Acknowledgments

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Summary points

The most effective form of acupuncture treatment for LBP is not known.

Many previous clinical studies have used standard acupuncture points.

This study in elderly patients showed a strong trend for deep needling at trigger points to be more effective than either shallow needling at trigger points or standard needling of standard points.

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A proposed experimental model of myofascial trigger points in human muscle after slow eccentric exercise

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Abstract

Background The purpose of this study was to develop an experimental model of myofascial trigger points to investigate their pathophysiology.

Methods Fifteen healthy volunteers who gave informed consent underwent repetitive eccentric exercise of the third finger of one hand (0.1Hz repetitions, three sets at five minute intervals) until exhaustion. Physical examination, pressure pain threshold, and electrical pain threshold of the skin, fascia and muscle were measured immediately afterwards and for seven days. Needle electromyogram (EMG) was also recorded in a subgroup of participants.

Results Pressure pain thresholds decreased to a minimum on the second day after the exercise, then gradually returned to baseline values by the seventh day. On the second day, a ropy band was palpated in the exercised forearm muscle and the electrical pain threshold of the fascia at the palpable band was the lowest among the measured loci and tissues. Needle EMG activity accompanied with dull pain sensation was recorded only when the electrode was located on or near the fascia of the palpable band on the second day of exercise.

Conclusion These results suggest that eccentric exercise may yield a useful model for the investigation of the myofascial trigger points and/or acupuncture points. The sensitised nociceptors at the fascia of the palpable band might be a possible candidate for the localised tender region.

Keywords

Myofascial trigger point, acupuncture point, eccentric exercise, palpable band, fascia, controlled trial.

Introduction

The clinical value of the concept of myofascial trigger points has been widely recognised,[1,2] and the close relation between myofascial trigger points and acupuncture points has also been noted.[3,4]

Myofascial trigger points have been characterized by their location on a palpable taut band of skeletal muscle, and by the induction of local twitch responses, jump signs and particular patterns of referred pain.[1,5,6] However, it has been shown to be quite difficult to discriminate trigger points from the tender points found in fibromyalgia patients and spontaneous tender points in normal subjects.[1,7]

The palpable band has long been supposed to be the site of muscle contracture, based on the existence of contraction knots and the lack of electrical activity.[8] However, recent studies have demonstrated that spontaneous electrical activity (SEA) can be recorded from palpable bands in patients with the myofascial pain syndrome. The origin of this SEA is uncertain, and different possibilities such as endplate potential and intrafusal fibre activity in muscle spindles have been proposed.[6,9] Uncertainty also exists in discussion about the acupuncture points: some acupuncture points have been characterized by their tenderness (called '*Ah shi*' points) which are often situated on a palpable band.[10] In addition, electrical activity that has been recorded from acupuncture points has been proposed to originate in spinal reflex activity,[11] or in intrafusal fibre activity in muscle spindles.[12] Thus, myofascial trigger points and acupuncture points seem to have some similarities, although their pathophysiology is still not fully understood.

In the present study, we investigated the localised tenderness of experimentally induced

muscle pain, compared its characteristics with those of myofascial trigger points, and evaluated its usefulness as a model for trigger points and possibly some acupuncture points. We used repetitive eccentric exercise to generate delayed onset muscle soreness (DOMS): eccentric exercise involves a muscle lengthening under load, and is particularly associated with the production of DOMS.

Methods

Subjects

Fifteen healthy volunteers (five male and ten female), ranging in age from 18 to 48 years (mean 22.6 years) who gave informed consent, were involved. All were in good health and not engaged in any training programmes involving exercise of the extensor digitorum muscle. The first group of seven subjects (five male and two female) underwent three sets of investigations in random order (crossover design): 1) a control procedure in which pain thresholds were measured (see below) without exercise; 2) a series of assessments in which pain thresholds of the tender area were measured after exercise; 3) a series of assessments in which the distribution of pain thresholds around the tender area after exercise were measured, again after exercise. Each set of investigations was performed at intervals of six months or more, since the induction of DOMS leads to a resistance to further development of the condition for some time. The second group consisted of the remaining eight subjects (all female) who underwent a single series of EMG recordings daily after exercise.

Procedure of eccentric exercise

The subject was seated, with one forearm supported as far as the wrist on a mat on top of a desk. A moveable 475g weight, consisting of a metal nut threaded on to a long-shaft bolt, was placed on the middle finger of one hand. The position of the weight was adjusted until the subject could retain the finger in a horizontal position for at least 10 seconds. The subject was then asked to hold this position as long as possible, and each time the finger bent 20° downward at the metacarpophalangeal joint, the finger was manually reset to the original horizontal position by the experimenter. This exercise continued repeatedly until exhaustion of the volunteer's extensor muscle. Three sets of this loading exercise were performed, separated by five minute rest periods. During the exercise, the electromyogram (EMG) of the extensor digital muscle was monitored and displayed on an oscilloscope to indicate when other muscles were being recruited to assist the tiring extensor digitorum.

Pressure Pain and Electrical Pain Threshold Measures

Pressure pain threshold (PPT) was determined by pressing the skin over the muscle with a finger pressure algometer (Aikoh Engineering Corp, Model 9500) which has a probe 6mm in diameter.[13] The measurement was repeated three times where a sensation of tenderness was first elicited and the minimum value was employed as the threshold value.

Electrical pain thresholds (EPTs) of skin, fascia and muscle were measured by a pulse algometer (Unique Medical Co Ltd, UPA-100).[14,15] A stainless steel needle electrode insulated with acrylic resin (180μm in diameter, impedance 391±30kΩ at 1kHz; Nishin Medical Institute) was used as a cathodal monopolar stimulating electrode. The needle was inserted manually and held in a guide tube attached to skin with adhesive tape. The needle was inserted progressively in steps of 0.5-1.0mm in order to measure the pain thresholds of the skin, fascia and muscle. The location of the fascia was determined by the needling stiffness (physical resistance to the needle) with the help of ultrasonic echo imaging (LOGIQ™400, GE Medical Systems) to identify the depth of the border between subcutaneous tissue and muscle. A metal, anodal, surface electrode 10mm in diameter was attached to the skin 10mm away from the needle. The subjects were requested to press a button when they felt pain (pain threshold), which automatically triggered a digital display of the stimulus current and terminated the current stimulus pulse.

For the control session without exercise, the location for testing both PPT and EPT was the middle of the extensor digital muscle, where the focal muscle tenderness tended to be produced.

To assess the distribution of the pressure and

electrical pain thresholds, assessments were made at the tender region and at four points 10mm away from the focus (proximal, distal, medial and lateral).

For the three sets of examinations of these seven subjects (see *Subjects* above), the schedules for assessment were as follows: 1) for the control session, pressure and electrical pain thresholds were measured once daily for seven days; 2) for threshold levels, PPTs were measured before, immediately after, and one, two, three, four and seven days after the eccentric exercise. EPTs were measured on the second and seventh day after the exercise; 3) for assessment of pain distribution, measurements were made on the second and seventh day after the exercise.

Detection of palpable band

On the second day after the exercise, the forearm extensor muscles were examined for the presence of a palpable band by a well-trained licensed acupuncturist with four years' training and seven years' clinical experience. The subject was again seated with the forearm placed relaxed on a soft mat, while being examined with repeated light pressure with the fingertip. In several cases, but not all, the observer was blinded as to which arm had been exercised: in other cases, he was present during the exercise procedure.

Recordings of referred pain pattern

The pattern of the referred pain elicited by finger pressure at the most tender region on the palpable band was drawn on the skin surface then was copied on a clear sheet. When the subject could not recognize any patterns of referred pain, the subject was classified as 'no referred pain'.

Recordings of needle EMG activity

In the second group of eight subjects the electrical activity at the skin, fascia and muscle of the focal tender region and non-tender region of palpable band and 10mm away from the band were measured.

An insulated needle, as used for the stimulation above, was used as a recording electrode, together with an indifferent surface electrode. The EMG activity was amplified using a band pass filter of 0.1-10kHz (DAM-80, WPI), displayed on an oscilloscope (V-202F, Hitachi) and recorded on a data recorder (RD-135T, TEAC). Electrical activity was recorded for one minute or more at 1.0mm increments of depth. The unitary discharge that continued for at least 30 seconds with relatively regular intervals (1-60Hz) was classified as EMG(+). At the same time, the surface EMG was recorded from a pair of metal surface electrodes (10mm in diameter) placed on the skin 50mm distal from the needling point.

Measurements were made on the second day after exercise.

Statistical analysis

Pressure pain and electrical pain thresholds were shown as mean ± standard deviation (mean±SD). Non parametric multiple test of Tukey and Dunnet's multiple test (Yukms version 5; Yukms Company) were used for the statistical analysis. The level of statistical significance was defined as $P<0.05$.

Results

Immediately after the repetitive eccentric exercise, subjects reported warmth and tenderness of the working muscle of the forearm. The region of tenderness was gradually restricted to the muscle in a region about 50mm distal to the elbow, where a ropy taut band could be detected on the first and second days after the exercise. By the seventh day, the palpable bands and local tender regions were hardly detectable.

