Novel Classes of Responsive and Unresponsive C Nociceptors in Human Skin

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One hundred ninety-four cutaneous C-fibers were recorded from the human peroneal nerve; 118 units were found by mechanical stimuli and 76 units were detected by electrical stimulation through a surface electrode. Needle electrodes were then inserted for electrical intradermal stimulation in the innervation territory of the units. Afferent and efferent sympathetic C-fibers were identified by slowing of conduction velocity after activation either by physical or chemical skin stimuli, or by arousal maneuvers eliciting sympathetic reflexes.

In addition to mechano-heat–responsive C units (CMH) also found in previous studies, we here report on novel classes of C nociceptors in human skin, namely, units responding only to mechanical stimuli (CM), units responding only to heating (CH), and units that were insensitive to mechanical and heating stimuli and also to sympathetic provocation tests (CM$_i$H$_i$).

With the electrical search technique we found 45% CMH, 13% CM, 6% CH, 24% CM$_i$H$_i$, and 12% sympathetic units. Excitation by topically applied mustard oil occurred in 58% of CMH units, and in one-third of CM and CM$_i$H$_i$ units, respectively.

Some CM, CH, and CM$_i$H$_i$ units were sensitized to heating and/or to mechanical stimuli after topical application of mustard oil or capsaicin. These units then acquired responsiveness to a stimulus modality to which they previously were insensitive. Such recruitment of previously silent nociceptors implies spatial summation to the nociceptive barrage at central levels, and may contribute both to primary hyperalgesia to heat and pressure after chemical irritation, and to secondary hyperalgesia as a consequence of central sensitization.

[Key words: microneurography, human nociceptors, C-fibers, sensitization, pain, hyperalgesia]

Since the first successful microneurographic experiments on afferent C-fibers in humans (Hallin and Torebjörk, 1970; Torebjörk and Hallin, 1970), most units recorded from the cutaneous

innervation territories of the radial and peroneal nerves were found to respond to mechanical stimuli and heating (for reviews, see Vallbo et al., 1979; Handwerker and Kobal, 1993). These units were classified as C polymodal nociceptors (Bessou and Perl, 1969) or C mechano-heat (CMH) nociceptors (Campbell et al., 1989). However, it has long been recognized that there exists in the monkey skin a rich diversity of C nociceptors that respond differentially to mechanical, mechanothermal, or thermal stimuli (Georgopoulos, 1976). Recently, improved experimental techniques with electrical search stimuli (Meyer and Campbell, 1988) have led to the discovery of very high threshold or insensitive cutaneous nociceptors that were primarily activated by inflammation processes. They seem to be frequent in the hairy skin of monkey (Meyer et al., 1991; Davis et al., 1993) and rat (Handwerker et al., 1991b; Kress et al., 1992) and also in noncutaneous tissues such as the knee joint (Schaible and Schmidt, 1985, 1988; Grigg et al., 1986) and the urinary bladder of cat (Häbler et al., 1988, 1990). Some of these unresponsive units were activated and/or sensitized to subsequent mechanical and thermal stimulation by chemical irritant substances (Neugebauer et al., 1989; Handwerker et al., 1991b; Kress et al., 1992; Davis et al., 1993). Moreover, the study of different forms of hyperalgesias in man has led to the theoretical assumption of purely chemosensitive nociceptors in human skin (LaMotte, 1988, 1992; LaMotte et al., 1988, 1991).

The purpose of this study was to search for a spectrum of C nociceptors of various classes, including the insensitive or chemosensitive ones, in human skin. To this purpose we changed the experimental protocol from the traditional mechanical search stimuli to a search procedure employing electrical stimuli (or a combination of mechanical and electrical stimuli) to recruit units independently of their sensitivity to natural stimulation. In addition, a computerized version of a method utilizing interactions between naturally and electrically evoked discharges (Hallin and Torebjörk, 1974b; Torebjörk, 1974; Torebjörk and Hallin, 1974) allowed reliable testing of the responsiveness of individual C units in multiunit recordings frequently encountered in microneurography. By this method the yield of individual experiments was greatly increased.

Preliminary results have been published in abstract form (Handwerker et al., 1993).

Materials and Methods

The experiments were carried out in the microneurography laboratories at Uppsala and Erlangen. Of the 40 experimental sessions, 31 were performed with male and nine with female subjects. Age range was 19–29 years. None of the subjects showed signs of neurological or derma-

tological disease. All of them gave their informed consent and the study was approved by the local ethics committees.

Conventional microneurography techniques were employed to record from C-fibers in the peroneal nerve (Torebjörk, 1974). In 30 cases we recorded from cutaneous fascicles in the common peroneal nerve dorsolateral to the fibular head and in 10 cases from the superficial branch of this nerve proximal to the ankle.

