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PAIN, HYPERALGESIA AND ACTIVITY IN NOCICEPTIVE C UNITS IN HUMANS AFTER INTRADERMAL INJECTION OF CAPSAICIN

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SUMMARY

1. Capsaicin, the potent algesic substance in chilli peppers, was applied topically to, or injected intradermally into or outside, the receptive fields of 14 C mechanoheat (polymodal) nociceptor units in awake humans. The nociceptor discharges were recorded using microelectrodes inserted into the peroneal nerve. Simultaneously, the subjects estimated the magnitude of pain as a function of time during the first 1·5–3 min after injection. Magnitude estimates of pain produced by heat and/or mechanical stimuli were also obtained before and after capsaicin in order to assess the magnitude of cutaneous hyperalgesia.

2. An injection within or adjacent to, but not greater than 4 mm outside, the receptive fields of C nociceptor units evoked discharges. The magnitude of pain and the mean discharge rate of the units were both maximal on injection, declining rapidly over the next 1–3 min, which indicates that these nociceptors contribute to the magnitude and duration of pain evoked by capsaicin injection.

3. Reduced or abolished excitability in C nociceptors after capsaicin injection within the receptive fields correlated with analgesia at the injection site.

4. Capsaicin injection produced a wide surround area of mechanical hyperalgesia, i.e. pain on gently stroking the skin or abnormally intense pain on punctate stimulation. Nevertheless, the injections did not lower the thresholds or enhance the responses to such mechanical stimuli of C nociceptor units with their receptive fields in this hyperalgesic area.

5. Topical application of capsaicin evoked on-going discharges in four units tested. Both nociceptor response thresholds and pain thresholds were lowered for heat from 45 to 35 °C. A newly developed weak response to stroking the skin in two units after capsaicin was accompanied by faint pain.

6. On-going activity in sensitized C nociceptors and concomitant pain were effectively reduced by cooling the skin in the receptive area.

7. It is concluded that activity in C mechanoheat (polymodal) nociceptors contributes to the magnitude and duration of pain evoked by intradermal injection of capsaicin. The after-effects of capsaicin on C nociceptor excitability depend on concentration: high concentration (by injection) leads to desensitization, whereas

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low concentration (by topical application) leads to sensitization. On-going discharges and lowered response thresholds to heat in these units after topical application of capsaicin correlates with background pain as well as lowered pain thresholds to heat of the affected skin (primary hyperalgesia). The unchanged responsiveness of C nociceptors in the skin well outside the injection area indicates that central rather than peripheral sensitization accounts for the observed mechanical hyperalgesia in this region (secondary hyperalgesia).

INTRODUCTION

Capsaicin is a potent algesic substance which, when applied topically to or injected into the skin of humans, can produce pain, hyperalgesia and analgesia (e.g. Szolcsányi, 1977; Carpenter & Lynn, 1981; LaMotte, Shain, Simone & Tsai, 1991). Intradermal injection has been found to be a useful way of delivering different concentrations of capsaicin in a reproducible manner in both psychophysical studies of pain in humans and neurophysiological experiments in anaesthetized animals (LaMotte *et al.* 1991; Baumann, Simone, Shain & LaMotte, 1991; Simone, Oh, Sorkin, Owens, Chung, LaMotte & Willis, 1991). Both the magnitude and duration of pain increase with the dose (Simone, Baumann & LaMotte, 1989). The peak magnitude of pain from the highest dose of 100 μg is an average of 2·6 times more intense than the pain produced by locally heating the skin to 51 °C for 5 s.

An intradermal injection of caspaicin resulted in a dose-dependent lowering of the threshold to painful heat, and enhanced pain over that normally caused by heat stimuli in humans (Simone, Ngeow, Putterman & LaMotte, 1987; Simone *et al.* 1991). This cutaneous hyperalgesia to heat was confined to an area of about 1 cm diameter, centred approximately on the injection site. In addition, a much larger dose-dependent area of hyperalgesia to mechanical stimuli developed around the injection site, characterized by tenderness on lightly stroking the skin and by lowered pain thresholds and enhanced suprathreshold pain on punctate stimulation with von Frey filaments (LaMotte *et al.* 1991). Results of experiments in the same study supported the hypothesis that mechanical hyperalgesia depends on neural activity originating at the injection site, spreading radially through intracutaneous nerve fibres and resulting in the enhanced responses ('sensitization') of neurons in the dorsal horn of the spinal cord to mechanical stimuli. The sensitization of one set of neurons by activity in another was termed neurogenic hyperalgesia. Preliminary evidence suggests that a similar mechanism may contribute, in part, to the heat hyperalgesia surrounding the capsaicin injection site.

Within the small bleb produced by the capsaicin injection, the skin was hypoalgesic to pin prick and von Frey filaments for hours or up to a day after the injection (LaMotte *et al.* 1991).

The peripheral neural events contributing to the pain, analgesia and hyperalgesia produced by an intradermal injection of capsaicin are still somewhat unclear. One of the most commonly studied nociceptors is the C fibre mechanoheat (CMH or polymodal) nociceptor which responds well to mechanical and thermal noxious stimuli. These units responded less than expected or not at all to capsaicin injected intradermally into or outside their receptive fields in anaesthetized monkeys. Rather than enhancing the response to mechanical or heat stimuli, capsaicin decreased (or

did not change) this response, suggesting a major role for these nociceptors in the analgesia but not the hyperalgesia produced by the intradermal injection of capsaicin (Baumann *et al.* 1991).

These conclusions require the assumption that the response properties of CMH nociceptors and the peripheral neural mechanisms contributing to pain and altered pain states are similar in monkeys and humans. However, there is evidence of species differences. For example, the flare that surrounds a local cutaneous injury in human skin and is believed to be mediated by an axon reflex is not visible in monkey skin. Thus, if mechanical hyperalgesia in humans requires an axon reflex, as hypothesized by Lewis (1936), and involves CMH nociceptors, then their role is best evaluated in humans. Further, the role of these nociceptors in capsaicin pain and altered pain states is best evaluated by measuring sensations of pain and nociceptor responses in the same subject at the same time. This was carried out in the present study before and after intradermal injections of capsaicin within or at various distances away from the cutaneous receptive fields of CMH nociceptors in human volunteers.

