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CENTRAL CHANGES IN PROCESSING OF MECHANORECEPTIVE INPUT IN CAPSAICIN-INDUCED SECONDARY HYPERALGESIA IN HUMANS

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SUMMARY

1. Capsaicin, the algesic substance in chilli peppers, was injected intradermally in healthy human subjects. A dose of 100 μg given in a volume of 10 μl caused intense pain lasting for a few minutes after injection and resulted in a narrow area of hyperalgesia to heat and a wide surrounding area of hyperalgesia to mechanical stimuli (stroking) lasting for 1–2 h.

2. Nerve compression experiments with selective block of impulse conduction in myelinated (A) but not in unmyelinated (C) fibres indicated that afferent signals in C fibres contributed to pain from capsaicin injection and to heat hyperalgesia, whereas conduction in afferent A fibres was necessary for the perception of mechanical hyperalgesia.

3. Electrical intraneural microstimulation normally eliciting non-painful tactile sensations was accompanied by pain when the sensation was projected to skin areas within the region of mechanical hyperalgesia induced by capsaicin injection.

4. The threshold for pain evoked by intraneural microstimulation was reversibly lowered and pain from suprathreshold stimulation was exaggerated during the period of mechanical hyperalgesia, regardless of lidocaine anaesthesia of the cutaneous innervation territory of the stimulated fibres.

5. The results indicate that hyperalgesia to stroking on a skin area surrounding a painful intradermal injection of capsaicin is due to reversible changes in the central processing of mechanoreceptive input from myelinated fibres which normally evoke non-painful tactile sensations.

INTRODUCTION

Hyperalgesia was defined by Head (1893) as 'increased sensitiveness to pain' and later was taken to mean lowered threshold for pain and increase in pain evoked by suprathreshold stimuli. Distinctions have been made between primary hyperalgesia,

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occurring within an area of tissue injury, and secondary hyperalgesia, occurring outside the area of injury (Lewis, 1936, 1942). While there is a general agreement that primary hyperalgesia is, at least in part, due to peripheral sensitization of nociceptive nerve endings (for review see Raja, Meyer & Campbell, 1988), there is long-standing controversy regarding the neural mechanisms underlying secondary hyperalgesia. Lewis (1936, 1942) and Jung (1941) largely explained secondary hyperalgesia through peripheral mechanisms involving axon reflex release of pain-enhancing substances which could spread around injured tissue and render remote nociceptors hyperexcitable. By contrast, Hardy, Wolff & Goodell (1950) presented indirect evidence to support their contention that secondary hyperalgesia involved changes in signal processing in the central nervous system.

In order to resolve this controversy, a series of detailed psychophysical experiments in humans (Simone, Baumann & LaMotte, 1989; LaMotte, Shain, Simone & Tsai, 1991) were paralleled by neurophysiological studies in monkeys (Baumann, Simone, Shain & LaMotte, 1991; Simone, Oh, Sorkin, Owens, Chung, LaMotte & Willis, 1991) and in humans (this study and LaMotte, Lundberg & Torebjörk, 1991). In each experiment, the hyperalgesia was produced by an intradermal injection of capsaicin, the algesic substance in red pepper. Here we provide novel evidence, based on data from experiments with intraneural stimulation in normal human subjects, that secondary hyperalgesia to mechanical stimulation in an area surrounding a severely painful intradermal injection of capsaicin is due to dynamic changes in the central processing of mechanoreceptive input in myelinated fibres which normally evoke non-painful tactile sensations.

METHODS

Subjects

A total of twenty-nine experiments with intraneural microstimulation (INMS) were performed in six healthy human subjects, five males and one female, aged 24–34 years. Two experiments were conducted on the superficial branch of the peroneal nerve 5–7 cm proximal to the ankle, and twenty-seven on the peroneal nerve dorsolateral to the fibular head at knee level. Of the latter, four experiments were rejected because of unintentional movement of the stimulating microelectrode during the course of the experiment. Thus, the total number of reported experiments with INMS is twenty-five. In addition, three experiments involving compression of the superficial radial nerve at wrist level were performed on three healthy male subjects, aged 27–49 years.

The experimental protocols were approved by the University Ethical Committee, and informed consent was obtained from each subject according to the Declaration of Helsinki.

Procedure

The subject reclined on a chair with the leg comfortably supported. A microelectrode was inserted manually through the skin into the peroneal nerve trunk, which was localized by palpation or surface electrical stimulation. A reference electrode was inserted into subcutaneous tissue 1–2 cm outside the nerve. The position of the microelectrode tip within a cutaneous fascicle was ascertained by evoking sensations projected to the skin of the foot or lower leg while stimulating electrically through the electrode.

Electrodes and equipment

Lacquer-insulated tungsten electrodes, 200 μm in diameter, of the type designed for human microneurography (Vallbo & Hagbarth, 1968), were connected to a Grass S48 stimulator with stimulus isolation unit and were used for intraneural stimulation.

Intraneural microstimulation (INMS)

Square-wave pulses of 0·25 ms duration and amplitudes less than 0·30 V were delivered at a frequency of 3 Hz while gently adjusting the intrafascicular position of the microelectrode and attending to evoked sensations reported by the subject. Having reached an intrafascicular site where INMS evoked a weak, monofocally projected tactile sensation, detection thresholds were established for 3 s trains at 5, 20 and 50 Hz. The cutaneous area to which the tactile sensation was projected (projected field) was drawn with pen on the skin of the foot or leg. Stimulus intensity was then increased to suprathreshold levels, and projected fields and stimulation thresholds were noted for additionally recruited sensations, including pain. Thus, in eleven experiments at least one tactile threshold and one pain threshold were established, and they were repeatedly checked at intervals of 2–10 min throughout the experiments (examples shown in Figs 3–5).

In fourteen other experiments, stimulus trains of *constant* intensity at an arbitrary level above tactile threshold and well below initial pain threshold were given throughout the experimental session. The stimulus trains were either constant at 50 Hz for 3 s (example shown in Fig. 6) or the frequency and duration of trains varied from 5 Hz for 3 s to 50 Hz for 5 s (example shown in Fig. 7), or, exceptionally, the 50 Hz trains were prolonged to 20 s.

Capsaicin injection

After establishing that the stimulating electrode was in constant intraneural position, as evidenced by consistent thresholds and projections of evoked sensations on repeated control trials, capsaicin was injected intradermally 7–20 mm outside the peripheral border of the projected field of a tactile sensation, evoked by INMS. The capsaicin (Fluka) was dissolved in Tween-80 and physiological saline, according to the description of Simone *et al.* (1989). The dose of capsaicin was 100 μl given in a 10 μl volume via a 0·5 ml tuberculin syringe with a 28 gauge needle (Beckton-Dickinson, Lo-dose).