Search for C-fiber units. When the tip of the recording electrode had reached a stable position in a cutaneous nerve fascicle, the skin field innervated by this fascicle was mapped by gently stroking the skin and listening to the high-pitched sound from multifiber discharges in low-threshold mechanosensitive A-fibers. Subsequently, one of two search strategies was employed for identification of C-fibers, as follows.

Mechanical search strategy. The skin within the A-fiber territory was scratched with a small wooden stick, or folds of the skin were squeezed at an intensity that was barely painful. Afferent C-fiber responses were identified by their low-frequency signal content, their long latencies, and their typical afterdischarges.

Electrical search strategy. A pointed steel electrode with a tip area of 1 mm^2 was gently pressed to the skin and single electrical pulses of 0.2 sec duration were delivered from an isolated constant voltage stimulator (Grass S48 or S8). Electrode gel was used to reduce the impedance. Stimulus strength was adjusted approximately to the pain threshold of the subject and/or to an intensity inducing but slight twitches of underlying muscles (i.e., to 60–100 V). This electrical stimulus was used to search the fascicular territory carefully, until a C-fiber response was obtained.

Intracutaneous electrical stimulation. Once the innervation territory of a C unit was found, the respective skin site was encircled with a pen and a pair of uninsulated steel needles, 0.3 mm in diameter, were inserted intracutaneously with one needle inside the innervation territory and the other placed transversely at a distance of 5–10 mm. We then used the needle electrodes for continuously stimulating this and other adjacent C units at intervals of 3–4 sec at a moderately painful intensity (10–30 V, 0.2 msec). Mechanosensitive fibers could always be stimulated electrically if the needles were properly inserted within their receptive fields, and, in addition, the intracutaneous stimulation would also excite adjacent mechanoinsensitive units.

Identification of sympathetic and afferent C units. At low frequency of intracutaneous electrical stimulation at constant intensity, the latencies of the evoked C-fiber responses were fairly stable, except for slow shifts due to posttetanic effects on a newly recruited fiber (Torebjörk and Hallin, 1974) or due to gradual changes in tissue temperature (see Fig. 8), or abrupt flip-flop shifts due to stimulation of different branches of the same parent axon (Torebjörk, 1974; Torebjörk and Hallin, 1974). However, if one of the electrically excited C units was also activated by some additional stimulus, the latency of that particular unit would increase due to conduction slowing during the relative refractory period (Torebjörk and Hallin, 1974). This has turned out to be a very sensitive "marking" of the activated units and has allowed a reliable differentiation between efferent C units firing during sympathetic reflexes and afferent C units responding to defined natural stimulation of the skin (Hallin and Torebjörk, 1974b; Torebjörk, 1974; Torebjörk and Hallin, 1976). Electrically excited C units were usually tested in the following order.

(1) *Maneuvers eliciting sympathetic reflexes.* Electrically excited C units were regularly tested for possibly being sympathetic by maneuvers known to increase greatly the skin sympathetic sudomotor and vasoconstrictor outflow in conscious man (Torebjörk and Hallin, 1970; Delius et al., 1972; Hagbarth et al., 1972). Sympathetic reflexes were provoked through loud noises unexpected for the subject, or by inciting the subject to laugh or to perform a deep inspiration. The efficiency of these maneuvers was controlled by recording massive bursts of sympathetic discharges.

(2) *Natural stimulation of the skin.* For quantitative mechanical stimulation a set of calibrated von Frey nylon filaments was used (Stoelting Co., Chicago, IL). Heat stimuli were delivered from a light bulb either while measuring the skin temperature in the receptive field with a thermistor attached to the skin, or while employing a device by which the temperature was feedback controlled from a thermocouple gently attached to the skin (Beck et al., 1974). In both cases the skin temperature was slowly increased, typically by 0.25°C/sec, from an adapting temperature of 30–32°C. Heating was stopped on demand of the subject before a level was reached at which involuntary withdrawal reflexes would have been threatening. With this method we were able to raise the local skin surface temperature to 48°C and in some cases up to 52°C.

Cold stimuli were applied by placing small pieces of ice onto the receptive field.

Several ascending series of mechanical and thermal stimuli were performed to establish thresholds and suprathreshold responsiveness of the afferent units. For insensitive units the test area was expanded to cover a circle with a radius of at least 3 cm around the stimulating electrodes inserted in the skin.

(3) *Chemical irritants.* After completing the first series of natural stimuli, the area from which the respective unit was expected to be recruited was treated with mustard oil (100% solution of allyl-isothiocyanate; Merck, Darmstadt, Germany) for 3–5 min. For this purpose pieces of filter paper soaked in mustard oil were placed on the skin and covered with a plastic film to prevent evaporation. After removing the mustard oil the treated skin area was reddened. It was then tested again with natural stimuli. Thereafter, this skin area was similarly treated with a solution containing 1% capsaicin dissolved in ethanol for 30 min, and if the recording was still stable, natural stimuli were done before the experiment was terminated. Both chemical irritants caused moderate ongoing burning pain.

