Brain (1999), **122,** 2245–2257

Secondary hyperalgesia to punctate mechanical stimuli

Central sensitization to A-fibre nociceptor input

E. A. Ziegler,[1] W. Magerl,[1] R. A. Meyer[2] and R.-D. Treede[1]

[1]*Institute of Physiology and Pathophysiology, Johannes Gutenberg University, Mainz, Germany and* [2]*Department of Neurosurgery, Johns Hopkins University, Baltimore, USA*

Correspondence to: Professor Dr med Rolf-Detlef Treede, Institute of Physiology and Pathophysiology, Johannes Gutenberg University, Saarstrasse 21, D-55099 Mainz, Germany
E-mail: treede@mail.uni-mainz.de

Summary

Tissue injury induces enhanced pain sensation to light touch and punctate stimuli in adjacent, uninjured skin (secondary hyperalgesia). Whereas hyperalgesia to light touch (allodynia) is mediated by A-fibre low-threshold mechanoreceptors, hyperalgesia to punctate stimuli may be mediated by A- or C-fibre nociceptors. To disclose the relative contributions of A- and C-fibres to the hyperalgesia to punctate stimuli, the superficial radial nerve was blocked by pressure at the wrist in nine healthy subjects. Secondary hyperalgesia was induced by intradermal injection of 40 μg capsaicin, and pain sensitivity in adjacent skin was tested with 200 μm diameter probes (35–407 mN). The progress of conduction blockade was monitored by touch, cold, warm and first pain detection and by compound sensory nerve action potential. When A-fibre conduction was blocked completely but C-fibre conduction was fully intact, pricking pain to punctate stimuli was reduced by 75%, but burning pain to capsaicin injection remained unchanged. In normal skin without A-fibre blockade, pain ratings to the punctate probes increased significantly by a factor of two after adjacent capsaicin injection. In contrast, pain ratings to the punctate probes were not increased after capsaicin injection when A-fibre conduction was selectively blocked. However, hyperalgesia to punctate stimuli was detectable immediately after block release, when A-fibre conduction returned to normal. In conclusion, the pricking pain to punctate stimuli is predominantly mediated by A-fibre nociceptors. In secondary hyperalgesia, this pathway is heterosynaptically facilitated by conditioning C-fibre input. Thus, secondary hyperalgesia to punctate stimuli is induced by nociceptive C-fibre discharge but mediated by nociceptive A-fibres.

Keywords: capsaicin; A-fibre nociceptors; C-fibre nociceptors; nerve conduction block; secondary hyperalgesia; neuropathic pain

Abbreviations: AMH = A-mechanoheat nociceptors; HTM = high-threshold mechanoreceptors; LTM = low-threshold mechanoreceptors; LSD = least squares differences; SNAP = sensory nerve action potential

Introduction

Tissue injury leads to two types of hyperalgesia that differ with respect to their location and psychophysical characteristics (for review, see Treede *et al*., 1992; Meyer *et al*., 1994; Treede and Magerl, 1995). Primary hyperalgesia is limited to the area of injury and is characterized by enhanced pain to both mechanical and heat stimuli. Secondary hyperalgesia occurs in undamaged skin adjacent to an injury and is characterized by enhanced pain to mechanical, but not heat, stimuli (Raja *et al*., 1984; Dahl *et al*., 1993; Ali *et al*., 1996).

Two forms of secondary hyperalgesia have been described: hyperalgesia to light touch (also referred to as allodynia) and hyperalgesia to punctate stimuli. Secondary hyperalgesia to light touch stimuli is more difficult to induce, less frequent, less pronounced, of shorter duration and encompasses a smaller area than secondary hyperalgesia to punctate stimuli (LaMotte *et al*., 1991; Cervero *et al*., 1993; Liu *et al*., 1996; Eisenach *et al*., 1997; Magerl *et al*., 1998). The question arises whether these differences simply reflect quantitative differences in stimulus intensity or whether different mechanisms are involved. Noxious stimuli induce secondary hyperalgesia via central sensitization of neurons in the spinal cord dorsal horn (Simone *et al*., 1991; Thompson *et al*., 1993; Pertovaara, 1998). Secondary hyperalgesia to light

touch is mediated by the A-fibre low-threshold mechanoreceptors (LTM) that are normally responsible for touch sensations. This was shown most conclusively by selective intraneural microstimulation of LTMs, which elicited a sensation of touch in normal skin but became painful when the receptive fields were part of an area of secondary hyperalgesia after adjacent injection of capsaicin (Torebjörk *et al*., 1992). In turn, this LTM-mediated secondary hyperalgesia was completely abolished by A-fibre conduction blockade. However, hyperalgesia to punctate stimuli, but not to light touch, was fully preserved in a patient devoid of A-fibre LTMs following selective large-fibre sensory neuropathy (Treede and Cole, 1993). In addition, graded hyperalgesia can be demonstrated in response to wool fabrics (Cervero *et al*., 1994), which have been shown to selectively activate nociceptors in a graded fashion (Garnsworthy *et al*., 1988). Thus, hyperalgesia to punctate stimuli appears to be mediated by nociceptors. However, results of previous experiments using the differential nerve block technique were inconclusive as to whether A- and/or C-fibre nociceptors are involved (Kilo *et al*., 1994; Koltzenburg, 1996).

The aim of this paper was to investigate whether A- or C-fibre nociceptors are responsible for secondary hyperalgesia to punctate mechanical stimuli. For this purpose we combined the induction of secondary hyperalgesia by intradermal injection of capsaicin (LaMotte *et al*., 1991; Magerl *et al*., 1998) with a pressure nerve block, which impairs impulse conduction in myelinated fibres before that in unmyelinated fibres (Gasser and Erlanger, 1929; Sinclair and Hinshaw, 1950). Conventionally, conduction blockade of large-diameter A-fibres is inferred from loss of touch, while loss or alterations of cold sensitivity into paradoxical warmth or heat are used to indicate a block of small-diameter A-fibres (Fruhstorfer, 1984; Wahren *et al*., 1989). However, these sensory tests do not specifically test for conduction in A-fibre nociceptors. The short-latency response to noxious stimuli (first pain sensation) provides a more specific measure of A-fibre nociceptor function. In previous studies loss of first pain to heat or electrical stimuli has been used to describe the conduction blockade of A-fibre nociceptors (Price *et al*., 1977; Bromm and Treede, 1987). Since secondary hyperalgesia involves enhanced pain to mechanical rather than heat stimuli, in the present work we used first pain reaction times to calibrated pinpricks as an indicator of conduction in mechanosensitive A-fibre nociceptors.

The principal findings of these experiments were that pricking pain to punctate probes was predominantly mediated by A-fibre nociceptors, but burning pain to capsaicin injection was mediated mainly by C-fibre nociceptors. Secondary hyperalgesia to punctate stimuli after intradermal capsaicin was induced by activity in C-fibre nociceptors, but mediated by activity in mechanosensitive A-fibre nociceptors.

Methods

Subjects

Ten healthy subjects (seven female and three male, age range 23–55 years, median age 27 years) participated in the experiment. One of these subjects did not develop a complete A-fibre conduction blockade and was therefore excluded from data analysis. All subjects had either volunteered for similar experiments before or were familiarized beforehand to the environment and experimental procedures, in particular to the kind of test stimuli being used throughout the experiment. The experiments were approved by the Ethics Committee of the Johannes Gutenberg University, Mainz. Informed consent was signed by all subjects.

Induction and monitoring of secondary hyperalgesia

A 10 mM solution of capsaicin (40 µg in a 12.5 µl volume of normal saline containing 0.16% Tween 80; for details, see LaMotte *et al*., 1991) was injected intradermally into the skin of the hand dorsum, which is innervated by the superficial radial nerve (Fig. 1A). Subjects were asked to rate the magnitude of capsaicin-evoked pain for 5 min (every 10 s for the first minute and every 30 s thereafter) on a numerical rating scale ranging from 0 (non-painful) to 100 (most intense pain imaginable). Five minutes after the injection, the bleb formed by the injected volume and the flare elicited by the capsaicin injection were marked on the skin with a felt-tip pen. Each subject was injected twice: first in the right hand, which served as a control site, and later, under complete A-fibre conduction blockade, at a corresponding site on the left hand.

Two types of stimuli were used to test for the occurrence and magnitude of secondary hyperalgesia to light touch and to punctate stimuli. Hyperalgesia to light touch was tested with commercially available cotton buds (Q-tips) fixed alongside a flexible metal strip as described previously (LaMotte *et al*., 1991). The stiffness of the metal strip allowed the delivery of forces of ~1000 mN when it was just noticeably bent. Stimuli were applied as short (1 cm) strokes at constant velocity. The experimenter was initially trained to deliver uniform stimuli across subjects by applying stimuli on a precision balance. Hyperalgesia to punctate stimuli was tested by stimulus response functions of pricking pain to a series of five punctate probes (forces of 35, 62, 111, 206 and 407 mN). The probes were cylindrical stainless steel wires (200 µm tip diameter) mounted on plastic rods of different weights. The rods moved freely within a wider hand-held tube. Stimulation was done by positioning the steel wire tip perpendicular to the skin surface such that the weight of the inner rod rested solely on the wire tip.

