Pathological C-fibres in patients with a chronic painful condition

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Summary
Little is known about the contribution of C-afferent fibres to chronic painful conditions in humans. We sought to investigate the role of C-fibres in the pathophysiology of pain and hyperalgesia in erythromelalgia as a model disease for chronic pain. Erythromelalgia is a condition characterized by painful, red and hot extremities, and patients often report tenderness on walking. We made microneurographic recordings from single C-fibres in cutaneous fascicles of the peroneal nerve in patients suffering from this disease. All patients had had a pain attack recently and psychophysical signs of allodynia and punctate hyperalgesia were found. We obtained recordings from a total of 103 C-fibres and found significantly lower conduction velocities and increased activity-dependent slowing of the conduction velocity of afferent C-fibres in the patients compared with healthy controls. Furthermore, several units with biophysical properties of mechano-insensitive fibres were pathological, being spontaneously active or sensitized to mechanical stimuli. Since these fibres also mediate the axon reflex flare, their hyperexcitability might account not only for ongoing pain and tenderness but also for redness and warming in this pain syndrome. The changes in conductive properties found in the C-fibres of these patients could be the first signs of a small-fibre neuropathy. This is the first systematic study of single C-fibres in patients and it shows an active contribution of mechano-insensitive fibres to chronic pain.

Keywords: nociception; hyperalgesia; erythromelalgia; sensitization; pain

Abbreviations: QST = quantitative sensory testing

Introduction
Our knowledge about C-fibres in chronic pain so far has been based mainly on animal research or experimental studies on healthy human subjects. Microneurographic recordings from healthy subjects have shown that there are two main categories of C-afferents in humans: mechano-responsive and mechano-insensitive fibres (Weidner et al., 1999). The aim of this study was to explore the possibility of altered C-fibre function in patients suffering from a long-standing painful condition with concomitant mechanical hyperalgesia, a combination typical of various chronic pain states (Price et al., 1992; Koltzenburg et al., 1994; Ali et al., 2002). We chose erythromelalgia as a model condition for chronic pain. Erythromelalgia is a rare condition characterized by painful, red, hot extremities (Fig. 1), reminiscent of the ABC syndrome (angry backfiring C-fibres), as postulated by Ochoa (1986). The condition was first described by Mitchell (Mitchell, 1878). There are no objective criteria for applying the diagnosis, but characteristically symptoms are aggravated by heat and patients find relief from their symptoms by cooling the affected limbs (Davis et al., 2000; Mørk et al., 2000; Mørk and Kvernebo, 2000). Most patients report pain only during attacks provoked by increased environmental temperature, physical activity, alcohol or sleeping. Some patients experience constant pain with intermittent aggrava-
tion in attacks (Kvernebo, 1998). So far no single treatment has been shown to be effective in all cases, although there are reports of partial relief with a wide spectrum of medications, such as acetylsalicylic acid, lidocaine (Kuhnert et al., 1999), prostaglandin E1, sodium nitroprusside (Kvernebo, 1998), gabapentin (Cohen, 2000), cyproheptadine (Sakakibara et al., 1996) and venlafaxine (Rudikoff and Jaffe, 1997).

Most studies have focused on vascular mechanisms, but some involvement of the PNS has also been reported. In a retrospective study on 54 erythromelalgia patients, 39% had signs of axonal neuropathy. Sixty-three per cent of those with normal nerve conduction velocity and EMG had findings of abnormal sudomotor function (Sandroni et al., 1999). In a case report on an erythromelalgia patient, skin sympathetic nerve activity recorded from the tibial nerve showed a normal skin sympathetic reflex response but no vasoconstriction response (Sugiyama et al., 1991).

The currently assumed pathophysiological mechanisms propose maldistribution of blood perfusion as one main factor that leads to superficial hypoxia in the skin, and thereby pain (Kvernebo, 1998; Mørk et al., 2000). In this study we investigated the contribution of C-nociceptive fibres to this painful condition.