Data acquisition and analysis. C unit responses to intracutaneous electrical stimulation were recorded on line by a PC computer via an interface card (DAP, Microstar, USA) using the SPIKE/SPIDI software package (Forster and Handwerker, 1990). A suitable time segment of the recording following each electrical stimulus pulse was displayed and subsequent traces were written from top to bottom on the computer screen for on-line assessment of latency shifts of the activated C units (see Fig. 1). In addition, the recordings were stored on hard disk for off-line analysis. Prior to final off-line evaluation, a digital filter squaring the data points was applied, resulting in an enlargement of the spike-to-noise ratio. By that means the spikes were enhanced and the lower noise peaks attenuated. In addition, the program provides a facility to eliminate background noise not surpassing predetermined levels. In most figures only events are shown that surpassed preselected threshold levels marked by dotted lines in the specimen records. After such signal treatment, a careful reevaluation and final assessment was made regarding the identity and responsiveness of all stable C units that were reliably discriminated from the noise.

Results

Sample of C units

One hundred ninety-four C units were analyzed. Of these, 118 units were found with the mechanical and 76 units with the electrical search strategy. Conduction velocities ranged from 0.44 to 1.4 m/sec. The triphasic, mainly negative action potentials with durations of the order of 2 msec provide further evidence that recordings were obtained from C fibers.

Mechanoresponsive units

Seventy-five percent of the units out of the sample collected with the mechanical search technique and 58% of the units encountered by searching electrically were mechanically excited by stimulation with von Frey filaments up to 1.2 N. Figure 1 shows an example of three units recorded simultaneously. Whereas the unit labeled *a* was insensitive to mechanical (and heat) stimuli, units *b* and *c* were activated from the same skin site. Unit *b* had a lower mechanical threshold and was activated by a von Frey filament exerting a pressure of 15 mN, whereas unit *c* required a stronger filament to become excited. Both units showed graded responses to suprathreshold stimuli in the noxious intensity range, as seen by the pronounced conduction delays following stimulation with a rather stiff von Frey filament of 2.6 N.

The distribution of mechanical thresholds of 80 units activated by stimulation with von Frey filaments is represented in Figure 2.
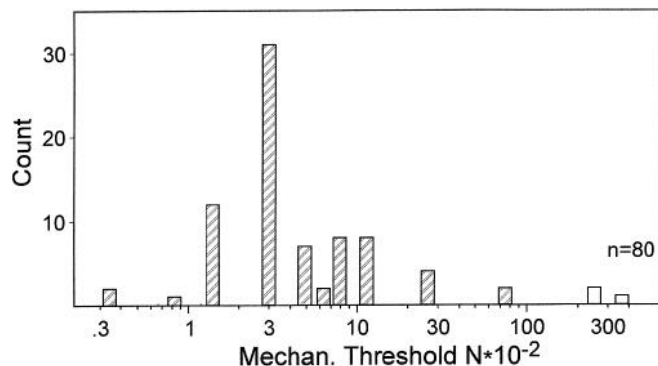
CMH and CM units

