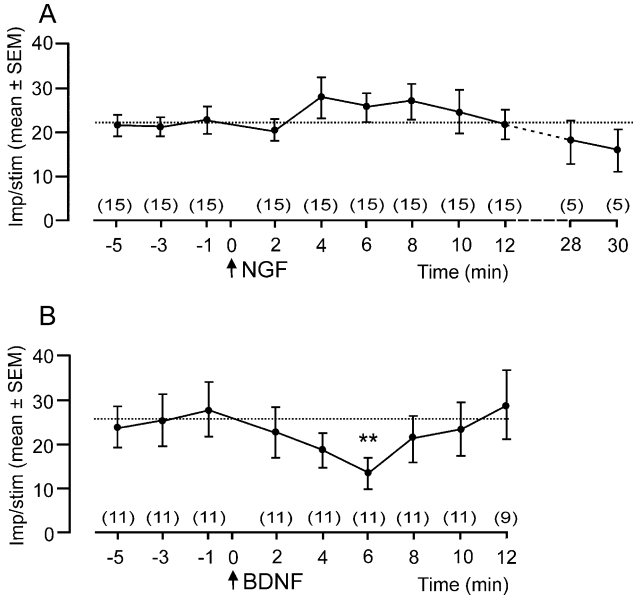

Fig. 6. Mean response magnitudes of group IV units to repeated pressure stimuli before and after intramuscular NGF or BDNF injection. (A) Mean response magnitudes of group IV units tested with NGF, all units (8 HTM, 7 LTM). The lowest P value obtained during the slight trend towards higher response magnitudes 4–10 min after NGF was $P=0.11$. (B) Mean response magnitudes of group IV units (6 HTM, 5 LTM) before and after BDNF injection. Dotted lines in A and B indicate the control value (=mean of the first three stimuli; −5, −3, and −1 min). $^{**}P<0.01$, Wilcoxon test of paired data. The figures in parentheses are the number of units tested. Arrows mark the time of intramuscular NGF or BDNF injection.

unit is shown in Fig. 5A. All responsive group IV units had HTM properties (10 out of 16 HTM units were excited by NGF, 62.5%). None of the 12 units with a low mechanosensitive threshold (LTM) showed a response to NGF. Fifteen units (8 HTM, 7 LTM) were tested repeatedly with quantitative mechanical stimulation. Within the first 8 min after NGF injection, there was a trend towards a sensitisation (Fig. 6A), but 12 min after NGF the activity was back to control level. Five units (4 HTM, 1 LTM) recorded for a period of 30 min after NGF injection likewise showed no signs of sensitisation.

3.2.4. Brain-derived neurotrophic factor (BDNF)

Eleven units were tested with intramuscular BDNF injections, six had a high mechanical threshold (HTM), five responded to moderate pressure (LTM). None of these units showed a response to BDNF. However, the mean response magnitudes to mechanical stimulation declined significantly 6 min after BDNF injection (Fig. 6B; all units: control value 25.91 imp/stim ± 5.22 SEM, 6 min post BDNF 13.45 imp/stim ± 3.52 SEM, $P<0.005$; HTM units: control 20.66 imp/stim ± 7.79 SEM, 6 min post BDNF 10.50 imp/stim ± 4.72 SEM, $n=6$, $P<0.05$; LTM units: control 32.20 imp/stim ± 6.37 SEM, 6 min post BDNF 17.00 imp/stim ± 5.37 SEM, $n=5$, $P<0.05$). Similar to TNF-α, the BDNF effect took several minutes to develop. Eight minutes after BDNF injection, however, the mean response magnitudes of all units had returned to control values (21.45 imp/stim ± 5.18 SEM).

3.2.5. Vehicle and hypertonic saline

Not a single neurone tested with the vehicle (PBS, $n=29$) showed a response (e.g. Fig. 4A). Vehicle injection did not change the mean response magnitudes to mechanical stimuli ($n=11$, control value 24.79 imp/stim ± 5.48 SEM; 6 min post vehicle injection 26.09 imp/stim ± 6.10 SEM).

Forty units that did not respond to TNF-α, IL-6, NGF or BDNF (see above) were tested with an ensuing injection of hypertonic saline. All units responded to the saline injection indicating that the test substances injected before had reached the receptive endings (Fig. 4B), but were not excitatory for that particular receptor.

4. Discussion

The data show that the cytokines TNF-α and IL-6, as well as the neurotrophins NGF and BDNF influence the impulse activity or mechanosensitivity of group IV muscle afferent units. These effects could be of functional importance when the agents are released from muscle or other cells, particularly under pathophysiological circumstances. NGF and IL-6 had an excitatory action on subpopulations of group IV units but did not acutely influence their sensitivity to pressure stimuli. These agents may contribute to the pain from an inflamed or injured muscle. TNF-α and BDNF did not excite group IV units but showed a desensitising influence, both reduced the response magnitudes to pressure stimuli for a few minutes after intramuscular injection. Both substances may constitute factors that counteract the well-known acute sensitising action of bradykinin, prostaglandin E_2, and other inflammatory agents.