Determination of area of mechanical hyperalgesia

When the subject reported that the pain from capsaicin injections had ceased, usually after 10–15 min, the presence of mechanical hyperalgesia was assessed by asking the subject to indicate whether gentle stroking of the skin with a cotton swab evoked pain or tenderness. The skin stroking started at least 5 cm away from the injection site and was repeated tangentially to the injection bleb at a progressively closer radius until the subject reported pain or tenderness. That site was marked on the skin with a felt-tip pen, and new series of skin stroking started from the periphery at a different angle, until after at least eight determinations the borders of secondary hyperalgesia were outlined on the skin. Such border determinations were repeated several times during the experiments to study the spatial changes of secondary hyperalgesia with time.

Analogue pain ratings

The subjects made continuous ratings of the magnitude of pain sensation evoked by INMS. The magnitude was indicated by moving a DC potentiometer lever along a 20 cm slot without marks, except that one end of the slot was to indicate no pain, and the other end maximal imaginable pain. Thus, the device was similar to a visual analogue scale, except that not only magnitude of pain, but also onset, offset and profile of pain could be recorded. Each subject was told to rate only the sensory magnitude of pain and not his reaction to pain, such as how unpleasant it was. If there was no pain, e.g. only tactile sensations, the subject was to indicate the absence of pain by keeping the lever at the bottom. Each subject was to rate pain continuously, indicating when the pain began and the time course of increases and decreases. The subjects had no clues as to exactly when intraneural stimuli were given, or what stimulus parameters were used during the INMS sessions.

The pain ratings obtained in this way were recorded on tape, together with corresponding stimulus patterns, for subsequent analysis.

Nerve compression block

The subject sat comfortably in a chair with the left arm supported in a horizontal position and was gripping a vertical handle to help stabilize the arm during the nerve compression. A 2·5 cm wide band was placed on the wrist over the superficial branch of the radial nerve. Two 2·1 kg

weights attached to the ends of the band hung down freely on both sides of the wrist. This arrangement produced steady compression of the nerve, without causing ischaemia. The mean duration of nerve compression was 45 min.

Data analysis

The margins of projected sensory fields and areas of secondary hyperalgesia were traced from the skin onto clear acetate and then retraced onto a digitizer and fed into an Apple Europlus Computer for calculation of areas. Statistical evaluation of results was performed using analysis of variance (ANOVA) with factorial analyses between groups. Fisher's method was used for comparisons, at the overall significance level of 95%. Data are presented as means $\pm$S.D.

RESULTS

Sensory events following capsaicin injection

Intradermal injection of 100 μl capsaicin was severely painful, usually described as intensely burning. After injection, spontaneous pain gradually declined to a low level within the first 5 min and usually disappeared completely within 15 min. At the centre of the little bleb produced by the injected fluid (about 4 mm diameter) there was insensitivity to heat, and mechanical stimuli were only felt (as pain) when the stimulus caused dislocation of the borders of the bleb. By contrast, hyperalgesia to warm (metal probe with a temperature of 40 °C) and mechanical stimuli (gently stroking with a cotton-tipped applicator) was evident in a narrow, roughly concentric zone typically having a radius of less than 1 cm surrounding the injection site. In a fairly broad area around the injection site, gentle stroking of the skin was felt as unpleasant soreness or overt burning pain but there was no hyperalgesia to warming in this region. The hyperalgesia to stroking generally peaked at 10–15 min after injection and then gradually decreased over the next hour, to disappear completely within 1–2 h after capsaicin injection. Its maximal area was smaller on the toes and forefoot, $14{\cdot}1 \pm 9{\cdot}2$ cm^2 ($n = 12$), than on the dorsum of the foot, $35{\cdot}5 \pm 11{\cdot}1$ cm^2 ($n = 10$), or on the ankle or calf, $56{\cdot}1 \pm 34{\cdot}8$ cm^2 ($n = 7$). These differences were statistically significant ($P < 0{\cdot}05$, Fisher's protected least significant difference).

What fibre types contribute to the various sensory events following capsaicin injection?

It has been demonstrated in direct microneurographic recordings from the superficial radial nerve in humans that a firm compression of the nerve for up to 45 min causes a progressive and selective block of impulse conduction in large and thin myelinated (A) fibres, whereas conduction in umyelinated (C) fibres remains virtually intact (Torebjörk & Hallin, 1973; Mackenzie, Burke, Skuse & Lethlean, 1975). At a time when impulse conduction is blocked in all A fibres, subjects can no longer perceive tactile or cold stimuli applied to the cutaneous territory of the compressed nerve, but they can still feel warmth and delayed burning pain as evidence of intact conduction in C fibres (Hallin & Torebjörk, 1976). We have used this model to elucidate the contribution of A and C fibres to the various sensory events after capsaicin injection. Compression of the left superficial radial nerve was performed at wrist level in three subjects. When they reported numbness to tactile and cold stimuli in the radial nerve territory on the dorsum of the hand, capsaicin was injected intradermally over the first interosseus muscle on the dorsum of both hands (Fig. 1). The injection was felt equally painful in the numb hand and in the intact control hand. Furthermore, hyperalgesia to heat close to the injection site developed on both sides, whereas hyperalgesia to tactile stimuli developed only on

the control side, and was totally absent on the blocked side. On release of nerve compression, hyperalgesia to stroking became apparent in the left hand within a few minutes as tactile sensibility recovered.

The results indicate that afferent signals in C fibres contribute to pain from capsaicin injection and to heat hyperalgesia, whereas conduction in afferent A fibres is necessary for the perception of mechanical hyperalgesia.