Mechanically responsive C units were subdivided in CMH units being also heat sensitive, and in CM units that were not excited
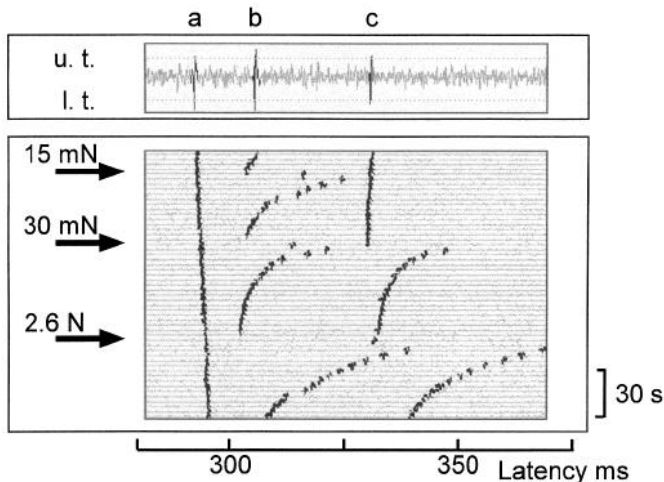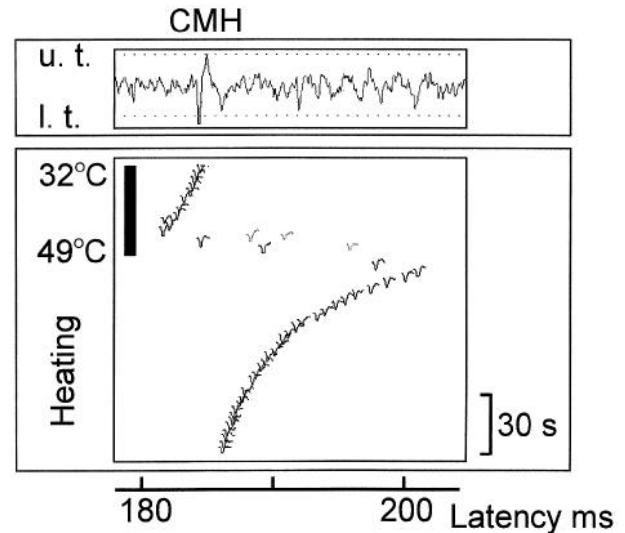
Figure 1. The top panel shows responses of C units to intracutaneous electrical stimulation. Three units (labeled a, b, and c) that exceed the upper (u.t.) and/or lower (l.t.) trigger levels are highlighted. The bottom panel shows, from top to bottom, successive recordings of the same units during electrical stimulation at 3 sec intervals (time scale to the right). Unit a did not respond to mechanical probing with different von Frey filaments (marked by arrows to the left). Unit b responded to stimuli of 15 mN and stronger, as indicated by marked increases of latency after each activation. Unit c responded to 30 mN and stronger stimuli. The latency after the electric shocks is indicated below.
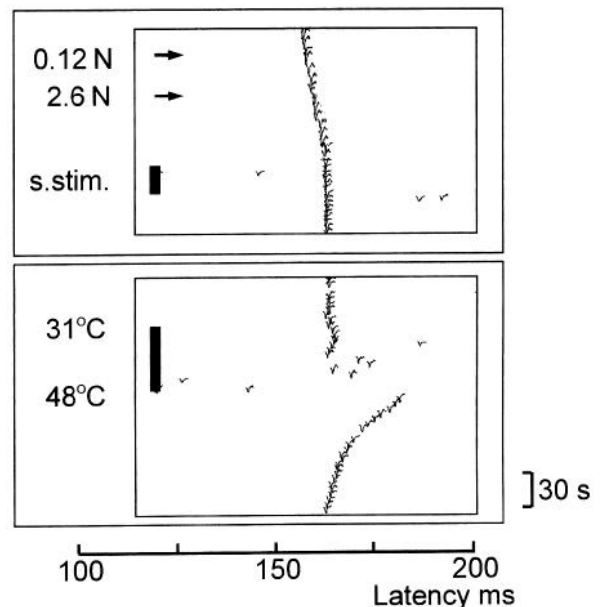

Figure 3. Mechano-heat–sensitive C unit (CMH) stimulated by heating the skin from 32°C to 49°C at 0.25°C/sec (thick vertical bar) while responding to electrical stimulation in the receptive field as shown in the top panel. Only spikes exceeding the upper (u.t.) and/or lower (l.t.) trigger levels are shown as successive recordings in the bottom panel. Note the acceleration of the conduction velocity due to warming before the unit started to respond, and then the dramatic slowing of conduction due to heat activation of the unit.

by heating up to the subject's tolerance level (see Materials and Methods). CMH units were more frequently found (n = 87) than CM units (n = 45) and accounted for about 66% of all mechanoresponsive units. The mechanical thresholds of CMH and CM units were similar. Thresholds of CMHs ranged from 3.4 to 750 mN with a median of 30 mN, thresholds of CMs from 14 mN to 360 mN, also with a median of 30 mN. Thus, there was no statistical difference between the groups. Both CMH and CM units exhibited greater increases in latency in response to suprathreshold stimulation with von Frey filaments of increasing stiffness, implying graded responses into the noxious intensity range.

An example of a CMH unit stimulated with radiant heat is shown in Figure 3. In this experiment the skin temperature was first adapted to 32°C and then raised to 49°C at 0.25°C/sec. Heating the receptive field of the CMH unit first led to a shortened conduction delay due to warming of the terminal conduc-
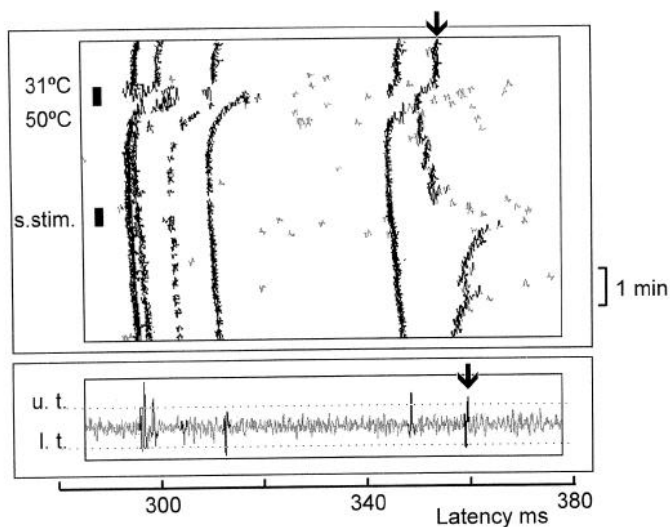
tive nerve membrane. With further increase in temperature, there was instead a pronounced increase in latency, clearly "marking" that the unit had been activated by the heat stimulus. Further examples of responses in a CMH unit are shown in Figure 7.