METHODS

Subjects

Microelectrode recordings were obtained from the common peroneal nerve just below the knee. Recordings were made on different occasions from eight healthy volunteers, six males and two females. Each subject gave informed consent to the procedures used in the experiments. The experiments were approved by the University Ethical Committee.

Microelectrode recording procedure

The lacquer-coated tungsten microelectrodes used here are the type commonly used for human microneurography (Vallbo & Hagbarth, 1968). Single-unit potentials were initially amplified via a preamplifier located close to the microelectrode. These signals were further amplified, filtered, displayed on an analog storage oscilloscope, audiomonitored, and stored on FM tape for off-line analysis. A switch in the preamplifier allowed the recording electrodes to be connected either to the amplifier or to the output of a constant-voltage stimulator (Grass S48), equipped with a stimulus isolation unit.

The subject reclined in a chair that provided comfortable support for the leg. The peroneal nerve was located by palpation and/or by surface electrical stimulation. A reference electrode was inserted into subcutaneous tissue close to the nerve trunk and the recording microelectrode was manually inserted through the skin and into the nerve. The electrode tip was advanced in small steps until it was successfully positioned within a cutaneous nerve fascicle as signalled by both the subject's sensory reports in response to low intensity electrical stimuli delivered through the electrode, and the discharges evoked in sensory afferent fibres by distal skin stimulation when the electrode was switched from the stimulating to the recording mode.

Identification of cutaneous C nociceptors

Responses in a single C mechanoheat (CMH) nociceptive unit were initially evoked by lightly scraping the skin with a sharp piece of plastic or by lightly pinching the skin between the experimenter's fingers. The receptive field was mapped by marking a dot on the skin with a felt-tip pen at each spot where impulses were evoked by a von Frey-type nylon filament (exerting a bending force of 225 mN). Two needle electrodes were inserted into the receptive field and square-wave pulses of 0·25 ms duration were delivered at a constant frequency of 0·3 Hz and $\leqslant$ 100 V. The conduction velocity was determined by the latency of the electrically evoked response and the distance between stimulating and recording electrodes. A unit was classified as a C fibre if the conduction velocity was $\leqslant$ 2 m/s. The fibre was further established as an afferent as opposed to a sympathetic efferent if transient increases in the latency of electrically evoked responses occurred during natural stimulation of the skin (Hallin & Torebjörk, 1974) and if there was no response to arousal stimuli that normally evoke reflex sympathetic responses (Delius, Hagbarth, Hongell &

Wallin, 1972). Finally, the unit was classified as a mechanoheat nociceptor if it responded selectively to mechanical and heat stimuli which typically evoked a faint or moderate sensation of pain in the subject (e.g. pricking nylon filaments, or a heat stimulus of 45 or 50 °C, applied for 5 s), but poorly or not at all to lower intensity painless stimuli such as those evoking only touch or warmth. The heat stimulus was applied with a contact Peltier thermode (contact diameter 1 cm and rise time of 2 °C/s) (LaMotte *et al.* 1991). The stimulus temperature was maintained to within ±0·1 °C of a desired value, measured by a thermocouple at the skin–thermode interface.

Preparation and delivery of capsaicin

A colloidal mixture of 1% capsaicin (Fluka) ($3{\cdot}3 \times 10^{-2}$ M) in Tween-80 and saline was prepared for injection as described previously (Simone *et al.* 1989) and then injected through a Millipore filter (0·2 μm pore size) into a sterile injection vial for storage. At the time of injection, a volume of 10 μl was drawn up from the vial into a 28-gauge hypodermic needle (Lo-dose, Beckton-Dickinson, 0·5 ml capacity) and injected into the skin in one of several locations relative to a fibre's receptive field.

The injection produced a slight elevation of the skin (a bleb) of about 4 mm in diameter and induced a red flare with a maximal area of 10–30 cm^2, as judged by visual inspection. The injection site was located so that the edge of the bleb was 3–30 mm outside, adjacent to, adjacent to but partially inside, or entirely within the receptive field. In some experiments an injection outside was followed by an injection adjacent to or inside the receptive field.

In four experiments, a solution of 1% capsaicin in 50% dimethyl sulphoxide (DMSO) in saline was prepared in a small screw-capped bottle for storage. During the experiments, a small dam was built around the nociceptor's receptive field with dental rubber cement (Reprosil) and the capsaicin solution was applied topically to the skin inside the dam with a cotton-tipped applicator. After several minutes another application was given.

Psychophysical measurements

The subject was instructed to assess the intensity of pain evoked by each stimulus by choosing a number that was proportional to this level according to the method of magnitude estimation (Stevens, 1975). If no pain was felt, the subject assigned the number zero. The assessment was to be based only on the sensation of pain and not the subject's reaction to the pain, such as how unpleasant or how tolerable it was. In order to assess the level of pain caused by capsaicin, the maximum level experienced during the previous 15 s was reported every 15 s after capsaicin was injected for a total of 1·5–3 min.

Testing procedure

The nylon filament (225 mN) was applied for 2 s to each of three test spots evenly spaced within the receptive field. Each spot was stimulated three times in rotation (interstimulus intervals of about 15 s) and the total number of evoked impulses for each was averaged. The numerical magnitude estimates of pain evoked by each stimulus were also averaged. Next, a cotton swab attached to a springy strip of metal (a coping saw blade) was stroked tangentially across the receptive field at approximately 3 cm/s along a 3 cm path. The cotton swab was calibrated to give an average force of 100 mN when the metal was just noticeably bent during a stroke. The total number of impulses and the magnitude estimate of pain during the stroke were recorded. This was repeated three times at interstimulus intervals of about 15 s and the responses were averaged.