The stroking light touch stimulus and each force of the punctate stimulus were applied five times in a randomized sequence, resulting in a total of 30 pain ratings per test. Subjects were instructed to distinguish pain from the perception of touch or pressure by the presence of a sharp or slightly pricking or burning sensation. Subjects were instructed to use the same criteria for pain ratings to test stimuli as they did for the rating of capsaicin-evoked pain.

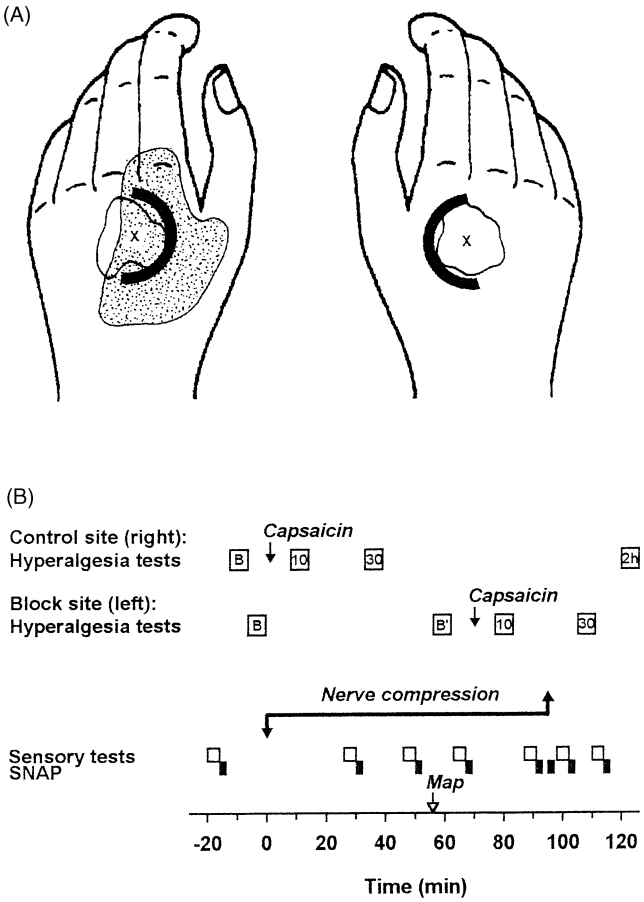

Fig. 1 (**A**) Spatial relationship of the autonomous zone of the left superficial radial nerve (dotted area) as determined by A-fibre conduction blockade, capsaicin injection site (X), axon reflex flare (open areas) and skin areas for testing of secondary hyperalgesia (filled semicircles). (**B**) Experimental protocol. Secondary hyperalgesia to punctate and stroking stimuli was studied following capsaicin injections (40 μg, 12.5 μl) into the dorsum of the left hand (pressure nerve block) and right hand (control). Hyperalgesia tests consisted of stimulus–response functions for pricking pain with punctate stimulators (200 μm diameter, five stimulus intensities, randomized) and soft stroking with a Q-tip; numbers inside the boxes indicate time after capsaicin injection (B = baseline, B′ = baseline under A-fibre conduction blockade). Progress of conduction blockade of the left superficial radial nerve by pressure at the wrist was checked at regular intervals by sensory testing (which included cold, touch and first pain detection; open squares) and by recording the compound SNAP (filled rectangles). The autonomous zone of the superficial branch of the radial nerve was mapped after the detection of touch had been lost (open arrow). Note that in the left hand the 10 min test for hyperalgesia was done under fully established A-fibre conduction blockade, while the 30 min test was done after release of the blockade and complete recovery of A-fibre function.

To avoid stimulation of the zone of primary hyperalgesia, all stimuli were applied along the circumference of a semicircle of radius 15 mm surrounding the injection site (Fig. 1A).

Induction and monitoring of nerve block

Differential conduction blockade of myelinated nerve fibres was achieved by means of pressure to the left superficial radial nerve. The hand rested on a soft layer of tissue in an intermediate position between pronation and supination. Care was taken to hold the hand, arm and shoulder in a position that could be comfortably maintained for 2 h. A 2.5 cm wide rubber band was put across the forearm just proximal to the wrist with its two ends hanging on either side of the arm. The two ends were connected and loaded with one 1.2-kg weight (Bromm and Treede, 1987). The nerve compression that is produced in this way leads to preferential A-fibre blockade, which has been verified in human subjects by microneurography (Torebjörk and Hallin, 1973; Mackenzie *et al*., 1975; Yarnitsky and Ochoa, 1989). This type of nerve block avoids any compression of major blood vessels and ensuing ischaemia of the hand (see section headed Effects of A-fibre blockade on capsaicin-evoked pain and skin responses, under Results).

The progress of the nerve conduction blockade was monitored quantitatively by several detection tasks that functionally characterize different types of peripheral sensory nerve fibres: (i) touch detection and compound sensory nerve action potential (SNAP) for large-diameter A-fibre mechanoreceptors (ii) cold detection for small-diameter A-fibre thermoreceptors; (iii) first pain reaction times for A-fibre nociceptors; (iv) innocuous heat ('warm') detection for C-fibre thermoreceptors; (v) second pain reaction times and capsaicin-evoked pain for C-fibre nociceptors.

Each sensory test started with 16 applications to the skin of a 4 mN von Frey hair. This force was chosen to achieve a touch detection rate close to but below 100% (average detection rate 94% in normal skin) in order to detect the earliest occurrence of a drop in detection rate. Then, cold and warm sensation was tested by touching the skin with a glass tube filled with cold or warm water (eight times each, in a randomized fashion; area of skin contact 1.5 cm^2). The cold and warm glass tubes were kept in water baths at 7° and 57°C, respectively. Short-duration (⩽2 s) skin contact resulted in effective skin cooling of 7.3 ± 0.4°C [mean ± SEM (standard error of the mean)] and warming of 8.6 ± 0.6°C as tested in six subjects (baseline temperature 29.8 ± 1.3°C). Baseline skin temperatures of both hands were measured with an infrared temperature scanner (Dermatemp, DT-1000; EXERGEN, Newton, Mass., USA).

First pain reaction time to a weighted needle pinprick (Chan *et al*., 1992; Magerl *et al*., 1998) was measured to the nearest 10 ms. The needle was the active electrode of an electrical circuit including the subject's skin. Touching the skin with the loaded 21 gauge needle (load 125 mN) closed the circuit and started a timer, which was stopped by pressing a reaction time button. If some stimuli were not perceived because of the nerve conduction blockade, more stimuli were added until a full set (17 perceived trials) of reaction times was obtained.

Finally, the compound SNAP was recorded using an orthodromic electrical stimulation technique. The stimulating electrode was placed in the area innervated by the superficial radial nerve and the recording electrode was placed

just proximal to the nerve block on top of the nerve (stimuli: 200 μs pulse width at four times the perception threshold; mean intensity 6 mA; 100 epochs averaged at a rate of 3/s).

Experimental protocol

The protocol for capsaicin injections, tests for punctate and stroking hyperalgesia, and block monitoring (sensory tests and electroneurographic measurements) is presented in Fig. 1B. Initially, a complete set of sensory tests was done on the dorsum of the subject's left hand to exclude any kind of sensory abnormality before the nerve block. Then, pain perception to punctate and stroking stimuli was tested on both hands. Pressure nerve block of the left superficial radial nerve commenced immediately after testing baseline pain perception. Since block development needs >1 h, this time was used to induce and test hyperalgesia at the control site. Capsaicin was injected intradermally into the dorsum of the right hand and pain perception to punctate and stroking stimuli was tested 10 and 30 min after capsaicin.

Monitoring of sensory loss at the side of nerve block was started at 30 min and repeated at intervals of 20 min. When sensory tests indicated that detection of both touch and cold had largely gone (<20% of stimuli detected), the autonomous zone of the superficial branch of the radial nerve was mapped (56 ± 2 min after block onset). Stimuli were moved centripetally from skin well outside the blocked area towards the numb skin until the subject was unable to detect the von Frey hair and cold stimulus, or until the cold stimulus evoked a sensation of paradoxical heat rather than cold (Fruhstorfer, 1984; Wahren *et al*., 1989; Hansen *et al*., 1996). The autonomous zone of the superficial radial nerve measured 13.5 ± 1.0 cm^2. All further tests were now restricted to this zone. Pain perception to punctate and stroking stimuli was tested again under established A-fibre conduction block within the autonomous zone.