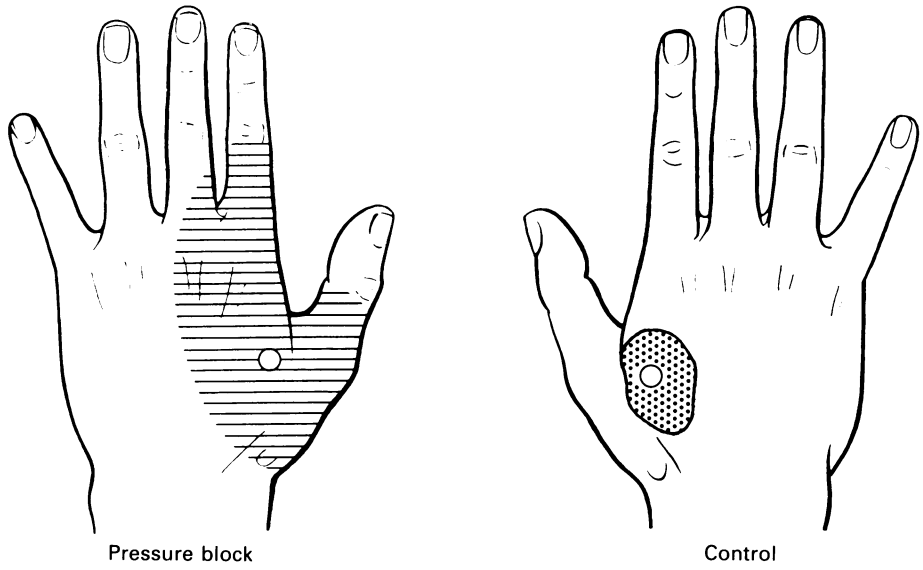

Fig. 1. Effect of A fibre block on secondary hyperalgesia. Left, 45 min of firm compression of the superficial radial nerve led to numbness to tactile and cold stimuli (horizontal lines) while warmth and heat pain were perceived normally, indicating block of impulse conduction in A fibres but not in C fibres. At this stage, capsaicin injection (open circle) was perceived as equally painful in the blocked (left) and control (right) hands, and primary hyperalgesia in response to heat close to the injection site developed in both hands, indicating that these sensory events were related to C fibre input. Secondary hyperalgesia to mechanical stimuli appeared in the control hand (dotted area, right) but was absent on the blocked side, until the A fibre block was released. Thus, a painful C fibre input is important for inducing the central changes that lead to secondary hyperalgesia, but additional input in A fibres is necessary for these central changes to manifest themselves as secondary hyperalgesia.

What type of A fibres conduct the peripheral signals for mechanical hyperalgesia?

In order to shed some light on this question, experiments with intraneural electrical stimulation were performed in cutaneous fascicles of the peroneal nerve. The stimulating electrode was manoeuvred into intrafascicular sites in which 3 s trains at 50 Hz evoked purely tactile (non-painful) sensations projected to fairly small areas of skin on the dorsum of the foot or on the lateral calf. Having reached this site, the electrode was left in permanent position, and capsaicin was injected 7–20 mm outside the border of the projection area of the tactile sensation. The area of mechanical hyperalgesia was mapped with cotton swabs every 5–10 min and intraneural stimulation at constant intensity was performed intermittently between sessions of mechanical testing. A typical experiment is illustrated in Fig. 2. In this

case, tactile sensation from intraneural stimulation was projected to a 7×20 mm^2 skin area on the dorsum of the foot (Fig. 2*A*), and capsaicin was injected 10 mm distally. Fourteen minutes after injection, mechanical hyperalgesia covered a 4×5 cm^2 skin area which overlapped the projection of tactile sensation from

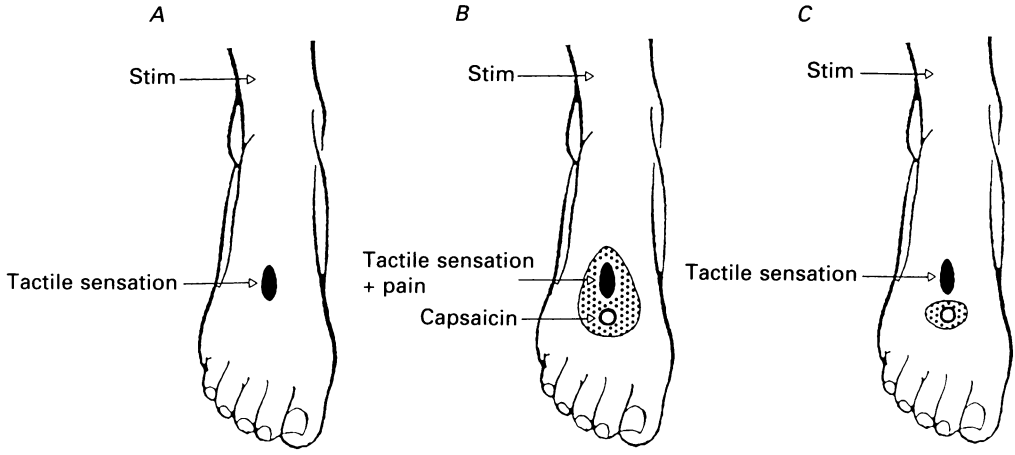

Fig. 2. Reversible change in conscious perception of a constant afferent input associated in time and space with development of secondary hyperalgesia. *A*, intraneural electrical stimulation at fixed intensity in the superficial peroneal nerve evoked a purely tactile (non-painful) sensation projected to a small skin area on the dorsum of the foot (filled area). *B*, after injection of capsaicin (open circle) and development of secondary hyperalgesia (dotted area) overlapping the sensory projection field, the intraneural stimulation was perceived as a tactile sensation accompanied by pain. *C*, 39 min after capsaicin injection, when the area of secondary hyperalgesia had retracted from the sensory projection field, the intraneural stimulation was again perceived as purely tactile, without any pain component, as in the control situation.

intraneural stimulation (Fig. 2*B*). Interestingly, the tactile sensation from INMS was now accompanied by an additional sore, painful sensation projected to the same skin area. Such pain was regularly reported after each stimulus train until, at 39 min, the area of mechanical hyperalgesia had decreased and no longer covered the projection of sensations from INMS (Fig. 2*C*). At this time, only tactile sensation and no pain was reported in response to intraneural stimulation, just as at the beginning of the experiment.

This striking correlation between reports of pain from otherwise non-painful intraneural stimulation and hyperalgesia on mechanical stimulation after capsaicin injection, when the area of mechanical hyperalgesia overlapped the projected fields of evoked sensations from INMS, was observed in eleven experiments out of fourteen. The quality of pain associated with INMS was usually reported as a burning soreness (seven experiments), and sometimes as stinging (two experiments) or unspecified (two experiments). The pain was projected to the same area as the tactile sensation in seven experiments, to the injection site in two experiments, to the flare area in one experiment, and to the periphery of the hyperalgesic area in one experiment. Typically, the painful component of the evoked sensation appeared with a delay of 0·5 to several seconds after the tactile component.