In a few experiments, the threshold for heat pain was determined. The Peltier thermode was centred over the receptive field and the skin adapted to a base temperature of 28 or 30 °C for 30 s. Then stimuli of 35, 45 and occasionally 50 °C were delivered; each was 5 s in duration and followed by a return to base temperature for 30 s. The total number of impulses and the estimate of the maximum magnitude of pain evoked by each stimulus were recorded.

Beginning with the injection or topical delivery of capsaicin, the subject estimated the maximum magnitude of pain felt during each successive interval of 15 s until 3 min had elapsed. Then test stimuli the same as those delivered prior to capsaicin were administered and neural activity and magnitude estimates of pain were again obtained.

Data analysis

Student's t test was used for statistical analysis and $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

Seventeen injections of capsaicin were performed outside and/or adjacent to or inside the cutaneous receptive fields of eleven CMH nociceptive units (CMHs). In addition, capsaicin was applied topically to the receptive fields of one of these units and to those of three additional CMHs. Table 1 contains a summary of the subjects and units tested, the conduction velocities of the fibres, the location of capsaicin application, the responses to capsaicin and the presence or absence of changes in response to mechanical and heat stimuli. The mean conduction velocity for the twelve fibres tested was $0{\cdot}9 \pm 0{\cdot}2$ m/s. Six units received more than one injection of capsaicin (consecutively denoted as 'a, b...' after the unit identification number) and topical capsaicin was applied to one unit after an injection outside its receptive field (unit 1).

Responses of CMH nociceptive units and magnitude ratings of pain on intradermal injection of capsaicin

All CMHs responded to capsaicin injection when the edge of the bleb was inside or within 4 mm of the border of the unit's receptive field, as determined using the von Frey filament. One unit (No. 5) gave a delayed response to an injection 8 mm distal to the receptive field, while six others injected 7–30 mm outside the receptive field failed to respond. The responses were weak when capsaicin was injected outside the receptive field (Nos 5, 8 and 9a) and stronger after an injection adjacent (proximal or distal) to or inside the receptive field. The highest discharge rate observed was 243 impulses during the first 3 min following injection within the receptive field (No. 9b). The discharge pattern was usually irregular (Fig. 1). Three units exhibited occasional bursts with maximal instantaneous frequencies in the range of 30–60 impulses/s (Fig. 2).

Magnitude estimates of pain on capsaicin injection were obtained simultaneously with recorded responses of a CMH unit in nine experiments during the first 1·5 min after injection and, for three of these, these measurements were continued up to 3 min. The unit responses and associated pain estimates are plotted in Fig. 3 from the start of injection. The needle was withdrawn within 20 s of the start of injection. Discharge rates were lower and delayed in onset after an injection outside the receptive field in comparison with those after injection adjacent to or inside the receptive field. The latter typically reached a maximum within the first 15–30 s of the beginning of the injection. Similarly, magnitude estimates of pain reached a maximum within the first 30 s (typically within the first 15 s). Pain estimates and rates of discharge declined after reaching the maximum.

The discharge rates of the CMHs, when averaged for the nine experiments in which pain estimates were also obtained for at least 1·5 min after injection, showed a similar time course as the averaged normalized magnitude estimates (Fig. 4). This suggests that the CMH activity contributed to the pain caused by the capsaicin injection.

Effects of remote injection of capsaicin on sensations of pain and responses of CMH nociceptors evoked by mechanical and heat stimulation of the receptive fields

In nine experiments in which capsaicin was injected 3–30 mm outside the border of the receptive field (first nine entries in Table 1), the skin was stimulated with the nylon filament or stroked with the cotton swab (see Methods). There was no

TABLE 1. Summary of effects of capsaicin on CMH responses

Subject	Unit No.	CV (m/s)	Location (inj/top)	Cap response	No. of impulses	Change in response to		
						23 g vF	Stroke	Heat
R.F.	1a	0·5	30 mm dist.	0	0	0	0 NR	0
A.R.	2a	1·0	12 mm prox.	0	0	0	0 NR	NT
B.K.	3a	0·8	10 mm prox.	0	0	0	0 NR	NT
T. N.	4a	NT	9 mm prox.	0	0	0	0	NT
T.N.	5	1·5	8 mm dist.	+	57/3 min	—	0	—
A.R.	6a	0·8	7 mm prox.	0	0	0	0 NR	NT
M.W.	7a	1·0	7 mm dist.	0	0	0	+	0
A.R.	8	NT	4 mm dist.	+	6/1·5 min	0	—	NT
M.B.	9a	1·0	3 mm lat.	+	8/1·5 min	0	0 NR	NT
B.K.	3b		Adj. prox.	+	154/1·5 min	0, —	+, 0, NR	NT
A.R.	2b		Adj. dist.	+	61/1·5 min	0, —	NT	NT
A.R.	6b		Adj. prox.	+	42/1·5 min	—	0 NR	NT
R.F.	10	0·7	Adj. prox.	+	63/3 min	—	NT	—
T.N.	11	1·0	Inside RF	+	77/1·5 min	—	NT	—
M.B.	9b		Inside RF	+	243/3 min	—	0 NR	—
M.W.	7b		Inside RF	+	91/1·5 min	0, —	NT	NT
T.N.	4b		Inside RF	+	21/1·5 min	—	NT	NT
B.J.	12	0·7	Topical	+	52/3 min	0	+	+
B.J.	13	0·9	Topical	+	38/3 min	0	0	+
K.G.	14	1·0	Topical	+	103/3 min	0	+	+
R.F.	1b		Topical	+	28/3 min	NT	NT	NT

