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Topical menthol—a human model for cold pain by activation and sensitization of C nociceptors

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Summary

Although cold hyperalgesia is a frequent symptom in patients with neuropathic pain, it is poorly understood. We investigated the mechanisms of cold pain by studying the effect of menthol on pain, temperature perception, touch sensation and skin perfusion. In 10 subjects, 40% L-menthol, and ethanol, serving as control, were topically applied to the forearm in a double-blinded two-way crossover study. Menthol induced significant pain and cold sensations, punctate and cold hyperalgesia and an increase in cutaneous perfusion. Other mechano-sensory and thermal tests were unchanged (touch, cold and warm detection thresholds, heat pain threshold; no dynamic and static hyperalgesia, no wind-up). To investigate the underlying mechanisms, the effects of menthol versus ethanol on the dorsum of the hand were tested during A fibre conduction blockade of the superficial radial nerve in another 10 subjects. The block itself led to hypoaesthesia for mechanical stimuli and anaesthesia for cold perception, but induced an increase in cold-mediated pain. This was due to lack of inhibition of C nociceptors normally exerted by concomitant activation of A fibres. Under these conditions, menthol-induced cold sensation and punctate hyperalgesia were abolished. However, menthol induced spontaneous pain with a trend to higher values than without block. Furthermore, the hyperalgesia to cold stimuli, that was already present during A fibre block, was further increased significantly by menthol. We suggested that menthol acts to sensitize cold-sensitive peripheral vasoactive C nociceptors and activates cold-specific A delta fibres. Punctate hyperalgesia is due to central sensitization based on the ongoing activity in the sensitized cold-sensitive peripheral C nociceptors. In conclusion, topical menthol is a human model for cold pain by exposing for the first time the mechanism of sensitized peripheral cold C nociceptors that may also be involved in neuropathic pain.

Keywords: menthol; cold pain; nociceptor; sensitization; A fibre conduction blockade

Abbreviations: CGRP = calcitonin gene-related peptide; CMR = cold- and menthol-sensitive receptor; NRS = numeric rating scale; TRP = transient receptor potential

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Introduction

Cold sensations, induced by a decrease in cutaneous temperature, become painful at further temperature reduction. Such cold pain is probably mediated by the activation of both thinly myelinated cold-specific A delta fibres, conducting painless cold sensations, and unmyelinated C fibres conducting pain (LaMotte and Thalhammer, 1982; Saumet *et al*., 1985). Several animal and human microneurographic recordings have classified C fibres responding to cold painful stimuli as polymodal nociceptors (Bessou and Perl, 1969; Torebjörk, 1974; Torebjörk and Hallin, 1973). Under pathophysiological conditions, normally innocuous skin cooling can induce pain. This cold hyperalgesia (allodynia) is a striking symptom in patients with neuropathic pain (Lindblom and Verrillo, 1979; Wahren and Torebjörk, 1992; Woolf and Mannion, 1999; Baron, 2000). However, little is known concerning the mechanisms underlying the perception of cold pain in humans (Davis, 1998). Psychophysiological studies suggest that cold-specific A delta fibres normally suppress the sensation of pain originating from C nociceptors. Blockade of A fibres unmasks cold-induced pain. It has been proposed that cold hyperalgesia is due to a change in the central decoding of afferent input due to lack of inhibition

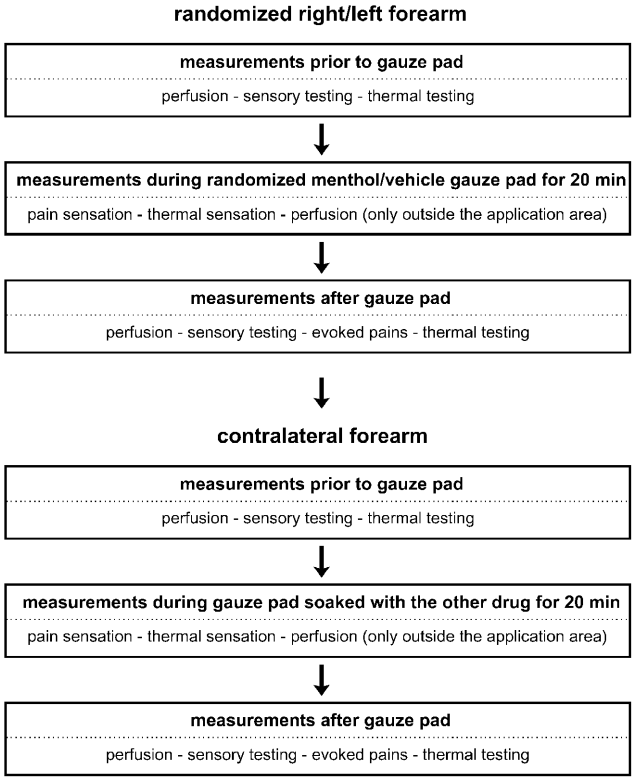

Fig. 1 Diagram of the experimental procedure to investigate the influence of menthol on pain and on thermal sensation, on perception evoked by mechanical and thermal stimuli and on perfusion (experiment I). In 10 subjects, 40% L-menthol, and ethanol serving as control, were topically applied to the forearm in a double-blinded two-way crossover study.

normally exerted by concomitant activation of myelinated fibres (Fruhstorfer, 1984; Wahren *et al*., 1989; Yarnitsky and Ochoa, 1990). In support of this hypothesis are patients with neuropathy who suffer from cold pain hyperalgesia in combination with cold hypoaesthesia (Ochoa and Yarnitsky, 1994). However, there are also patients suffering from cold-induced pain without any deficit in cold perception (Kashihara and Yabuki, 1987; Verdugo and Ochoa, 1992). Therefore, the question arises of whether any mechanism exists that directly involves C nociceptors in mediating cold-induced hyperalgesia.

As heat-sensitive C nociceptors have a role in neuropathic pain (LaMotte *et al*., 1991; Koltzenburg and Torebjörk, 1995; Fields *et al*., 1998; Julius and Basbaum, 2001; Baumgärtner *et al*., 2002), it is feasible that sensitized C nociceptors also have a role in cold pain. In recent studies on cultured cold-sensitive neurons of rats, a cold- and menthol-sensitive receptor (CMR 1) was identified that was present primarily in small-diameter neurons, which typically are involved in sensing pain (McKemy *et al*., 2002). This receptor belongs to the transient receptor potential (TRP) family as demonstrated by DNA analysis and is therefore named TRPM8 in a new nomenclature (Jordt *et al*., 2003). The capsaicin-sensitive vanilloid receptor type 1 (TRPV1, formerly named VR 1) also belongs to this family of receptors (Caterina *et al*., 1997, 2000; Clapham, 2002; McKemy *et al*., 2002; Peier *et al*., 2002). Recent electrophysiological *in vitro* studies in mice identified a subgroup of menthol-sensitive C fibres containing calcitonin gene-related peptide (CGRP) that is a typical feature of vasoactive nociceptors (Beacham *et al*., 2002).

In the present study, we sought to determine whether menthol can be used as a human model for cold pain by activating and sensitizing nociceptive afferents. For this purpose, the influence of menthol on pain and cold sensation, on the perception evoked by mechanical and thermal stimuli and on skin perfusion was investigated in volunteers in a double-blinded placebo-controlled two-way crossover trial. In a second series of experiments, menthol effects were tested during A fibre blockade to identify the neuronal pathways involved.

Methods

Subjects

In 10 healthy subjects (five women, five men, mean age 39.3 ± 5.9 years, range 22–69), the effect of topically applied menthol versus ethanol was tested. In a second series of experiments, the effects of topical menthol versus ethanol were investigated when A fibre conduction was blocked in 10 healthy subjects (five women, five men, mean age 39.3 ± 6.0 years, range 22–69). Six subjects participated in both experiments. In four of them, a further A fibre block was performed without any previous topical drug application. All experiments were performed in the supine position in a room at a constant temperature (20.4 ± 0.2°C) and a relative humidity of 30.2 ± 1.4%. None of the subjects were on any medication. The aim of the study and the nature of the tests were explained to the subjects according to the Declaration of Helsinki. The study was approved by the local ethical committee of the University Clinic of Kiel. All volunteers gave informed consent to participate in the study.