Since tactile sensations are normally believed to derive from stimulation of large myelinated fibres (Torebjörk & Ochoa, 1980; Ochoa & Torebjörk, 1983), and since the electrode position and stimulus parameters were kept constant throughout the experiments, the reported addition of pain from INMS would suggest a dynamic

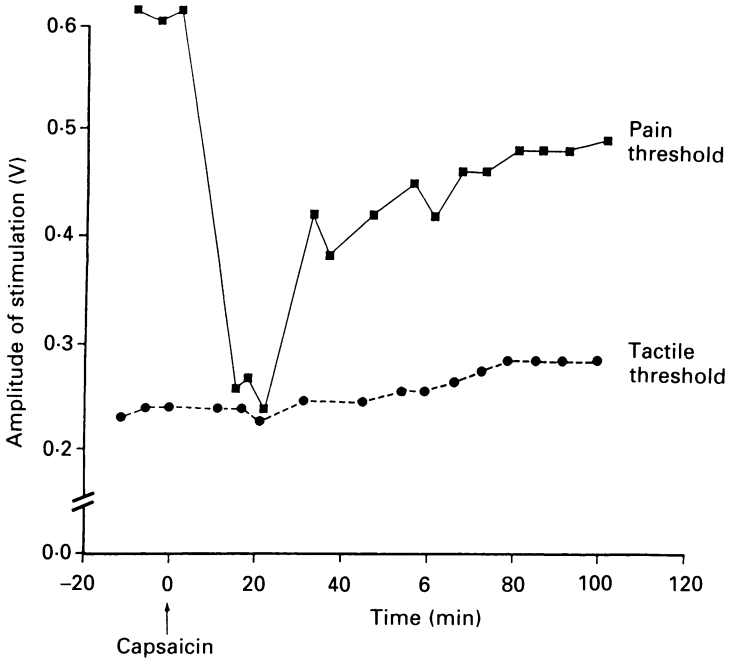

Fig. 3. Drop in pain threshold, as tested by electrical intraneural stimulation, after intradermal injection of capsaicin. After establishing reproducible threshold values for evoking tactile and pain sensations (left), capsaicin was injected (at time 0) about 2 cm outside the overlapping projected fields of touch and pain. As secondary hyperalgesia spread around the injection site and invaded the projected sensory fields, the threshold for evoking pain dropped dramatically, almost to tactile threshold (at 16–22 min after injection), and then gradually increased again without reaching control level at 100 min, when the experiment was terminated. At that time, there was still mild secondary hyperalgesia to touch in the projected fields. Notice that the tactile threshold was fairly consistent throughout the experiment, indicating that no major shifts in electrode position had occurred.

change in the central processing of afferent impulses in large mechanoreceptive fibres concomitant with the development of mechanical hyperalgesia.

Dynamic changes in pain threshold during mechanical hyperalgesia. Effect of spatial summation

In most experiments, testing of tactile and pain thresholds in response to intraneural electrical stimulation was avoided during the first 5–10 min after capsaicin injection, since the subjects had some spontaneous pain remaining which might have interfered with the sensations from INMS and hence made the threshold assessments difficult. Figure 3 shows how the pain threshold could drop dramatically to almost tactile threshold level, when tested about 15–20 min after capsaicin

injection, and how the pain threshold then slowly increased again without completely reaching control level at 100 min after injection, when the experiment was terminated. At 45 min a test stimulus was given at an intensity which was barely painful before capsaicin injection. This evoked considerable pain, making the subject

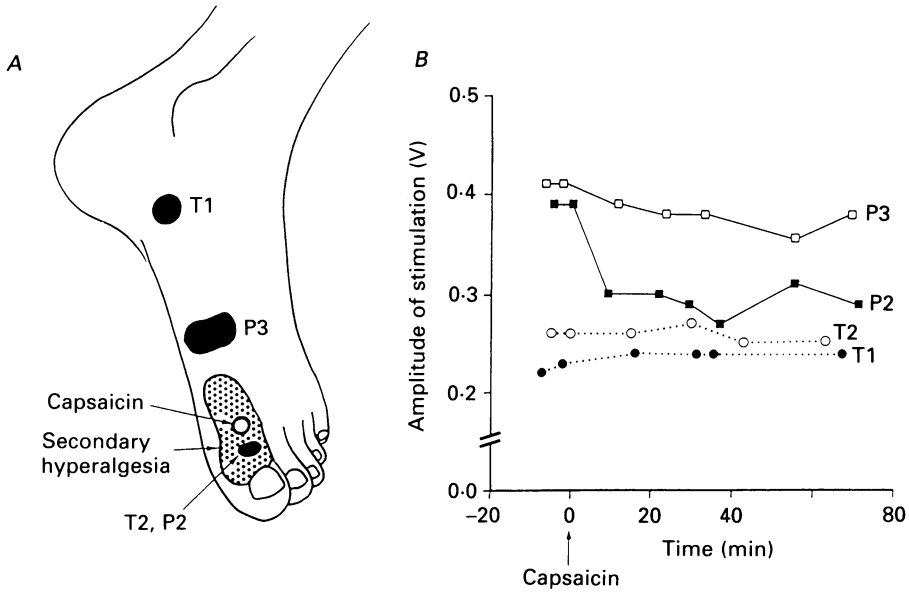

Fig. 4. Somatotopic organization of central changes in the pain threshold associated with secondary hyperalgesia. *A*, filled areas indicate projected fields of successively recruited sensations at increasing amplitudes of intraneural stimulation. T1 represents the area of a tactile sensation recruited at the lowest threshold; T2 and P2 are overlapping projections of tactile and pain sensations with higher thresholds, and P3 is a pain projection recruited at the highest stimulus intensity. Capsaicin injection is indicated by an open circle, and the dotted area marks the extension of secondary hyperalgesia. *B*, electrical thresholds for evoking tactile sensations T1 and T2 and pain sensation P3 were fairly constant after capsaicin injection and comparable to the pre-injection control, whereas the threshold for evoking pain sensation P2 projected to the area of secondary hyperalgesia dropped markedly.

cry out. This effect could hardly be due to a shift in the electrode position, since the tactile threshold was fairly constant.

Reversible lowering of the pain threshold, in response to intraneural electrical stimulation (with little or no effect on the tactile threshold during the period of mechanical hyperalgesia), was observed in seven experiments of eleven. Results of this kind support the notion that mechanical hyperalgesia is associated with a dynamic and long-lasting change in the central processing of afferent input from the skin. The fact that the pain threshold could drop almost to the level of the tactile threshold is consonant with the idea that large calibre myelinated fibres convey the signals associated with hyperalgesia.