The columns from left to right contain the following data: subject initials, unit number (a and b signify that a unit was exposed to capsaicin twice), conduction velocity (CV), location relative to border of receptive field and mode of capsaicin exposure, responsiveness to capsaicin (Cap), number of impulses after start of capsaicin exposure, and change in responsiveness to pointed stimulation with von Frey (vF) filament (23 g), stroking and heat. NT and NR mean not tested and no response, respectively. For unit B.K. 3b the symbols +, 0, NR indicate that its responsiveness to stroking was enhanced in one part of the receptive field, unchanged in another and completely depressed in a third (see Fig. 6). For other units in this column the symbols 0 NR indicate no response to stroking either before or after capsaicin.

significant change in the number of impulses evoked by punctate stimulation (Fig. 5*A*) after injection of capsaicin (when the flare covered the receptive field) nor was there an increased response to stroking (Fig. 5*B*). In general, there were no changes in the size of the receptive fields as mapped by stimulation with the nylon filament. In contrast, the mean magnitude estimate of pain from the punctate stimulus increased significantly ($P < 0·05$) after capsaicin (Fig. 5*C*). Similarly, stroking that was not perceived as painful by any subject prior to the injection, evoked pain in every subject after capsaicin (Fig. 5*D*). Responses to heat were tested for only three units. Two of these showed no change in responsiveness to heat after injection of

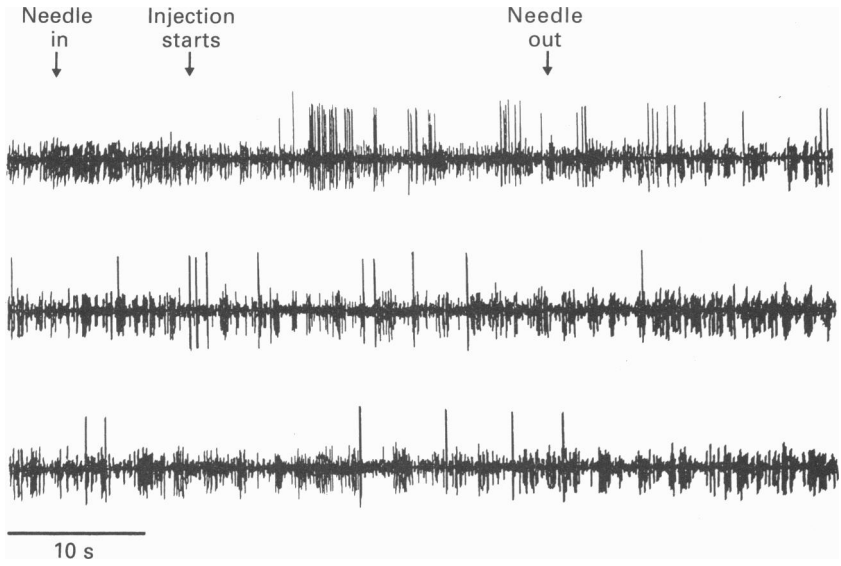

Fig. 1. Responses of a CMH nociceptor unit (No. 1a) to an injection of capsaicin adjacent and proximal to the border of its receptive field. Responses were recorded up to 3 min after the start of the injection. Polarity of action potentials distorted by filtering in Figs 1, 2 and 6.

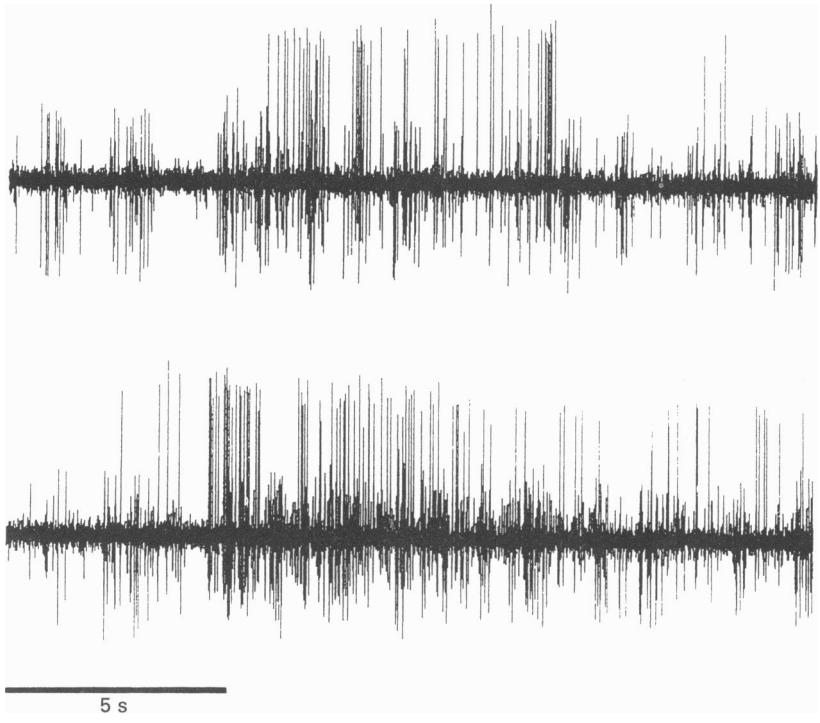

Fig. 2. Bursting discharges (upward deflections) in a CMH nociceptor unit (No. 9b) after the needle was withdrawn from capsaicin injection inside the receptive field. Sympathetic discharges of lower amplitude are also seen in the record.