Changes in pressure pain threshold

The PPTs at the centre of the measuring area, where a palpable band was usually formed after the eccentric exercise, are shown in Figure 1. In the control session, the PPT did not change significantly during the experimental periods for seven days (Dunnett's multiple test, P=0.75-0.99). After exercise, on the other hand, the PPTs gradually decreased to a minimum on the second day, then recovered by the seventh day. The mean value for PPT before the exercise was 972±178 arbitrary units (AU; 1AU=1.8g), decreasing significantly to 274±57AU on the second day (Dunnett's multiple test, $P<0.01$).

Spatial distribution of the PPTs on and around the palpable band is demonstrated in Figure 2, and the values (Table 1) show that a significant difference between the tender locus and other

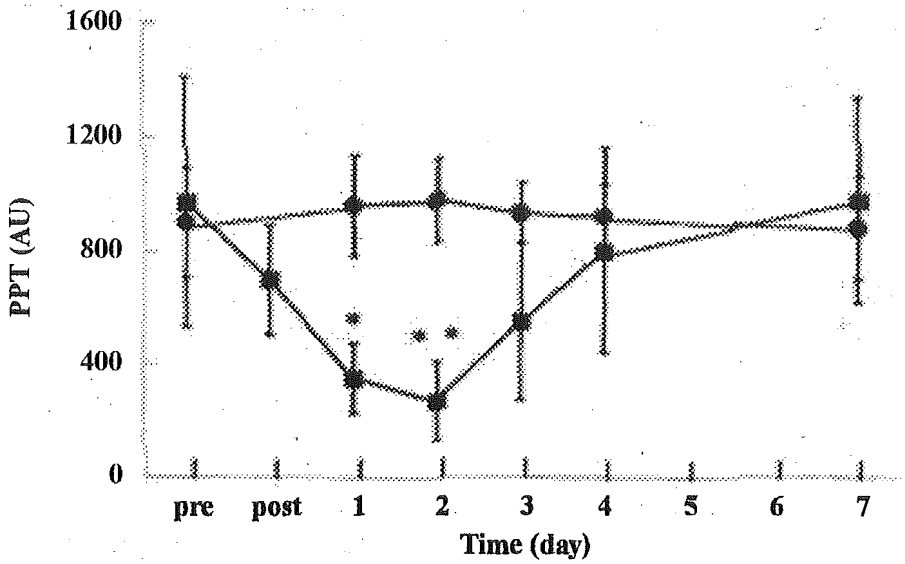

*Figure 1 This figure shows the effects of eccentric exercise of extensor digitorum on the pressure pain threshold (PPT) in seven subjects (mean±SD arbitrary units (AU)). Circles indicate values in the control session without exercise, and squares indicate values in the experimental session with exercise. Asterisks indicate significant difference compared with the baseline threshold (Dunnet's multiple test, *P<0.05, **P<0.01); **pre: pre-exercise; post: post-exercise.***

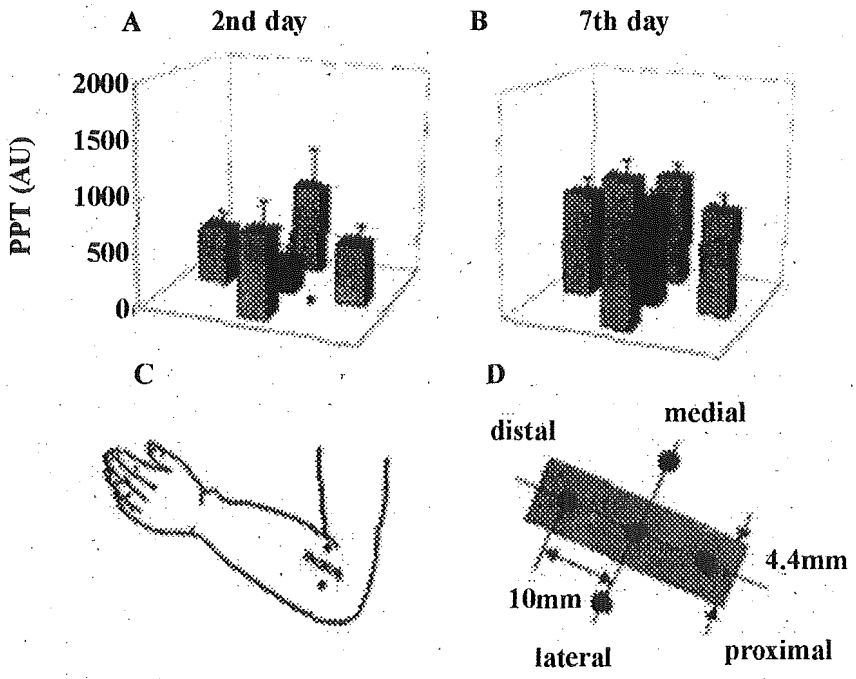

Figure 2 This figure shows the distribution of pressure pain thresholds on and around the palpable band. A and B show the distribution of pressure pain thresholds (PPTs) on the second and seventh days after the exercise. The distribution of PPTs on the sites of PPT measurement are illustrated schematically in C and D.

Table 1 **Pressure pain thresholds (mean±SD, arbitrary units) in and around the palpable bands**

Location	centre	distal	proximal	medial	lateral
Second day	296±32**	534±61	561±70	839±148	766±112
Seventh day	944±128	982±118	973±136	1057±175	1345±118

** Significantly different from all four other values (Tukey, P<0.01)

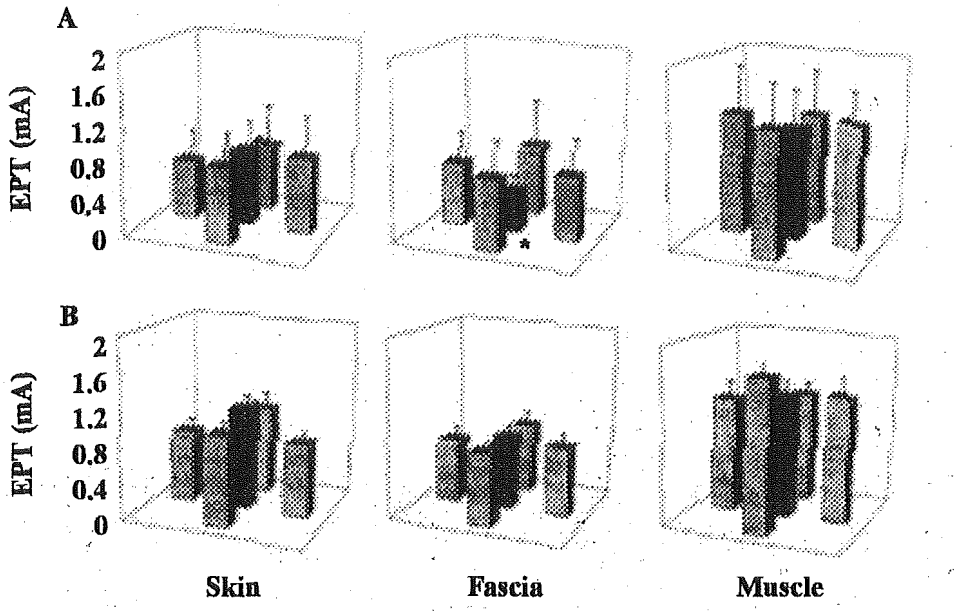

Figure 3 This figure shows the distribution of electrical pain thresholds (EPTs) of the different tissues. (A) shows the readings taken on the second day, and (B) shows the readings taken on the seventh day. The location of measuring sites were the same as in Fig 2. The asterisk shows significant difference from ***other four sites (nonparametric Tukey's multiple test, P<0.05).***

Table 2 **Electrical pain thresholds (mean±SD mA) in sites at and 10mm away from the palpable band on the second day after exercise**

Location	palpable band	distal	proximal	medial	lateral
Skin	0.84±0.11	0.65±0.11	0.77±0.19	0.85±0.18	0.84±0.15
Fascia	0.45±0.08**	0.71±0.11	0.82±0.21	0.73±0.15	0.79±0.15
Muscle	1.27±0.14	1.39±0.21	1.29±0.21	1.29±0.21	1.41±0.18

** Significantly lower than values for other sites in fascia (Tukey's multiple test, P=0.01)

points was detected on the second day (Tukey, P<0.01). There were no differences on the seventh day. The distribution of PPT before exercise was not examined because the precise location of the palpable band could not be predicted.

Changes in Electrical Pain Thresholds

Figure 3 shows the EPT of the skin, fascia and muscle, on the second day (A), and on the seventh day (B), at the tender locus on the palpable band, and four surrounding sites. The values are given in Table 2, which indicates that there was a significantly lower EPT for the fascia only, at the tender locus on the second day (Tukey's multiple test, P=0.01). There was no difference on the seventh day. There was no significant difference among the five EPTs of the skin and muscle.