Menthol

A 1 ml aliquot of a solution containing 400 mg of L-menthol (40%) dissolved in 90% ethanol (Kieler Hofapotheke, Kiel, Germany) was placed on a gauze pad, which was applied to the skin. L-Menthol (angle of rotation –50.4°) was used, because it is suggested to be the active stereoisomer (Green, 1985; Swandulla *et al*., 1987). To protect the ethanol from evaporation, the gauze pad was covered by an adhesive film (Suprasorb F, Lohmann & Rauscher, Rengsdorf, Germany) and fixed by a 2.5 cm wide rubber band to ensure adequate contact between the solution and the skin. The pressure on the skin was so mild that it affected neither the comfort of the subjects nor the cutaneous perfusion. After removing the gauze pad, the skin was wiped to remove any remaining menthol.

In both series of experiments (experiment I and II), the effect of menthol was compared with that of the vehicle of 90% ethanol serving as placebo control. A gauze pad soaked with 1 ml of ethanol was applied to the skin in exactly the same way as menthol.

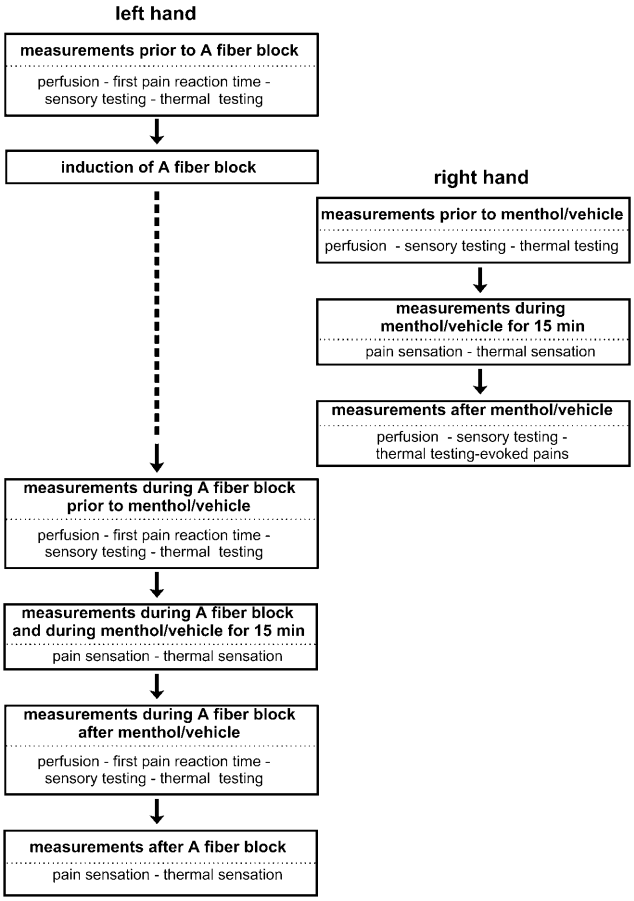

Fig. 2 Diagram of the experimental procedure to investigate the influence of menthol on pain and on thermal sensation, on perception evoked by mechanical and thermal stimuli and on perfusion during A fibre blockade of the left superficial radial nerve in 10 subjects (experiment II). Investigations of the menthol effect in the innervation territory of the unblocked superficial radial nerve at the right hand served as control. In a further series of experiments, the influence of ethanol was also investigated in the same subjects during A fibre blockade with the identical experimental protocol at a later point in time. One individual did not develop a complete A fibre block after 85 min of nerve compression and was therefore excluded from the data analysis.

Effects of menthol on pain and thermal sensations, cutaneous perfusion, sensory and thermal testing and mechanically evoked pains (experiment I, Fig. 1)

Application of menthol and vehicle

A 2.5 × 5 cm^2 gauze pad was placed on the volar forearm 10 cm distal to the elbow for 20 min. In each of the 10 individuals, two gauze pads (one per forearm) containing menthol and vehicle, respectively, were tested in a randomized double-blinded two-way crossover procedure. Neither the individuals nor the investigators were aware whether menthol or vehicle was applied first, because substances were encoded by a technical assistant. To avoid identifying menthol by its characteristic smell, subjects and investigators sniffed at a vial containing 10% menthol prior to each swab application so that the smell of menthol was present regardless of whether menthol or vehicle was tested. The right and left forearms were alternated for the first application in each experiment. The inter-trial interval between the two applications was ~1 h.

Pain and thermal sensations

The subjects were instructed to report any sensation of pain during the 20 min of gauze pad application. The pain was quantified by rating the intensity on a numeric rating scale (NRS 0–10, with 0 representing 'no pain' and 10 being 'the maximum pain that can be imagined'). The quality of pain was noted by using descriptors from the McGill Pain Questionnaire (Melzack, 1975; Stein and Mendl, 1988).

Furthermore, the subjects had to rate any sensation of temperature at the application site. The temperature was quantified by rating intensity on an NRS ranging from –10 to +10 (with –10 representing 'the maximum coolness that can be imagined', 0 representing 'neutral temperature sensation' and +10 representing 'the maximum warmth that can be imagined').

Cutaneous perfusion

Skin temperature was measured with an infrared thermometer immediately prior to and after topical menthol and vehicle at the site of application. In the same area, cutaneous perfusion was measured non-invasively by laser Doppler flowmetry showing relative blood flow changes in arbitrary perfusion units (Periflux pf 4001 and integrating probe pf 413, time constant 0.2 s; Perimed, Stockholm, Sweden). During the 20 min of menthol and vehicle application, a laser Doppler probe (integrating probe pf 413) was placed ~3 mm distant from the edge of the gauze pad for continuous measurement of skin blood flow outside the application area.

Mechano-sensory testing and mechanically evoked pains

Before and after topical menthol and vehicle application, touch detection and pain thresholds to punctate stimuli were measured using a set of 17 von Frey hairs with a bending force ranging from 0.26 to 1080 mN (SENSELab monofilaments; Somedic, Stockholm, Sweden). Using the method of limits, the subjects reported their sensation after three repetitive stimuli with 0.2 Hz of each von Frey hair until they felt pain. Each single stimulus was applied for 1 s. Thresholds were determined as the average reading of two successive stimulation series.

After gauze pad removal, mechanically evoked pains were investigated: the area of punctate hyperalgesia (gentle pressure with a standardized von Frey hair of a bending force of 166 mN), dynamic hyperalgesia (brush-evoked allodynia: gentle moving with a cotton swab on the skin) and static hyperalgesia (pressure-induced hyperalgesia: gentle static pressure with a cotton swab) were investigated by repetitive mechanical stimulation of the skin at a distance of 0.5 cm with a frequency of 1 Hz until the subject noticed that the stimulus became painful (Koltzenburg *et al*., 1992; Ochoa and Yarnitsky, 1993; Ziegler *et al*., 1999). Each single stimulus was applied for 1 s. The border between non-painful and painful stimulation was marked with a pen. By a fan-like procedure, the area of hyperalgesia was determined from all around. The measurements were performed twice to determine exactly the area of hyperalgesia, which was then drawn on a plastic film and measured with a digital planimeter. To determine the intensity of hyperalgesic pain, three

repetitive applications of the corresponding stimuli were performed within the hyperalgesic area with a frequency of 0.2 Hz. Each single stimulus was applied for 1 s. The subjects rated the intensity for this type of hyperalgesia by giving one averaged NRS value (0–10) for these three stimulations. Wind-up was induced by consecutive stimulation with a standardized von Frey hair (166 mN) at 1 Hz for 20 s. Subjects rated pain of the first and the last stimulus. None of the stimuli evoked pain prior to gauze pad application.

Thermal testing

Perception thresholds for thermal sensations (warm, cold, heat and cold pain) were measured by a thermotest device (Somedic, Stockholm, Sweden) prior to and after menthol and vehicle (Fruhstorfer *et al*., 1976; Yarnitsky and Ochoa, 1991). A Peltier type thermode (2.5×5 cm^2) was applied exactly in the area of gauze pad application. The temperature of the thermode could either rise or fall depending on the direction and intensity of the current flow through the Peltier device. The method of limits was used by applying ramp stimuli with a velocity of 1°C/s starting from 32°C (baseline temperature). The temperature of the thermode increased for detection of warm and heat pain thresholds and decreased to determine cold and cold pain detection thresholds. The subjects were asked to press a button immediately the respective thermal sensation was perceived. The velocity of temperature change to achieve the baseline temperature of the thermode after a stimulus was 3°C/s. The period between the achievement of the baseline temperature and the beginning of the following stimulus (inter-stimuli interval) was 0 s for warm and cold and 20 s for heat and cold pain detection thresholds. Thresholds were determined as the average reading of five successive stimuli. The five measurements of a given perception were completed before testing the next perception. To protect the skin from injury, the increase of the thermode temperature was limited to 50°C, even if the button was not pressed. The limit for decreasing temperatures was 4°C.