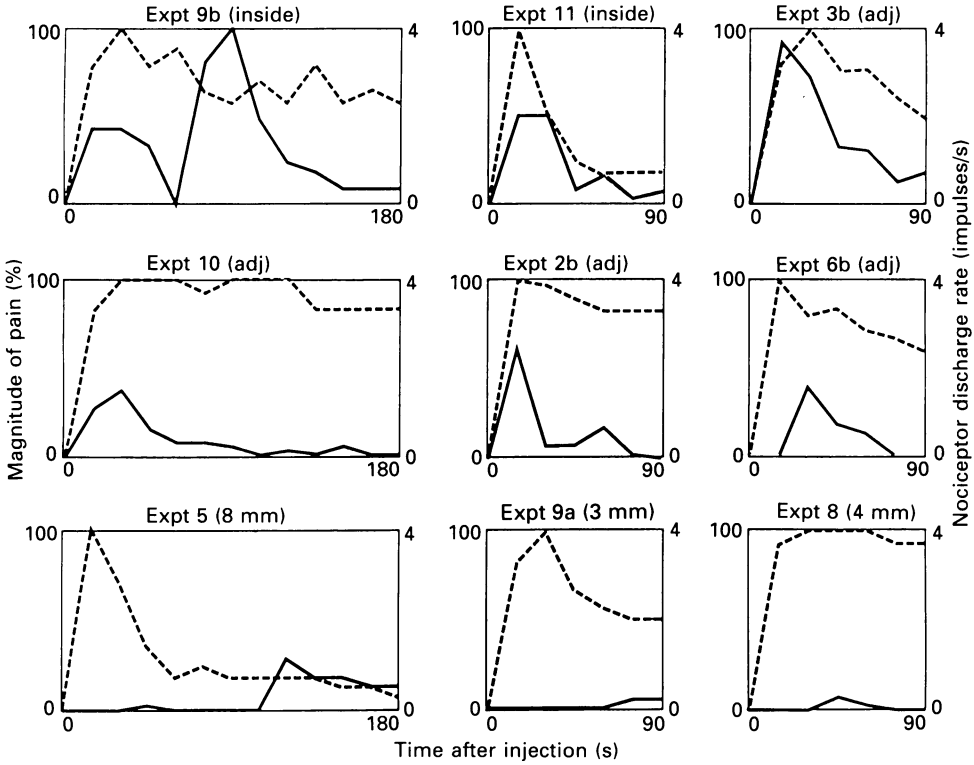

Fig. 3. Comparisons of discharge rates (impulses/s) in each CMH nociceptor (continuous lines) and the magnitude estimates of pain (dashed lines) obtained simultaneously after an injection of capsaicin. The capsaicin was injected outside, adjacent to or inside the receptive field. The unit number and site of injection according to Table 1 is indicated above each panel. The abscissa indicates the time (s) from the start of injection. The magnitude estimates of pain are expressed as a percentage of the maximum obtained for that subject. The data in the left column were collected up to 3 min after the start of the injection and the data in the two right columns were collected for 1·5 min.

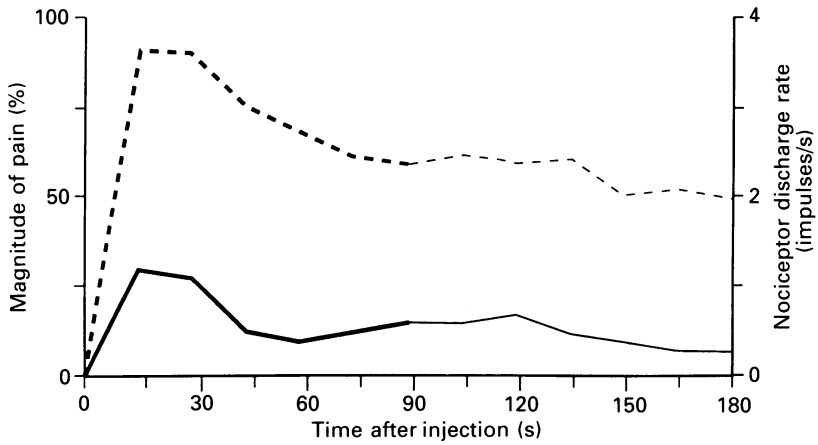

Fig. 4. Averaged normalized pain ratings (dashed line) and mean number of impulses (continuous line) for nine units recorded for 1·5 min after the start of capsaicin injection (thick lines) and for three of these for up to 3 min after injection (thin lines).

capsaicin and one unit was depressed. Pain ratings in response to heat were unchanged, or in one case slightly increased. Thus, there were no indications of a remote sensitization of CMH nociceptors by injections of capsaicin even though their receptive fields were located in hyperalgesic skin, covered by flare. A summary of the changes in response for individual units is given in Table 1.

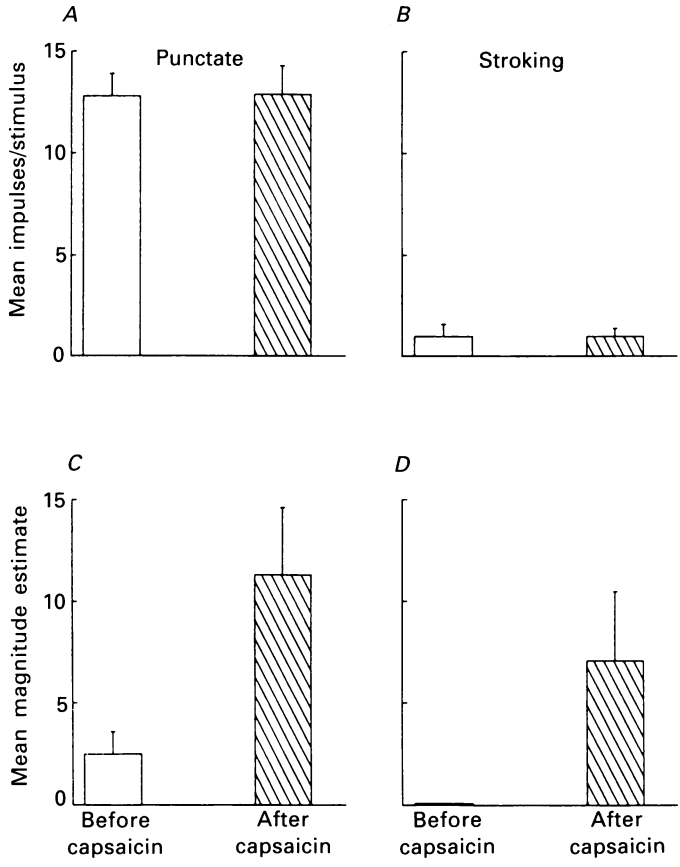

Fig. 5. The effects of an injection of capsaicin remote to the CMH receptive field on mechanically evoked responses and pain sensations. The upper panels show the mean number of impulses evoked by punctate stimulation with the nylon filament (*A*) and stroking with the cotton swab (*B*) before and after injection of capsaicin (open and hashed bars, respectively). The lower panels show corresponding mean magnitude estimates of pain on the same punctate (*C*) and stroking (*D*) stimuli before and after capsaicin injection. Data were obtained from nine experiments comprising eighty-one observations for punctate stimuli, and from twenty-seven observations for stroking.