Before capsaicin injection in the dorsum of the left hand, sensory status was checked again after the autonomous zone had been mapped. Capsaicin was injected well inside the confines of the autonomous zone (mean distance from border 6.2 ± 1.0 mm). Ten minutes after the injection, pain perception to stroking and punctate stimuli was tested, followed by another sensory test to verify that A-fibre conduction blockade was still present and complete and that C-fibre function was still intact. Afterwards, the block was released at 96 ± 3 min. Recovery of A-fibre conduction was monitored by the rapid increase in SNAP during the first minute after the block had been lifted. Recovery of SNAP and sensory performance was also checked 5 and 15 min after block release to verify that A-fibre function was completely restored. Pain perception to punctate and stroking stimuli was tested in between both sensory tests 10 min after block release, i.e. 30 min after capsaicin injection. Finally, experiments were completed with another test of pain perception to punctate and stroking stimuli of the control right hand dorsum ~2 h after capsaicin.

Data evaluation and statistics

Sensory tests for block monitoring were analysed as simple detection rates for touch, cold and warm sensation. Detection rate of first pain to punctate mechanical stimuli was determined as the proportion of reaction times that were <500 ms. This cutoff was derived from the intersection of two normal distributions (log-transformed reaction times before and after A-fibre conduction blockade) (Fig. 3B; cf. Campbell and LaMotte, 1983). Compound SNAPs varied considerably across subjects and were analysed as the percentage of control values taken before nerve blocks. Parameters used to characterize the loss of A-fibre function (sensory testing and SNAP) were contrasted by comparing the time required for 50% decay, as interpolated from the decay curves.

To test for the presence of secondary hyperalgesia, differences in the incidence of pricking pain between hyperalgesic and normal skin were compared by Yates' corrected χ^2 test. Population thresholds were estimated from 50% incidence levels, which were interpolated from the recruitment curves. The magnitude of pain ratings to punctate stimuli was not normally distributed, and the standard deviation was strongly correlated to the mean across the different levels of stimulation force. Thus, all pain ratings were transformed into decadic logarithms. To avoid the loss of zero values due to the logarithmic transformation, a small constant (0.1) was added to all raw data (zero and non-zero values) (Bartlett, 1947). This strategy of data handling was established in a previous study on hyperalgesia to punctate stimuli (Magerl *et al*., 1998). Discrimination of the five intensities of punctate probes in both hands was tested by two-way repeated measures ANOVA (analysis of variance; main factors: stimulus magnitude and left versus right hand, for baseline data only). Differences in the magnitude of pain rating between hyperalgesic and normal skin were tested separately for the right (control) and left hand dorsum by two-way repeated measures ANOVA (main factors: stimulus magnitude and time of pain rating before and at various times after capsaicin). Alterations in pricking pain perception and/or hyperalgesia introduced by the nerve conduction blockade were tested by three-way repeated measures ANOVA (main factors: stimulus magnitude, time of pain rating before and 10 and 30 min after capsaicin, and control versus nerve block). The magnitudes of differences between the various levels were estimated from the mean log shift of stimulus–response functions and tested by least squares differences (LSD) *post hoc* tests. Data are presented as mean ± standard error of the mean unless specified otherwise. Probabilities of $P < 0.05$ were considered as being statistically significant.

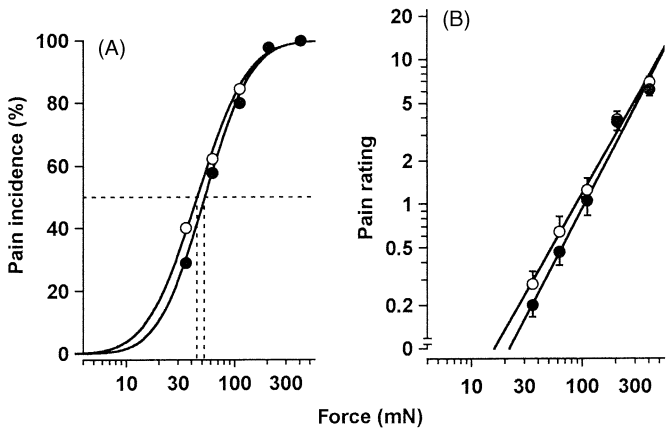

Fig. 2 Stimulus–response functions for pricking pain to the punctate probes (diameter 200 μm) in both hands before initiation of conduction block (open circles, control side; filled circles, side of blockade). (**A**) The incidence of reported pain increased as a function of stimulus force. The 50% thresholds for the two hands were similar. (**B**) The pain ratings (mean ± SEM, $n = 9$) increased as a function of stimulus force. The stimulus–response functions for the two hands were similar. The five force intensities were each delivered five times. The order of stimulus delivery was randomized.

Results

Pricking pain from the punctate probes

The punctate probes produced pain in all of the subjects before the conduction blockade. The incidence of pain reports increased with increasing force, and the recruitment of pain reports matched a cumulative probability function that was very similar in the two hands before capsaicin injection (Fig. 2A). The force associated with 50% probability of pain was 45 mN on the right hand and 52 mN on the left hand. The ratings of pricking pain at baseline were almost identical in the two hands ($P = 0.52$, Fig. 2B). The pain ratings correlated closely with the force delivered by the probe, and the stimulus–response functions were found to be linear in double logarithmic space ($r = 0.69$ for the right and $r = 0.73$ for the left hand) (Fig. 2B). The slopes of these regression lines were slightly positively accelerating (1.35 ± 0.10 for the right hand and 1.47 ± 0.09 for the left hand). The different intensities of the punctate stimuli, which were spaced by a factor of ~2, were easily discriminated [ANOVA: $F(4,40) = 23.65$, $P < 0.001$].

Characterization of A-fibre conduction blockade

Figure 3A shows the changes in the parameters used to monitor A- and C-fibre function under the pressure nerve block. Testing ~30 min after initiation of the nerve compression revealed that loss of SNAP and of touch and cold detection was minor (<20%). The loss of function occurred mainly between 30 and 60 min after the initiation of nerve compression. The time course of conduction blockade was very similar for all non-nociceptive A-fibre functions: the time to 50% decay of function was 42 ± 3 min for touch, 39 ± 4 min for cold and 38 ± 4 min for SNAP ($n = 9$, all differences $P > 0.30$) (Fig. 3B). In contrast, 50% loss of first pain detection occurred significantly later, at 57 ± 3 min ($\Delta T = 15 \pm 2$ min versus touch, 18 ± 4 min versus cold and 19 ± 4 min versus SNAP; all $P < 0.001$) (Fig. 3B). The tenth subject did not develop a complete A-fibre conduction blockade (this subject was not included in the data analysis). Notably, in this subject first pain sensation remained completely unchanged in the presence of an almost complete blockade of touch and cold sensation.

The function of nociceptive A-fibres was inferred from the reaction time to touching the skin with a sharp needle-tip (Fig. 3C). These first pain reaction times were normally distributed in logarithmic space (on average 2.25 ± 0.13 log units = 180 ms; mean ± standard deviation). For the sensory testing done 65 ± 2 min (mean ± standard error of the mean) after initiation of nerve compression, the reaction time increased dramatically, indicating a blockade of nociceptive and non-nociceptive A-fibre functions (on average 3.06 ± 0.14 log units = 1150 ms; mean ± standard deviation, $P < 0.001$). In the nine subjects with complete A-fibre blockade, most of the pinprick stimuli (153/194 = 79%) were still detected and warm perception was never affected (only 1/504 stimuli not detected), indicating that C-fibre function was fully intact at all times. All functional A-fibre test parameters returned to normal very rapidly after the pressure block had been released, except for touch, for which detection rates remained ~10% below control values.

Effects of A-fibre blockade on capsaicin-evoked pain and skin responses

Injection of capsaicin into the dorsum of the right hand caused a strong burning pain (peak pain rating upon injection 84 ± 6 on a scale of 0–100; mean ± SEM) that declined exponentially ($\tau = 88$ s) and was almost gone after 5 min (Fig. 4A). Injection of capsaicin into the dorsum of the left hand under A-fibre conduction blockade evoked an intense burning pain of magnitude and time course similar to those in control skin (peak pain rating upon injection 89 ± 6, τ = 92 s) (Fig. 4A). The size of the wheal produced by the capsaicin injection, which reflected the mechanical effect of the injected volume rather than active wheal formation (LaMotte *et al*., 1991), was 37 ± 4 mm^2 in the left versus 33 ± 3 mm^2 in the right hand. The area of flare following capsaicin injection on the side of the blockade was identical to that on the control side (9.7 ± 0.3 versus 9.2 ± 0.9 cm^2). Initial skin temperatures were similar in the two hands (left 29.5 ± 0.9°C, right 30.1 ± 0.9°C) and decreased slightly in a similar way during the experiment (ΔT left –2.7 ± 0.7°C, right –2.1 ± 0.6°C at the time of blockade release; not significant), indicating intact perfusion of the blocked hand.