The sensations elicited by the current pulse stimulation apparently varied with the tissues stimulated. In the skin, volunteers reported

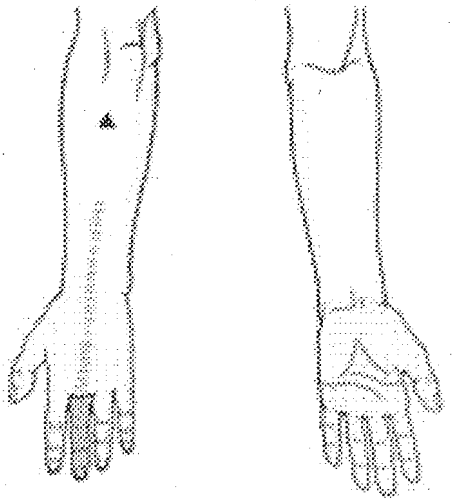

Figure 4 This is a schematic illustration of the pattern of referred pain from the palpable band. Referred pain was elicited by mechanical stimulation of the most localised tender region (▲). More common areas of referred pain are represented by denser shading. Note that the dorsal part of the middle finger is the most frequent area of the pain referral.

localised sharp pain and sometimes burning. In the fascia, dull and uncomfortable pain tended to be reported, and in the muscle, pulling pain.

Development of palpable band

Immediately after the eccentric exercise, the working muscle tended to be swollen and became hard to touch. On the second day after exercise, a clear ropy palpable band could be detected at the musculotendinous attachment area of the working muscle. The band was detected in all 15 exercised subjects by a well-trained observer. In several cases, the observer was asked to detect which of the two arms contained the palpable band in a blinded manner, and in all cases he detected the band correctly. The mean width and length of the palpable band on the second day were 4.4±0.6mm and 33.0±1.9mm respectively. The existence of a taut band was also confirmed during the manual needle insertion for measuring the electrical pain threshold. Resistance was increased when the needle penetrated the palpable band. On the seventh day after exercise, the bands had softened and become diffuse, and could hardly be identified.

Induction of referred pain

Application of an intense pressure to the tender locus usually induced various pain sensations projected over the distal area accompanied by dull and uncomfortable sensations. These referred pain sensations were felt most intensely in the hand, and formed a line that extended along the dorsum of the forearm, wrist, hand, and the middle finger, as shown schematically in Figure 4. The tender locus of the palpable band had the lowest threshold for inducing referred pain.

During the measurement of EPT, referred pain was also frequently reported when the stimulating needle electrode was inserted into the palpable band. The fascia at the tender focus most readily induced referred pain, and stimulation of the skin and muscle hardly provoked the referred pain.

Needle EMG activity at the tender region

In general, no spontaneous EMG activity was recorded from the relaxed muscle of the subjects. In the control session, we could not detect any sustained EMG activity from the fascia or muscle, only a transient injury burst response. On the other hand, sustained unitary EMG activity was frequently recorded when the recording needle was situated close to the fascia at the tender locus on the palpable band. Usually the discharges continued for 30 seconds with regular frequency (1-60Hz), but in one case they continued for more than 10 minutes. Table 3 shows that sustained EMG activity was only recorded from the tender region on the palpable band.

Table 3 **Incidence of the presence EMG (+), or absence EMG (-), of sustained EMG activity recorded at different electrode positions**

Position of electrode	EMG(+)	EMG(-)	total
Tender region on the palpable band	7	1	8
Non-tender region on the palpable band	0	8	8
Non-tender region beside the palpable band	0	8	8

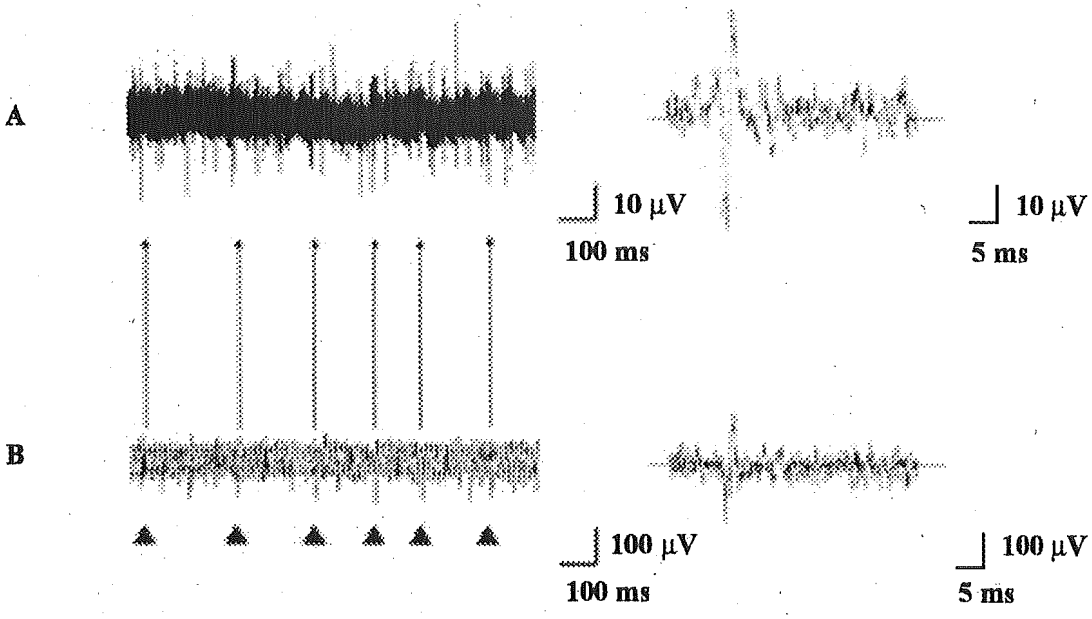

Figure 5 This figure shows simultaneous recordings of sustained EMG activity with the needle and surface electrodes. The needle electrode was placed at the palpable band and surface electrode 50mm away from the needling site. A shows the needle EMGs and B shows the surface EMG recordings (time scales are different in the right and left figures). Solid triangles mark spike activity in the surface EMG, and arrows indicate the spike discharges of needle EMGs. The synchronous appearance of the spike activities ***should be noted.***

Figure 5 demonstrates an example of EMGs recorded from an insulated needle electrode at the palpable band (A) and from a surface electrode on the skin 50mm distal to the needle electrode over the same muscle (B). Synchronized unitary discharges were observed at the two recording sites simultaneously, and also disappeared at the same time.

When EMG activity was recorded, the subjects frequently reported strong dull pain sensation which subjectively appeared to be associated with the frequency of the EMG activities.

Discussion

Repetitive eccentric exercise produced a localised tender region with a palpable ropy band on the second day, with decreased pain threshold of the fascia at the locus. Intense pressure applied to the tender locus on the palpable band also elicited referred pain. Sustained electrical activity was frequently recorded from the palpable band, and was accompanied by a deep, dull pain sensation.

The experimental model of localised tender region

Eccentric contraction is known to produce tissue injury more easily than concentric contraction,[16] because muscle can bear a greater load under eccentric contraction, and therefore the evoked tension of a single muscle fibre is greater.[17]

The majority of previous studies on experimental DOMS have reported only muscle soreness,[18,19] or have given a rough illustration of the distribution of tenderness on the muscle.[20] No previous reports have described a palpable band or a localised tender region, as we describe. Our findings may be due to the size of the muscle studied, and the methods of examination and pain measurement. Previously, relatively large muscles such as the quadriceps femoris or biceps brachialis have been used, whereas we exercised a small forearm muscle and then carefully palpated in order to find the most tender region along the length of a taut band. We identified a reduction in the PPT which was greatest at the tender region in the palpable band, and the EPT of the fascia at that site was selectively decreased. The distribution of

the EPTs in the fascia was similar to that of the PPTs, so the tender structure detected by the pressure algometer was presumably the fascia. This is the first report of a localised tender region on a rope-like taut band appearing after eccentric exercise and of a selective decrease in the pain threshold at the fascia of the extensor digitorum muscle.