Effects of capsaicin injection adjacent to or inside the receptive fields on responses of CMH nociceptors to mechanical and heat stimulation

The number of impulses evoked by punctate stimulation was significantly ($P < 0{\cdot}005$) reduced in every unit tested after injection of capsaicin adjacent to or inside the receptive field. The mean number of impulses decreased from $14{\cdot}5 \pm 1{\cdot}7$ before capsaicin to $2{\cdot}6 \pm 1{\cdot}3$ after (see also Table 1). In three experiments, there was a greater decrease in response for the test spot(s) closer to the injection (Fig. 6).

Similarly, for one unit the responses to stroking were either depressed, unchanged or slightly enhanced in different parts of the receptive field, depression being prominent close to the injection site. Three nociceptors, one injected adjacent to and the others inside the receptive field, became unresponsive to both mechanical and heat

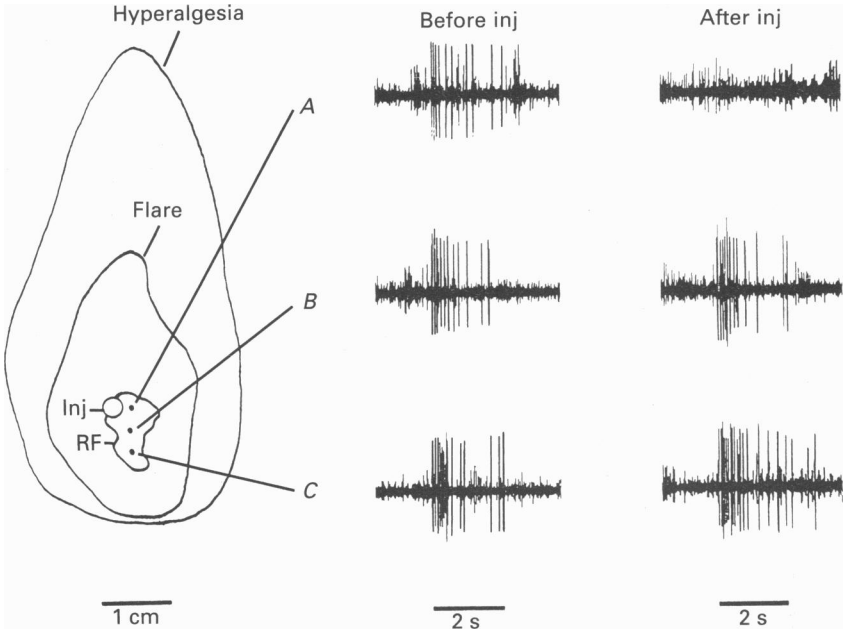

Fig. 6. Responses of a CMH unit to punctate mechanical stimulation applied to three different loci within its receptive field after an adjacent injection of capsaicin. 'Inj' and 'RF' refer to injection site and receptive field, respectively. The extent of the maximal flare area as determined by visual inspection is also illustrated. The recordings show that responses at the stimulation site closest to the injection (*A*) were eliminated while those evoked at adjacent sites (*B* and *C*) were relatively unaffected.

stimulation. However, the C fibres could still be excited by electrical stimulation through needle electrodes inserted intracutaneously in the receptive fields.

Magnitude estimates of pain in response to mechanical stimulation at the site of the injection bleb were obtained in only two of these experiments. The level of pain was decreased after the injection of capsaicin inside the receptive field on both occasions.

The possibility of a novel population of purely chemosensitive nociceptors

The CMH nociceptors in the present study were identified by lightly scraping or poking the skin with innocuous but mildly painful mechanical stimuli. It is likely, therefore, that the population was biased in that it would not contain any units that do not respond to such stimuli.

A few presumably chemosensitive C units were discovered serendipitously during studies of CMH units. In one experiment, electrical stimuli applied through needles inserted into the receptive field of a CMH nociceptor evoked discharges in an

additional C fibre (with a conduction velocity of 1 m/s). Responses in this fibre could not be evoked by either heat (50 °C) or mechanical (von Frey type and needles) noxious stimuli but occurred in bursts for almost 3 min after injection of capsaicin. Since manoeuvres that elicit sympathetic reflexes (rapid inspiration, loud noises) failed to affect the on-going discharge, the unit was tentatively classified as a C chemonociceptor on the basis of preliminary evidence of such units reported for the monkey and the rat (Davis, Meyer, Cohen & Campbell, 1989; Baumann *et al.* 1991; Handwerker, Kilo & Reeh, 1989).

In another experiment, a C fibre conducting at 0·9 m/s began to discharge with repetitive high frequency bursts of about 18 impulses/s after capsaicin injection. These responses continued for about 80 s and thereafter occurred only occasionally for up to 10 min after injection. Prior to the injection, this fibre had only responded to electrical stimulation in the skin, but not to mechanical test stimuli. After capsaicin it occasionally responded to the cotton swab (applied within 1 cm of the injection site) with prolonged after-discharges. It is possible that this fibre was a C chemonociceptive afferent that became mechanically sensitized by capsaicin. One such fibre has been observed in the monkey (Baumann *et al.* 1991). Several other, less well documented, observations were made of units that appeared *de novo* only after an injection of capsaicin. These observations remain anecdotal and are mentioned primarily to serve as a reminder that other types of nociceptors exist that were not searched for in the present series of experiments.