**Methods**

**Patients**

One male patient and eight female patients with erythromelalgia, aged 23–60 years, participated in the study after giving their informed consent (Table 1). The study was carried out at the Department of Clinical Neurophysiology, University Hospital, Uppsala and was approved by the local ethics committee at the hospital. None of the patients had diabetes or any of the systemic diseases sometimes associated with erythromelalgia (Cohen, 2000). It is known that the condition may be hereditary. This seemed to be the case for Patient 3, whose mother had symptoms so severe that she had had both legs amputated. Patients 7 and 8 were also mother and daughter. As seen in Table 1, the onset of the disease in the second generation of patients was at the age of 5 years for Patient 3 and 11 years for Patient 8. All the patients had experienced symptoms in their feet and two had additional symptoms in their hands. Two of them had constant pain that was aggravated during attacks, but most of them had no continuous pain, but only pain attacks, often precipitated by warm weather or physical activity (Fig. 1). The attacks were more severe in the evening and during the night. Cooling the affected extremities during the attacks partly relieved the pain. The patients had no major thick-fibre impairment, as determined by normal motor and sensory nerve conduction velocities, amplitudes and F-responses. Quantitative sensory testing (QST) with thermal stimuli showed no clear thin-fibre alteration.

**Healthy subjects**

A sample of 97 characterized C-fibres, recorded from cutaneous fascicles of the peroneal nerve in 61 healthy subjects aged 19–32 years, was used as normal material. These findings have been published in detail (Weidner et al., 1999).

![Fig. 1 Symptoms of erythromelalgia. The left foot of Patient 1 during an attack of erythromelalgia after physical exercise. The sole and the heel are red, hot and very tender to pressure, and there is ongoing burning pain, relieved by cooling.](image-url)

**Table 1 Characteristics of patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Disease duration (years)</th>
<th>Idiopathic or hereditary</th>
<th>Location of recording</th>
<th>Symptoms during recording</th>
<th>Medication with some effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>60</td>
<td>2</td>
<td>Idiopathic</td>
<td>Foot</td>
<td>No</td>
<td>Venlafaxine</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>35</td>
<td>9</td>
<td>Idiopathic</td>
<td>Leg and foot</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>23</td>
<td>18</td>
<td>Hereditary</td>
<td>Foot</td>
<td>Yes</td>
<td>Misoprostol</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>54</td>
<td>7</td>
<td>Idiopathic</td>
<td>Foot</td>
<td>Yes</td>
<td>Venlafaxine</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>28</td>
<td>8</td>
<td>Idiopathic</td>
<td>Foot</td>
<td>Constant pain</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>30</td>
<td>13</td>
<td>Idiopathic</td>
<td>Foot</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>46</td>
<td>6</td>
<td>Hereditary</td>
<td>Foot and toe</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
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<td>13</td>
<td>Hereditary</td>
<td>Leg and foot</td>
<td>Yes, 2nd experiment</td>
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<tr>
<td>9</td>
<td>F</td>
<td>30</td>
<td>3</td>
<td>Idiopathic</td>
<td>Foot</td>
<td>No</td>
<td>Misoprostol</td>
</tr>
</tbody>
</table>
Measuring areas of allodynia and punctate hyperalgesia

Hypersensitivity to gentle touch was assessed by lightly stroking the affected limbs with a cotton swab to delineate the borders of allodynia (LaMotte et al., 1991). The borders of hyperalgesia to punctate stimuli (LaMotte et al., 1991) were determined with a 260 mN von Frey filament.

Microneurographic recording technique

Experiments were performed in a quiet shielded room at a room temperature of 21–23°C. The patient sat with foot and lower leg exposed below knee level. Skin temperature on the dorsum of the foot, measured with an infrared thermometer during 10 recording sessions, was 28.6–33.3°C (mean 31.4°C).

Methods of microneurography employed in this study have been described in detail elsewhere (Vallbo and Hagbarth, 1968; Torebjörk, 1974; Schmidt et al., 1995). Micro-electrodes were inserted at the level of the fibular head into cutaneous branches of the peroneal nerve. Single C-units were sought in the skin of the lower leg and the dorsum of the foot by the use of transcutaneous electrical stimuli from an insulated constant current stimulator (0.2 ms, 10–50 mA; Digitimer DS7, Digitimer Ltd, Hertfordshire, UK). They were identified by action potentials that could be recorded at a long latency after each electrical stimulus. When a stable C-fibre response was encountered, two needle electrodes, 0.2 mm in diameter, were inserted 5 mm apart for repetitive intracutaneous electrical stimulation at 0.25 Hz for the whole experiment. Single C-fibres could be tracked over hours with this method. Additional natural activity could be assigned to a particular fibre by applying the ‘marking’ method.