CH units

Six units in the entire material, and 6% of the units found with the electrical search procedure, responded to heating with

Figure 4. Heat-sensitive C unit (CH) not responding to mechanical probing with different von Frey filaments (arrows) or to sympathetic provocation (s.stim.) shown in the top panel, but activated by heating the skin from 31°C to 48°C as indicated in the bottom panel. The figure shows successive responses to electrical stimulation in the receptive field at 3 sec intervals as in Figures 1 and 3.

Figure 2. Mechanical thresholds of 80 C units activated with von Frey filaments. Force on the horizontal logarithmic scale is expressed as N × 10^{-2}. Units with mechanical thresholds exceeding 1.6 N were regarded as mechanoinsensitive (open columns).

Figure 5. *Top,* Five CMH units with latencies of 295–350 msec to intracutaneous electrical stimuli were responsive to heating the skin from 31°C to 50°C, but unaffected by sympathetic provocation tests (*s.stim.*). By contrast, a sympathetic unit (*arrow*) at a latency around 360 msec was not activated by heating as indicated by the passive shortening of latency. Instead the unit was spontaneously active in the absence of any intentional stimulus, as shown by the irregular increases in latency, and sympathetic reflex stimulation induced a very obvious response. *Bottom,* Only spikes exceeding the upper and lower trigger levels are shown.

thresholds ranging from 45° to 48°C but were not activated by mechanical stimulation even with a rather stiff von Frey filament (1.6 N). An example is shown in Figure 4. There were no latency shifts in response to sympathetic provocations for any of these units, excluding that the heat responses would have been due to sympathetic reflexes.

Sympathetic units

Twenty-one sympathetic units were identified. They constituted 11% of all units tested, and 12% of the units found with the electrical search strategy. None of them responded to mechan-
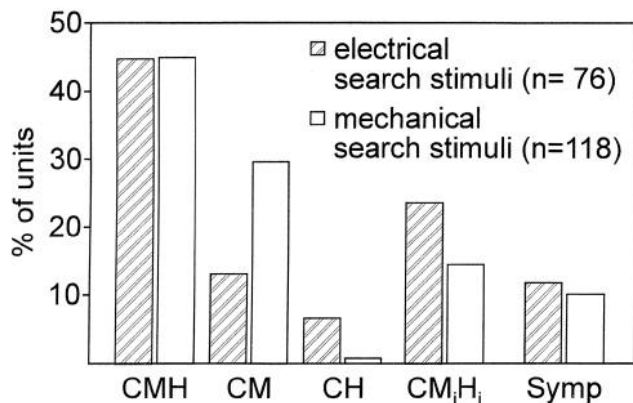
Figure 6. Proportions of C units of different classes found by the electrical (*hatched bars*) and the mechanical search strategy (*open bars*). For details, see Materials and Methods. It is obvious that the mechanical search technique favored detection of mechanosensitive units. With the less biased electrical search technique, 45% were mechano-heat sensitive (*CMH*), 13% were mechano-sensitive (*CM*), 6% were heat sensitive (*CH*), 24% were insensitive to mechanical and heat stimuli (*CM$_i$H$_i$*), and 12% were sympathetic efferent units (*Symp*).
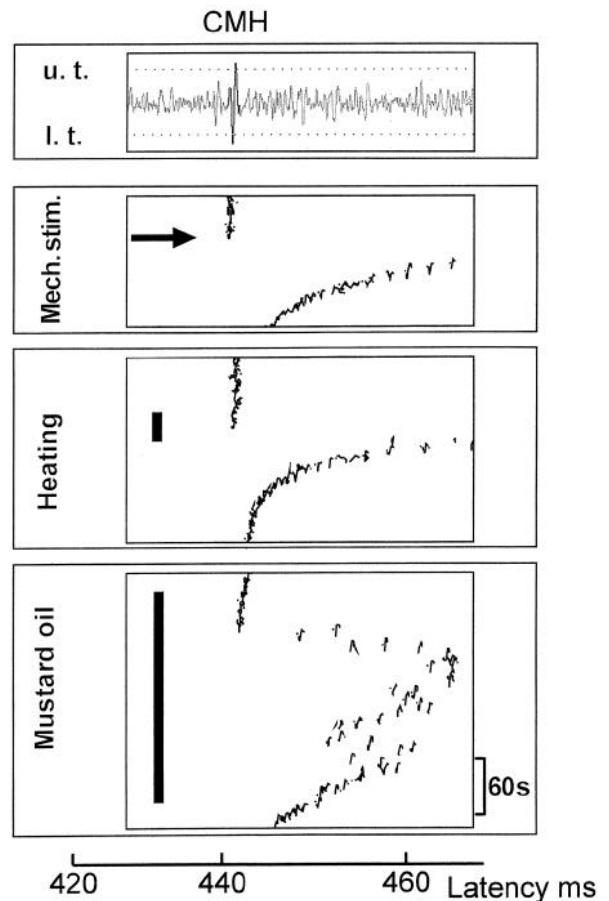
Figure 7. CMH unit (*top panel*) responding to mechanical stimuli (*second panel*) and to heating (*third panel*). Notice the irregular increases in latency of this unit during topical application of mustard oil (*bottom panel*), implying chemosensitivity.