Influence of A fibre blockade on menthol effects (experiment II, Fig. 2)

To investigate which nerve fibres topical menthol acts upon, the effect of menthol was tested after blockade of A fibres of the superficial radial nerve in 10 subjects. One individual did not develop a complete A fibre block after 85 min of nerve compression and was therefore excluded from the data analysis.

Experimental protocols

Three series of experiments were performed to investigate (i) menthol effects during A fibre blockade; (ii) vehicle effects during A fibre blockade; and (iii) effects of release of A fibre block on spontaneous sensations without any previous drug application.

Menthol effects during A fibre blockade (Fig. 2). Mechano-sensory and thermal measurements and cutaneous perfusion at the site of menthol application were performed as described above on the dorsum of both hands in the innervation territory of the intact superficial radial nerve. In preliminary tests, an A fibre blockade was performed to determine the innervated area. Furthermore, in comparison with the investigations at the forearm (experiment I), the surface of the thermode was reduced to 2.5×3.5 cm^2 to ensure that the innervation territory of the superficial radial nerve was tested exclusively. To investigate spatial distribution and pain intensity of punctate hyperalgesia, the first von Frey hair that induced pain was used in the case of a lowered pain threshold after menthol. No standardized von Frey hair was taken for this measurement, as baseline pain thresholds to punctate stimuli were higher than at the volar forearm. Additionally, the first pain reaction time was measured at the left hand by touching the skin with a needle that was fixed in a flexible plastic holder to induce a reproducible light needle pinprick stimulation. The subjects indicated the first pain sensation acoustically and this was recorded with a stopwatch by the investigator. The average value out of three light needle pinprick stimulations was taken for analysis. Prior to this, two test stimulations were applied to familiarize the subjects with the method. The first pain sensation is conducted by A delta fibre nociceptors (Ziegler *et al*., 1999).

Afterwards, A fibre conduction block of the superficial radial nerve block was performed at the left hand (see below).

During the induction of a sufficient conduction block at the left hand that lasted at least 45 min, menthol was applied for 15 min at the right intact hand as control (Fig. 2). Compared with experiment I, the size of the menthol gauze pad was also reduced to 2.5×3.5 cm^2 covering exactly the area of previous testing. During the application period of 15 min, subjects rated menthol-induced pain and thermal sensation on an NRS as described above. Thereafter, cutaneous perfusion, mechano-sensory and thermal thresholds and mechanically evoked pains were measured again.

After a sufficient A fibre block at the left hand, pain and thermal sensations evoked by menthol application for 15 min were determined. Prior to and after menthol application, cutaneous perfusion, mechano-sensory and thermal thresholds, and mechanically evoked pains were measured (Fig. 2).

Finally, the block was released and subjects were asked to report any spontaneous sensation at the dorsum of the left hand.

Vehicle effects during A fibre blockade (Fig. 2). For control purposes, the influence of ethanol was also investigated in the same subjects during A fibre blockade with the identical experimental protocol at a later point of time. The blinding procedures as described above were also performed in these experiments so that neither the individuals nor the investigators were aware of whether menthol or vehicle was applied.

Effects of release of A fibre block on spontaneous sensations without any previous drug application. A further A fibre blockade was performed at a later point of time in four of the nine subjects who were asked to report any spontaneous sensation at the dorsum of the left hand after the block was released without any previous topical drug application.

Induction of A fibre block. Blockade of A fibres was achieved by means of pressure to the left superficial radial nerve as described previously (Bromm and Treede, 1987; Ziegler *et al*., 1999). The hand rested on soft blankets in an intermediate position between pronation and supination. Care was taken that the position was comfortable for the subjects, because the experiments lasted ~2 h. A 2.5 cm wide rubber band was placed across the forearm just proximal to the wrist and loaded with a weight of 1.2 kg. Human microneurography studies have demonstrated that this kind of nerve compression led to preferential A fibre blockade (Torebjörk and Hallin, 1973; Mackenzie *et al*., 1975). Furthermore, no major blood

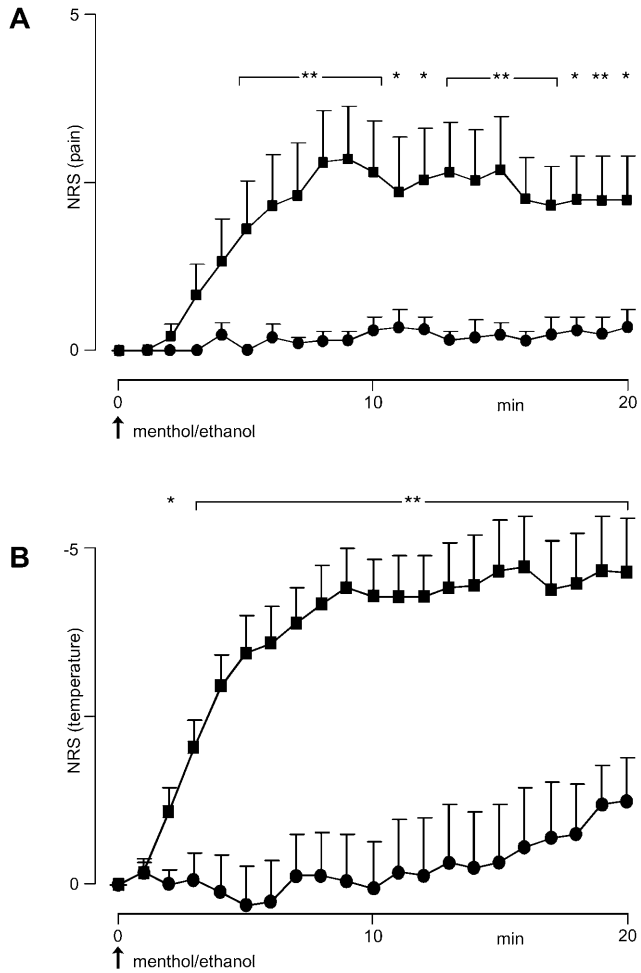

Fig. 3 Group data (10 subjects) showing menthol-induced (filled squares) and ethanol-induced (filled circles) pain (**A**) and thermal (**B**) sensations in a double-blinded two-way crossover procedure. A gauze pad soaked with 1 ml of a solution containing 400 mg of L-menthol (40%) dissolved in 90% ethanol placed to the volar forarm for 20 min induced a significant sensation of burning pain (**A**) and coldness (**B**) in comparison with the vehicle of 90% ethanol that was applied to the skin in exactly the same way. NRS = numerical rating scale (**A**, 0–10; **B**, –10 to +10), mean ± SEM, $*P < 0.05$; $**P < 0.01$.

vessels are affected and the evoked sensations are restricted purely to the effects of nerve blocking without inducing any pain.

Monitoring of A fibre block. The progress of nerve conduction blockade was monitored by testing the cold detection threshold (function of cold-specific A delta fibres) with a thermotest device as decribed above. The first measurement was performed 45 min after induction of A fibre block and was repeated every 10 min. The block was estimated as sufficient when the cold detection threshold was below 10°C. At this point, three further measurements were performed for monitoring and determination of the efficacy of A fibre blockade. (i) The first pain reaction time was measured again as described above to investigate the function of nociceptive A delta fibres. (ii) The area of anaesthesia for light mechanical touch was determined by gentle moving a cotton swab over the skin to investigate the function of mechanosensitive A beta fibre afferents. At the end of the experiment, this area was marked on the skin with a pen and drawn on a plastic film to measure the size with a digital planimeter. (iii) The thermal thresholds for warm perception and heat pain as well as for cold-induced pain were determined to investigate the function of C fibre afferents.

Data acquisition and analysis

Quantity (NRS) of pain and thermal sensations during menthol and vehicle application were documented at 1 min intervals. Continuous blood flow measurements outside the gauze pad area during menthol and vehicle application in experiment I were fed into a computer. Data are given as mean values ± SEM.

The Wilcoxon test was used to compare paired data. P values of <0.05 were regarded as statistically significant.

Results

Effects of menthol on pain and thermal sensations, thermal and sensory testing, mechanically evoked pains and cutaneous perfusion (experiment I)

Pain sensation

Topical menthol on the volar forearm induced a spontaneous pain sensation in eight of 10 subjects. Seven subjects described the pain as burning, one subject as dull. The pain occurred with a latency of a few minutes and reached an intensity plateau of about NRS 3 after 8 min. The pain induction was significant compared with vehicle application that induced pain of a much lesser extent occasionally in only two out of 10 subjects (Fig. 3A).

Thermal sensation

All subjects noticed a significant feeling of coldness at the site of menthol application. Cold sensation occurred within the first 2 min and reached an intensity plateau of about NRS –4.5 after 9 min. Vehicle mediated only a mild cold sensation occasionally in three out of 10 subjects and a feeling of warmth occasionally in one subject (Fig. 3B).