The marking method

During iterative intracutaneous electrical stimulation at a constant frequency of 0.25 Hz for several minutes, the C-fibre responses stabilized at latencies characteristic for each unit. It has been shown that even a single additional spike induced in a C-fibre by a conditioning stimulus produces an increase of ~1 ms in the delay of the subsequent electrically induced spike (Schmelz et al., 1995). This marking technique is very useful in showing which C-fibre in a multiunit recording responds to a particular stimulus (Torebjörk, 1974; Schmidt et al., 1995). In this study we used the marking technique to determine whether C-units were spontaneously active and to determine the responsiveness of afferent C-nociceptors to mechanical and heat stimuli and the responsiveness of efferent sympathetic C-units during induction of sympathetic reflexes.

Conduction velocity measurements

The latencies of C-fibre responses to the first electrical impulse after a rest period of at least 2 min were used in computing the conduction velocities. The shortest distance between the stimulating needles and the recording needle in the nerve was assessed using a measuring tape.

Activity-dependent slowing

Activity-dependent slowing of conduction velocity is a well-known characteristic of unmyelinated nerve fibres, and is due to prolonged changes in membrane properties after excitation (Torebjörk and Hallin, 1974). It can be used to differentiate functional subtypes of C-fibres in rats (Thalhammer et al., 1994) and humans (Serra et al., 1999). It is much more pronounced for mechano-insensitive C-nociceptors than for mechano-responsive C-nociceptors and sympathetic efferent fibres in human skin, particularly at low stimulation frequencies (Weidner et al., 1999). This feature has been used for reliable separation between these unit classes in healthy subjects. After a rest period of at least 2 min, intracutaneous electrical stimuli were applied in a train consisting of 20 pulses at 0.125 Hz, immediately followed by a second train of 20 pulses at 0.25 Hz and a third of 30 pulses at 0.5 Hz. The accumulated increase in latency during the entire stimulation protocol (total slowing) was measured in ms.

Transcutaneous electrical thresholds

It has been shown that transcutaneous electrical thresholds are much higher for cutaneous mechano-insensitive C-nociceptors than for mechano-responsive C-nociceptors, and there is no overlap in healthy subjects (Weidner et al., 1999). Electrical thresholds were obtained using a Perspex holder containing in its cavity a round cotton disc, 5 mm in diameter, soaked with saline. This cotton disc was gently pressed to the skin by hand (Magler et al., 1990). A constant-current stimulator (Digitimer DS7) was used to deliver single pulses of 0.2 ms. A large (5 × 10 cm) metal plate attached to the skin on the lower leg served as reference electrode.

Mechanical stimulation

A set of calibrated von Frey nylon monofilaments (Stoelting, Chicago, IL, USA) was used to quantify mechanical responsiveness. The forces exerted by the filaments were 1.5–750 mN. Fibres unresponsive to 750 mN were regarded as mechano-insensitive.

Heat stimulation

Heat stimuli were delivered by radiation from a halogen bulb focused on the target area and feedback controlled from a thermocouple gently attached to the skin (Beck et al., 1974). To test responsiveness to heating, the skin temperature was slowly increased by 0.25°C s⁻¹, from an adapting temperature of 32°C to a maximum of 50°C. Heating could be stopped by the patient before the pain tolerance limit was reached.
**Histamine stimulation**

Histamine was applied iontophoretically in three patients. A cotton swab soaked with a solution of 1% histamine dihydrochloride dissolved in distilled water was gently pressed against the skin by a silver anode 6 mm in diameter. A constant current of 1 mA was delivered for 20 s between this applicator and a large cathode applied to the skin outside the territory of the peroneal nerve.

**Sympathetic provocation**

Sympathetic units were identified by their responses related to arousal stimuli, e.g. unexpected loud noise or mental stress, or during deep inspiration. These manoeuvres are known to elicit sympathetic reflexes in human skin nerves (Hallin and Torebjörk, 1970; Hagbarth et al., 1972). The efficiency of these manoeuvres was controlled by recording the background activity of sympathetic burst discharges.

**Classification of C-fibres**

According to the receptive and reflex properties assessed as described above, C-fibres were classified into three main classes: efferent sympathetic fibres (s); afferent mechano-responsive (m); and mechano-insensitive fibres (i, also called ‘sleeping nociceptors’). Heat-responsive fibres are called ‘CMH’ in the mechano-responsive group and ‘CH’ in the mechano-insensitive group. Fibres without heat response up to 50°C are called ‘CM’ and ‘CMiHi’ (Schmidt et al., 1995). For the present study, heat classes had to be disregarded since patients could not usually tolerate temperatures near 50°C, the maximum for testing. Therefore this assessment was not comparable between the patients and normal subjects.