Effects of A-fibre blockade on pricking pain

After ~1 h of nerve compression (59 ± 2 min), pricking pain to the punctate stimuli on the left hand was strongly

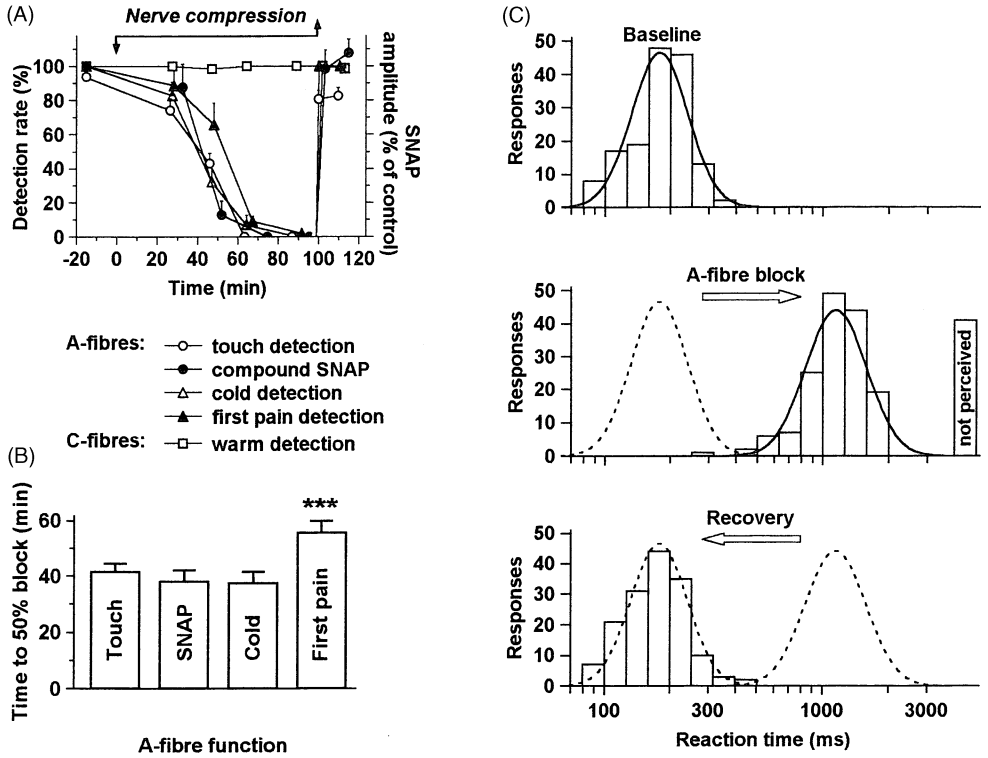

Fig. 3 Complete conduction block of nociceptive and non-nociceptive A-fibres by superficial radial nerve compression ($n = 9$). (**A**) Time course of sensory testing results (mean detection rate ± SEM) for A- and C-fibre functions. A-fibre functions decreased during the course of the block. C-fibre function remained fully intact throughout the block, as indicated by a stable incidence of warm detection and the presence of second pain to pinpricks. (**B**) Bar graph (mean ± SEM) showing the time taken by different sensory functions to decrease to 50% of baseline values. All parameters for non-nociceptive A-fibre functions, including cold detection, were reduced to 50% of baseline values ~40 min after onset of the block. In contrast, loss of first pain (reaction times to pinprick < 500 ms) occurred significantly later (paired *t* test, $n = 9$). (**C**) Histograms of reaction times to pinprick stimuli before nerve blockade (top), at fully established A-fibre conduction blockade (middle) and after blockade release (bottom). Reaction times appeared to be normally distributed when transformed into decadic logarithms (secondary normal distribution). Before nerve blockade, the reaction times were fast (<500 ms, average = 180 ms). The reaction times were slow under fully established A-fibre nerve blockade (1150 ms on average), but returned rapidly to the control value of 180 ms after blockade release.

diminished, to 25% of baseline values (LSD *post hoc* test, $P < 0.001$) (Fig. 4B). As indicated by the shift in reaction times to pinpricks (Fig. 3C), the remaining pain evoked by the punctate probes was mediated by C-fibres.

Effects of A-fibre blockade on punctate hyperalgesia

Ten minutes after the capsaicin injection in the right hand, a marked increase in pain perception to punctate stimuli applied adjacent to the capsaicin injection site was observed [ANOVA: $F(3,80) = 21.4$, $P < 0.001$] and pain ratings had increased to 192% above baseline values (LSD *post hoc* test, $P < 0.001$). There was almost no diminution of secondary hyperalgesia on the control hand 2 h after capsaicin (−6.5%, $P = 0.43$ versus 30 min). This stable state of hyperalgesia enabled us to compare skin sensitivities at various times after capsaicin directly, most importantly in the left hand 10 min after capsaicin and fully established A-fibre conduction block and at 30 min, when sensitivity had fully recovered after block release.

In contrast to the control hand, pain ratings to the punctate stimuli 10 min after the capsaicin injection to the left hand did not increase, but further decreased to 18% of baseline (additional reduction, $P < 0.01$). Twenty minutes after the capsaicin injection in the left hand the nerve compression was released, and cutaneous sensibility quickly returned to normal (Fig. 3A). Thirty minutes after the capsaicin injection, the pain ratings to the punctate stimuli on the left hand had increased significantly, to 227% of baseline values before the initiation of nerve compression (LSD *post hoc* test, $P < 0.001$). The pain ratings for the left hand now matched precisely the pain ratings for the control right hand 30 min after capsaicin ($P = 0.78$). We conclude that the barrage in the non-blocked C-fibre nociceptors had led to a similar degree of central sensitization for the two hands. The

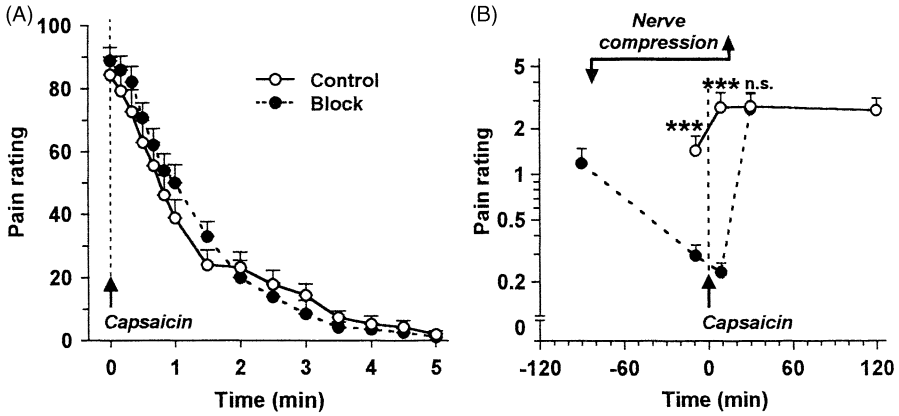

Fig. 4 Effects of complete A-fibre block on capsaicin-evoked pain and punctate hyperalgesia. (**A**) Pain ratings to intradermal injection of 40 μg capsaicin with (filled circles) and without (open circles) A-fibre conduction blockade. Peak pain ratings and time courses of exponential decay (τ = 88 and 92 s, respectively) of capsaicin-evoked pain were similar. (**B**) Mean pain ratings to stimulation with five mechanical punctate probes (averages across all stimulus intensities). The abscissa shows time with respect to capsaicin injection for both hands. Capsaicin injection in normal skin (open circles) caused a long-lasting increase in pain ratings to punctate stimuli without appreciable loss of the hyperalgesia effect over the 2 h observation period. Before the onset of nerve compression, pain to punctate stimuli in the left hand (closed circles) was not different from that in the right control hand at baseline. Under A-fibre conduction blockade, the pain to punctate stimuli was profoundly diminished, and the capsaicin injection did not result in any signs of hyperalgesia. However, hyperalgesia was fully expressed, as shown by enhanced pain ratings after block release (mean ± SEM, $***P < 0.001$, LSD test block versus control site).

resulting punctate hyperalgesia was not apparent during the A-fibre conduction blockade, but became apparent when the A-fibre block was released.