Effects of topical application of capsaicin on evoked responses of CMH nociceptors and corresponding sensations of pain

Spontaneous activity and effect of cooling

In four experiments, capsaicin was applied topically to the receptive field (Table 1). The CMHs began responding within 30 s of beginning the application at a low irregular discharge rate in the order of 28–103 impulses/3 min (Table 1), accompanied by reports of spontaneous burning pain. The discharge, which continued for each unit throughout the experiment, was shown to depend on skin temperature. A typical example is shown in Fig. 7. When the skin was cooled from 33 to 10 °C by a thermode placed over the receptive field, the on-going discharge and the burning pain were nearly abolished. Rewarming the skin to 32 °C brought back the discharge and the pain.

Responses to mechanical stimulation

In three experiments with two subjects, the discharges from CMH nociceptors and magnitude estimates of pain were obtained in response to mechanical stimulation of the receptive field before and 15–60 min after topical treatment with capsaicin. In two of these experiments, the subject reported an increase in the magnitude of pain evoked by the punctate stimulus after capsaicin while in the third, no pain was evoked either before or after. The number of impulses evoked by stimulating each locus after capsaicin lay within the range of values obtained prior to capsaicin treatment.

Stroking the skin evoked newly developed weak responses in two C nociceptors

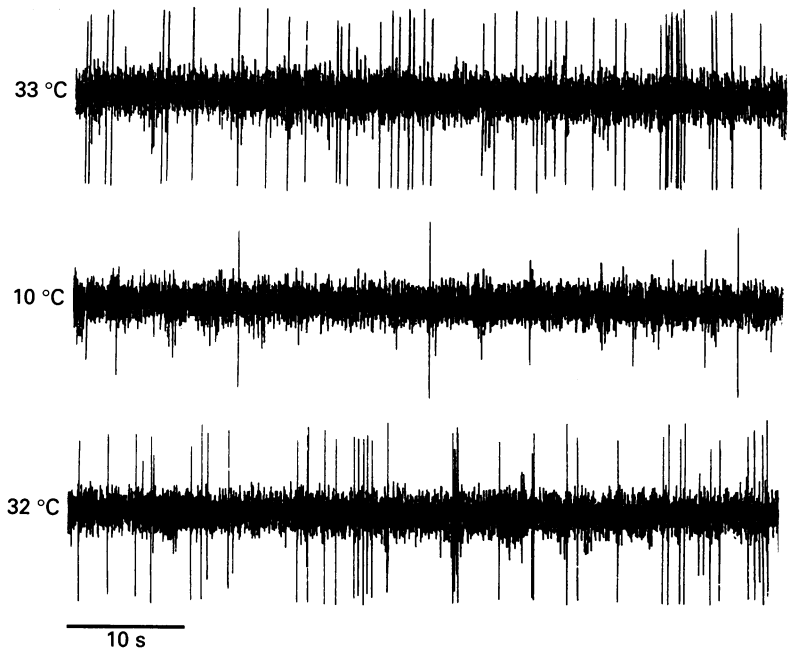

Fig. 7. The effects of cooling and then rewarming the receptive field of a CMH nociceptor on the background discharges elicited by a topical application of capsaicin. A thermode controlled the temperature of the capsaicin-treated skin.

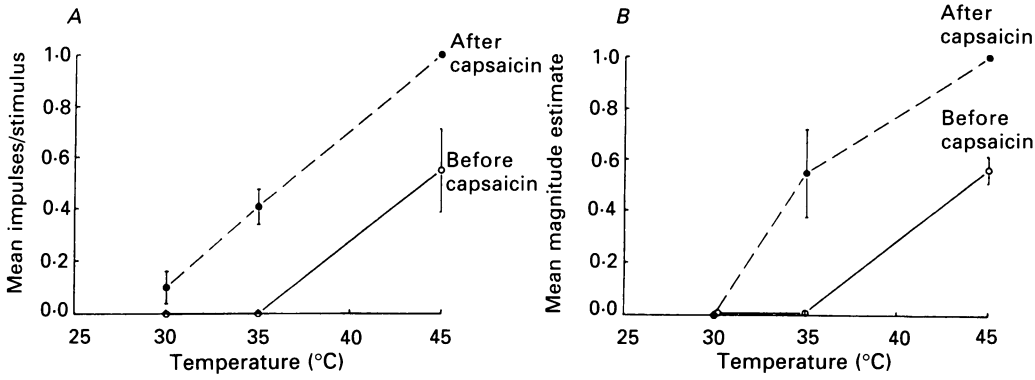

Fig. 8. Mean normalized number of discharges per stimulus (±S.E.M.) in CMH nociceptors (*A*) and mean normalized pain ratings (*B*) evoked by the same heat stimuli delivered before (continuous lines) and after (dashed lines) a topical application of capsaicin.

after capsaicin compared with the control, accompanied by faint sensations of pain. In a third experiment, there was no response by the CMH unit and no sensation of pain, either before or after capsaicin.

Responses to heat

In three experiments, heat stimuli of 30, 35 and 45 °C, each lasting for 5 s, were delivered on a base temperature of 28 °C before and about 30 min after applying capsaicin to the receptive field. In each experiment, a lowered threshold and

increased discharge rate of the C nociceptor was accompanied by a lowered heat pain threshold and increased magnitude of pain elicited by suprathreshold heat stimuli (Fig. 8). Response thresholds decreased from 45 °C before capsaicin to 35 °C for two CMHs and to 30 °C for the other after capsaicin. Similarly, the pain threshold decreased from 45 to 35 °C.

DISCUSSION

Proportion of CMH nociceptors responding to intradermal injection of capsaicin

All of the CMH nociceptors recorded from the human peroneal nerve responded to an injection of 100 μg capsaicin adjacent to or within the receptive fields. This is in contrast to findings in the monkey, where only 40% of those CMH units injected adjacent to or within their receptive fields responded to capsaicin (Baumann *et al.* 1991). Both studies are in agreement, however, as to the inability of capsaicin to evoke a response in most CMH nociceptors when injected well outside their receptive fields.