ical or heat stimuli. In contrast to afferent C units, most of them were spontaneously active, as seen by latency increases in the absence of any intentional maneuver. An example is illustrated in Figure 5. In this figure responses of six C units to electrical intracutaneous stimulation can be distinguished and five of them could be identified as CMH units responding to radiant heat (and to mechanical) stimulation. However, the slowest unit was spontaneously active as indicated by the more variable conduction delays. This unit did not respond to heat but showed marked increases in latency during sympathetic reflex provocations. On repeated reflex provocation the sympathetic C fiber responses tended to decrease, as a sign of habituation. This was in contradistinction from responses in mechanoresponsive C units that showed no signs of fatigue if von Frey stimuli for 2 sec were repeated at intervals of 30 sec or more.

Cooling

Responses of afferent C units to cold stimuli were difficult to evaluate with the present technique. Both the direct action of the lowered temperature and the partial inactivation of the membrane in the relative refractory period after cold-induced discharges would lead to lower conduction velocities. For some CM and CMH units a low-frequency response to strongly cooling the skin to temperatures below 20°C could be neither excluded nor verified with certainty, since it was not possible to

corroborate the identity of units discharging during cooling with those excited by the electrical intracutaneous stimulation.

CM_iH_i units

With the mechanical search technique 17 units, and with the electrical search strategy 18 units, were encountered that did not respond to mechanical stimulation with von Frey filaments exerting forces up to 1.6 N, which is far beyond the threshold of CMHs described in pervious microneurographic studies (Torebjörk, 1974; Van Hees and Gybels, 1981; Adriaensen et al., 1983). These units were also insensitive to heating to levels of 48°C or higher, that is, temperatures that also surpass the thresholds of previously described human CMH units (Torebjörk, 1974; Gybels et al., 1979; Torebjörk et al., 1984). None of the units were activated by sympathetic reflex provocations. Two of these unresponsive units and one CH unit showed increased latencies during stimulation by the strongest von Frey filaments exerting forces of 2.5 and 3.6 N (see Fig. 2). However, since such strong stimulation induced movements of the intracutaneous needle electrodes, we were not sure if these latency shifts were provoked by mechanical irritation from the needles or by shifting the site of the electrical stimulation along the nerve terminals. Therefore, 1.6 N was taken as the upper limit of mechanical responsiveness in this study. The insensitive units were labeled CM_iH_i, with the subscript "i" standing for "insensitive."

Sampling bias

Figure 6 summarizes the classes of C units found with both search strategies. It is seen that mechanosensitive units were more frequent, and insensitive units were less often found with the mechanical as compared with the electrical search technique. These differences are statistically significant (χ^2, $p < 0.05$).

Conduction velocities

Mean conduction velocities of CMH units were found to be higher (0.97 m/sec) than those of all other unit types, and this difference was statistically significant (ANOVA and Newman-Keuls post hoc test, $p < 0.04$). The conduction velocities of the other unit classes were not significantly different from each other (CM, 0.84 m/sec; CH, 0.81 m/sec; CM_iH_i, 0.80 m/sec; sympathetic, 0.77 m/sec).

Excitation of C units by mustard oil

Sixty units were topically exposed to mustard oil for 3–5 min. Twenty-four of them were activated. Figure 7 shows an example of irregular increases in latency of the electrically evoked response in a CMH unit during mustard oil application.

Eleven of 19 CMH units responded to mustard oil (58%) and 5 of 14 of the CM units (36%), respectively. Seven of 22 CM_iH_i units (32%) were also excited. None of two sympathetic units tested were activated by that treatment.