The effect of the nerve block on the incidence and magnitude of pain over the range of stimulating forces is shown in Fig. 5. The probability of pain reports was significantly increased after capsaicin injection in the control right hand, and the 50% population threshold was lowered from 45 to 28 mN (Fig. 5A). In contrast, the probability of pain reports decreased substantially under the A-fibre conduction blockade, and the 50% population threshold increased from 52 to 160 mN (Fig. 5B). However, after the block was released, the probability of pain reports increased dramatically, and the 50% population threshold decreased to 34 mN, which was almost identical to that for the control hand. In the control hand, the capsaicin injection led to a significant increase in pain ratings at most levels of stimulus force, and the stimulus–response function was shifted to the left but maintained the same slope (Fig. 5C). Under established A-fibre conduction blockade, the pain ratings were lowered at all stimulus intensities, and the steepness of the regression line was also significantly reduced (0.89 ± 0.10 versus 1.47 ± 0.09, $P < 0.001$) (Fig. 5D). When pain to punctate stimuli was tested again 10 min after capsaicin injection, pain ratings were further reduced, but the exponent of the power function was unchanged (0.84 ± 0.13). Conversely, after the conduction block had been released, pain ratings were again significantly higher than baseline at all stimulus levels and the stimulus–response function was again parallel to the baseline, as for control skin. In the control hand, only three subjects reported any pain to light touch after the capsaicin injection: two subjects at 10 min and one subject at 30 min, but none at 2 h. Altogether, in the control hand only 3/135 (2%) of the light touch stimuli were perceived as painful. Thus, hyperalgesia to light stroking stimuli was not statistically significant at any time and clearly differed from hyperalgesia to punctate stimuli. Pain to light touch was never reported under established A-fibre blockade, but, as for the control hand, one subject reported some pain to stroking with the Q-tip 30 min after the nerve block had been released.

Discussion

This study has shown that intradermal injection of capsaicin into the dorsum of the hand elicits a stable state of secondary hyperalgesia for at least 2 h that is characterized by a leftward shift of the stimulus–response function for punctate mechanical stimuli that elicit a pricking pain sensation. We have also demonstrated that non-ischaemic pressure nerve blockade affects nociceptive A-fibres significantly later than cold fibres. When conductivity in both small-diameter A-fibre subtypes had been lost but while C-fibres were still unaffected, intradermal injection of capsaicin produced pain that was equal in magnitude to the pain produced without a nerve block, but no hyperalgesia to punctate stimuli could be detected. After the block had been released, punctate hyperalgesia was found to be fully developed. These findings indicate that C-fibre discharges induce central sensitization and that A-fibre nociceptors mediate secondary hyperalgesia to punctate stimuli.

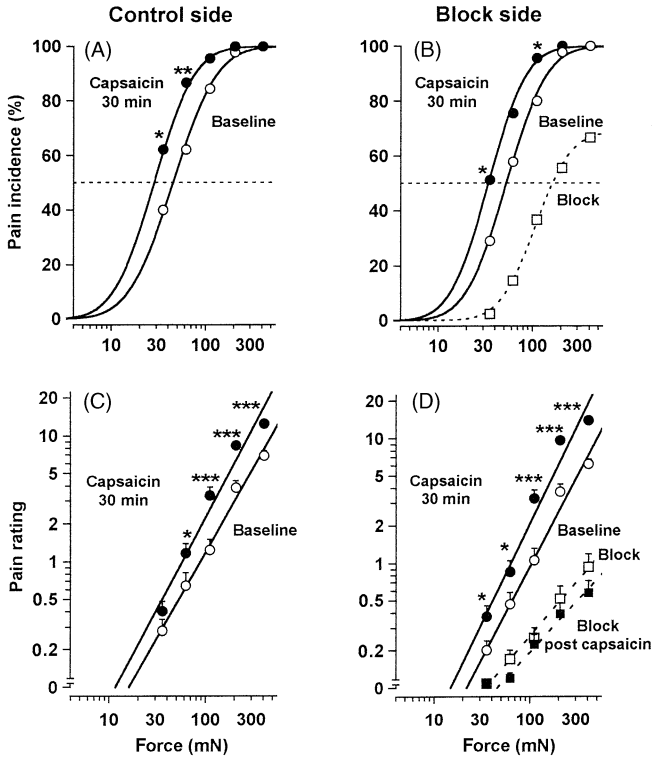

Fig. 5 Modification of stimulus–response functions of pricking pain in the zone of secondary hyperalgesia and during A-fibre conduction blockade. Probe diameter was 200 µm. Incidences of pain reports (top panels) and magnitudes of pain ratings (bottom panels) are plotted as a function of stimulus force. Data for the control hand are plotted in the left column and data for the A-fibre blocked hand in the right column. (**A**) The population threshold (force at which 50% of stimuli were reported to be painful) in the control right hand was interpolated as 45 mN. Thirty minutes after capsaicin injection, the recruitment function was significantly shifted to the left and the population threshold had decreased to 28 mN. (**B**) The recruitment function of pain reports on the left hand was significantly shifted to the right by A-fibre conduction blockade (threshold = 160 mN). Note that, under A-fibre blockade, the fitted function is flatter than the baseline function and apparently does not approach the 100% detection level. This suggests that, under established A-fibre conduction blockade, pricking pain detection will be incomplete even at stimulus levels beyond those used in the experiment. After capsaicin injection, the recruitment function was unchanged (data not shown). However, after the blockade was released it was shifted to the left from the baseline function to the same extent as in control skin (threshold = 34 mN). (**C**) Stimulus–response functions of pricking pain to punctate stimuli in the right control hand before and 30 min after capsaicin injection. Stimulus–response function after capsaicin was significantly shifted to the left and upwards. (**D**) Stimulus–response functions of pricking pain to punctate stimuli in the left hand. Under A-fibre conduction blockade, pain ratings were significantly diminished (open squares) and had not increased 10 min after capsaicin injection (filled squares). After the blockade had been released, the stimulus–response function (30 min after capsaicin) was shifted to the left with respect to the baseline, indicating a mechanical hyperalgesia that was similar to that in the control hand. Note that the stimulus–response functions under A-fibre blockade (squares) had a reduced slope. Open symbols = before capsaicin; closed symbols = after capsaicin; circles = normal conducting nerve; squares = A-fibre nerve conduction blockade. Mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus baseline; χ^2 tests in **A** and **B**, LSD tests in **C** and **D**.

Completeness of differential A-fibre blockade

According to classical studies, differential blockade of A-fibres can be achieved by nerve compression, whereas local anaesthetics at low concentration yield a differential blockade of C-fibres (summarized in Sinclair and Hinshaw, 1950). Later studies demonstrated that compression nerve blocks differentiate poorly between different types of A-fibres (Yarnitsky and Ochoa, 1990), as shown by the nearly simultaneous disappearance of cold sensation (mediated by small-diameter A-fibres) and the detection of light touch (mediated primarily by large-diameter A-fibres). We have again confirmed the similar susceptibility of small- and large-diameter A-fibres to a pressure block. However, the A-fibre nociceptors responsible for first pain sensation were blocked significantly later (by almost 20 min or a 40% longer duration of nerve compression). One miconeurographic study has shown that, during the stage of impaired cold discrimination, the average intrafascicular neurogram can retain fibre potentials of conduction velocities 5–15 m/s (Mackenzie *et al*., 1975). Although cold fibres and A-fibre nociceptors have similar conduction velocities in the Aδ range, they differ with respect to other properties, such as absolute and relative refractory periods (Raymond *et al*., 1990). Additionally, some A-fibre nociceptors are sensitive to desensitization by epicutaneous capsaicin (Beydoun *et al*., 1996) or conduction blockade by perineural capsaicin (Petsche *et al*., 1983), while cold fibres are not affected.

Several lines of evidence argue strongly against the hypothesis that our pressure block had started to affect C-fibres. (i) In rabbit peripheral nerve, C-fibres were unaffected after 2 h of pressure that completely blocked large-diameter fibres within 23 min (Dahlin *et al*., 1989). (ii) In human microneurography, multi-unit C-fibre activity was not blocked after up to 2 h of pressure block (Torebjörk and Hallin, 1973; Mackenzie *et al*., 1975). (iii) C-fibre-mediated heat pain was unaffected by 40 min of compression ischaemia that blocked cold sensation after 20 min; thresholds for warm sensation showed a gradual increase over this period (Yarnitsky and Ochoa, 1991). (iv) In our data, warm detection and capsaicin-evoked pain were unaffected by pressure block. (v) Pinprick detection latency was shifted from one normal distribution around 180 ms to another normal distribution around 1150 ms, and only a small number of stimuli were not detected.

The relative resistance of A-fibre nociceptors to pressure nerve blockade implies that studies using this technique to differentiate between A- and C-fibre nociceptors have to be interpreted with caution when the detection of cold stimuli or paradoxical heat sensation is used as the criterion for complete A-fibre blockade. Since A-fibre nociceptors mediate first pain to heat, mechanical or electrical stimuli, reaction time tasks using these stimuli are appropriate tests for the completeness of a differential A-fibre blockade. The cut-off between first-pain and second-pain reaction times is close to 500 ms for laser radiant heat stimuli (Campbell and

LaMotte, 1983; Bromm and Treede, 1987) and electrical stimuli (Price *et al*., 1977). We have now used calibrated pinpricks (Chan *et al*., 1992; Magerl *et al*., 1998) that are adequate to activate cutaneous nociceptors (Garell *et al*., 1996) in a similar reaction time task and have also found a cut-off of 500 ms. This technique is easy to use and is recommended for future studies on the differential sensory functions of A- and C-fibre nociceptors.