Contribution of capsaicin-evoked activity in CMH nociceptors to pain sensation

It was obvious from the single-unit recordings that the C nociceptor responses to capsaicin injection varied greatly depending on the distance between the receptive field and the injection site, and this in turn could contribute to considerable variability in comparisons between discharge profiles in individual units and magnitude ratings of pain (Fig. 3). However, when the population response in nine units was compared to averaged pain ratings after capsaicin injection, the two curves are almost parallel (Fig. 4), suggesting that the input from C polymodal nociceptors contributes to pain from capsaicin injection. This notion is further supported by the parallel waxing and waning of pain with increase and decrease of temperature-dependent on-going activity in C polymodal nociceptors after topical application of capsaicin.

An interesting question is why pain from intradermal injection of 100 μg of capsaicin is so intense, on average judged to be 2·6 times more intense than the pain evoked by a heat stimulus of 51 °C for 5 s (Simone *et al.* 1989). One possible explanation might be that the discharge pattern in C polymodal nociceptors on capsaicin injection is irregular, with bursts of impulses at maximal instantaneous frequencies of up to 60 impulses/s. It is conceivable that such high instantaneous firing rates during bursts of impulses could give rise to considerable pain due to temporal summation (Jørum, Holm, Lundberg & Torebjörk, 1990), even though the average firing frequency was fairly low. It is also possible that several types of nociceptors are activated by capsaicin. Candidate nociceptors in addition to the CMHs could include purely chemosensitive units as well as the C heat nociceptors that respond both to noxious heat and to capsaicin but not to mechanical or cold stimuli (Baumann *et al.* 1991). In the monkey, the C heat nociceptors have been found to respond with greater discharge rates to an intradermal injection of 100 μg of capsaicin than to a heat stimulus of 51 °C.

In the present study, a few C units that responded to capsaicin but not to prior stimulations with noxious thermal or mechanical stimuli might be classified as putative chemonociceptors. In the future, one could search for purely chemosensitive

units in human nerves with electrical stimuli (Meyer & Campbell, 1988) in addition to the usual mechanical or heat stimuli, as has recently been carried out in monkeys (Davis *et al.* 1989) and rats (Handwerker *et al.* 1989).

Depressed sensitivity in CMH nociceptors and analgesia at the capsaicin injection site

An intradermal injection of capsaicin adjacent to or within the receptive field, rather than enhancing the responses of CMH nociceptors to cutaneous stimulation, typically reduced or eliminated such responses. A similar result was obtained in a study in which capsaicin was injected adjacent to or within the cutaneous receptive fields of CMH and A mechanoheat nociceptors in the monkey (Baumann *et al.* 1991). In that study, responses of both types of units to a 100 g von Frey filament stimulus at a capsaicin injection site within the receptive field were significantly reduced, while responses to stimulation of an adjacent site a few millimetres away were unaffected. This spatially restricted 'desensitizing' effect caused by capsaicin injection was observed in the present experiments as well. These results suggest that the desensitization of mechanoheat nociceptors in humans may account for the presence of analgesia to pinprick at the capsaicin injection site.

Contribution of the sensitization of CMH nociceptors to primary hyperalgesia within a cutaneous area treated with topical capsaicin

Cutaneous hyperalgesia directly within an area of injury is termed 'primary' hyperalgesia while the hyperalgesia outside the injury is 'secondary' hyperalgesia (Lewis, 1936; Hardy, Woolf & Goddell, 1950). It is well known that topical application of capsaicin produces primary hyperalgesia particularly to heat stimuli within the treated area which lasts for hours (e.g. Szolcsányi, 1977; Carpenter & Lynn, 1981; Culp, Ochoa, Cline & Dotson, 1989). As shown by Konietzny & Hensel (1983) and verified here, human CMH nociceptors are sensitized particularly to heat by topical application of capsaicin, suggesting that sensitization of nociceptors in the periphery is one mechanism underlying primary hyperalgesia. This does not rule out that central sensitization may also contribute. The marked inhibitory effect of cooling on the on-going discharge in sensitized CMH nociceptors as shown in this study may be relevant to explain, in part, the pain-relieving effect of cooling reported by some patients with chronically sensitized C nociceptors (Cline, Ochoa & Torebjörk, 1989). However, it must be emphasized in this context that cooling also inhibits pain by central gating mechanisms (Bini, Cruccu, Hagbarth, Schady & Torebjörk, 1984; Wahren, Torebjörk & Jørum, 1989).

Secondary hyperalgesia surrounding a capsaicin injection and the absence of sensitization of CMH nociceptors

While CMH nociceptors become sensitized by capsaicin in low concentrations (such as after topical application), high concentrations (such as after intradermal injection) primarily depress the responsiveness of these nociceptors. From the results of psychophysical experiments with intradermal injections of capsaicin in humans, it was concluded that the area of primary hyperalgesia resulting from sensitization of nociceptors directly exposed to low concentrations of capsaicin was relatively

small (LaMotte *et al.* 1991). Instead, part of the area of heat hyperalgesia and most of the area of mechanical hyperalgesia were believed to be of the secondary type. In both the present experiments with CMH nociceptors in humans and those with CMHs in monkeys, a capsaicin injection outside (and not adjacent to) the receptive field failed to alter the sensitivity of these nociceptors. Thus, the 'secondary' cutaneous hyperalgesia surrounding the injection site cannot be explained on the basis of a sensitization or enhanced responsiveness of mechanoheat-sensitive nociceptive units. Rather, several pieces of evidence from both psychophysical (LaMotte *et al.* 1991) and neurophysiological experiments in monkeys (Baumann *et al.* 1991) and humans (Torebjörk, Lundberg & LaMotte, 1991) point to the sensitization of certain spinothalamic neurons within the dorsal horn of the spinal cord (Simone *et al.* 1991) as being responsible for the enhanced pain and lowered pain thresholds to mechanical stimulation of the hyperalgesic skin surrounding a capsaicin injection.

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