**Data acquisition and analysis**

Signals from the recording electrodes were amplified and recorded on-line with a personal computer, using an interface card (DAP 3200a; Microstar Laboratories, Bellevue, WA, USA) and the SPIKE/SPIDI software package (Forster and Handwerker, 1990).

Standard ANOVA (analysis of variance), ANCOVA (analysis of covariance) and linear regression tests (StatSoft Tulsa, OK, USA) were performed. When appropriate, non-parametric statistics were applied. Differences were regarded as significant at P < 0.05. The appropriate corrections (Bonferroni) for repetitive testing were used.

**Results**

**Allodynia and hyperalgesia**

Allodynia to gentle stroking with a cotton swab was found in three of the patients. Such touch-evoked pain was restricted to the dorsum of the toes, the dorsum of the foot, and sometimes up to the ankle joint. In one case, the gentle scratching of the skin which was necessary to adjust the position of the recording needle within the nerve was so painful that the experiment had to be terminated. Punctate hyperalgesia was found in all the patients and included the dorsum of the foot and in some patients also the distal parts of the lower leg. All patients had had a pain attack within few days before the experiment.

**Microneurography**

Microneurographic recordings were performed in cutaneous fascicles of the peroneal nerve in eight of nine patients with erythromelalgia. For ethical reasons the experimenters tried to minimize the duration of the experiments as much as possible, especially when the patients had ongoing pain. For this reason the experimental protocol was not complete for some of the units.

A total of 103 C-units were recorded in patients with erythromelalgia. The mean conduction velocity of these units was 0.81 ± 0.02 m s⁻¹. Compared with the 96 units found in healthy subjects (0.94 ± 0.02 m s⁻¹), the conduction velocity in the patients was significantly lower (ANOVA, P < 0.05).

Sixty-five of the units found in the patients were tested both for conductive properties (conduction velocity and total slowing) and for mechanical responsiveness. These units were classified according to their reaction to mechanical and sympathetic stimuli as mechano-responsive (m; n = 30), mechano-insensitive (i; n = 24) or sympathetic units (s; n = 11).

**Sympathetic units**

The sympathetic units (n = 11) had a conduction velocity of 0.75 ± 0.01 m s⁻¹ and a total slowing of 16.0 ± 1.9 ms. In the normal material (n = 8) the corresponding numbers were 0.78 ± 0.02 m s⁻¹ and 14.5 ± 1.5 ms. There were no statistically significant differences between patients and healthy subjects in conduction velocity or total slowing in sympathetic fibres (Fig. 2).

**Mechano-insensitive units**

The 24 units not responding to mechanical stimulation and not characterized as sympathetic units were classified as mechano-insensitive C-nociceptive fibres. Their mean conduction velocity was 0.67 ± 0.02 m s⁻¹ and the mean total slowing was 62.6 ± 3.5 ms. Compared with the mechano-insensitive fibre group in healthy subjects (n = 32, conduction velocity 0.86 ± 0.03 m s⁻¹, total slowing 40.8 ± 2.7 ms), the patient group had lower conduction velocity (ANOVA, P < 0.05) and a higher degree of activity-dependent slowing (ANOVA, P < 0.05) (Fig. 2).

The electrical thresholds of mechano-insensitive fibres in healthy subjects are usually high. The results for the patients were difficult to compare with those seen in normal subjects since the tolerance level of the patients for transcutaneous
stimulation was lower. We were therefore not able to test with such high currents as we usually do in healthy subjects. In patients, electrical thresholds >30 mA were therefore assessed in only 10 of the units, as depicted in Fig. 3. Seven of these were mechano-insensitive. Only one of the mechano-insensitive units (unit C in Fig. 3) showed spontaneous activity, with two bursts of action potentials in a patient with the only slightly elevated temperature of 31.5°C on the dorsum of the foot. It did not respond to sympathetic reflex provocations. The unit had a high degree of total slowing (82 ms), low conduction velocity (0.6 m s⁻¹), an electrical threshold of 60 mA and a heat threshold >46.7°C. We have never observed such spontaneous discharges in any mechano-insensitive fibres in healthy subjects. Seven other C units showed ongoing activity, but we were unable to obtain a reliable classification.