Mechanically evoked pain is predominantly mediated by A-fibre nociceptors

According to electrophysiological data on the response properties of cutaneous nociceptors to mechanical stimuli in the cat and the monkey (Slugg *et al*., 1995; Garell *et al*., 1996), the punctate probes used in our study provided stimuli that were adequate to activate A- and C-fibre nociceptors. The shape of the stimulators is particularly important, since it has been found that smoothing the edge of the probes significantly reduces nociceptor excitation (Garell *et al*., 1996) and pain (Greenspan and McGillis, 1991; Magerl *et al*., 1998) to probes of identical diameter and force. Mechanical thresholds of A- and C-fibre nociceptors in the hairy skin of the human hand are in a similar range (Adriaensen *et al*., 1983). However, A-fibre nociceptors in the cat and monkey exhibit steeper stimulus–response functions than C-fibres, which may even saturate at high stimulus strengths (Slugg *et al*., 1995; Garell *et al*., 1996). During complete A-fibre blockade, pricking pain evoked by punctate mechanical probes was dramatically reduced, but not abolished (Fig. 5). This suggests that pricking pain sensation in normal skin is mainly mediated by A-fibre nociceptors whereas the remaining mechanically induced pain under established A-fibre conduction blockade is mediated by C-fibres.

Capsaicin-evoked pain is predominantly mediated by C-fibre nociceptors

Capsaicin, the active ingredient in hot chilli peppers, has been shown to activate both A-fibre and C-fibre nociceptors (Szolcsányi *et al*., 1988; Baumann *et al*., 1991; for review, see Holzer, 1991). We found that capsaicin-induced pain under A-fibre blockade was identical to that on the unblocked control site (see also Torebjörk *et al*., 1992). Thus, the capsaicin-evoked activity in A-fibre nociceptors does not seem to contribute much to sensation. However, the largest group of C-fibres, the polymodal C-nociceptors, respond only weakly to capsaicin whereas high discharge rates have been found in chemosensitive C-fibre nociceptors (Baumann *et al*., 1991; Schmelz *et al*., 1997). Another class of nociceptors that respond vigorously to capsaicin are the mechanically insensitive A-fibres (Ringkamp *et al*., 1997). This may be the same fibre class that mediates first pain to heat (Treede *et al*., 1998). Because of their very high mechanical thresholds, these fibres may not have been activated by our test stimuli. We therefore do not know whether they were affected by our pressure block. Hence, we suggest that capsaicin-evoked pain relies mainly on input from C-fibres, with a possible contribution from A-fibre mechanically insensitive afferents.

Central sensitization is induced by C-fibre discharges

Since capsaicin was injected in the presence of a complete A-fibre blockade, the remaining C-fibre discharge must have been responsible for the induction of secondary hyperalgesia to punctate stimuli, i.e. for the sensitization of pain-signalling spinal cord neurons. Glutamate acting at NMDA (*N*-methyl-D-aspartate) receptors and substance P acting at NK1 (neurokinin 1) receptors are assumed to be important mediators for the induction of central sensitization (McMahon *et al*., 1993; Urban *et al*., 1994; Chizh *et al*., 1997; Woolf *et al*., 1998). Whereas glutamate is released by all primary afferents, substance P is restricted to A- and C-fibre nociceptors (Duggan *et al*., 1988), with the notable exception of nociceptive A-fibre high-threshold mechanoreceptors (Aδ-HTMs) (Lawson *et al*., 1997), which mediate the hyperalgesia to punctate stimuli (see below). NK1-antagonists prevent capsaicin-induced sensitization to brushing stimuli (Dougherty *et al*., 1994). Thus, the neuropeptide substance P appears to be a critical factor in capsaicin-induced sensitization of central nociceptive neurons to mechanical stimuli.

Hyperalgesia to punctate stimuli is mediated by A-fibre nociceptors

Under the critical condition when A-fibre-nociceptive afferents were blocked, intradermal injection of capsaicin was not accompanied by punctate hyperalgesia. As hyperalgesia to the punctate probes in the secondary zone was evident after the block was released, we postulate that central sensitization had been induced by the capsaicin-evoked C-fibre discharge, but was not detectable during the block because the nociceptive A-fibres that are responsible for its mediation were not conducting. This finding conclusively demonstrates that C-fibres do not mediate punctate hyperalgesia (the small remaining C-fibre-mediated pain evoked by our punctate stimuli was not facilitated by capsaicin). Moreover, we have recently demonstrated psychophysically that the C-fibre-mediated wind-up of pain perception is not altered by experimentally induced secondary hyperalgesia (Magerl *et al*., 1998).

These results appear to be at variance with a previous nerve block study on punctate hyperalgesia in which the magnitude of punctate hyperalgesia to stiff nylon filaments (with bending forces of $>$400 mN) was not changed 22 h after a freeze lesion of the skin (Kilo *et al*., 1994). Apart from the different conditioning stimulus, their criteria for an

A-fibre block were based only on the absence of touch and cool perception. Punctate hyperalgesia might not have been abolished because nociceptive A-fibres were still conducting. In another study in which capsaicin (100 μg) was injected intradermally after the senses of touch and cool had been lost (LaMotte *et al*., 1991), two out of five subjects failed to develop hyperalgesia either to stroking or to punctate stimulation (225 mN von Frey hair, 580 μm in diameter); the remaining three subjects reported only a second pain to stroking. In this case the A-fibre block was probably more complete, although first pain detection was not tested. Other previous nerve block studies investigated only the effects on hyperalgesia to stroking stimuli (Campbell *et al*., 1988; Torebjörk *et al*., 1992; Koltzenburg *et al*., 1994).

Several lines of evidence suggest that hyperalgesia to punctate stimuli is not mediated merely by more intense stimulation of LTMs but rather by the facilitation of the same A-fibre nociceptors that also mediate the pricking pain to these stimuli in normal skin (see above). A graded increase in pain was observed in the zone of secondary hyperalgesia across a wide range of stimulus forces in which the discharge in A-fibre-LTMs would already be saturated (Leem *et al*., 1993). Under pressure nerve block, punctate hyperalgesia remained partially present when brush-evoked secondary hyperalgesia had already been eliminated (Kilo *et al*., 1994). Likewise, hyperalgesia to punctate stimuli was normally expressed in a patient completely devoid of A-fibre-LTMs but with intact A-fibre nociceptors (Treede and Cole, 1993). In addition, wool fabrics, which selectively activate nociceptors in a graded fashion (Garnsworthy *et al*., 1988), produce a graded pain in the zone of secondary hyperalgesia (Cervero *et al*., 1994).

A model of secondary hyperalgesia based on heterosynaptic facilitations

Secondary hyperalgesia is widely accepted to result from sensitization of nociceptive neurons in the dorsal horn of the spinal cord (Simone *et al*., 1991). Most models of secondary hyperalgesia are single-neuron models in which a critical neuron, namely the nociceptive projection neuron, is thought to be sensitized by the concerted action of neurokinins and excitatory amino acids (e.g. McMahon *et al*., 1993; Urban *et al*., 1993). However, these models are difficult to reconcile with the majority of psychophysical findings and recent electrophysiological data.

Spinal facilitation in secondary hyperalgesia must be heterosynaptic, because the facilitated pathway is different from the facilitating pathway (Treede and Magerl, 1995). Moreover, central sensitization is limited to mechanoreceptor input, since hyperalgesia to heat was absent (Raja *et al*., 1984; LaMotte *et al*., 1991) or heat pain perception was even reduced in secondary hyperalgesia (Ali *et al*., 1996). In the present study, we demonstrate that not all mechanical inputs are facilitated. Only A-fibre mechanoreceptive input was facilitated; C-fibre-mediated mechanical pain, which was isolated by the differential nerve block technique, was not facilitated at all. Considering the restrictions mentioned above, the fibre population that is facilitated in secondary hyperalgesia must be limited to subgroups of A-fibres that are either insensitive to heat or have high heat thresholds. The A-fibre HTMs and the type I A-mechanoheat nociceptors (AMHs) fulfil these criteria. Thus, as illustrated in Fig. 6, in secondary hyperalgesia the facilitated primary afferent pathways (A-fibre LTM, A-fibre HTM, type I AMH) do not overlap with those of the facilitating pathways (chemosensitive/capsaicin-sensitive C-fibre nociceptors, polymodal C-fibre nociceptors, mechanically insensitive afferents, type