Figure 4 illustrates topographical features of the central dynamic changes, which seemed to be restricted to cells receiving input from certain skin regions close to the capsaicin injection. As seen in Fig. 4*A*, INMS at increasing stimulus strength

recruited several sensations projected to separate skin areas on the foot. At threshold intensity for conscious detection (0·23 V) a tactile sensation was projected distal to the medial malleolus (T1). On increasing the stimulus intensity to 0·25 V another tactile sensation was recruited, projected to the dorsum of the big toe (T2). Further increase in stimulus intensity to 0·39 V evoked pain in this area (P2) and, finally, at 0·41 V another sensation of pain was projected to the medial plantar border of the foot (P3). After establishing that these projections and thresholds were consistent on repeated trials, capsaicin was injected 12 mm proximal to the tactile projection field (T2) at the base of the big toe. When the intense pain from the injection had subsided, and mechanical hyperalgesia overlapped the T2 projection field on the big toe, thresholds for tactile sensations and pain were repeatedly tested for the different projection territories (Fig. 4*B*). It was found that the thresholds for tactile sensations projected to areas T1 and T2 were virtually unchanged throughout the experiment, as was the threshold for pain projected to area P3. This is strong evidence that the electrode position was unchanged. Notably, the threshold for evoking pain projected to the hyperalgesic area P2 dropped from 0·39 to 0·30 *V* and remained at this lower level for the rest of the experiment (70 min), during which time hyperalgesia to tactile stimuli remained in this region. Furthermore, the pain projected to the big toe on intraneural stimulation had the same sore quality as the pain evoked by gently stroking the skin.

These results show that the central changes are topographically restricted to cells receiving input from a localized peripheral skin area surrounding the initial painful lesion.

Local anaesthetic block in the cutaneous projection field

Even though the weight of the presented evidence speaks in favour of a central change in the processing of afferent signals as a cause for mechanical hyperalgesia, it is still conceivable that peripheral mechanisms might be involved, perhaps by antidromic intraneural stimulation of afferent fibres which might activate sensitized nociceptive fibres by some kind of peripheral coupling (Meyer, Raja & Campbell, 1985), thereby contributing to lowering of the pain threshold and hyperalgesia. To test this possibility, three experiments were performed in which 1% lidocaine was infiltrated intradermally in the area to which tactile and painful sensations from INMS were projected, once mechanical hyperalgesia and lowering of the pain threshold had been established. In each case, the area of anaesthesia did not include the capsaicin injection site. As shown in Fig. 5, the lidocaine block, which should interrupt any coupling between fibres in the periphery, did not influence the pain threshold, as tested with INMS, which was lowered during the typical time period (90 min) for secondary hyperalgesia, regardless of the lidocaine block which made part of the hyperalgesic skin anaesthetic.

Dynamic changes in latency and magnitude of pain sensations during mechanical hyperalgesia

It was noted in several experiments that the reaction time for pain responses evoked by intraneural stimulation was fairly short a few minutes after capsaicin injection and that the latency progressively increased thereafter. An illustrative example is

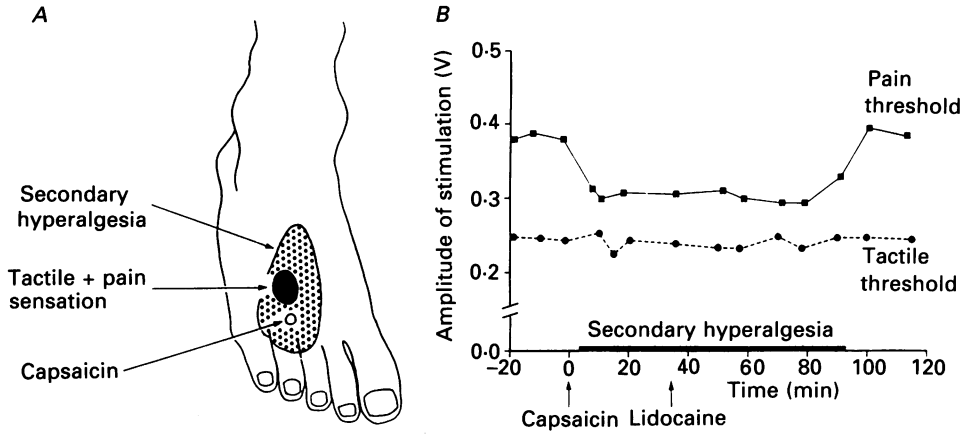

Fig. 5. No effect on pain threshold during lidocaine block in the sensory projected fields. *A*, after establishing control thresholds for tactile and pain sensations evoked by INMS, capsaicin was injected 2 cm distal to the overlapping projected fields for touch and pain (time 0). *B*, a reversible drop in pain threshold was observed for 90 min after the injection, concomitant with the period of secondary hyperalgesia (thick bar on the horizontal time scale). This lowering of the pain threshold was not influenced by anaesthetizing the skin at the sensory projected fields with 1% lidocaine (35 min after injection).

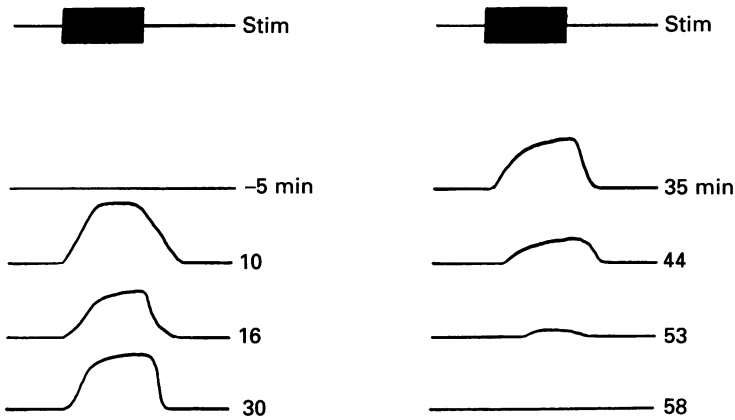

Fig. 6. Progressive increase in latency and decrease in magnitude of pain ratings obtained during the period of secondary hyperalgesia. Top traces indicate the onset and duration of intraneural stimulus trains at 50 Hz for 3 s delivered at constant amplitude. Traces below show pain ratings obtained at different times (minutes) relative to injection of capsaicin. Five minutes before injection (−5 min) such a train evoked no pain. After injection, pain was signalled at a latency of 300 ms at 10 min, its latency gradually increased to 1·5 s at 53 min, and the magnitude of pain gradually decreased to zero at 58 min.