Thermal testing

Topical menthol induced cold hyperalgesia by a significant decrease in cold pain threshold (Fig. 4A). Further thermal thresholds were not affected by menthol or vehicle, except for a vehicle-induced moderate decrease of heat pain threshold that just reached statistical significance (44.5 ± 0.8°C prior to versus 43.3 ± 0.8°C after ethanol, $P = 0.05$). This might be because ethanol mediates a nociceptor response via the vanilloid receptor 1 (Trevisani *et al*., 2002). However, compared with heat pain threshold after menthol, there was no significant difference, indicating a very mild effect.

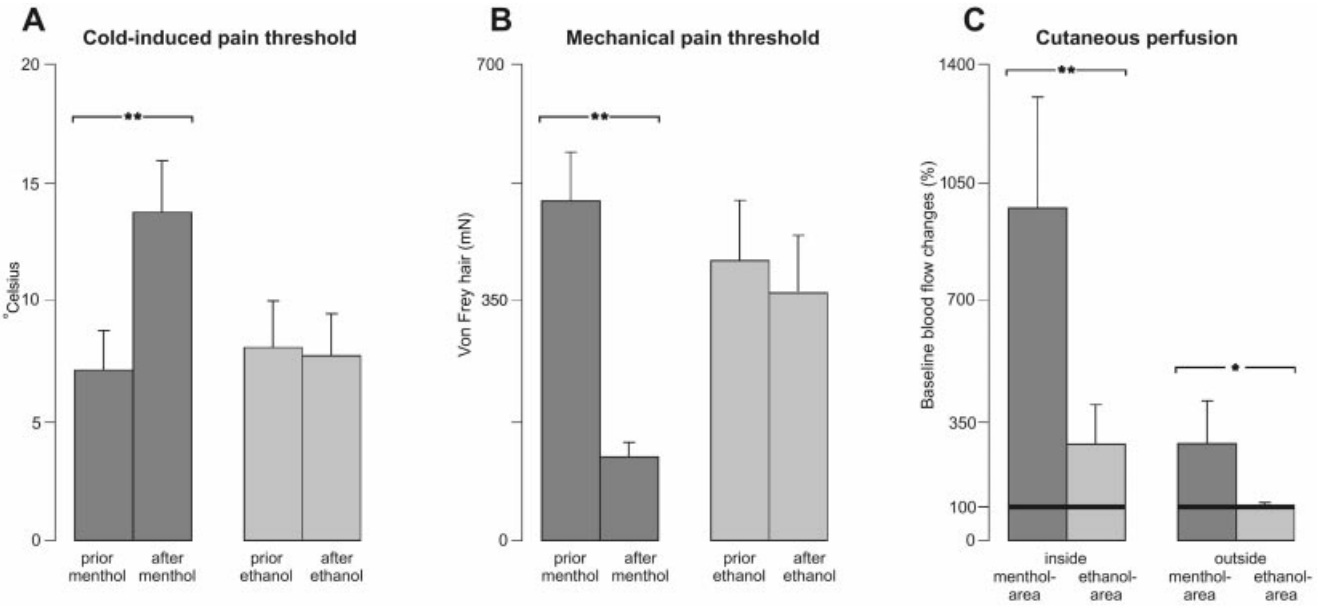

Fig. 4 Effects of menthol (dark columns) and ethanol (light columns) on cold-induced pain thresholds (**A**), mechanical pain thresholds (**B**) and cutaneous perfusion (**C**) at the volar forearm in 10 subjects. Mean values ± SEM prior to and after application of a gauze pad that was soaked with menthol and ethanol, respectively, were compared. Menthol, but not ethanol, induced cold hyperalgesia (**A**) and punctate hyperalgesia (**B**) by significantly decreasing the cold pain threshold measured by thermotesting (°C) and the mechanical pain threshold tested with von Frey hairs (mN). Furthermore, menthol induced a significant cutaneous vasodilatation measured by laser Doppler flowmetry both at the site and outside the area of application compared with ethanol (**C**). For calculation of relative blood flow changes (%), the baseline blood flow prior to gauze pad application was set at 100% (marked by thick lines within the columns). $*P < 0.05$; $**P < 0.01$.

Mechano-sensory testing and mechanically evoked pains

Touch detection thresholds to innocuous punctate stimuli were not affected either by topical menthol or by vehicle (0.7 ± 0.1 mN prior to and after menthol; 0.5 ± 0.1 mN prior to versus 0.7 ± 0.1 mN after ethanol). However, menthol induced punctate hyperalgesia by a significant reduction in pain threshold to punctate stimuli (10 out of 10 subjects) (Fig. 4B). Cutaneous stimulation with a standardized von Frey hair of a bending force of 166 mN induced significant pain after menthol in comparison with vehicle (NRS 2.4 ± 0.7 versus 1.0 ± 0.7, $P = 0.009$). Furthermore, it revealed a significant area of punctate hyperalgesia in eight out of 10 subjects after menthol but not after vehicle (7.3 ± 2.8 versus 1.3 ± 1.2 cm^2, $P = 0.009$). Although this area was on average smaller than the area treated with menthol, the detailed analysis demonstrated that in only three out of 10 subjects was the area of hyperalgesia completely within the menthol-treated area. In three subjects, the hyperalgesic area spread beyong the treatment area at some sites, and in two subjects the larger area of hyperalgesia spread completely beyond the treatment area.

Neither menthol nor vehicle induced any significant dynamic hyperalgesia (menthol and ethanol each one out of 10 subjects), static hyperalgesia (menthol zero and ethanol one out of 10) or wind-up (menthol and ethanol each one out of 10).

Cutaneous perfusion

Starting from a baseline temperature of 31.5 ± 0.4°C, both menthol and vehicle induced a decrease in skin temperature of ~1°C at the application site that just reached statistical significance for menthol compared with baseline temperature ($P = 0.04$). Compared with skin temperature after ethanol, there was no significant difference, indicating an unspecific effect probably due to application of a moist gauze pad.

In nine out of 10 subjects, skin was reddened at the application site after menthol was removed. Laser Doppler demonstrated a significant increase in cutaneous blood flow both at the site of menthol action (10 out of 10 subjects) and to a lesser extent outside the application area (eight out of 10 subjects). Vehicle induced cutaneous redness in only two out of 10 subjects without any significant alterations in skin perfusion (Fig. 4C).

Influence of A fibre blockade on menthol effects (experiment II)

Control application of menthol and vehicle

Topical menthol at the dorsum of the right hand reproduced the results found in experiment I by inducing pain and cold sensations (Fig. 5), a cold hyperalgesia (Fig. 6A), a punctate hyperalgesia (Fig. 6B) and an increase in skin perfusion (baseline change 368.9 ± 85.9%, $P = 0.004$). The intensity of punctate hyperalgesic pain was rated with an NRS of 2.0 ± 0.5

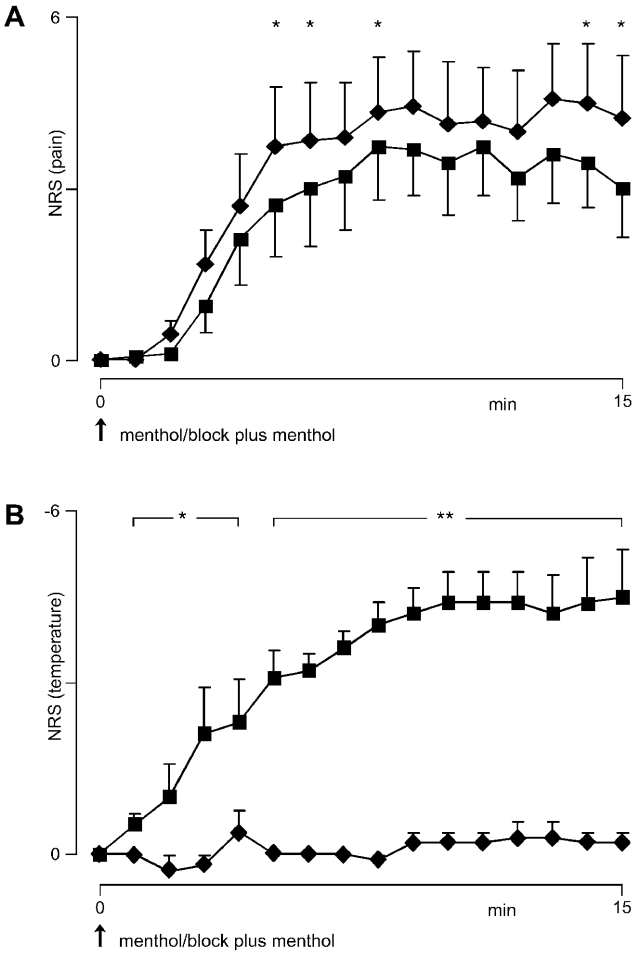

Fig. 5 Group data (nine subjects) showing menthol-induced pain (**A**) and thermal (**B**) sensations following A fibre blockade of the left superficial radial nerve (filled rhombus) in comparison with the menthol-induced sensations on intact nerve fibres at the right hand (filled squares). A gauze pad soaked with 1 ml of a solution containing 40% L-menthol was placed in the innervation territory of the superficial radial nerve for 15 min. Menthol-induced pain ratings showed a clear trend to higher values, with some reaching statistical significance (**A**), and menthol-induced sensations of coldness were significantly abolished (**B**) following A fibre blockade. NRS = numerical rating scale (**A**, 0–10; **B**, –10 to +10), mean ± SEM, $*P < 0.05$; $**P < 0.01$.

and the area of punctate hyperalgesia measured 3.2 ± 1.5 cm^2 on average.