Sensitization of C units by mustard oil

Twenty-five C units were tested with natural skin stimuli after application of mustard oil. Out of seven CM units tested, three units became heat responsive. One of three CH units tested was excited by mustard oil and became sensitized to mechanical stimuli afterward. In the class of CM_iH_i units, 2 of 15 tested units became responsive to heating but not to mechanical stimuli, a further unit was sensitized to mechanical stimulation but not to heat, and one unit was sensitized to both (Fig. 8). Thus,
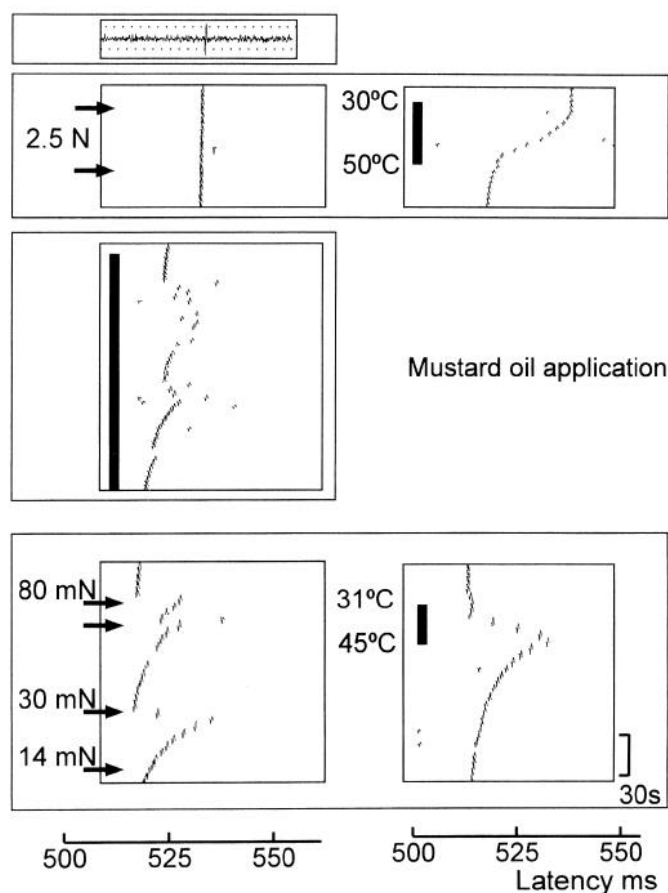

Figure 8. Sensitization of a CM_iHi unit (*top panel*), which was initially unresponsive to strong mechanical stimulation with von Frey filament up to 2.5 N and to heating from 30°C to 50°C (*second panel*). The unit was excited by topical application of mustard oil (*third panel*). After 5 min of mustard oil application, the unit was responsive to von Frey filaments down to 30 mN and to heating from 31° to 45°C (*bottom panel*). The shift in baseline latency is due to increase in skin temperature.

4 of 15 CM_iH_i units became sensitized to physical stimuli after treatment with mustard oil. Only two of these four units had been excited during application of this irritant substance.

Excitation and sensitization by capsaicin

For 15 units (of which 14 were not sensitized after mustard oil) the recordings were stable enough to allow subsequent testing with capsaicin applied topically for 30 min. Two CM units were neither excited by capsaicin nor sensitized afterward. Two CH units were not excited by capsaicin but became responsive to mechanical stimuli afterward. One CM_iH_i was excited by capsaicin but not sensitized. Of another 10 CM_iH_i units none responded to capsaicin but two units became sensitized to mechanical and heat stimuli. A third sensitized unit is shown in Figure 9. This CM_iH_i unit was not activated by mustard oil, but this treatment rendered the unit responsive to stimulation with a von Frey filament of 250 mN. Subsequent application of capsaicin possibly led to a low level of excitation. After this treatment the unit was further sensitized to mechanical stimuli and responded to heat, albeit with a rather high threshold of about 48°C.

Altogether, of 24 CM_iH_i units exposed to chemical irritants, 15 could be tested afterward. Of these 15 units, six were sen-
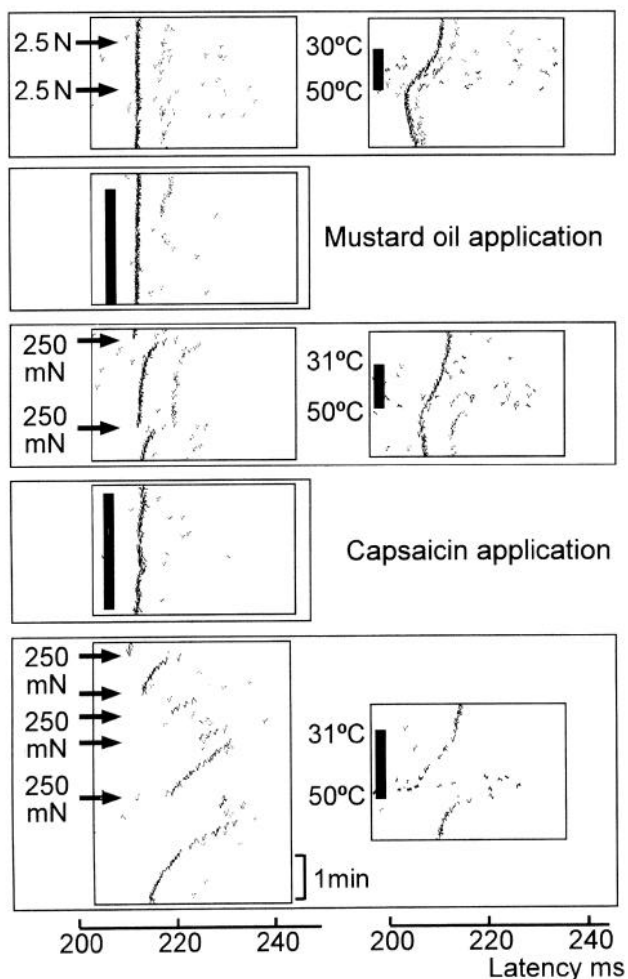
Figure 9. Sensitization of a CM_iH_i unit that did initially not respond to mechanical probing (2.5 N), heating (50°C), and mustard oil application (*top two panels*). After the latter procedure heat insensitivity was unchanged, but the unit could be activated by nylon filaments exerting forces of 250 mN (*third panel*). After capsaicin application (*fourth panel*) the unit was further sensitized to mechanical stimuli and in addition heat sensitivity developed (*bottom panel*).