shown in Fig. 6. In this experiment, 50 Hz trains at 0·25 V for 3 s evoked tactile but no pain responses before capsaicin injection. The latency for evoking the tactile responses was as short as 300 ms on stimulation in the peroneal nerve at knee level. Ten minutes after capsaicin injection, a pain response with a similar short latency

was evoked by the same stimulus train. With time, the latencies of the pain responses became progressively longer until, at 53 min after injection, the latency was 1·5 s. In addition, the amplitude of the pain rating had decreased to less than one-tenth, and the duration to less than half of the 10 min rating. Fifty-eight minutes after the

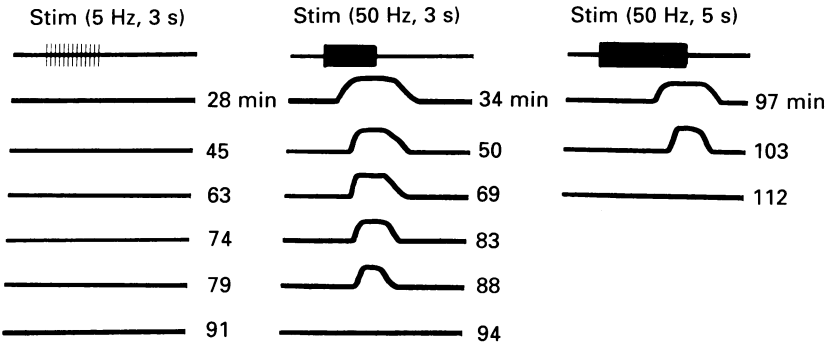

Fig. 7. Effect of temporal summation on pain responses. Upper traces in the three columns indicate onset and duration of stimulus trains given at constant amplitude at 5 Hz for 3 s (left column), 50 Hz for 3 s (middle column) and 50 Hz for 5 s (right column). Pain ratings obtained for each type of stimulus train at different times (minutes) after capsaicin injection are shown below. Left, no pain was reported in response to low-frequency stimulation. Middle, pain was reported in response to 50 Hz stimulation. Note progressive increase in latency of pain rating from 1 s at 34 min to 2 s at 88 min after injection. At 94 min, this stimulus evoked no pain. Right, increase in 50 Hz train duration from 3 to 5 s temporarily revived pain responses, which appeared with a latency as long as 4 s at 103 min after injection. At 112 min even this stimulus was non-painful.

injection there was no pain evoked by the stimulus trains, only tactile sensation as had occurred in the control situation.

Effects of temporal summation on pain responses during mechanical hyperalgesia

A typical finding in all experiments was that INMS with 5 Hz trains for 3 s at a stimulus intensity above the tactile threshold did not cause pain, whereas 20 Hz and particularly 50 Hz trains of the same intensity and duration often (eleven experiments of fourteen) did cause pain when mechanical hyperalgesia was present. An example of this phenomenon is illustrated in Fig. 7. Trains of 5 Hz did not evoke any pain sensations in several tests performed at 28–91 min after capsaicin injection (Fig. 7, left panel). In contrast, 50 Hz stimuli of the same amplitude and duration evoked pain responses 34–88 min after injection (Fig. 7, middle panel). However, no pain was reported at 94 min. After increasing the train duration from 3 to 5 s, pain responses were again evoked at 97 and 103 min, but not at 112 min (Fig. 7, right panel). Note the progressive increase in latency of the pain responses, from 1 s at 34 min (middle panel) to 4 s at 103 min (right panel). Note also that the pain ratings often outlasted the duration of the stimulus trains by 1–2 s, and that this 'overshoot' became progressively less pronounced with time after capsaicin injection. In two experiments, prolonged (20 s) 50 Hz trains were tried at a late stage after capsaicin injection when 5 s trains no longer evoked pain. Notably, pain could be evoked at the ends of these trains, with latencies as long as 18–19 s.

These results clearly demonstrate that temporal summation of afferent input is necessary for pain to occur, and that there are dynamic changes in the efficacy of a constant temporal input during the time course of secondary hyperalgesia.

Analysis of negative findings

Table 1 summarizes the results obtained in six subjects, arranged in sequential order of experimental sessions for each subject. Plus signs indicate that lowering of

TABLE 1. Summary of INMS results in six subjects, identified by initials to the left, and arranged in sequential order of experimental sessions as indicated on top. Lowering of pain threshold and/or increased pain sensitivity to INMS (+) occurred in eighteen experiments, whereas no such changes (−) could be demonstrated in seven experiments

	1	2	3	4	5	6	7	8	9	10
T.N.	+	+	−	−	−	−	+	+	+	−
M.B.	+	+	+	+	+	+	+			
C.P.	+	−	−							
J.L.	+	+								
C.L.	+	+								
B.K.	+									

the pain threshold and/or increased pain sensitivity to INMS were demonstrated during the period of mechanical hyperalgesia after capsaicin injection relative to the control, while minus signs indicate that no change in the pain responses to INMS was observed in spite of evidence of mechanical hyperalgesia.

It is seen that pain from INMS was enhanced in each subject in their first experimental session, and for four out of five subjects tested in their second session. However, subject C.P. failed to demonstrate enhanced pain in his second and third sessions, and so did subject T.N. in his third session. To test whether repeated tests would influence the results in any systematic way, several experiments were performed in two subjects. Subject M.B. always exhibited enhanced pain sensitivity after capsaicin injection in seven serial experiments, whereas subject T.N. showed evidence of pain enhancement in five experiments and no enhancement in another five, without serial trend.

From these observations, we conclude that positive and negative findings in this study were not due to individually linked constitutional factors and were not due to training or other effects related to repetition of experiments.

We also analysed whether the distances or locations of capsaicin injections differed relative to the projections of tactile sensation from INMS in experiments which revealed positive *versus* negative results. It was found that positive or negative results could occur regardless of whether capsaicin injections were performed proximally, distally, medially or laterally to the projected field of tactile sensation evoked by INMS. Furthermore, the mean distance $\pm$ S.D. between the nearest border of the injection bleb and the projected tactile field was $9{\cdot}1 \pm 4{\cdot}9$ mm for experiments yielding positive results and $8{\cdot}3 \pm 3{\cdot}7$ mm for those yielding negative results. These differences were not significant.

Could it be that the initial pain from capsaicin injection differed between the two groups? An analysis of pain rating during injection revealed high ratings, between 50

and 80% of the maximum tolerable, in all subjects. There was no trend towards low ratings in those experiments which yielded negative results; if anything, the initial pain ratings were slightly higher than in those yielding positive findings.