The mechanisms of localised tenderness

The facts that the tender region was not spontaneously painful and that pain was evoked only when light pressure was applied suggest that the sensitisation of nociceptors in the damaged tissue might be a possible cause of the restricted tenderness. The time-course of development of DOMS has been shown to be similar to that of inflammatory processes,[21-23] and the production of various inflammatory mediators such as prostaglandin E_2 (PGE_2), bradykinin and histamine in the damaged tissues has been reported.[21] Recently, we found that the application of indomethacin, a prostaglandin synthesis inhibitor, suppressed the development of DOMS in rabbits.[24] An increase of prostaglandin E_2 in the muscle of the DOMS subject has also been demonstrated by microdialysis.[25] These mediators sensitise the nociceptors and may activate silent nociceptors in the muscle.[26-28]

The polymodal-type receptor is proposed as a possible candidate for the formation of the tender point.[29] It is responsive to mechanical (acupuncture), thermal (moxibustion), and chemical stimulation (various chemical substances are produced by acupuncture and moxibustion). Excitation of the polymodal-type receptors produces flare and oedema (neurogenic inflammation), and such phenomena are frequently observed after acupuncture and moxibustion. The sensation elicited by selective activation of the polymodal-type receptors is similar to the *de qi* sensation, and sensitisation of the polymodal-type receptors may be a possible cause of tender points and myofascial trigger points.[29]

Central sensitisation should be considered as another possible explanation for the tenderness. A decrease in the threshold of nociceptive dorsal horn neurons in acute inflammation is well known.[30] Expansion of the receptive field and appearance of a new receptive field were induced by intramuscular injection of chemical algesics.[31] These phenomena, however, do not seem to be the major cause of the formation of localised tender regions. In the present study we found that the tender region on the palpable band was highly restricted to a spot-like area less than 2mm in diameter and located on the fascia. These very localised changes in the electrical pain threshold of a particular tissue are difficult to attribute to the effects of central sensitising mechanisms as they are currently understood.

Formation of the palpable band

In the present study, a ropy palpable band was detected on the second day after the exercise, and disappeared, or was hardly detectable, by the seventh day. Clinically, the development of the palpable band is considered to be a characteristic feature of a myofascial trigger point and is readily detected by well-trained examiners.[32] Fricton et al found that experienced raters were more reliable in detecting the trigger point than inexperienced raters.[5] It should be noted that the training in palpation is important.

The palpable band was initially regarded as a localised muscle contracture induced by the increase of intracellular calcium ion within the muscle cell,[33] then the idea developed into the more sophisticated energy crisis theory.[1;34] This theory postulates an initial release of intracellular calcium from the sarcoplasmic reticulum or inflow from the extracellular fluid through the injured membrane. The increase of intracellular calcium ions causes sustained contraction of the muscle, which may inhibit the local circulation and thus induce a shortage of oxygen and ATP in the tissues. The lack of energy may inhibit calcium re-uptake into the sarcoplasmic reticulum by the calcium pump, which would perpetuate the vicious cycle. While the increased excitability of the motor neuron is undoubtedly a possible mechanism for the increase in muscle tension and rigidity, the lack of EMG activity from the palpable band has been well established,[33] and it seems that muscle contraction is unlikely to be the cause of the palpable band. Localised oedema developing in deep tissues was proposed as another possibility.[29] In the present model,

eccentric exercise undoubtedly produced muscular tissue injury. When the localised muscle fibres are damaged, causing an accumulation of extracellular water and an increase in intra-tissue pressure, this may be detected as a taut band.

Referred pain phenomena, electrical activity and local twitch response

It has been well established that intramuscular injection of hypertonic saline induces particular referred pain patterns depending on the sites of injection.[35] This phenomenon has been well established in recent studies with human subjects.[36;37] Our data clearly demonstrate that the localised tender region is the most sensitive to elicit at the localised tender region and that needle insertion at the fascia also frequently provokes referred pain.

Recently, the participation of spinal neurons in referred pain was demonstrated in anaesthetised rats,[30;31] as an intramuscular injection of bradykinin induced a new receptive field of nociceptive dorsal horn neurons. This suggests possible mechanisms of referred pain phenomena from the muscle.

Lack of electrical activity in the palpable band has long been commonly accepted, and various mechanisms were proposed based on that observation. Recently, however, spontaneous electrical activity (SEA) has been recorded from myofascial trigger points in human subjects.[9;38] The cause of the SEA has not been clarified yet. This SEA was not considered to be the result of spastic hyperexcitability of motor neurons because it was recorded from very restricted sites in the muscle. Simons pointed out the similarity of the SEA to the end-plate potentials in clinical electromyogram studies.[6;39] On the other hand, Hubbard argued that the SEA was activity of the intrafusal muscle of the muscle spindle elicited by the sympathetic nerve.[9]

In the present study we could record the sustained EMG activity at the restricted tender region on the palpable band, and the EMG activity was accompanied by strong deep pain sensation. In other words, the electrical activity seemed to be the result of the insertion of a recording electrode into the tender region which produced deep pain sensation. The fact that the synchronised unitary EMG discharges were recorded simultaneously from the surface electrode and needle electrode (Fig 4) strongly suggests that the recorded sustained EMG activity was the reflex activity evoked by the nociceptive inputs from the fascia. Our recent study demonstrated that sustained EMG activity recorded from the eccentrically exercised rabbit palpable band was clearly suppressed by acupuncture needling to the muscle 50mm away from the band.[40] Although we could not exclude the possibility of the endplate potential, the majority of the sustained EMG activity in the present study might be the reflex activities elicited by nociceptive inputs produced by the needle insertion to the tender region of palpable band.

The local twitch response (LTR) elicited by palpation of the taut band is a useful indicator of a myofascial trigger point, and the LTR was considered to be a spinal reflex elicited by the noxious inputs from the trigger point.[38;41] In our study, similar LTR was frequently observed during the insertion of the needle electrode. Thus, the features of the restricted tender region in our study and those of the trigger point are quite similar. Therefore, some trigger points may be explained as the result of DOMS-like phenomena.

The relationships between myofascial trigger point and acupuncture point

The present experimental model was based on the similarity of myofascial trigger points and acupuncture points. The characteristics of the trigger point such as localised tender points on the palpable band and a typical referred pain and local twitch response are also clinically useful indicators of the acupuncture points. Melzack et al found that the location of acupuncture points are coincident with trigger points in 100% of cases, allowing for a difference of 3cm. Typical referred pain patterns of the trigger point also resemble the meridian patterns. Moreover, sustained electrical activity was frequently recorded from trigger points and acupuncture points.[10;42]

The present model may be useful for further investigation of myofascial trigger points and certain acupuncture points.

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Editorial comment

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This paper's passage from submission to publication has presented the editors with a dilemma: whether to continue remorselessly in a reiterative process of review, dispute and revision over several months, or whether to publish the paper and let it stimulate. The findings themselves have been clearly described in the paper, and we are left with differences of opinion that cannot easily be resolved without doing more studies. Therefore, out of respect to the authors' standing in the world of acupuncture physiology, and to the potential importance of this claimed model of the myofascial trigger point, we decided to publish the paper after two sets of revisions; and, out of respect and gratitude to our reviewers (we used four altogether, three of whom specialise in this area of research) we want to summarise the limitations of the paper that they have pointed out, and other possible reasons for caution in accepting this model in its present form and at the present time.

One set of limitations concerns the likelihood that the changes seen in delayed onset muscle soreness (DOMS) are really similar enough to trigger points to provide a meaningful model. DOMS usually affects whole muscles and a number of tender areas can be found. The whole muscle may develop the characteristics which could appear to be the 'taut band' of a myofascial trigger point, but some reviewers questioned whether a taut band in the middle finger portion of the extensor digitorum could be differentiated by palpation from the whole muscle. They commented that the damage in DOMS affects the entirety of the muscle, and therefore is significantly different from the focal symptoms associated with trigger points. They suggested that it was plausible that the 'palpable band' that the authors report in their paper might be a swollen entire muscle in the forearm, which becomes hard because the fascia which envelops it does not allow the oedema to dissipate. The tender sites in DOMS have not been shown previously to refer pain [which does not mean they cannot, of course]. The clinical picture of DOMS is different from that of trigger points: DOMS settles in a week or so, and actually confers some protection against further episodes of DOMS. Trigger points, in contrast, tend to persist at least in latent form and are more likely to be made worse by further insults such as over-exercise. We are in the land of uncertainty since nobody knows the pathophysiological mechanisms of DOMS or trigger points. An associated problem is an ethical one about using this model in volunteers: the pain of DOMS is difficult to relieve, which may be an acceptable price to pay for a volunteer running a marathon, but hardly fair on those who help trigger point research!

Another area of doubt is how to interpret the various features of the electrical activity reported here. Firstly, the authors report that they recorded the maximal electrical activity from the fascia. It has to be said that the basis for claiming the needle was exactly at the fascia can be challenged – the ultrasound examination was not simultaneous, so the judgement probably relied largely on the 'feel' of the needle-tip, which is subjective. Secondly, the claim that sustained needle EMG activity arose in the fascia needs further explanation and exploration. Fascia seems unlikely to be the source of action potentials, nor is it likely to be a source of other spontaneous electrical activity, though it might *conduct* action potentials from remote muscles (which could be identified by further studies recording simultaneously from intramuscular needles). One reviewer doubted whether the

recordings made simultaneously from the skin could be interpreted correctly without fully analysing their characteristics, particularly the time-course (which would differ from direct recordings, since it is conducted through tissues).

We know that many significant scientific discoveries are greeted by disbelief. Progress relies on the exchange of ideas that provoke others to comment and criticise, and to conduct more experimental studies. We therefore publish this article in the hope of stimulating just such a response.