Mechano-responsive units (m)
Thirty of the units were activated by mechanical stimulation with von Frey bristles of ≈750 mN. Their conductive properties were characterized by a mean conduction velocity of 0.86 ± 0.03 m s⁻¹ and a total slowing of 19.6 ± 3.5 ms, while units activated by mechanical stimulation in the healthy subjects (n = 56) had conduction velocity 1.01 ± 0.02 m s⁻¹ and a total slowing of 5.8 ± 0.5 ms. The patient group had a significantly lower conduction velocity (ANOVA, P < 0.05) and a significantly greater total slowing (ANOVA, P < 0.05) (Fig. 2). Eighteen of the units were tested for electrical threshold (15.3 ± 3.6 mA). In healthy subjects the electrical threshold was 4.2 ± 0.4 mA. The electrical threshold was significantly higher in the patients (ANOVA, P < 0.05). Heat thresholds were tested in 20 of the 30 units and could be determined in 15 (range 36.4–49.9°C). For five fibres, the heat device was switched off before the unit was activated.

The different conductive properties and electrical thresholds of afferent C-fibres in patients and controls are depicted in Fig. 3. In healthy subjects, the characteristic combination of low electrical thresholds and moderate activity-dependent slowing separated mechano-responsive units from the mechano-insensitive fibres without overlap (Fig. 3, left panels). The variations within the group of mechano-responsive units was low, so that a narrow range can be tentatively given which includes this entire nociceptor class in the control material (shaded area in Fig. 3). In the patients, we observed more pronounced activity-dependent slowing and higher electrical thresholds for this unit type, which considerably exceeded the range observed in controls (Fig. 3, right panels, within dotted border). Most of the mechano-responsive units (with the obvious exceptions of units A, B and D, as marked in Fig. 3) could still be differentiated from the mechano-insensitive group, which was also shifted towards higher amounts of total slowing. However, the distinction was less clear (dotted lines in Fig. 3).

The increased total slowing of afferent C-fibres in patients might be just a consequence of their reduced conduction velocities. However, patients still showed a significantly greater pronounced activity-dependent slowing of afferent C-fibres when we controlled for the slower conduction velocity (P < 0.05) or the longer conduction time (P < 0.05) in an
ANCOVA, although part of the variance was explained by conduction velocity.

Furthermore, outliers contributed to the low conduction velocity and high slowing and electrical threshold of mechano-responsive fibres in the patients. Several fibres showed a total slowing that we have never observed in mechano-responsive units of healthy subjects. At least three mechano-responsive fibres in the patients (fibres A, B and D in Fig. 3) had a pronounced total slowing and fairly high transcutaneous electrical thresholds within the range normally restricted to mechano-insensitive units. The mechanical thresholds of the three sensitized units were 110, <260 and <750 mN. For one of these units the response pattern to increasing stimulus frequencies and mechanical stimulation is compared with that of normal fibres of either class in Fig. 4. Furthermore, units A and B exhibited a long-lasting response to histamine iontophoresis (>15 min), which has previously been seen exclusively in mechano-insensitive itch fibres in the peroneal nerve of healthy subjects (Schmelz et al., 1997).

**Discussion**

Microneurography was performed in eight patients with erythromelalgia. In comparison with a large sample of units obtained from healthy subjects, decreased conduction velocity and increased activity-dependent slowing was found in afferent C-nociceptive fibres, but not in efferent fibres.
Furthermore, we detected signs of sensitization and spontaneous activity in mechano-insensitive nociceptors.

Altered conductive properties in afferent C-fibres in erythromelalgia

Our results show reduced conduction velocity and increased activity-dependent slowing of afferent C-fibres in patients with erythromelalgia. This could be attributed partly to the greater age of the patients compared with the control group, but then one would expect that this effect should also have altered the conductive properties of the sympathetic fibres, which was not the case (Fig. 2). Furthermore, age was not correlated with conduction velocity, either in the patients or in the control group.

One of the main symptoms in erythromelalgia is an elevated temperature in the affected limbs during pain attacks. Some of our patients did experience erythromelalgia symptoms during recording (Table 1), but increased skin temperature should increase the conduction velocity and not decrease it, as we saw in these patients. We therefore ascribe this general change to an effect of the disease itself and suggest that lowered conduction velocity and a higher degree of activity-dependent slowing among afferent fibres are the first signs of small-fibre neuropathy in these patients.