As expected from experiment I, topical ethanol at the dorsum of the right hand induced neither any relevant pain nor any relevant cold sensation. No evoked pains (Fig. 6A and B) and no significant increase in cutaneous perfusion were found.

Monitoring of efficacy of A fibre block

After an A fibre block duration of 58 ± 5 min in the menthol experiments and of 63 ± 5 min in the vehicle experiments, the cold detection threshold was shifted below 10°C in all subjects, which was a marker for an effective blockade of cold-specific A delta fibres. First pain reaction time increased from 0.57 ± 0.04 to 1.02 ± 0.11 s ($P = 0.004$) for the menthol sites and from 0.6 ± 0.08 to 1.3 ± 0.04 s ($P = 0.004$) for the vehicle sites. This doubling of the first pain reaction time demonstrated a significant blockade of A delta nociceptive afferents. Although these measurements were performed manually with a stopwatch, the values are consistent with the data from Ziegler *et al*. (1999) who established this method with a semi-electronic system. As a marker for blockade of mechanosensitive A beta fibre afferents, an anaesthetic area for light mechanical touch developed (55.4 ± 3.7 cm^2 in the menthol and 49.2 ± 4.1 cm^2 in the vehicle experiments). Furthermore, touch detection threshold for punctate stimuli within this area increased from 0.7 ± 0.4 to 7.4 ± 5.1 mN ($P = 0.009$) for the menthol sites and from 0.5 ± 0.2 to 7.4 ± 4.6 mN ($P = 0.004$) for the vehicle sites. The area of patch application was completely within this zone in each experiment. In summary, all these parameters indicate an effective blockade of A fibre afferents.

Warm detection threshold increased significantly from 34.3 ± 0.4 to 36.0 ± 1.1°C ($P = 0.005$) for the menthol sites and from 34.6 ± 0.5 to 35.5 ± 0.6°C ($P = 0.02$) for the vehicle sites, indicating that C fibres were also affected by the blockade as also found by other investigators (Wahren *et al*., 1989). However, the involvement of C fibres was moderate, because the discrimination for warm sensation was still intact and the heat pain threshold was unaltered.

Effects of A fibre block on cold-induced pain, punctate stimuli-induced pain and perfusion

In agreement with the findings of other investigators, the cold-induced pain threshold was significantly decreased during A fibre blockade (Fig. 6C) (Fruhstorfer, 1984; Wahren *et al*., 1989; Yarnitsky and Ochoa, 1990). It is suggested that this is due to the lack of central inhibition of cold C nociceptors by cold-specific A delta fibres.

Cutaneous perfusion and pain detection threshold to punctate stimuli (Fig. 6D) were not affected by the A fibre conduction block.

Effects of menthol and vehicle during A fibre conduction block

Topical menthol still induced a significant burning pain sensation during A fibre block with a clear trend to increased values, with some reaching statistical significance in comparison with control application of menthol without A fibre blockade (Fig. 5A). The cold-induced pain threshold that was already decreased due to A fibre blockade as explained above was further decreased significantly (Fig. 6C). The menthol-mediated increase in skin perfusion was also intact (baseline change 429.1 ± 111.5%, $P = 0.006$).

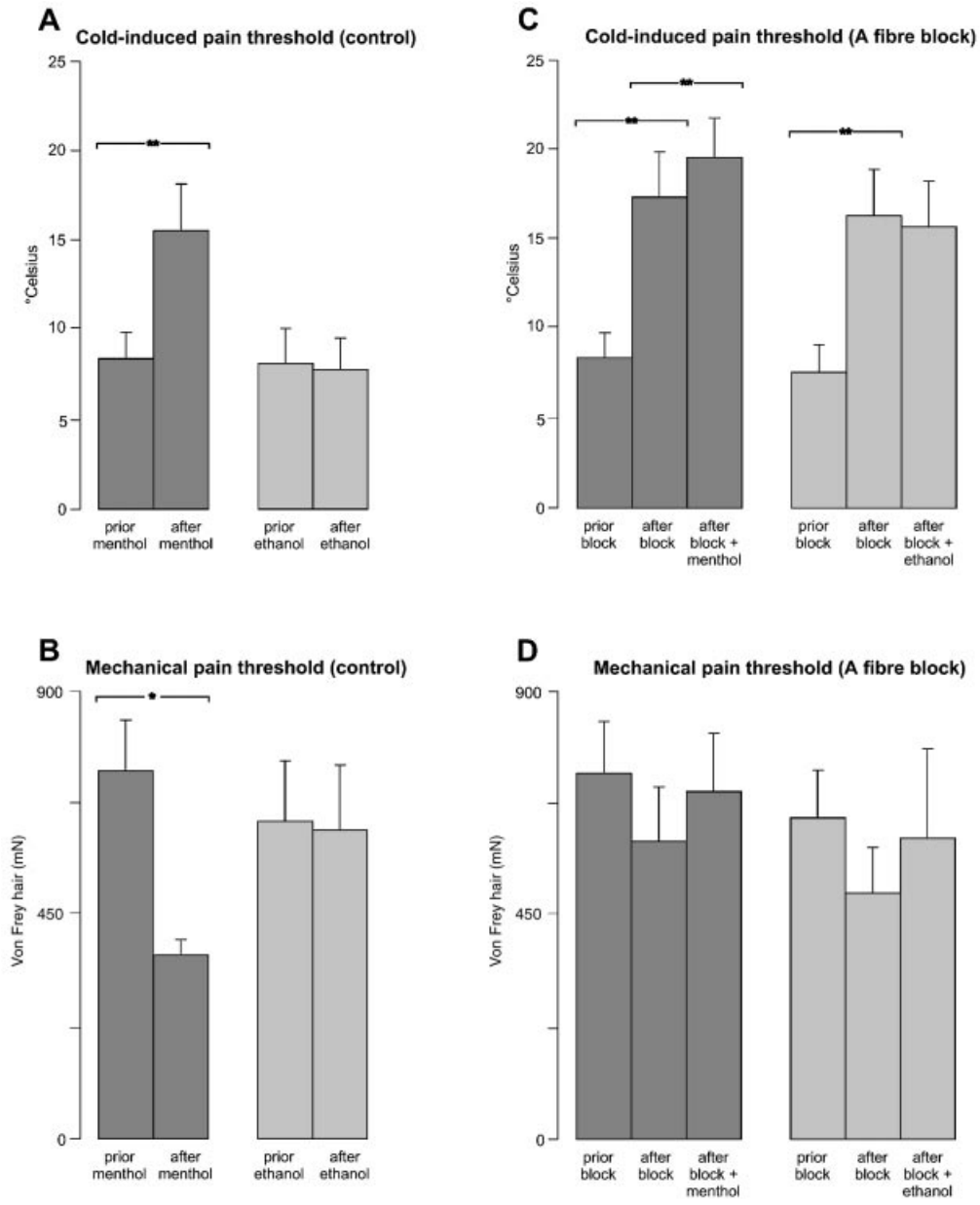

Fig. 6 Effects of menthol (dark columns) and ethanol (light columns) on cold-induced pain thresholds and punctate hyperalgesia during A fibre blockade at the dorsum of the hand in the innervation territory of the superficial radial nerve in nine subjects. Without any nerve block, the control application of menthol, but not ethanol, induced cold hyperalgesia (**A**) and punctate hyperalgesia (**B**) by significantly decreasing the cold pain threshold measured by thermotesting (°C) and the mechanical pain threshold tested with von Frey hairs (mN). The A fibre block itself without any drug application led to a significant decrease in cold-induced pain thresholds (**C**, 'prior block' versus 'after block'). During A fibre blockade, menthol, but not ethanol, induced a significant further decrease in the cold-induced pain threshold (**C**, 'after block' versus 'after block + menthol'). Mechanical pain thresholds tested with von Frey hairs (mN) were not affected significantly either by A fibre blockade, by menthol or by ethanol during the nerve block (**D**). Mean ± SEM, $*P < 0.05$; $**P < 0.01$.