DISCUSSION

The technique of intraneural microstimulation in intact nerves in awake human subjects lends itself to the study of central mechanisms of evoked sensations, since peripheral and perhaps sensitized receptors are bypassed by stimuli delivered at mid-axon level. However, such stimulation elicits impulses which propagate both orthodromically and antidromically, and it is conceivable that antidromic impulses might activate sensitized nociceptive nerve endings by some kind of peripheral coupling (Meyer *et al.* 1985), thereby contributing to the lowering of the pain threshold and hyperalgesia. This possibility was ruled out by anaesthetizing the cutaneous innervation territory of the stimulated fibres (i.e. the region to which sensation evoked by INMS was projected), which should interrupt any coupling in the periphery. The fact that pain thresholds tested by INMS remained lowered regardless of such peripheral blocks strongly supports the notion that the sensory changes reported here are due to central rather than peripheral mechanisms. It should be made clear, in this context, that the lidocaine blocks were restricted to just one part of the entire area of mechanical hyperalgesia and did not involve the capsaicin injection site. Thus, to the extent that central lowering of the pain threshold is contingent on some low level of afferent input from the injured area, such input was not completely interrupted by the limited anaesthetic block.

The outcome of the nerve compression block experiments indicates that capsaicin injection activates afferent C fibres which evoke severe pain and create reversible changes in the central processing of afferent inputs. One such change, mechanical hyperalgesia to stroking, is demonstrable only in the presence of intact conduction in myelinated fibres (cf. LaMotte *et al.* 1991). Several lines of evidence from the INMS experiments suggest that some of these central changes involve altered processing of input from low-threshold mechanoreceptor units with large diameter nerve fibres. Thus, tactile sensations evoked by INMS, and normally attributed to stimulation of low-threshold mechanoreceptive afferents (Torebjörk & Ochoa, 1980; Vallbo, 1981; Ochoa & Torebjörk, 1983; Schady & Torebjörk, 1983; Vallbo, Olsson, Westberg & Clark, 1984; Torebjörk, Vallbo & Ochoa, 1987) became accompanied by pain when the area of hyperalgesia to stroking overlapped the area of projected sensation evoked by INMS. Since the stimulation parameters were held constant and the projected field of the tactile component of the evoked sensation remained unchanged, we have no reason to believe that the appearance of a painful component additional to the tactile sensation would be due to spatial recruitment of nociceptive fibres, or to any change in position of the stimulating electrode. Instead, the appearance and disappearance of this painful component of the evoked sensation, which coincided spatially and temporally with the appearance and disappearance of mechanical hyperalgesia, is thought to be due to reversible changes in the central processing of an unchanged input from low-threshold mechanoreceptive afferents. Further support for the notion that fast-conducting mechanoreceptive fibres are implicated is

supplied by the finding that the pain threshold could drop almost to the level of the tactile threshold in some INMS experiments (Fig. 3), and that the minimum reaction time for the pain component of the evoked sensation could be as short as for the tactile component. Finally, in some experiments the painful component could be evoked towards the end of a 20 s long train at 50 Hz. This is probably incompatible with sustained C fibre stimulation, since it has been shown by directly monitoring the neural responses to constant supramaximal intraneural stimulation that C fibres fail to respond within 30 s even when the train frequency is kept as low as 10 Hz (Torebjörk, Schady & Ochoa, 1984).

It appears that this change in the central processing of mechanoreceptive input is somatotopically organized. Thus, the pain threshold to INMS was lowered for projection within the area of mechanical hyperalgesia but apparently not for projection outside that area (Fig. 4). It is interesting to note, in this context, that the maximal area of hyperalgesia to stroking was significantly smaller distally on the foot as compared to more proximal regions of the lower leg. It is hypothesized that this difference may be related to the central terminations in the spinal cord of those afferent C fibres that cause the central changes, being more restricted for distal than for proximal innervation territories of the limb.

A striking feature of the presented results is the marked influence of temporal summation of the afferent input on the pain component evoked by INMS during the period of mechanical hyperalgesia. In experiments in which the spatial content of the afferent input was kept constant and only the temporal pattern was varied, it was found that low-frequency trains (5 Hz) typically did not evoke pain, whereas higher frequencies of 20 or 50 Hz generally did. As the pain threshold progressively increased towards the end of the period of hyperalgesia, the latency for evoking the pain response typically increased, in some instances by as much as 19 s. Such long reaction times for pain are not compatible with normal conduction in primary afferent nociceptive fibres of any type. Instead, the findings indicate that pain progressively builds up as a consequence of repetitive release of neuromodulatory substances which have fairly long durations of action on central nociceptive neurons.

Not only temporal but also spatial summation of afferent input was important for eliciting pain during the period of mechanical hyperalgesia. This was noted in experiments in which the number of activated fibres was varied, but the temporal pattern was kept constant. The stimulus intensity needed to reach the pain threshold was typically higher towards the end of the period of hyperalgesia, indicating that spatial summation of afferent input in more fibres was required.

The need for sufficient temporal and spatial summation of afferent input to detect central changes in the pain threshold may explain why such changes were not found in every experiment. If the intraneural stimulus intensity was too low to allow enough spatial summation or the frequency and duration of stimulus trains were inadequate for enough temporal summation, the central changes in the pain threshold would remain undetected. Furthermore, errors in the ability of the subjects to precisely localize sensations projected to the skin of the lower leg and foot occur. For tactile stimuli, such errors are in the order of 1 cm, and for C fibre pain the errors are in the order of 2 cm (Jørum, Lundberg & Torebjörk, 1989). It is conceivable that some subjects mislocated the tactile sensations evoked by INMS and that the

capsaicin injection was made too far from the projected field for mechanical hyperalgesia to overlap that region. This may perhaps have contributed to some of the negative results.

In conclusion, our results indicate that secondary hyperalgesia felt in response to stroking the skin area surrounding a severely painful intradermal injection of capsaicin is due to reversible changes in the central processing of mechanoreceptive input from myelinated fibres which normally evoke non-painful tactile sensations. If such central changes can occur after temporary experimental injury in normal human subjects, such changes are also likely to be of pathophysiological importance in patients with chronic pain. Indeed, there are clinical observations obtained from selective block of impulse conduction in myelinate nerve fibres (Wallin, Torebjörk & Hallin, 1976; Torebjörk & Hallin, 1979; Campbell, Raja, Meyer & Mackinnon, 1988), reaction time measurements (Lindblom & Verillo, 1979) and nerve stimulation experiments (Price, Bennett & Rafi, 1989; Torebjörk, 1990) which suggest that hyperalgesia to tactile stimuli in some patients with pain after peripheral nerve injury is associated, in part, with activation of fast-conducting afferents.