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Responses of C-fiber low threshold mechanoreceptors and nociceptors to cold were facilitated in rats persistently inflamed and hypersensitive to cold

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Abstract

Cold allodynia is an annoying symptom in conditions of chronic inflammation such as rheumatoid arthritis. To examine whether primary afferent nerve activities are changed in association with hypersensitivity to cold, we recorded single nerve activities from the sural nerve in persistently inflamed rats in vivo. Inflammation was induced by an injection of complete Freund's adjuvant (CFA) solution into the tibio-tarsal joint. Inflamed rats showed an increased number of paw shakes to paw immersion in 25 °C water (pre-inflammation: 1.15 ± 0.58, 2-week inflammation: 4.70 ± 1.15). We also recorded cutaneous C-fiber activities under pentobarbital anesthesia and studied their responses to thermal and mechanical stimuli. The response of C-low threshold mechanoreceptors to cooling (total discharges between 27 and 23 °C) increased 1.8-fold (control group: 5.17 ± 1.04 impulses, inflamed group: 9.38 ± 1.47 impulses). In addition, the proportion of C-nociceptor units responding to cold down to 2 °C was significantly greater in the inflamed group (9 out of 18 units; threshold: 10.0 ± 2.6 °C) than in the intact group (1 out of 14 units; threshold: 4.0 °C). These results suggest that the facilitated responses of these primary afferents are associated with cold hypersensitivity in chronically inflamed conditions.

Keywords: Cold allodynia; Cold hyperalgesia; Rheumatoid arthritis; Persistent inflammation; Nociceptors; Low threshold mechanoreceptors

1. Introduction

Exposure to a cold environment often induces pain in patients with chronic inflammatory conditions such as rheumatoid arthritis, and this pain is a serious problem that interferes in their everyday lives (Jahanshahi et al., 1989; Jamison et al., 1995; Drane et al., 1997). Many complain that their pain becomes worse even with mild temperature decreases that are surely not noxious. This cold allodynia/hyperalgesia in chronic inflammation is often explained by a putative but not experimentally proven mechanism; namely, that tissue ischemia resulting from cooling-induced vasoconstriction sensitizes or excites nociceptors. Rat adjuvant-induced arthritis, the histopathology of which resembles that observed in rheumatoid arthritis, has been used to investigate inflammation-induced changes for nearly half a century (Lewis et al., 1985); however, few behavioral studies exist regarding changed cold sensitivity. Persistent inflammation has been shown to induce hyperalgesia to noxious cold in rats (≤10 °C) (Perrot et al., 1993; Jasmin et al., 1998), but there has been little investigation of the effects of mild cold on pain (Sato et al., 2000). Cold allodynia in neuropathic pain, which contains inflammatory components, is now attracting the attention of researchers, most of whom are focusing on its central mechanism (Vrinten et al., 2000; Yashpal et al., 2001). Thus, virtually no research on peripheral mechanisms of cold allodynia/hyperalgesia has been done. This contrasts sharply with the many studies on the peripheral mechanisms of mechanical and/or heat hyperalgesia in inflammation (Kocher et al., 1987; Schaible and Schmidt, 1988; Mizumura and Kumazawa, 1996; Andrew and Greenspan, 1999; Koltzenburg et al., 1999; see Mizumura, 1998 for review).

Several types of primary afferents are known to respond to cooling. Cold receptors, which are a type of thermoreceptor, respond vigorously even to a slight decrease in temperature (Hensel et al., 1960; Iggo, 1969; Spray, 1986). Nociceptors respond predominantly to noxious stimuli such

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as intense mechanical and/or heat stimuli, and some of them respond also to noxious cold (<15 °C) (Bessou and Perl, 1969; Shea and Perl, 1985; Simone and Kajander, 1996, 1997). Low threshold mechanoreceptors consist of rapidly conducting Aβ- and slowly conducting C-fibers. C-low threshold mechanoreceptors (CLTMs) respond both to innocuous mechanical stimulus and to small skin temperature decreases (by <2 °C) (Bessou and Perl, 1969; Bessou et al., 1971; Shea and Perl, 1985).

In the current study, we examined first the existence of cold allodynia in rats with adjuvant monoarthritis, a model which has previously been used to investigate the mechanism of inflammatory pain (Butler et al., 1992; Neto et al., 2000), and then performed single nerve fiber recordings in vivo using these animals, focusing on C-fibers. We found that nociceptive behavior in response to innocuous cold (25 °C) was increased in the monoarthritic rats, and that the cold responses of two types of C-primary afferent fibers (CLTM units and C-nociceptors) were facilitated in these rats.

Preliminary reports have appeared in abstract form (Takahashi et al., 2002).

2. Materials and methods

Forty-two adult male Sprague-Dawley rats (254–400 g, SLC Inc., Japan) were used in this study; 20 for behavioral experiments and 22 for electrophysiological experiments. The rats were divided into two groups: a control group that received a saline injection and a persistently inflamed group that received an injection of complete Freund's adjuvant (CFA) (described below). The animals were kept in a temperature-controlled room (24 ± 1 °C) on a 12-h alternating light:dark cycle. Water and food were provided ad libitum. We carried out all experiments with the approval of the Animal Care Committee, Nagoya University.

2.1. Induction of persistent inflammation

We induced monoarthritis according to the method developed by Butler et al. (1992), with some modification. Briefly, CFA, a suspension of heat-killed *Mycobacterium butyricum* (60 mg, Difco, Detroit, USA) in paraffin oil (6 ml) was mixed with 0.9% NaCl (4 ml) and Tween 80 (1 ml), and autoclaved. The inflamed group ($n = 22$) received an injection of 0.05 ml of this CFA solution into the left tibio-tarsal joint, while the control group ($n = 20$) was injected with 0.05 ml of 0.9% NaCl under anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The day of these injections was designated day 0.

2.2. Behavioral experiment

We employed the cold-immersion test (Attal et al., 1990, 1994; Perrot et al., 1993) to observe the response to innocuous cold (25 °C) stimulus. The number of paw shakes was considered to be an indicator of pain, because a pilot study showed almost no shakes in intact animals with a bath temperature of 25 °C, and an increase by about 3 when the temperature was lowered to 15 °C. The response to cold was tested from days −7 to 21, and this time was divided into four periods: 'Pre' (days −7 to −1), '1 week' (days 1–7), '2 week' (days 8–14) and '3 week' (days 15–21). Measurements were taken twice for each period and then averaged. The rats were held with a towel and the right paw (non-treated paw) was fixed with a piece of surgical tape. They tried to escape from this fixation at first, but became accustomed to it after the second trial and remained calm. The left paw (treated paw) was slowly immersed into the 25 °C water bath down to the ankle and left immersed for 30 s. The normal temperature of the plantar surface of the rats' hind paw was approximately 30 °C. We recorded all the behavioral experiments on videotape, and later counted the number of brisk paw shakes during the water immersion. The number of paw shakes in the Pre period was subtracted from that at 1, 2 and 3 weeks, respectively, to cancel individual behavioral differences.

2.3. Electrophysiological experiment

2.3.1. Surgical procedure

As will be described in Section 3, inflamed rats showed increased paw shaking behavior shortly after the hind paw was immersed into the 25 °C water bath. This observation suggests that cutaneous receptors are responsible for this phenomenon, since it should take some time until the temperature decrease reaches the deeper ankle joint. Therefore, cutaneous receptors were examined in this study.

We recorded the single nerve activity from the sural nerve using the method of Leem et al. (1993) with some modification. Recordings were taken from animals between 2 and 3 weeks after CFA injection, when behavioral cold hypersensitivity was observed. Approximately four fibers were recorded per experiment. Anesthesia was initially induced with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), and then maintained by constant infusion of sodium pentobarbital (17 mg/kg/h)) through a cannula inserted into the jugular vein. A tracheostomy was performed for unobstructed breathing. Blood pressure and rectal temperature were maintained at >80 mmHg and at 37.5 ± 0.5 °C, respectively. A dorsal midline incision from the ankle to the knee was made to expose the sural nerve, and the skin was sewn to a fixed metal ring to make a pool with warm mineral oil. The sural nerve was separated from adjacent tissues and cut proximally at its junction with the sciatic nerve. Hairs of the lateral hind paw, including the toe and the heel, were removed using depilatory cream (DEPILA, Taisho Pharmacy, Japan) to search for the receptive fields of cutaneous receptors.

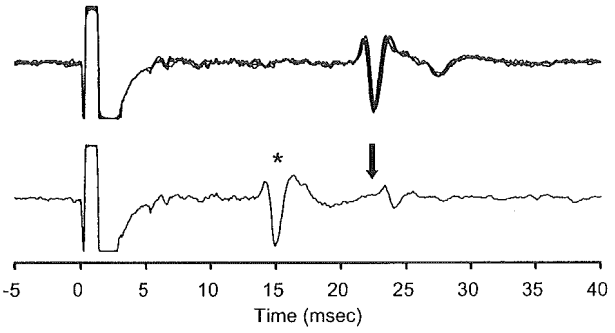

Fig. 1. Sample recordings showing the procedure for confirming single-unit activity (collision test). This unit was a C-low threshold mechanoreceptor with a conduction delay of 22.8 ms (conduction distance: 20 mm). Electrical stimulation was applied at time 0. The top panel shows an overlay of four traces. In the bottom panel, the preceding activity evoked by mechanical stimulation (*) cancelled the subsequent action potential that should appear (↓).