However, the sensation of coldness was significantly decreased (Fig. 5B). Only two subjects reported occasionally a moderate feeling of coldness, whereas in seven subjects temperature sensation was completely abolished. Furthermore, the pain threshold to punctate stimuli was not decreased, indicating a lack of punctate hyperalgesia during A fibre block (Fig. 6D). Also, no other mechanically evoked pains (dynamic, static and wind-up) were found.

Ethanol application induced no further change of the cold-induced pain threshold that was already significantly

decreased due to A fibre blockade (Fig. 6C), indicating that the menthol-induced increase of cold hyperalgesia during A fibre blockade was specific and not due to prolonged duration of the block. As expected, ethanol induced no relevant pain, no relevant cold sensation, no significant increase in cutaneous perfusion nor any change of pain threshold to punctate stimuli (Fig. 6D) similar to values evoked by vehicle applied at intact skin.

Release of A fibre blockade

The A fibre conduction block was released by removing the weighted rubber band 96 ± 4 min after initialization in the menthol experiments and 101 ± 6 min after initialization in the vehicle experiments. In both experimental series, the subjects perceived a cold sensation at the dorsum of the hand within the first few minutes (NRS –3.4 ± 0.4 versus –3.4 ± 0.3, NS). Subjects also reported a cooling sensation after block release without any previous topical drug application (NRS –3.0 ± 0.2). However, the cooling sensation after block release lasted ~20 min in the menthol experiments, but only a few minutes in the experiments with vehicle as well as in the experiments without any previous drug application. In none of the experiments was any pain sensation reported, but all subjects noticed a short-lasting tingling sensation after block release at the dorsum of the hand.

Discussion

The results of the present study demonstrate that topical menthol on human skin elicits sensations of pain and coldness, an increased cutaneous perfusion and cold and punctate hyperalgesia. Blockade of A fibres inhibited cold sensations and punctate hyperalgesia.

An interaction between menthol and nociception was suggested in previous studies, although they have not consistently demonstrated menthol-induced pain sensations (Green, 1992; Yosipovitch *et al*., 1996). Earlier investigators used a lower concentration of L-menthol (5–10%) and the duration of application was shorter, which may explain their different results. The 40% L-menthol that was used in the present study is the highest concentration of L-menthol that can be dissolved in 90% ethanol.

Menthol activates C nociceptors

Menthol induced a significant burning pain sensation that was not abolished by blockade of A fibres, suggesting that C nociceptors encoded this painful sensation. The clear trend to higher pain ratings with some values reaching statistical significance following A fibre block is probably due to the additional lack of central inhibition of cold C nociceptors by cold-specific A delta fibres (Fruhstorfer, 1984; Wahren *et al*., 1989; Yarnitsky and Ochoa, 1990). The menthol reactivity of cold-sensitive sensory neurons with nociceptive properties has been identified in mouse dorsal root ganglia cultures. About 15% of cold-responsive cells react not only to menthol but also to the vanilloid receptor agonist capsaicin (Beacham *et al*., 2002). The molecular cloning of the capsaicin receptor (TRPV1) and its identification as an ion channel gated by capsaicin, noxious heat and acids led to the conclusion that sensitivity to capsaicin is a functional hallmark of nociceptors (Caterina *et al*., 1997; Caterina and Julius, 2001; Julius and Basbaum, 2001). Recent investigations on excitatory ion channels identified a cold- and menthol-sensitive TRP channel (TRPM8) that is activated within the range of 8–28°C. The receptor is expressed in ~10% of all trigeminal and dorsal root ganglia neurons of rats, primarily within small-diameter cells (McKemy *et al*., 2002). About 50% of TRPM8-expressing neurons also expressed TRPV1 and can therefore be categorized as cold- and heat-responsive neurons. Accordingly, ~50% of the menthol-sensitive neurons were also activated by capsaicin (McKemy *et al*., 2002; Reid *et al*., 2002). Interestingly, Story *et al*. (2003) identified a TRP-like channel, ANKTM1, that is present in <4% of sensory neurons. ANKTM1 is found exclusively in cells that also express TRPV1 and peptide markers of nociceptors, such as CGRP (Jordt *et al*., 2003). However, ANKTM1 is activated near 17°C and is insensitve to menthol. Therefore, TRPM8 and not ANKTM1 is suggested to be the mediator for cold pain in the present study.

Cutaneous perfusion measurements in the present study demonstrated an increase in blood flow and a visible flare in the area of menthol application. In previous menthol studies, such local vasodilation was described as a skin irritation that might be due to a local skin reaction (Eccles, 1994, 2000). However, our observation of a significant blood flow increase also 3 mm distant from the site of menthol application might indicate a neurogenic vasodilatation, although a local skin reaction cannot be excluded completely. Such ‘antidromic’ or ‘axon reflex vasodilatation’ is produced by antidromically activated axon branches of nociceptive C fibres that release vasoactive substances, i.e. CGRP and substance P (Lewis, 1927; Szolcsanyi, 1988; Holzer, 1992; Schmelz *et al*., 2000). These vasoactive nociceptors are sensitive to capsaicin (Simone *et al*., 1989; LaMotte *et al*., 1991). The existence of a menthol-sensitive subclass of vasoactive nociceptors has been postulated recently by Beacham *et al*. (2002) who found CGRP-containing small fibre afferents reacting to menthol.

Nociceptor sensitization by menthol

Menthol led to cold hyperalgesia in the area of application by decreasing the cold pain threshold. Following A fibre block, the cold hyperalgesia, that was already induced due to the lack of central inhibition of cold C nociceptors by cold-specific A delta fibres (Fruhstorfer, 1984; Wahren *et al*., 1989; Yarnitsky and Ochoa, 1990), further increased significantly due to menthol. These results clearly indicated for the first time a sensitization of peripheral nociceptors to cold stimuli. In contrast to previous investigations on peripheral

nociceptors sensitized to heat, no static (pressure-induced) hyperalgesia was found in the present study (Koltzenburg *et al*., 1992; Schmidt *et al*., 2000). However, we did not define the pressure of the applied stimulation during testing of static hyperalgesia. Therefore, a moderate menthol-induced static hyperalgesia that was not detected by the applied method cannot be excluded.

Hyperalgesia to cold as well as to heat and mechanical stimuli was also observed following a mild freeze injury to the skin (Beise *et al*., 1998; Kilo *et al*., 1994). However, in contrast to the present study, primarily noxious cold stimuli below 0°C were investigated. Furthermore, Beise *et al*. (1998) suggested that cold hyperalgesia of a burning pain quality was due to central disinhibition of C nociceptor input following reduced cold fibre activity, similar to the results of other investigators (Fruhstorfer, 1984; Wahren *et al*., 1989; Yarnitsky and Ochoa, 1990).

Apart from the peripheral site of action, it has been demonstrated from animal studies that wide dynamic range neurons and high-threshold cells in the superficial and deep dorsal horn are cold sensitive and contribute to thermal hyperalgesia by enhanced responses to cold and heat stimuli (Kenshalo *et al*., 1982; Craig and Bushnell, 1994; Craig and Serrano, 1994; Khasabov *et al*., 2001).

Evidence for involvement of central mechanisms in the present menthol experiments comes from menthol-induced mechanical hyperalgesia to punctate stimuli that disappeared after A fibre conduction block. Studies with capsaicin that sensitizes peripheral heat nociceptors suggest that punctate hyperalgesia is mediated by central sensitization of nociceptive A fibre high-threshold mechanoreceptors. The central facilitation of these A fibre nociceptors is mediated by ongoing activity in primary sensitized C nociceptors (Ziegler *et al*., 1999). This might be also an explanation for menthol-induced punctate hyperalgesia. However, capsaicin-induced punctate hyperalgesia is not only found in the primary zone of capsaicin application, but also spreads into the surrounding unaffected skin (secondary zone), whereas menthol-mediated hyperalgesia is predominantly restricted to the primary zone. This may be due to a lesser C nociceptor activation and sensitization by menthol in comparison with capsaicin. This may also explain why neither dynamic mechanical hyperalgesia nor the wind-up phenomenon were found in the menthol model. It might also be possible that central pathways of menthol-sensitive nociceptors differ from capsaicin-sensitive neurons.