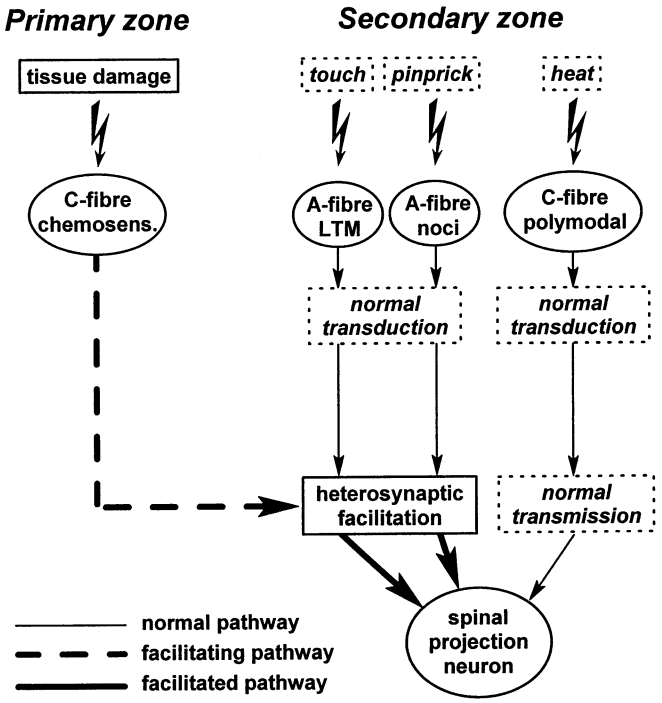

Fig. 6 Model for cutaneous secondary hyperalgesia. The facilitating pathway involves chemosensitive (capsaicin-sensitive) C-nociceptors in injured tissue (including polymodal C-fibre nociceptors, mechanically insensitive afferents, chemoheat nociceptors and chemospecific nociceptors). These afferents are known to discharge vigorously to capsaicin injection and to induce sensitization of nociceptive neurons in the dorsal horn of the spinal cord. This sensitization is heterosynaptic and leads to facilitation of two other inputs onto spinal cord nociceptive projection neurons (both of which are mechanosensitive). The first facilitated pathway (responsible for secondary hyperalgesia for light touch) involves low-threshold mechanoreceptors (A-fibre LTM) that normally signal touch sensation. The second facilitated pathway (responsible for secondary hyperalgesia for punctate stimuli) involves A-fibre nociceptors, including high-threshold mechanoreceptors (A-fibre HTM) and A-fibre nociceptors with high heat thresholds (type I AMHs). The absence of secondary hyperalgesia under established A-fibre conduction blockade suggests that C-fibre inputs themselves are not facilitated. The absence of heat hyperalgesia in the secondary zone suggests that inputs from heat-sensitive nociceptors in general are not facilitated, including polymodal A- and C-fibre nociceptors and A-fibres that normally signal first pain to heat (type II AMHs).

II A-fibre nociceptors, chemo-heat nociceptors, chemospecific nociceptors). The facilitation of input from the LTMs leads to secondary hyperalgesia to light touch; the facilitation of input from A-fibre nociceptors leads to punctate hyperalgesia. Notably, gating of non-nociceptive input from low threshold mechanoreceptors changes the quality of perception, since it adds a component of pain to the sensation of touch (see also Torebjörk *et al*., 1992). In contrast, hyperalgesia to punctate stimuli involves facilitation of a nociceptive input, which is already painful in normal skin. Consequently, in hyperalgesia to punctate stimuli there is an increase in perceived pain with no change in quality of perception.

The psychophysical findings are consistent with electrophysiological data from nociceptive projection cells of the spinal dorsal horn in the rat, cat and monkey. Transmission of heat-evoked input after adjacent capsaicin injection was either unchanged (Simone *et al*., 1991; Pertovaara, 1998) or even transiently inhibited (Dougherty *et al*., 1998) at the same time when a clearcut sensitization to mechanical stimuli was present. Likewise, substance P sensitizes spinal nociceptive neurons to mechanical stimuli but not to heat stimuli (Liu and Sandkühler, 1995). Thus, central sensitization of the nociceptive pathways probably involves a more complex circuitry than is assumed by a single-neuron model. To account for this complexity, we have recently proposed that the critical sensitized neurons may be a subclass of spinal interneurons that receive input from C-fibre nociceptors located inside the zone of tissue injury (Treede and Magerl, 1995) and that lead to facilitation of input from low-threshold A-fibre mechanoreceptors from adjacent non-injured skin, such that psychophysically light touch now elicits a sensation of burning pain (Torebjörk *et al*., 1992). We have now expanded this model by proposing a second facilitated pathway (for A-fibre nociceptors) that accounts for punctate hyperalgesia (Fig. 6). This model also acknowledges the fact that there is rarely a direct monosynaptic pathway from nociceptive primary afferents to spinal nociceptive projection neurons (e.g. Jasmin *et al*., 1997). Alternatively, it has been proposed that different inputs may act at microdomains, specialized regions of the postsynaptic membrane similar to the dendritic spines of hippocampal neurons (Meller, 1994; Dougherty *et al*., 1998). Another recent concept, based on dorsal root reflexes, suggests that secondary hyperalgesia may be explained by the mechanisms of primary afferent depolarization, implying a link that is presynaptic to the first spinal synapse (Cervero and Laird, 1996).

Clinical implications

The observations in experimentally induced secondary hyperalgesia are reminiscent of the different types of mechanical hyperalgesia seen in patients with neuropathic pain. Dynamic hyperalgesia (tested by a soft brush) was abolished in all patients under A-fibre block (the criterion being loss of touch and cold sensation) (Ochoa and Yarnitsky, 1993; Koltzenburg *et al*., 1994). In contrast, static hyperalgesia (tested by pinching or pressing the skin) remained in 15 of 18 patients. This was interpreted to indicate that hyperalgesia to static mechanical stimuli may be mediated by C-fibres, similar to the static hyperalgesia in the primary zone after topical application of capsaicin (Kilo *et al*., 1994). However, in these studies the function of A-fibre nociceptors was not tested explicitly. As detailed above, we have shown that A-fibre nociceptors are significantly more resistant to nerve block than all other A-fibres, and that many of their axons may still conduct when all other A-fibre-related functions are already completely blocked. Thus, the static hyperalgesia in neuropathic patients would also be consistent with mediation by A-fibre nociceptors. Hyperalgesia to punctate mechanical stimuli such as von Frey hairs has also been observed in patients with neuropathic pain (Frost *et al*., 1988; Koltzenburg *et al*., 1994). According to the results of the present study, this type of mechanical hyperalgesia is mediated by A-fibre nociceptors. In conclusion, the two separate primary afferent channels that mediate experimentally induced secondary hyperalgesia are also likely to operate in the hyperalgesia of patients suffering from neuropathic pain.

Acknowledgements

We wish to thank Dr J. Ellrich for help with electroneurographic methods and Dr P. N. Fuchs for critical comments. This publication contains essential parts of the thesis of E.A.Z., which will be submitted to the Medical Faculty of the Johannes Gutenberg University, Mainz, Germany. This study was supported by the Deutsche Forschungsgemeinschaft (grant Tr 236/6), a NATO collaborative research grant (CRG 95032540495) and an NIH grant (NS 14447).

References

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

Ali Z, Meyer RA, Campbell JN. Secondary hyperalgesia to mechanical but not heat stimuli following a capsaicin injection in hairy skin. Pain 1996; 68: 401–11.

Bartlett MS. The use of transformations. Biometrics 1947; 3: 39–52.

Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. J Neurophysiol 1991; 66: 212–27.

Beydoun A, Dyke DBS, Morrow TJ, Casey KL. Topical capsaicin selectively attenuates heat pain and A delta fiber-mediated laser-evoked potentials. Pain 1996; 65: 189–96.

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

Campbell JN, LaMotte RH. Latency to detection of first pain. Brain Res 1983; 266: 203–8.

Campbell JN, Raja SN, Meyer RA, Mackinnon SE. Myelinated afferents signal the hyperalgesia associated with nerve injury. Pain 1988; 32: 89–94.

Cervero F, Laird JM. Mechanisms of touch-evoked pain (allodynia): a new model [see comments]. [Review]. Pain 1996; 68: 13–23. Comment in: Pain 1997; 72: 288–9.

Cervero F, Gilbert R, Hammond RG, Tanner J. Development of secondary hyperalgesia following non-painful thermal stimulation of the skin: a psychophysical study in man. Pain 1993; 54: 181–9.

Cervero F, Meyer RA, Campbell JN. A psychophysical study of secondary hyperalgesia: evidence for increased pain to input from nociceptors [see comments]. Pain 1994; 58: 21–8. Comment in: Pain 1995; 60; 351–2.

Chan AW, MacFarlane IA, Bowsher D, Campbell JA. Weighted needle pinprick sensory thresholds: a simple test of sensory function in diabetic peripheral neuropathy. J Neurol Neurosurg Psychiatry 1992; 55: 56–9.

Chizh BA, Cumberbatch MJ, Herrero JF, Stirk GC, Headley PM. Stimulus intensity, cell excitation and the N-methyl-D-aspartate receptor component of sensory responses in the rat spinal cord in vivo. Neuroscience 1997; 80: 251–65.

Dahl JB, Brennum J, Arendt-Nielsen L, Jensen TS, Kehlet H. The effect of pre- versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin. Pain 1993; 53: 43–51.