The diagnosis of small-fibre neuropathy is difficult. Patients describe burning pain and sensory changes. On clinical examination, diminished sensation to temperature and pinprick might be found (Holland et al., 1998; Periquet et al., 1999). When there is no thick-fibre involvement, conventional neurography and EMG are normal. We therefore rely on indirect evidence of the function of the fibres, such as thermal thresholds in QST and different tests for autonomic function, such as the quantitative sudomotor autonomic reflex test (QSART) and cardiovascular autonomic testing (Tobin et al., 1999). A decrease in intraepidermal fibre density, assessed by skin biopsy, can support a clinical suspicion of small-fibre neuropathy (Holland et al., 1998; Periquet et al., 1999). The patients in our study did not show clear signs of small-fibre neuropathy in QST, but their symptoms with burning pain and allodynia are also seen in patients with small-fibre neuropathy (Holland et al., 1998). Indeed, preliminary results from an ongoing study on erythromelalgia patients in Oslo reveal that a subgroup of these patients have pathological QST and hence another sign of probable small-fibre affection (K. Ørstavik, C. Mørk, K. Kvernebo and E. Jørum, unpublished results). Other studies on patients with erythromelalgia have also shown signs of thin-fibre involvement, but these have measured solely the function of the sympathetic fibres, either with QSART (Sandroni et al., 1999) or by microneurographic recording of sympathetic mass activity (Sugiyama et al., 1991).

None of the routine clinical methods test the conductive properties of the small fibres. There have been experimental studies on patients with diabetes. Microneurographic studies on sympathetic fibres of patients suffering from diabetic neuropathy have shown normal conduction velocities of postganglionic sympathetic fibres, but a decrease in the number of fibres was suggested (Fagius and Wallin, 1980). In a microneurographic study on one patient with erythromelalgia, normal skin sympathetic nerve activity was found, but this did not result in vasoconstriction (Sugiyama et al., 1991). Recording from afferent C-fibres in diabetic patients has not shown any signs of altered conductive or receptive properties (Serra et al., 1999). In a model of diabetic polyneuropathy in the rat, there was no significant effect on conduction velocity (Chen and Levine, 2001).

Thus, to our knowledge this is the first report of altered properties of afferent C-fibres in a chronic painful condition. Decreased conduction velocity and increased activity-dependent slowing are possible pathological features of a small-fibre neuropathy.
Ionic basis for altered axonal properties

The observed pathological changes of C-axons in the patients could be caused by a variety of different mechanisms. Depolarization of the axons could promote the hyperexcitability and generation of spontaneous activity observed in this study. It could also reduce hyperpolarization-activated current ($I_{h}$) and thereby increase activity-dependent slowing (Grafe et al., 1997), as reported for patients with diabetic neuropathy (Horn et al., 1996). Although depolarization is thought to increase conduction velocity, inactivation of sodium channels could counteract this increase, perhaps even resulting in a reduction in conduction velocity. However, if depolarization causes reduced conduction velocity, activity-dependent hyperpolarization should again counteract this depolarization-induced slowing. The opposite was observed in our study. Likewise, hyperpolarization of the axons could explain the reduced conduction velocity and would also increase electrical thresholds. The observed increase in activity-dependent slowing could not be explained directly by hyperpolarized axons. However, if the hyperpolarization were due to a reduction in $I_{h}$, increased activity-dependent slowing could be explained. $I_{h}$ normally counters activity-dependent slowing (Takigawa et al., 1998); thus, its reduction would promote it, and this is also compatible with lower conduction velocity. Even so, spontaneous activity and hyperexcitability cannot be explained by decreased $I_{h}$. Altered composition of voltage-gated sodium channels, i.e. higher peripheral expression of tetrodotoxin-resistant compared with tetrodotoxin-sensitive channels, could also explain the lower conduction velocity and higher transcutaneous threshold. A changed expression pattern was indeed observed in the lower conduction velocity and higher transcutaneous thresholds. A changed expression pattern was indeed observed in the lower conduction velocity and higher transcutaneous thresholds. A changed expression pattern was indeed observed in this study. The observed increase in activity-dependent slowing could also explain the higher peripheral expression of tetrodotoxin-resistant channels, had their innervation territories within or close to the symptomatic area. Recurrent periods of hypoxaemia with concomitant release of inflammatory mediators might have led to the lowered threshold for mechanical stimulation in normally mechano-insensitive fibres. From animal studies, it has long been known that such peripheral sensitization may occur and contribute to nocifensive behaviour interpreted as primary hyperalgesia (Schaible and Schmidt, 1988). Also in humans, there are two microneurographic case reports on patients with different pain syndromes showing prolonged afterdischarges and possibly lowered mechanical thresholds of polymodal C nociceptors (Cline et al., 1989; Ochoa et al., 2002). Sensitization of ‘sleeping’ nociceptors has also been described in human models of pain and hyperalgesia and has been hypothesized to be an underlying mechanism of primary mechanical hyperalgesia (Schmidt et al., 1995, 2000). However, until now there has been no direct evidence for the involvement of sleeping nociceptors in the pathophysiology of chronic pain.