How specific are menthol-sensitive C nociceptors?

The results of the present study indicate that menthol-sensitive nociceptors in humans mediate a burning pain sensation, are vasoactive, can be sensitized to cold stimuli and are involved in punctate hyperalgesia. These characteristics have not been described for any nociceptor before, and one might speculate that menthol-sensitive nociceptors represent a specific subclass of C fibre afferents. Indeed, cold-specific nociceptors were found in the cat's cornea that were excited by small temperature decreases in the range between 30 and 8°C (Gallar *et al*., 1993). However, most studies demonstrated that noxious low temperature activates polymodal C nociceptors that also respond to mechanical and heat stimuli (Bessou and Perl, 1969; Torebjörk, 1974; Torebjörk and Hallin, 1974; LaMotte and Thalhammer, 1982). Microneurographic recordings from mechano-heat-responsive C nociceptors innervating hairy skin in humans revealed that ~40% exhibited an additional response to noxious low temperature (Campero *et al*., 1996). Therefore, we suggest that menthol acts on a subpopulation of polymodal C nociceptors that are provided with both the cold- and menthol-sensitive TRP channel (TRPM8) and the heat- and capsaicin-sensitive TRP channel (TRPV1). Alternatively, menthol might act on mechano-insensitive C nociceptors, because recent animal and human research demonstrated that only these afferents are involved in neurogenic inflammation, but not polymodal C nociceptors (Lynn *et al*., 1996; Schmelz *et al*., 2000). On the other hand, it is unknown whether mechano-insensitive C nociceptors respond to noxious cold. However, the lack of menthol-induced heat hyperalgesia in our experiments is consistent with both hypotheses, because the TRPV1 was not gated by menthol.

Menthol acts on cold-specific A delta fibre afferents

It is well established that cold sensation is mediated by small myelinated A delta fibres (Darian-Smith *et al*., 1973; Mackenzie *et al*., 1975; Adriaensen *et al*., 1983; Fowler *et al*., 1988; Yarnitsky and Ochoa, 1991). Therefore, the inhibition of menthol-induced cold sensation after conduction blockade of A fibres provides strong evidence that menthol acts on cold-specific A delta fibre afferents (Hensel and Zottermann, 1951; Wang *et al*., 1993). At the molecular site of menthol action, it has been shown that the TRPM8 is expressed by small-diameter neurons without co-expression of the TRPV1 as an indicator for cold-specific non-nociceptive afferents, presumably A delta fibres (McKemy *et al*., 2002).

The cold sensation returned during the first minutes after release of A fibre blockade, although menthol has been removed some time previously. This effect was not specific for menthol, because it was also observed after vehicle application and even without any previous drug application after A fibre block release. Therefore, it is suggested that this cold sensation resulted from spontaneous activity in cold-specific A delta fibre afferents that took place due to 'functional regeneration' after the nerve block. Similarly, there was always a tingling sensation after release of the blockade due to 'functional regeneration' of A beta fibre afferents. However, the cold sensation after releasing the

block lasted much longer when menthol was applied previously. This might indicate that menthol sensitizes cold-specific A delta fibres leading to prolonged spontaneous activity at room temperature in our experiments. This hypothesis is supported by other studies showing that menthol potentiates responses of trigeminal fibres and dorsal root ganglia cells to cold by shifting their thermal activation thresholds to higher temperatures (Hensel and Zottermann, 1951; McKemy *et al*., 2002; Reid and Flonta, 2002).

Two subjects in the present study still reported occasionally a menthol- and vehicle-induced moderate cold sensation despite sufficient A fibre blockade. This might be due to activation of a small group of cold-specific C fibre afferents responding to innocuous low temperature (Campero *et al*., 2001; Jiang *et al*., 2002). An incomplete selectivity of the block would be an alternative but unlikely explanation, because the subjects met all the criteria for a complete A fibre blockade including the loss of any cold sensation during thermotesting.

Clinical implications

So far, the only model to explain cold hyperalgesia is the blockade of A fibres. Through this model, the central suppression of C nociceptors by cold-specific A delta fibres is abolished, resulting in a decreased cold-induced pain threshold (Fruhstorfer, 1984; Wahren *et al*., 1989; Yarnitsky and Ochoa, 1990). This mechanism of central interaction between nociceptors and cold-specific afferents would explain cold pain hyperalgesia combined with cold hypoaesthesia, e.g. in large fibre neuropathy (Ochoa and Yarnitsky, 1994), and is suggested to play a crucial role in central pain syndromes and paradoxical heat sensation (Craig and Bushnell, 1994; Craig *et al*., 1996, 2000; Susser *et al*., 1999; Morin *et al*., 2002). In contrast, menthol-induced cold hyperalgesia is due to sensitization of vasoactive peripheral C nociceptors. Furthermore, there is evidence for central sensitization mediating mechanical punctate hyperalgesia. We suggest that this mechanism is also involved in the pathophysiology of clinical cold pain. Indeed, determination of thermal thresholds in 465 patients with different neuropathies revealed that cold pain hyperalgesia in combination with cold hypoaesthesia is found in only 2% of the patients, whereas 9% were characterized by cold pain hyperalgesia without hypoaesthesia, indicating sensitization of cold nociceptors (Verdugo and Ochoa, 1992). Therefore, it is speculated that there are two different groups of patients with cold pain: in one group, A delta fibres were degenerated and the patients developed pain to cold stimuli due to lack of inhibition of C nociceptors normally exerted by concomitant activation of cold specific A fibres; in the other group of patients, cold pain is due to sensitization of cold-sensitive C nociceptors. Interestingly, these mechanisms of degeneration and sensitization play an important role in the pathophysiology of heat-sensitive nociceptors in neuropathic pain (Fields *et al*., 1998; Baumgärtner *et al*., 2002). To prove this hypothesis, the effect of menthol should be tested in combination with the thermotest in patients with cold pain in further studies.

In conclusion, this is the first study demonstrating that topical menthol is a model to induce cold pain by activation and sensitization of cold-sensitive C nociceptors and activation of cold-specific A delta fibres. These mechanisms might be involved in cold hyperalgesia in patients with neuropathy.

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References

Adriaensen H, Gybels J, Handwerker HO, Van Hees J. Response properties of thin myelinated (A-delta) fibers in human skin nerves. J Neurophysiol 1983; 49: 111–22.

Baron R. Peripheral neuropathic pain: from mechanisms to symptoms. Clin J Pain 2000; 16 (2 Suppl): S12–20.

Baumgärtner U, Magerl W, Klein T, Hopf HC, Treede RD. Neurogenic hyperalgesia versus painful hypoalgesia: two distinct mechanisms of neuropathic pain. Pain 2002; 96: 141–51.

Beacham DW, Parkinson MK, Beggs SA, Huerta JJ, Koltzenburg M. Cold and menthol responsive sensory neurons from mouse dorsal root ganglia. In: Abstracts of the 10th World Congress on Pain. San Diego: IASP Press; 2002. p. 127–8.

Beise RD, Carstens E, Kohlloffel LU. Psychophysical study of stinging pain evoked by brief freezing of superficial skin and ensuing short-lasting changes in sensations of cool and cold pain. Pain 1998; 74: 275–86.

Bessou P, Perl ER. Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. J Neurophysiol 1969; 32: 1025–43.

Bromm B, Treede RD. Pain related cerebral potentials: late and ultralate components. Int J Neurosci 1987; 33: 15–23.

Campero M, Serra J, Ochoa JL. C-polymodal nociceptors activated by noxious low temperature in human skin. J Physiol 1996; 497: 565–72.

Campero M, Serra J, Bostock H, Ochoa JL. Slowly conducting afferents activated by innocuous low temperature in human skin. J Physiol 2001; 535: 855–65.

Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 2001; 24: 487–517.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997; 389: 816–24.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000; 288: 306–13.

Clapham DE. Signal transduction. Hot and cold TRP ion channels. Science 2002; 295: 2228–9.

Craig AD, Bushnell MC. The thermal grill illusion: unmasking the burn of cold pain. Science 1994; 265: 252–5.

Craig AD, Serrano LP. Effects of systemic morphine on lamina I spinothalamic tract neurons in the cat. Brain Res 1994; 636: 233–44.

Craig AD, Reiman EM, Evans A, Bushnell MC. Functional imaging of an illusion of pain. Nature 1996; 384: 258–60.

Craig AD, Chen K, Bandy D, Reiman EM. Thermosensory activation of insular cortex. Nat Neurosci 2000; 3: 184–90.