2.3.2. *Recording and stimulating procedures*

We teased each bundle of nerve fibers on a small mirror with forceps under a dissecting microscope until we obtained single fiber activities. Bipolar platinum stimulating electrodes were placed 14–25 mm distal to the recording site. Gold wires were used for the recording- and indifferent-electrodes. Nerve activity was amplified and stored in DAT tapes and later analyzed on a PC computer with a template-matching program (Forster and Handwerker, 1990). To ensure that the recording was taken from the same single unit, we used the 'collision method' (Ritchie and Douglas, 1957) (Fig. 1). The conduction velocity was then measured and the sensory properties of the unit were examined. Units conducting at <2.0 m/s were considered to be C-units, as in other studies in the rat (Handwerker et al., 1991).

2.3.3. *Identification of the receptor type of a cutaneous afferent unit*

We classified C-afferent fibers according to the criteria used by Leem et al. (1993) and Cain et al. (2001). A series of search stimuli was applied to the hind paw as follows: first, we applied weak mechanical stimuli such as cotton ball stroke and mild extension of the skin with a finger or forceps. Next, we applied a small drop of acetone to find cooling-sensitive units. An acetone drop produced a decrease of about 5 °C in the skin temperature. If these innocuous stimuli failed to excite a unit, we applied stronger mechanical stimuli (pinching the skin with fingers or with blunt-tipped forceps). After an active unit was found, the location and size of the receptive field was accurately determined with homemade nylon monofilaments (von Frey hairs) at the strength of about twice the threshold and marked with a felt-tip pen. These nylon monofilaments have a diameter of 0.5 mm and strength ascending in quasi-logarithmic order (14.4, 34.0, 46.1, 53.9, 74.8, 126.7, 264.3, 284.9 mN). We defined the mechanical threshold of a unit as the lowest strength that consistently evoked action potentials. Next, we applied thermal stimuli to the receptive field using a feedback-controlled Peltier thermode (DPS-777, Dia Medical, Japan) with a contact area of 12.6 mm^2. We exercised special care to ensure that the thermode contacted the entire receptive field evenly. After the thermode was placed properly with armatures and a resting period of at least 120 s at a baseline temperature of 32 °C had elapsed, the skin was cooled down to 2 °C over 120 s. The stimulus temperature was set at 6 °C in some cases. Then, after a 180 s interval, a 30 s heat stimulus was applied to increase the temperature to 49 °C.

Nerve fibers were classified as CLTM units if they responded to an innocuous mechanical stimulation such as slow brushing of the receptive field or stretching of the skin surrounding the receptive field. Neither stimulus evoked any discharges in C-nociceptors, which made the fiber discrimination process easy. CLTM fibers typically had a field-like oval shaped receptive field (short diameter: 1–3 mm; long diameter: 2–4 mm) and responded vigorously to a rather small temperature decrease (about 2 °C) caused by stimuli such as an acetone drop (Bessou et al., 1971; Shea and Perl, 1985; Leem et al., 1993). Fibers were classified as nociceptors if they did not respond to weak mechanical stimuli such as brushing or stretching of the skin but predominantly to noxious stimuli (pinching the skin with fingers or with blunt-tipped forceps). Nociceptors were further divided into four subclasses with regard to their thermal sensitivity: fibers that did not show any response to either cold (down to 2 °C) or heat (up to 49 °C) stimuli were classified as C-mechanical units. Fibers that responded only to heat stimulus were classified as C-mechanoheat units, most of which are polymodal receptors (Bessou and Perl, 1969), and those that responded only to cold stimulus were classified as C-mechanocold units. Fibers that responded to both heat and cold were classified as C-mechanoheat-cold units. Criteria for a positive response were as follows: (1) the instantaneous frequency of a spike exceeded the resting discharge rate (discharges were counted every second (DR), and mean DR during the 120 s pre-stimulation period (MDR) was calculated) +2 S.D. of MDR, and (2) subsequent spike(s) appeared during the next 2 °C temperature decrease/increase. When a unit responded to the stimulus, the temperature that satisfied criterion 1 was taken to be the threshold temperature. No attempt was made to find C-heat nociceptors (fibers that respond only to noxious heat stimuli). Finally, fibers that continuously fired (at approximately 1–10 Hz) at the baseline skin temperature of 32 °C (some units did not have any firing at 32 °C) and/or responded vigorously to a slight decrease in temperature were classified as C-cold units (Hensel et al., 1960; Iggo, 1969). These units typically did not respond to mechanical stimulation. Finally, the mechanical response was re-checked using the nylon monofilament of threshold strength to confirm that the unit was still alive.

2.4. Data analyses

Statistical analyses were performed using Prism software (GraphPad Software Incorporated, version 3.02, 2000). The results were regarded as significant when $P < 0.05$. We applied the Fisher's exact probability test to examine the differences between the control and inflamed groups in the proportion of cold sensitive nociceptors (i.e. C-mechanocold and C-mechanoheat-cold) among all nociceptors, and the proportion of paradoxical discharge-positive units among all C-cold units. Student's *t*-test was used for the analyses of the difference between the control and inflamed groups. Data are expressed as mean ± S.E.M.

3. Results

3.1. Inflammation

The intra-articular injection of CFA led to swelling and erythema in the entire treated hind paw distal to the ankle, which included the skin area from which we recorded nerve activities. While the erythema diminished gradually and finally disappeared about 7 days post-injection, paw swelling persisted for the entire experimental period (up to 3 weeks). Animals tended to lift their inflamed paw to avoid contact with the floor of the cage soon after they recovered from the anesthesia, and this behavior lasted throughout the experiment. The toes were flexed and showed resistance to extension from about 2 weeks post CFA injection, suggesting that inflammation spread over the entire paw (Fig. 2). No sign of inflammation was observed in the contralateral paw.

3.2. Inflammation-induced behavioral change to cold

The number of paw shakes in response to stimulation to the hind paw with a normally non-noxious temperature (25 °C) was 1.65 ± 1.12 in the control group and 1.15 ± 0.58 in the inflamed group at Pre. There was no significant difference between them ($P > 0.05$). CFA injection into the tibio-tarsal joint induced increased sensitivity to 25 °C cold (Fig. 3). The number of paw shakes, most of which were observed shortly after immersion into the cold water,

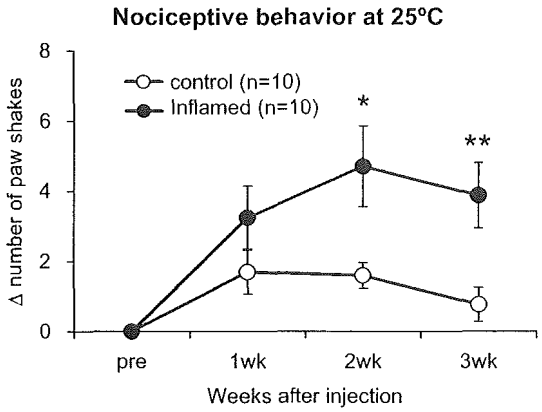

Fig. 3. Increased pain behavior in response to immersion in 25 °C water. *Ordinate*: Change in the number of paw shakes during 30 s immersion in 25 °C water compared with the Pre value. The numbers of paw shakes at Pre were not different between the control and inflamed groups. *Abscissa*: Weeks after injection of CFA. The number of paw shakes in the inflamed group tended to increase after inoculation of CFA, and became significantly larger than that in the control group 2 and 3 weeks after inoculation (comparison between the control and inflamed groups at each week, Student's *t*-test). (*) $P < 0.05$; (**) $P < 0.02$. Results are mean ± S.E.M.

gradually increased after CFA injection and the change from Pre reached a maximum value of 4.70 ± 1.15 at 2 weeks. In the control group, the number of paw shakes increased slightly by 1.70 ± 0.63 at 1 week, but after that it decreased and remained at almost the same level as at Pre. The number of paw shakes in the inflamed group was significantly larger than that in the control group at 2 and 3 weeks ($P = 0.028$ and 0.011, respectively) (cold allodynia).