REFERENCES

BAUMANN, T. K., SIMONE, D. A., SHAIN, C. & LAMOTTE, R. H. (1991). Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibres that contribute to capsaicin-induced pain and hyperalgesia. *Journal of Neurophysiology* **66**, 212–227.

CAMPBELL, J. N., RAJA, S. N., MEYER, R. A. & MACKINNON, S. E. (1988). Myelinated afferents signal the hyperalgesia associated with nerve injury. *Pain* **32**, 89–94.

HALLIN, R. G. & TOREBJÖRK, H. E. (1976). Studies on cutaneous A and C fibre afferents, skin nerve blocks and perception. In *Sensory Functions of the Skin in Primates, with Special Reference to Man. Wenner–Gren International Symposium*, vol. 27, ed. ZOTTERMAN, Y., pp. 137–149. Pergamon Press, Oxford.

HARDY, J. D., WOLFF, H. G. & GOODELL, H. (1950). Experimental evidence on the nature of cutaneous hyperalgesia. *Journal of Clinical Investigation* **29**, 115–140.

HEAD, H. (1893). On disturbances of sensation with special reference to the pain of visceral disease. *Brain* **16**, 1–132.

JØRUM, E., LUNDBERG, L. E. R. & TOREBJÖRK, H. E. (1989). Peripheral projections of nociceptive unmyelinated axons in the human peroneal nerve. *Journal of Physiology* **416**, 291–301.

JUNG, R. (1941). Die allgemeine Symptomatologie der Nervenverletzungen und ihre physiologischen Grundlagen. *Nervenarzt* **14**, 493–516.

LAMOTTE, R. H., LUNDBERG, L. E. R. & TOREBJÖRK, H. E. (1991). Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. *Journal of Physiology* **448**, 749–764.

LAMOTTE, R. H., SHAIN, D., SIMONE, D. A. & TSAI, E.-F. (1991). Neurogenic hyperalgesia: Psychophysical studies of underlying mechanisms. *Journal of Neurophysiology* **66**, 190–211.

LEWIS, T. (1936). Experiments relating to cutaneous hyperalgesia and its spread through somatic nerves. *Clinical Science* **2**, 373–423.

LEWIS, T. (1942). *Pain*. Macmillan Press, New York.

LINDBLOM, U. & VERILLO, R. T. (1979). Sensory functions in chronic neuralgia. *Journal of Neurology, Neurosurgery and Psychiatry* **42**, 422–435.

MACKENZIE, R. A., BURKE, D., SKUSE, N. F. & LETHLEAN, A. K. (1975). Fibre function and perception during cutaneous nerve block. *Journal of Neurology, Neurosurgery and Psychiatry* **38**, 865–873.

MEYER, R. A., RAJA, S. N. & CAMPBELL, J. N. (1985). Coupling of action potential activity between unmyelinated fibers in the peripheral nerve of monkey. *Science* **227**, 184–187.

OCHOA, J. & TOREBJÖRK, E. (1983). Sensations evoked by intraneural microstimulation of single mechanoreceptor units innervating the human hand. *Journal of Physiology* **342**, 633–654.

PRICE, D. D., BENNETT, G. J. & RAFI, A. (1989). Psychophysical observations on patients with neuropathic pain relieved by a sympathetic block. *Pain* **36**, 273–288.

RAJA, S. N., MEYER, R. A. & CAMPBELL, J. N. (1988). Peripheral mechanisms of somatic pain. *Anesthesiology* **68**, 571–590.

SCHADY, W. J. L. & TOREBJÖRK, H. E. (1983). Projected and receptive fields: A comparison of projected areas of sensations evoked by intraneural stimulation of mechanoreceptive units, and their innervation territories. *Acta Physiologica Scandinavica* **119**, 267–275.

SIMONE, D. A., BAUMANN, T. K. & LaMOTTE, R. H. (1989). Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. *Pain* **38**, 99–107.

SIMONE, D. A., OH, U., SORKIN, L. S., OWENS, C., CHUNG, J. M., LaMOTTE, R. H. & WILLIS, W. D. (1991). Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic neurons. *Journal of Neurophysiology* **66**, 228–246.

TOREBJÖRK, H. E. (1990). Clinical and neurophysiological observations relating to pathophysiological mechanisms in reflex sympathetic dystrophy. In *Sympathetic Reflex Dystrophy*, ed. STANTON-HICKS, M., JÄNIG, W. & BOAS, R. A., pp. 71–80. Kluwer Academic Publications, Boston.

TOREBJÖRK, H. E. & HALLIN, R. G. (1973). Perceptual changes accompanying controlled preferential blocking of A and C fibre responses in intact human skin nerves. *Experimental Brain Research* **16**, 321–332.

TOREBJÖRK, H. E. & HALLIN, R. G. (1979). Microneurographic studies of peripheral pain mechanisms in man. In *Advances in Pain Research and Therapy*, vol. 3, ed. BONICA, J. J., LIEBESKIND, J. C. & ALBE FESSARD, D. G., pp. 121–131. Raven Press, New York.

TOREBJÖRK, H. E. & OCHOA, J. L. (1980). Specific sensations evoked by activity in single identified sensory units in man. *Acta Physiologica Scandinavica* **110**, 445–447.

TOREBJÖRK, H. E., SCHADY, W. & OCHOA, J. L. (1984). A new method for demonstration of central effects of analgesic agents in man. *Journal of Neurology, Neurosurgery and Psychiatry* **47**, 862–869.

TOREBJÖRK, H. E., VALLBO, Å. B. & OCHOA, J. L. (1987). Intraneural microstimulation in man. Its relation to specificity of tactile sensations. *Brain* **110**, 1509–1529.

VALLBO, Å. B. (1981). Sensations evoked from the glabrous skin of the human hand by electrical stimulation of unitary mechanosensitive afferents. *Brain Research* **215**, 359–363.

VALLBO, Å. B. & HAGBARTH, K.-E. (1968). Activity from skin mechanoreceptors recorded percutaneously in awake human subjects. *Experimental Neurology* **21**, 270–289.

VALLBO, Å B., OLSSON, K. A., WESTBERG, K.-G. & CLARK, F. J. (1984). Microstimulation of single tactile afferents from the human hand: sensory attributes related to unit type and properties of receptive fields. *Brain* **107**, 727–749.

WALLIN, G., TOREBJÖRK, H. E. & HALLIN, R. G. (1976). Preliminary observations on the pathophysiology of hyperalgesia in the causalgic pain syndrome. In *Sensory Functions of the Skin in Primates, with Special Reference to Man. Wenner–Gren International Symposium*, vol. 27, ed. ZOTTERMAN, Y., pp. 489–502. Pergamon Press, Oxford.