Spontaneous activity

As a third major pathological finding, we observed spontaneous activity of a mechano-insensitive unit. This unit was found in a patient who also reported constant pain during the experiment. Seven other units exhibited ongoing activity, which made it difficult to judge whether they responded to sympathetic reflex activation or mechanical stimulation. These units were not included in the present data, which may represent a very conservative estimate of the spontaneously active C-nociceptors in the patients. In a sample of several hundred nociceptors recorded in a large number of experiments in healthy volunteers over the last 10 years, we have never observed spontaneous activity in C-nociceptors. Ongoing activity could be caused by the insufficient blood supply of the nociceptor environment in superficial skin layers that is presumed to be a characteristic of erythromelalgia (Littleford et al., 1999; Mørk et al., 2000). This might result in hypoxia, low pH and/or release of inflammatory mediators, all of which are thought to induce ongoing activity in C-nociceptors (Kocher et al., 1987; Steen et al., 1992; Steen et al., 1995, 1996).

Alldynia and punctate hyperalgesia

Touch-evoked allodynia in an area surrounding a lesion (secondary zone) has been shown to be dynamically linked with the magnitude and duration of ongoing pain from the site of the lesion (primary zone), both in experimental models (LaMotte et al., 1991) and in patients with neuropathic pain (Koltzenburg et al., 1994). There is evidence to suggest that previously established criteria would be classified as mechano-insensitive units in healthy subjects (units A, B and D in Fig. 3). The three units with the most pronounced activity-dependent slowing, i.e. the most probable sensitized mechano-insensitive fibres, had their innervation territories within or close to the symptomatic area. Recurrent periods of hypoxaemia with concomitant release of inflammatory mediators might have led to the lowered threshold for mechanical stimulation in normally mechano-insensitive fibres. From animal studies, it has long been known that such peripheral sensitization may occur and contribute to nocifensive behaviour interpreted as primary hyperalgesia (Schaible and Schmidt, 1988). Also in humans, there are two microneurographic case reports on patients with different pain syndromes showing prolonged afterdischarges and possibly lowered mechanical thresholds of polymodal C nociceptors (Cline et al., 1989; Ochoa et al., 2002). Sensitization of ‘sleeping’ nociceptors has also been described in human models of pain and hyperalgesia and has been hypothesized to be an underlying mechanism of primary mechanical hyperalgesia (Schmidt et al., 1995, 2000). However, until now there has been no direct evidence for the involvement of sleeping nociceptors in the pathophysiology of chronic pain.

Sensitized mechano-insensitive C-fibres

Here we demonstrate for the first time a pathological mechano-responsiveness in several C-nociceptors, which by
touch-evoked allodynia is due to central sensitization (Torebjörk et al., 1992) induced and maintained by ongoing activity in mechnano-insensitive nociceptors (Schmelz et al., 2000b).

Our findings are in line with this interpretation. Ongoing activity and sensitization of mechnano-insensitive C-fibres both increased the neural input necessary to maintain central sensitization in our patients. Mechanical thresholds for three sensitized fibres were lowered to 110, <260 and <750 mN respectively, which was far above the gentle forces exerted by the cotton swab that was used to test for touch-evoked allodynia. Thus, we have no evidence to suggest that the sensitized mechnano-insensitive units would be stimulated. Instead our findings are fully compatible with the central sensitization hypothesis, i.e. the activity of Aβ fibres is perceived as painful because of temporarily altered central processing.

Hyperalgesia to punctate stimuli with a stiff von Frey hair has a larger area and a longer time course (up to 24 h) than touch-evoked pain after injection of capsaicin into the skin (LaMotte et al., 1991). Thus, it may persist long after the pain in the primary zone has subsided. It is also thought to be due to central sensitization. In line with this, we found punctate hyperalgesia in all patients. In the three patients with touch-evoked allodynia, the areas of punctate hyperalgesia exceeded the areas of allodynia. As the areas of inflamed skin vary with temperature and amount of exercise, there is no clear border between primary hyperalgesia (peripheral nociceptor sensitization) and secondary hyperalgesia (central sensitization). The force used to map the outer borders of punctate hyperalgesia, 260 mN, was within the range of the lowered mechanical thresholds of some of the sensitized mechnano-insensitive nociceptors. Thus, punctate hyperalgesia as observed in our patient could be attributed to either peripheral or central sensitization.