3.3. Inflammation-induced alteration in the property of primary afferent neurons

3.3.1. Fiber population

Ninety single C-primary afferent fibers were analyzed in this study. Of them, 41 units (12 CLTMs, 17 C-nociceptors and 12 C-cold units) were recorded from the control group and 49 units (13 CLTMs, 28 C-nociceptors and 8 C-cold units) from the inflamed group. Only the nociceptors tested their cold response down to the lowest temperature of 2 °C (14 units in the control and 18 units in the inflamed

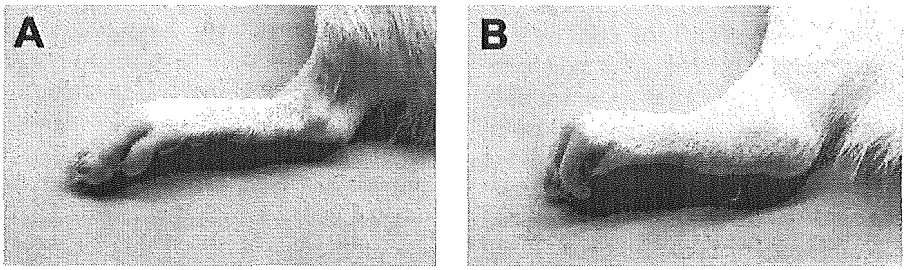

Fig. 2. Photographs of the rat hind paw 3 weeks after an intra-articular injection of saline (A) or CFA (B). Note massive swelling at the tibio-tarsal joint and flexion of toes in the inflamed animal.

Table 1
Proportions of different kinds of nociceptive units in the control and inflamed groups

	Control	Inflamed
CM	2 (14.3)	1 (5.6)
CMH	11 (78.6)	8 (44.4)
CMC	0 (0.0)	1 (5.6)
CMHC	1 (7.1)	8 (44.4)

Values are numbers of responding fibers, with percents in parentheses. Only the units tested for their cold response down to 2 °C were recruited for this study population. CM: C-mechanical units; CMH: C-mechanoheat units; CMC: C-mechanocold units; CMHC: C-mechanoheat-cold units.

groups) were classified into one of the four subgroups of nociceptors. The proportions of different types of nociceptive units in the control and inflamed groups are shown in Table 1.

In the control group, most of the nociceptive units were C-mechanoheat units (11 of 14, 78.6%); the remaining units consisted of two C-mechanical (14.3%) and one C-mechanoheat-cold (7.1%) units. We did not encounter C-mechanocold units in the control group, possibly because of the sample size of this experiment. In contrast, the proportion of C-mechanoheat-cold units was greater in the inflamed group (8 of 18, 44.4%), and that of C-mechanoheat units was smaller (8 of 18, 44.4%). The remaining units in the inflamed group consisted of one C-mechanical (5.6%) and one C-mechanocold (5.6%) unit. The proportion of cold sensitive units (i.e. C-mechanocold and C-mechanoheat-cold) among the total number of nociceptive units was significantly greater in the inflamed group ($P = 0.011$).

Among units that were excluded from this classification because they were cooled down to only 6 °C, 2 of 3 units responded to cold in the control group (thresholds: 7.2 and 16.6 °C), and 4 of 10 units in the inflamed group (mean threshold: 12.1 ± 1.9 °C). The proportion of cold sensitive units was still significantly higher in the inflamed group even when all these units were included ($P = 0.048$).

3.3.2. C-low threshold mechanoreceptors

The receptive fields of all units recorded were at the hairy skin of the lateral hind paw, including the toe and the heel. A sample recording is shown in Fig. 4 (top trace). Most of them, irrespective whether they were recorded from the control or inflamed group, had little or no firing when nothing was touching the receptive field. However, when the Peltier thermode at the baseline temperature (32 °C) was fixed to the receptive field, 7 out of 13 CLTM units in the inflamed group showed persistent firings (~0.29 Hz), while only 2 out of 12 did in the control group. Two units showed burst-like spontaneous activity in one inflamed rat, but none did in the control group. The resting discharge rate before cold stimuli in the inflamed group was significantly greater than that in the control group (0.12 ± 0.03 and 0.04 ± 0.02 impulses/s, respectively; $P = 0.016$), as shown in Fig. 5.

All CLTM units in both groups responded to weak mechanical stimuli such as slow brushing of the receptive field or stretching of the skin. Thus, even the weakest von Frey hair used (14.4 mN) was clearly above the threshold and induced vigorous discharges. Further attempts to determine the precise mechanical threshold were not made. There was no significant difference in the conduction velocities of the control and inflamed groups (0.71 ± 0.02 and 0.76 ± 0.05 m/s, respectively; $P > 0.05$).

CLTM units showed a typical response pattern to cold stimuli, responding shortly after the cooling ramp was started, when the stimulus temperature was still in the innocuous range of cold. There was no significant difference in cold threshold between the control and inflamed group (26.6 ± 1.6 and 27.6 ± 0.9 °C, respectively; $P > 0.05$). Their discharge rates peaked before the temperature reached the noxious level, then declined and finally ceased firing at noxiously cold temperatures (Fig. 4, top trace). It is worth noting that some units in the inflamed group showed prolonged response down to the noxious cold range.

The response of CLTM fibers to 120 s cold stimulation in the inflamed group was 2.2 times larger than that in the control group (control group: 28.0 ± 5.5 impulses, inflamed group: 60.5 ± 11.8 impulses; $P = 0.020$). Fig. 6 shows averaged cold responses of CLTM units from the baseline temperature of 32 °C to 20 °C. The temperature used in the behavioral experiment (25 °C) was included in this temperature range and the temperature change was linear (temperature decrease: 0.9 °C/s) there. When the response of CLTM fibers is expressed as impulses per second at every second, these fibers started to respond 5–6 s after the onset of cold stimulus (30.0–29.1 °C) and showed the maximum cold response 10–11 s after the onset (24.3–25.3 °C), which were at the innocuous level, in both the control (1.58 ± 0.31 impulses/s) and inflamed (2.46 ± 0.51 impulses/s) groups. The response was significantly larger in the inflamed group than in the control one ($P < 0.05$) at 5, 9, 10 and 12 s after the onset of cold stimulus (correspond to 30.0, 26.2, 25.3 and 23.4 °C, respectively). The total number of spikes at 27–23 °C, which was close to the temperature where cold allodynia was detected, was significantly greater in rats with inflammation than in control rats (9.38±1.47 impulses and 5.17±1.04 impulses, respectively; $P = 0.027$).

When the heat stimulus was applied with the Peltier thermode, only one CLTM unit in the inflamed group showed discharges exceeding the level of the resting discharge rate +2 SD, with a heat threshold of 47.5 °C. The greatest supra-threshold response was 3.3 Hz (instantaneous frequency) at the maximum stimulation temperature of 49 °C.

It is noteworthy that most CLTM units responded during the cooling phase after the cessation of the heating. The threshold temperature of this discharge (range: 34.8–42.3 °C) was higher than that when the skin temperature was decreased from the baseline temperature of 32 °C