Dahlin LB, Shyu BC, Danielsen N, Andersson SA. Effects of nerve compression or ischaemia on conduction properties of myelinated and non-myelinated nerve fibres. An experimental study in the rabbit common peroneal nerve. Acta Physiol Scand 1989; 136: 97–105.

Dougherty PM, Palecek J, Palecková V, Willis WD. Neurokinin 1 and 2 antagonists attenuate the responses and NK1 antagonists prevent the sensitization of primate spinothalamic tract neurons after intradermal capsaicin. J Neurophysiol 1994; 72: 1464–75.

Dougherty PM, Willis WD, Lenz FA. Transient inhibition of responses to thermal stimuli of spinal sensory tract neurons in monkeys during sensitization by intradermal capsaicin. Pain 1998; 77: 129–36.

Duggan AW, Hendry IA, Morton CR, Hutchison WD, Zhao ZQ. Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. Brain Res 1988; 451: 261–73.

Eisenach JC, Hood DD, Curry R, Tong C. Alfentanil, but not amitriptyline, reduces pain, hyperalgesia, and allodynia from intradermal injection of capsaicin in humans. Anesthesiology 1997; 86: 1279–87.

Frost SA, Raja SN, Campbell JN, Meyer RA, Khan AA. Does hyperalgesia to cooling stimuli characterize patients with sympathetically maintained pain (reflex sympathetic dystrophy)? In: Dubner R, Gebhart GF, Bond MR, editors. Proceedings of the Vth World Congress on Pain. Amsterdam: Elsevier Science; 1988. p. 151–6.

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

Garell PC, McGillis SL, Greenspan JD. Mechanical response properties of nociceptors innervating feline hairy skin. J Neurophysiol 1996; 75: 1177–89.

Garnsworthy RK, Gully RL, Kenins P, Mayfield RJ, Westerman RA. Identification of the physical stimulus and the neural basis of fabric-evoked prickle. J Neurophysiol 1988; 59: 1083–97.

Gasser HS, Erlanger J. The role of fiber size in the establishment of a nerve block by pressure or cocaine. Am J Physiol 1929; 88: 581–91.

Greenspan JD, McGillis SL. Stimulus features relevant to the perception of sharpness and mechanically evoked cutaneous pain. Somatosens Mot Res 1991; 8: 137–47.

Hansen C, Hopf HC, Treede R-D. Paradoxical heat sensation in patients with multiple sclerosis. Evidence for a supraspinal integration of temperature sensation. Brain 1996; 119: 1729–36.

Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. [Review]. Pharmacol Rev 1991; 43: 143–201.

Jasmin L, Carstens E, Basbaum AI. Interneurons presynaptic to rat tail-flick motoneurons as mapped by transneuronal transport of pseudorabies virus: few have long ascending collaterals. Neuroscience 1997; 76: 859–76.

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. Afferent mechanisms mediating pain and hyperalgesias in neuralgia. In: Jänig W, Stanton-Hicks M, editors. Progress in pain research and management, Vol. 6: Reflex sympathetic dystrophy: a reappraisal. Seattle: IASP Press; 1996. p. 123–50.

Koltzenburg M, Torebjörk HE, Wahren LK. Nociceptor modulated central sensitization causes mechanical hyperalgesia in acute chemogenic and chronic neuropathic pain. Brain 1994; 117: 579–91.

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

Lawson SN, Crepps BA, Perl ER. Relationship of substance P to afferent characteristics of dorsal root ganglion neurones in guinea-pig [see comments]. J Physiol (Lond) 1997; 505: 177–91. Comment in: J Physiol (Lond) 1997; 505: 1.

Leem JW, Willis WD, Weller SC, Chung JM. Differential activation and classification of cutaneous afferents in the rat. J Neurophysiol 1993; 70: 2411–24.

Liu X-G, Sandkühler J. The effects of extrasynaptic substance P on nociceptive neurons in laminae I and II in rat lumbar spinal dorsal horn. Neuroscience 1995; 68: 1207–18.

Liu M, Max MB, Parada S, Rowan JS, Bennett GJ. The sympathetic nervous system contributes to capsaicin-evoked mechanical allodynia but not pinprick hyperalgesia in humans. J Neurosci 1996; 16: 7331–5.

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

Magerl W, Wilk SH, Treede R-D. Secondary hyperalgesia and perceptual wind-up following intradermal injection of capsaicin in humans. Pain 1998; 74: 257–68.

McMahon SB, Lewin GR, Wall PD. Central hyperexcitability triggered by noxious inputs. [Review]. Curr Opin Neurobiol 1993; 3: 602–10.

Meller ST. Thermal and mechanical hyperalgesia: a distinct role for different excitatory amino acid receptors and signal transduction pathways? [Review] APS Journal 1994; 3: 215–31.

Meyer RA, Campbell JN, Raja SN. Peripheral neural mechanisms of nociception. In: Wall PD, Melzack R, editors. Textbook of pain. 3rd ed. Edinburgh: Churchill Livingstone; 1994. p. 13–44.

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

Pertovaara A. A neuronal correlate of secondary hyperalgesia in the rat spinal dorsal horn is submodality selective and facilitated by supraspinal influence. Exp Neurol 1998; 149: 193–202.

Petsche U, Fleischer E, Lembeck F, Handwerker HO. The effect of capsaicin application to a peripheral nerve on impulse conduction in functionally identified afferent nerve fibres. Brain Res 1983; 265: 233–40.

Price DD, Hu JW, Dubner R, Gracely RH. Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain 1977; 3: 57–68.

Raja SN, Campbell JN, Meyer RA. Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin. Brain 1984; 107: 1179–88.

Raymond SA, Thalhammer JG, Popitz-Bergez F, Strichartz GR. Changes in axonal impulse conduction correlate with sensory modality in primary afferent fibers in the rat. Brain Res 1990; 526: 318–21.

Ringkamp M, Peng YB, Campbell JN, Meyer RA. Intradermal capsaicin produces a vigorous discharge in mechanically-insensitive A-fiber nociceptors of the monkey [abstract]. Soc Neuroscience Abstr 1997; 23: 1258.

Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjörk HE. Differential sensitivity of mechanosensitive and -insensitive C-fibers in human skin to tonic pressure and capsaicin injection [abstract]. Soc Neuroscience Abstr 1997; 23: 1004.

Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991; 66: 228–46.

Sinclair DC, Hinshaw JR. A comparison of the sensory dissociation produced by procaine and by limb compression. Brain 1950; 73: 480–98.

Slugg RM, Meyer RA, Campbell JN. Mechanical receptive field structure for cutaneous C- and A-fiber nociceptors in the monkey determined with graded intensity stimuli [abstract]. Soc Neuroscience Abstr 1995; 21: 1161.

Szolcsányi J, Anton F, Reeh PW, Handwerker HO. Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin. Brain Res 1988; 446: 262–8.

Thompson SW, Woolf CJ, Sivilotti LG. Small-caliber afferent inputs produce a heterosynaptic facilitation of the synaptic responses evoked by primary afferent A-fibers in the neonatal rat spinal cord in vitro. J Neurophysiol 1993; 69: 2116–28.

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, Lundberg LE, LaMotte RH. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol (Lond) 1992; 448: 765–80.

Treede R-D, Cole JD. Dissociated secondary hyperalgesia in a subject with a large-fibre sensory neuropathy. Pain 1993; 53: 169–74.

Treede R-D, Magerl W. Modern concepts of pain and hyperalgesia: beyond the polymodal C-nociceptor. News Physiol Sci 1995; 10: 216–28.

Treede R-D, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. [Review]. Prog Neurobiol 1992; 38: 397–421.

Treede R-D, Meyer RA, Campbell JN. Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response properties. J Neurophysiol 1998; 80: 1082–93.

Urban L, Perkins MN, Campbell E, Dray A. Activity of deep dorsal horn neurons in the anaesthetized rat during hyperalgesia of the hindpaw induced by ultraviolet irradiation. Neuroscience 1993; 57: 167–72.

Urban L, Thompson SWN, Dray A. Modulation of spinal excitability: co-operation between neurokinin and excitatory amino acid neurotransmitters. [Review]. Trends Neurosci 1994; 17: 432–8.

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

Woolf CJ, Mannion RJ, Neumann S. Null mutations lacking substance: elucidating pain mechanisms by genetic pharmacology [published erratum appears in Neuron 1998; 21: 453]. [Review]. Neuron 1998; 20: 1063–6.

Yarnitsky D, Ochoa JL. Sensations conducted by large and small myelinated afferent fibres are lost simultaneously under compression-ischaemia block. Acta Physiol Scand 1989; 137: 319.

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. Differential effect of compression-ischaemia block on warm sensation and heat-induced pain. Brain 1991; 114: 907–13.

Received November 18, 1998. Revised June 7, 1999.
Accepted June 14, 1999