**Pathophysiological implications**

The currently assumed pathophysiological mechanism for erythromelalgia proposes maldistribution of the blood supply as a main factor (Kvernebo, 1998; Littleford et al., 1999; Mørk et al., 2000). According to this concept, blood flow via arteriovenous shunts is increased, whereas nutritive perfusion is decreased. The inadequately nutritive blood flow induces superficial hypoxia, leading to a fall in pH and the accumulation of vasodilatory mediators. However, when these vasodilatory mediators reach the arteriovenous shunts a vicious circle is initiated. Increased flow through these thermoregulatory shunts will further reduce nutritive perfusion and, in addition, increase temperature and worsen the hypoxia due to increased energy consumption. The abnormal excitability that we have found in these patients, manifested as spontaneous activity and mechano-responsiveness of otherwise ‘sleeping’ C-nociceptors, could provide a peripheral explanation for ongoing pain and the pain elicited by pressure and exercise reported frequently by erythromelalgia patients. The paradox that nerve fibres with increased neural accommodation (slowing) do not block conduction but should contribute to hyperexcitability can be resolved knowing that greater degrees of accommodation allow for higher intra-burst frequencies, predominantly of mechnano-insensitive nociceptors (Weidner et al., 2002). Abnormal excitability in these fibres could also contribute to the reddening in the symptomatic areas. It has been shown that sleeping mechnano-insensitive nociceptors, and not mechnano-responsive C nociceptors, mediate the axon reflex flare in human skin by the release of vasoactive neuropeptides (Schmelz et al., 2000a). Furthermore, activation of mechnano-insensitive C-fibres has been shown to be intimately linked to the induction and maintenance of secondary mechanical hyperalgesia (Schmelz et al., 2000b).

According to our findings, we suggest that these nociceptors are involved in the neurophysiological mechanisms for erythromelalgia. Beyond signalling pain centrally, nociceptors might actively contribute to the pathophysiological process in the periphery. Neuropeptides released by mechnano-insensitive C-nociceptors can cause vasodilation in the superficial and deep arterioles, as shown by laser Doppler scanning and infrared thermography. In the forearm skin, mean temperature increases of 2°C have been observed inside an experimentally induced axon reflex erythema (Forster et al., 1995).

Theoretically, neuropeptide-induced vasodilation should be beneficial if nutritive arterioles are dilated. However, there is no evidence for a preferential effect of neuropeptides on the nutritive vessels. By increasing skin temperature, the oxygen consumption of the skin will increase and hypoxia is thereby worsened. In addition, increased temperature has direct excitatory effects on the nociceptors (Reeh and Petho, 2000); moreover, it potentiates their responsiveness to chemical mediators such as bradykinin, histamine and low pH (Mizumura and Koda, 1999; Reeh and Petho, 2000). Such mediators not only activate nociceptors but also sensitize them to heat (Reeh and Petho, 2000; Liang et al., 2001). It is interesting to note that systemically applied local anaesthetics (lidocaine) have been reported to improve the clinical picture in an erythromelalgia patient dramatically (Kuhnert et al., 1999). Local cooling, the usual self-treatment of the patients, as well as systemic lidocaine, is known to have analgesic effects via the suppression of activity in unmyelinated nociceptors (Koppert et al., 1998, 2000; LaMotte et al., 1992). Thus, the beneficial effects of systemic lidocaine, not only on pain (Kuhnert et al., 1999) but also on vasodilation and skin temperature (Koppert et al., 1998), indicate an active pathophysiological role of nociceptors in erythromelalgia.

In conclusion, this is the first study to report systematic recordings from pathological C-nociceptive fibres in patients with a chronic painful condition. Impulse propagation was altered in afferent, but not efferent C-fibres, with lower conduction velocity and a more pronounced activity-dependent slowing, suggesting incipient afferent small-fibre
neuropathy. Mechanical sensitization and spontaneous activity in originally mechno-insensitive C-fibres correlates with the symptoms in the patients and suggests an active role of these ‘sleeping nociceptors’ in erythromelalgia.

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