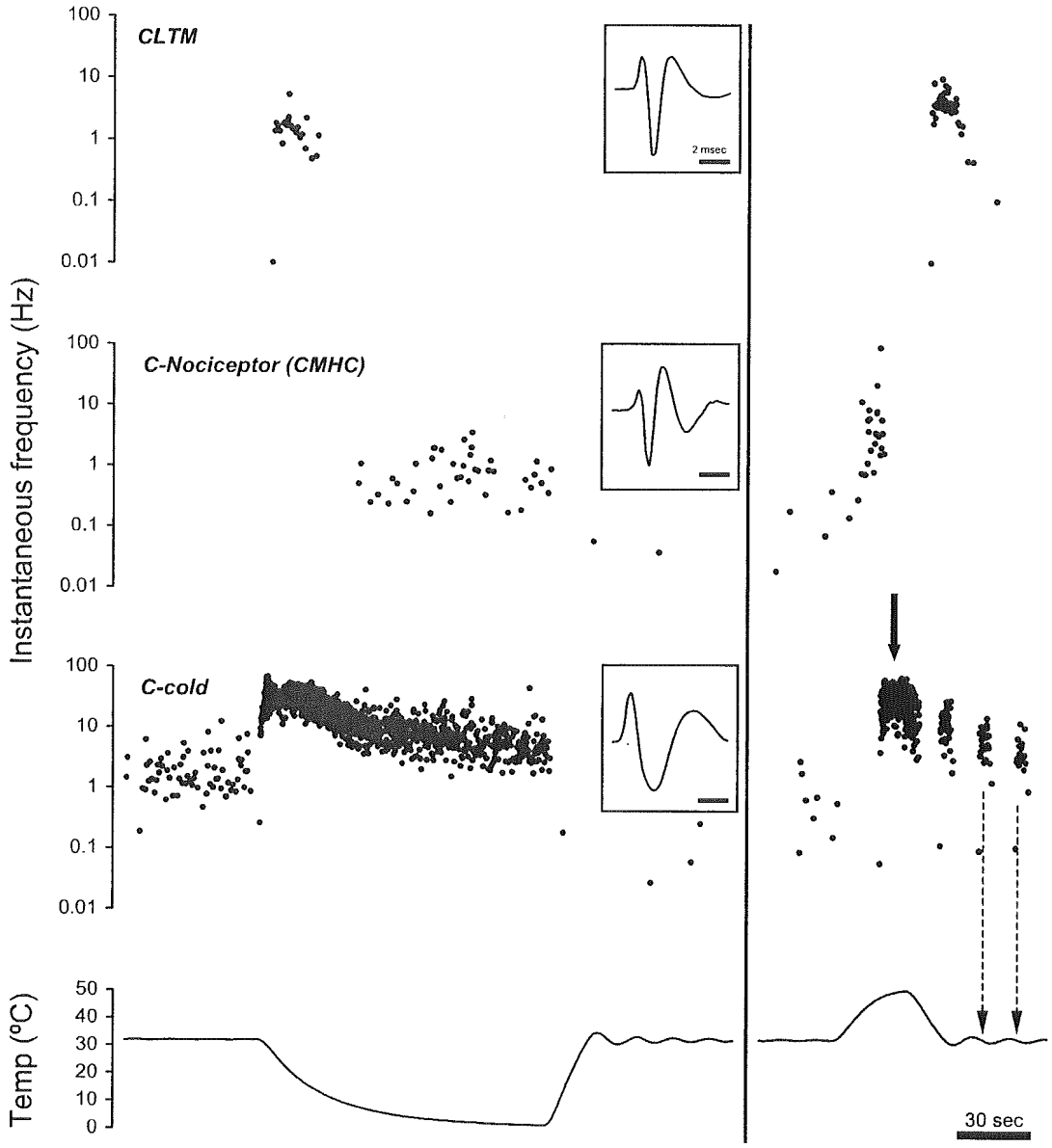

Fig. 4. Sample recordings of 3 receptor types of C-fibers. Primary afferent activity is illustrated by means of instantaneous frequency. Top trace: a C-low threshold mechanoreceptor (CLTM). Second trace: a C-nociceptor (in this case, a C-mechanoheat-cold unit). Third trace: a C-cold unit. Bottom trace: temperature recording. *Insets*: spike shape of each unit. The bars inside insets indicate 2 ms. The bar at bottom right indicates 30 s. Note the differences in the response pattern to each stimulus (left panel: cold stimulation down to 2 °C; right panel: 49 °C heat) among units. A CLTM unit (top trace), taken from the control group, responded to cold in an innocuous range (in this case, 28.7–17.2 °C). It did not respond to heat stimulus; activity was observed when the skin temperature was decreasing after the heat stimulation. The conduction velocity was 0.71 m/s. A C-nociceptor (second trace), taken from the inflamed group, typically showed excitation at a noxious range (in this case, ≤8.9 °C). This fiber also responded to heat, thus it was classified as a C-mechanoheat-cold unit. The conduction velocity was 0.68 m/s. A C-cold unit (third trace), taken from the inflamed group, had a continuous resting discharge at the normal skin temperature (32 °C) and showed an immediate and vigorous response to temperature decrease. This unit showed 'paradoxical discharge' to a heat stimulus (black arrow, threshold: 44.2 °C). The subsequent firings appeared in the temperature decrease phases of temperature fluctuation (broken arrows). The conduction velocity was 0.49 m/s.

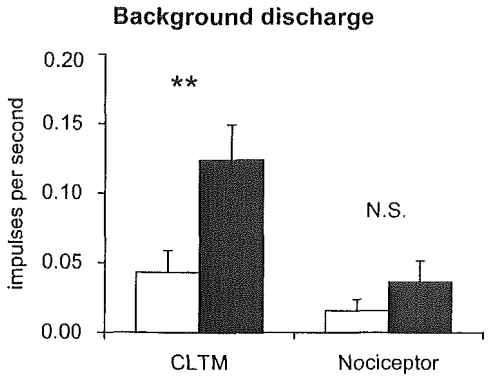

Fig. 5. Resting discharge rates in CLTM and C-nociceptor units. Resting discharge rates were measured after the Peltier thermode (32 °C) was attached to the skin, thus they were observed under some influence of pressure to the skin. Open bar: data from the control group; closed bar: the inflamed group. (**) $P < 0.02$; NS: $P > 0.05$ (Student's t-test).

(7.6–28.7 °C) (a typical example is shown in the top panel of Fig. 4). This observation suggests that CLTM units did not respond to the absolute temperature, but rather to the relative temperature decrease.

3.3.3. C-nociceptors

The majority of the C-nociceptors recorded had receptive fields at the hairy skin of the lateral hind paw, including the toe and the heel, and some at the glabrous skin adjacent to these areas. In contrast to CLTM units, these nociceptors had no response to innocuous cold, but typically responded to noxious cold (<10 °C) (Fig. 4). Usually, the maximum discharge rate in response to cold was less than 10 Hz (highest instantaneous frequency: ~32.8 Hz, median: 0.95 Hz). There was no significant difference in the number of resting discharges between the control and inflamed groups (0.02 ± 0.01 and 0.04 ± 0.02 impulses/s, respectively; $P > 0.05$) (Fig. 5). The median mechanical threshold of nociceptive units measured with nylon monofilaments was 34.0 mN for both groups (range: 14.4–284.9 mN for both groups). There was no significant difference in conduction velocities between the two groups (0.75 ± 0.02 and 0.76 ± 0.03 m/s, respectively; $P > 0.05$).

Cold sensitivity was identified in the fibers that were stimulated down to 2 °C (14 units for the control group and 18 for the inflamed group). Among these, only one unit (7.1%) was cold sensitive in the control group while nine (50%) were in the inflamed group; these incidences were significantly different ($P = 0.011$). The distribution of the cold threshold is shown in Fig. 7. The cold threshold of one unit in the control group was 4.0 °C, and the mean cold threshold in the inflamed group was 10.0 ± 2.6 °C (range: 2.4–26.7 °C). The supra-threshold cold response of nociceptors in the inflamed group was 0.19 ± 0.06 impulses/s (at 2–4 °C, range: 0.06–0.64 impulses/s), and that in the control group was 0.06 impulses/s. A statistical analysis of the response magnitude was not done because there was only one cold responder in the control group.

The heat threshold was not different between the groups (42.8 ± 0.7 °C, $n = 14$ in the control group and 42.9 ± 1.0 °C, $n = 23$ in the inflamed group; $P > 0.05$). In the supra-threshold response to heat, as well, no significant difference was found in any temperature sector (Fig. 8).

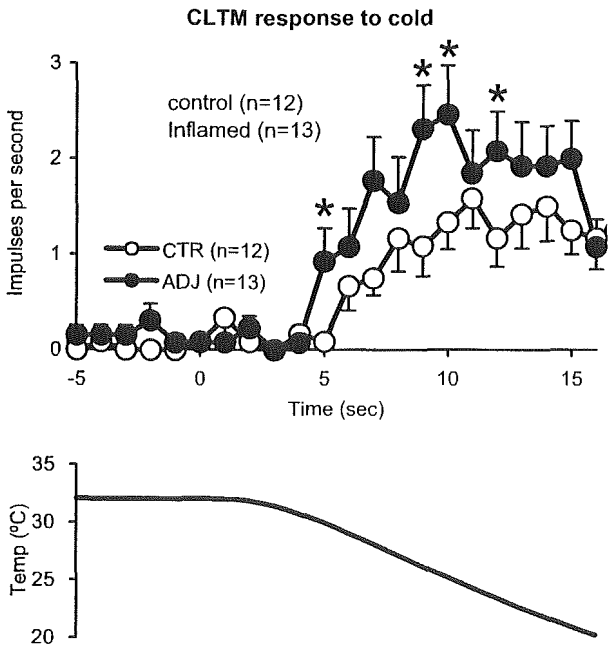

Fig. 6. Stimulus response curves for CLTM units during a cold ramp stimulation. Top panel: The averaged cold response of CLTM units. Open circle: CLTM units from the control rats ($n = 12$); closed circle: those from the inflamed rats ($n = 13$). Data are expressed as mean impulses/s ± S.E.M. Bottom panel: Temperature recording. (*) $P < 0.05$ (comparison between control and inflamed groups, Student's t-test).

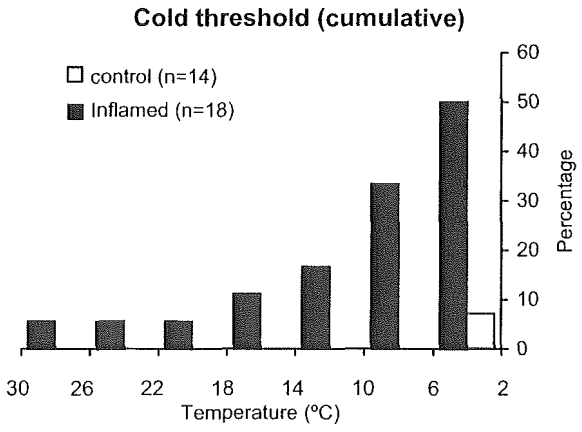

Fig. 7. Proportions of C-nociceptors excited during a ramp cold stimulation down to 2 °C. The proportion was cumulatively presented along the threshold temperature. Open bar: data from the control group ($n = 14$); closed bar: the inflamed group ($n = 18$). Note that only 7.1% of C-nociceptors responded to 2 °C cold in the control group, while 50% of them did in the inflamed group.