Darian-Smith I, Johnson KO, Dykes R. 'Cold' fiber population innervating palmar and digital skin of the monkey: responses to cooling pulses. J Neurophysiol 1973; 36: 325–46.

Davis KD. Cold-induced pain and prickle in the glabrous and hairy skin. Pain 1998; 75: 47–57.

Eccles R. Menthol and related cooling compounds. J Pharm Pharmacol 1994; 46: 618–30.

Eccles R. Role of cold receptors and menthol in thirst, the drive to breathe and arousal. Appetite 2000; 34: 29–35.

Fields HL, Rowbotham M, Baron R. Postherpetic neuralgia: irritable nociceptors and deafferentation. Neurobiol Dis 1998; 5: 209–27.

Fowler CJ, Sitzoglou K, Ali Z, Halonen P. The conduction velocities of peripheral nerve fibres conveying sensations of warming and cooling. J Neurol Neurosurg Psychiatry 1988; 51: 1164–70.

Fruhstorfer H. Thermal sensibility changes during ischemic nerve block. Pain 1984; 20: 355–61.

Fruhstorfer H, Lindblom U, Schmidt WC. Method for quantitative estimation of thermal thresholds in patients. J Neurol Neurosurg Psychiatry 1976; 39: 1071–5.

Gallar J, Pozo MA, Tuckett RP, Belmonte C. Response of sensory units with unmyelinated fibres to mechanical, thermal and chemical stimulation of the cat's cornea. J Physiol 1993; 468: 609–22.

Green BG. Menthol modulates oral sensations of warmth and cold. Physiol Behav 1985; 35: 427–34.

Green BG. The sensory effects of l-menthol on human skin. Somatosens Mot Res 1992; 9: 235–44.

Hensel H, Zottermann Y. The effect of menthol on the thermoreceptors. Acta Physiol Scand 1951; 24: 27–34.

Holzer P. Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds. Rev Physiol Biochem Pharmacol 1992; 121: 49–146.

Jiang CH, Mazieres L, Lindstrom S. Cold- and menthol-sensitive C afferents of cat urinary bladder. J Physiol 2002; 543: 211–20.

Jordt SE, McKemy DD, Julius D. Lessons from peppers and peppermint: the molecular logic of thermosensation. Curr Opin Neurobiol 2003; 13: 487–92.

Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature 2001; 413: 203–10.

Kashihara K, Yabuki S. Cold-induced dysaesthesia in acute idiopathic polyneuritis. J Neurol 1987; 235: 53–5.

Kenshalo DR Jr, Leonard RB, Chung JM, Willis WD. Facilitation of the response of primate spinothalamic cells to cold and to tactile stimuli by noxious heating of the skin. Pain 1982; 12: 141–52.

Khasabov SG, Cain DM, Thong D, Mantyh PW, Simone DA. Enhanced responses of spinal dorsal horn neurons to heat and cold stimuli following mild freeze injury to the skin. J Neurophysiol 2001; 86: 986–96.

Kilo S, Schmelz M, Koltzenburg M, Handwerker HO. Different patterns of hyperalgesia induced by experimental inflammation in human skin. Brain 1994; 117: 385–96.

Koltzenburg M, Torebjörk HE. Pain and hyperalgesia in acute inflammatory and chronic neuropathic conditions. Lancet 1995; 345: 1111.

Koltzenburg M, Lundberg LE, Torebjörk HE. Dynamic and static components of mechanical hyperalgesia in human hairy skin. Pain 1992; 51: 207–19.

LaMotte RH, Thalhammer JG. Response properties of high-threshold cutaneous cold receptors in the primate. Brain Res 1982; 244: 279–87.

LaMotte RH, Shain CN, Simone DA, Tsai EF. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 1991; 66: 190–211.

Lewis T. The blood vessels of the human skin and their responses. London: Shaw; 1927.

Lindblom U, Verrillo RT. Sensory functions in chronic neuralgia. J Neurol Neurosurg Psychiatry 1979; 42: 422–35.

Lynn B, Schutterle S, Pierau FK. The vasodilator component of neurogenic inflammation is caused by a special subclass of heat-sensitive nociceptors in the skin of the pig. J Physiol 1996; 494: 587–93.

Mackenzie RA, Burke D, Skuse NF, Lethlean AK. Fibre function and perception during cutaneous nerve block. J Neurol Neurosurg Psychiatry 1975; 38: 865–73.

McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002; 416: 52–8.

Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1975; 1: 277–99.

Morin C, Bushnell MC, Luskin MB, Craig AD. Disruption of thermal perception in a multiple sclerosis patient with central pain. Clin J Pain 2002; 18: 191–5.

Ochoa JL, Yarnitsky D. Mechanical hyperalgesias in neuropathic pain patients: dynamic and static subtypes. Ann Neurol 1993; 33: 465–72.

Ochoa JL, Yarnitsky D. The triple cold syndrome. Cold hyperalgesia, cold hypoaesthesia and cold skin in peripheral nerve disease. Brain 1994; 117: 185–97.

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, et al. A TRP channel that senses cold stimuli and menthol. Cell 2002; 108: 705–15.

Reid G, Flonta ML. Ion channels activated by cold and menthol in cultured rat dorsal root ganglion neurones. Neurosci Lett 2002; 324: 164–8.

Reid G, Babes A, Pluteanu F. A cold- and menthol-activated current in rat dorsal root ganglion neurones: properties and role in cold transduction. J Physiol 2002; 545: 595–614.

Saumet JL, Chery-Croze S, Duclaux R. Response of cat skin mechanothermal nociceptors to cold stimulation. Brain Res Bull 1985; 15: 529–32.

Schmelz M, Michael K, Weidner C, Schmidt R, Torebjörk HE, Handwerker HO. Which nerve fibers mediate the axon reflex flare in human skin? Neuroreport 2000; 11: 645–8.

Schmidt R, Schmelz M, Torebjork HE, Handwerker HO. Mechano-insensitive nociceptors encode pain evoked by tonic pressure to human skin. Neuroscience 2000; 98: 793–800.

Simone DA, Baumann TK, LaMotte RH. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. Pain 1989; 38: 99–107.

Stein C, Mendl G. The German counterpart to McGill Pain Questionnaire. Pain 1988; 32: 251–5.

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 2003; 112: 819–29.

Susser E, Sprecher E, Yarnitsky D. Paradoxical heat sensation in healthy subjects: peripherally conducted by A delta or C fibres? Brain 1999; 122: 239–46.

Swandulla D, Carbone E, Schafer K, Lux HD. Effect of menthol on two types of Ca currents in cultured sensory neurons of vertebrates. Pflügers Arch 1987; 409: 52–9.

Szolcsanyi J. Antidromic vasodilatation and neurogenic inflammation. Agents Actions 1988; 23: 4–11.

Torebjörk HE. Afferent C units responding to mechanical, thermal and chemical stimuli in human non-glabrous skin. Acta Physiol Scand 1974; 92: 374–90.

Torebjörk HE, Hallin RG. Perceptual changes accompanying controlled preferential blocking of A and C fibre responses in intact human skin nerves. Exp Brain Res 1973; 16: 321–32.

Torebjörk HE, Hallin RG. Identification of afferent C units in intact human skin nerves. Brain Res 1974; 67: 387–403.

Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B, et al. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 2002; 5: 546–51.

Verdugo R, Ochoa JL. Quantitative somatosensory thermotest. A key method for functional evaluation of small calibre afferent channels. Brain 1992; 115: 893–913.

Wahren LK, Torebjörk E. Quantitative sensory tests in patients with neuralgia 11 to 25 years after injury. Pain 1992; 48: 237–44.

Wahren LK, Torebjörk E, Jorum E. Central suppression of cold-induced C fibre pain by myelinated fibre input. Pain 1989; 38: 313–9.

Wang Y, Erickson RP, Simon SA. Selectivity of lingual nerve fibers to chemical stimuli. J Gen Physiol 1993; 101: 843–66.

Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 1999; 353: 1959–64.

Yarnitsky D, Ochoa JL. Release of cold-induced burning pain by block of cold-specific afferent input. Brain 1990; 113: 893–902.

Yarnitsky D, Ochoa JL. Warm and cold specific somatosensory systems. Psychophysical thresholds, reaction times and peripheral conduction velocities. Brain 1991; 114: 1819–26.

Yosipovitch G, Szolar C, Hui XY, Maibach H. Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. Arch Dermatol Res 1996; 288: 245–8.

Ziegler EA, Magerl W, Meyer RA, Treede RD. Secondary hyperalgesia to punctate mechanical stimuli. Central sensitization to A-fibre nociceptor input. Brain 1999; 122: 2245–57.