The present experiments did not study the mechanisms by which the cytokines and neurotrophins influence group IV units. There are two general possibilities: (1) a direct action through specific and unspecific receptors on the endings, or (2) an indirect action by stimulating the release of agents that act on the group IV units. The different cytokines bind to specific receptors, but all use the signal transducer gp130 for inducing intracellular effects (De Jongh et al., 2003). IL-6 binds to its specific receptor IL-6R (gp80, De Jongh et al., 2003), while two TNF-α receptors with different intracellular domains mediate TNF-α effects (Vandenabeele et al., 1995). Neurotrophins can signal through two different types of cell surface receptors, the trk receptorkinases (trk) and the p75 neurotrophin receptor (p75; Chao, 2003). NGF is known to activate numerous signalling pathways through trkA and p75, while BDNF binds to trkB and p75. None of these receptors is known to be directly linked to an excitatory ionic current. Nevertheless, some data in the literature point to a direct, rapid interaction of trk

neurotrophin receptors with ion channels (Blum et al., 2002; Chao, 2003; Kafitz et al., 1999, 2000; Kovalchuk et al., 2004; Merighi et al., 2004). In the rat, it has been shown that NGF acutely increases the intracellular calcium concentration in a subpopulation of dorsal horn neurones in lamina II (Merighi et al., 2004), and that BDNF as well as neurotrophin-4/5 depolarises hippocampal neurones as rapidly as the neurotransmitter glutamate (Kafitz et al., 1999). Other data indicate that trkA receptors are tightly coupled to the capsaicin-sensitive TRPV1 (VR1) ion channel (Galoyan et al., 2003) which opens in response to heat and extracellular protons (Caterina and Julius, 2001).

During tissue inflammation, there is a stronger NGF-induced expression of neuropeptides (Donnerer et al., 1992), acid-sensing ion channels (Mamet et al., 2003), and sodium channels (Gould et al., 2000) in sensory neurones. These neuroplastic changes could generate peripheral sensitisation and hyperexcitability, but they are too slow to explain the excitatory action of NGF on group IV units that occurred within a few seconds.

In human subjects, intramuscular injection of NGF was not associated with acute pain but caused long-lasting hyperalgesia and allodynia after a latency of more than 1 h (Svensson et al., 2003). In our animal experiments, NGF activated about 60% of the units with a high mechanical threshold, which should elicit acute pain. This discrepancy may be explained by the observation that in anaesthetised rats, only a few dorsal horn neurones are activated by group IV muscle afferent fibres (Hoheisel et al., 1994, 1997; Steffens et al., 2003). Therefore, the NGF-induced activation of higher centres may not be strong enough to elicit subjective sensations. Nevertheless, activity in muscle group IV fibres is known to be very effective in inducing spinal neuroplastic changes (Hoheisel et al., 1993, 1994; Wall and Woolf, 1984).

In our experiments, intramuscular injection of TNF-α had a short-lasting desensitising action on group IV units, while in other experimental studies intramuscular (Schäfers et al., 2003) and intraplantar (Cunha et al., 1992; Woolf et al., 1997) TNF-α injections evoked hyperalgesia within a few hours. These data suggest a dual action of TNF-α when released intramuscularly or in other deep tissues: it suppresses neuronal excitability early after release but contributes to neuronal hyperexcitability in a later phase. There is evidence in the literature indicating that the hyperalgesic action of TNF-α may be indirect in that it induces the upregulation of other inflammatory mediators that cause hyperalgesia (Schäfers et al., 2003; Woolf et al., 1997). The acute desensitising action of TNF-α on group IV muscle afferent units is in contrast to findings from C fibres innervating the rat skin. In the rat, subcutaneously injected TNF-α sensitised cutaneous C-fibre nociceptors to mechanical stimuli within 30 min (Junger and Sorkin, 2000) while in our time window (up to 10 min after injection) intramuscularly injected TNF-α caused a desensitisation of group IV units to mechanical stimulation. Like TNF-α, in our experiments BDNF had a hypoalgesic action in that it reduced the response magnitudes of HTM units to noxious mechanical stimuli, while in the rat skin BDNF caused thermal hyperalgesia (Shu et al., 1999). Thus, TNF-α and BDNF may have different effects on cutaneous and muscle nociceptors.

The finding that IL-6 did not excite muscular group IV units with a high mechanical threshold which presumably were nociceptive (see below) is in line with data from skin nociceptors. In the rat skin, IL-6 in combination with its soluble IL-6R—but not alone—sensitised nociceptors to heat (Obreja et al., 2002; Oprée and Kress, 2000), which was interpretated as indicating that cutaneous nociceptors did not express the IL-6R but only gp130.

HTM and LTM units—which were classified by hand in this study—differed significantly in their threshold to quantitative pressure stimuli indicating that both types represent distinct neurone populations with different functions. Possible functions of HTM and LTM group IV units were discussed in detail in a previous study of our group (Hoheisel et al., 2004): units with a high mechanical threshold (HTM) fulfill several criteria of a nociceptor while units that have a mechanical threshold in the innocuous range (LTM) may have non-nociceptive functions (Haouzi et al., 1999; Iggo, 1961; Light and Perl, 2003; McCloskey and Mitchell, 1972a,b; Mense and Meyer, 1985). The finding of the present study that background (ongoing) activity of LTM units was not higher than that of HTM afferents speaks against the assumption that LTM neurones are sensitised former HTM neurones. Despite the difference in mechanical threshold, both types of group IV muscle afferent units appear to share some chemical response profiles. All inflammatory substances tested so far excited subpopulations of both HTM and LTM units (Fock and Mense, 1976; Hoheisel et al., 2004; Kaufman et al., 1982). NGF, however, had an excitatory action exclusively on units with a high mechanical threshold, while IL-6 excited a few low-threshold mechanosensitive units but no high-threshold mechanosensitive ones. This finding is a further indication that LTM and HTM group IV muscle afferent units are functionally distinct.

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