sitized to natural stimuli, but only three of them were directly activated by the chemicals.

Discussion

Two decades ago, a technique was developed to differentiate electrically evoked sympathetic and afferent C-fiber responses in cutaneous human nerves (Hallin and Torebjörk, 1974b; Torebjörk and Hallin, 1974, 1976). The basis for this technique is the characteristic slowing of impulse conduction in thin fibers that is observed after repetitive firing induced either by electrical or natural skin stimulation, or by sympathetic reflex activation. The method is sensitive enough to allow detection even of one or a few extra impulses elicited by any maneuver, as indicated by a recognizable increase in the otherwise stable response latency to electrical pulses delivered at low frequency from needle electrodes inserted into the skin (Torebjörk and Hallin, 1974). This technique has the advantage of providing definite information on the identity of the units that are activated by sympathetic reflexes or various types of natural skin stimuli. Furthermore, it can be used for detailed mapping of the receptive

area of afferent units, for estimation of response thresholds, and for semiquantitative evaluation of suprathreshold response magnitude. Since C-fibers tend to aggregate and remain as neighbors for considerable distances in the human peroneal nerve (Jørum et al., 1989), several C-fibers with slightly different conduction velocities can be identified in each recording site, even if they have similar spike forms. Computer processing using dedicated software (Forster and Handwerker, 1990) greatly facilitates on-line and off-line analysis. As shown in this report, this technique has opened up the possibility of collecting large C unit samples in humans.

It has long been recognized that searching and identification of cutaneous afferent units by probing mechanically may have induced a sampling bias (Handwerker and Kobal, 1993). Even though we identified only one C unit by mechanical searching in each recording site, and then also analyzed any adjacent unit that was electrically excited from the surrounding skin area, it was obvious that this search strategy induced a considerable bias in favor of mechanosensitive C units (Fig. 6). However, searching with electrical stimuli delivered from a pointed probe that is systematically moved on the skin is not without bias either. This strategy will clearly favor detection of fibers with many widespread terminal branches in the skin, whereas fibers with just one or a few closely apposed endings are easily missed. This may be one explanation of why we have not encountered any warmth-specific C unit in our present material, since these units typically have small spot-like receptive fields in human skin (Hensel, 1976; Hallin et al., 1982). Like for efferent fibers, afferent axons with deep endings are not likely to be recruited by this superficial search strategy. Thus, the proportions of different unit classes discussed here are only tentative, and we realize that these proportions may vary depending on different experimental approaches.

About 12% of the C units were identified as efferent sympathetic by their spontaneous activity and by their participation in sympathetic reflex discharges. This may be an underestimation of the proportion of sympathetic fibers in cutaneous fascicles of the peroneal nerve. We could only identify efferent fibers that were spontaneously active or excited by the maneuvers used to elicit sympathetic reflexes, that is, sudomotor and vasoconstrictor units (Jänig et al., 1983). Other types, like piloerector and vasodilator units, have no spontaneous activity (at least not in the anesthetized cat; Jänig et al., 1983) and may be activated only during specific functional states of the organism, for example, during severe exposure to cold, hypoxia, or special emotional states (Jänig, 1984). Such silent sympathetic fibers would obviously not have been recognized in our experiments; they would remain unidentified, and classified as insensitive (see below).

It is unlikely that some of the units that we classified as mechanoresponsive and/or heat responsive were in fact sympathetic fibers. This is because sympathetic fiber endings did not respond directly to the stimuli we used. Furthermore, many of the units had activation thresholds well below pain thresholds, and hence their activation would not be likely to elicit strong sympathetic reflexes. This is particular true for von Frey stimulation, which readily activates C nociceptors below pain threshold (Van Hees and Gybels, 1981). Finally, sympathetic reflex responses decreased (habituated) with repetition (Hallin and Torebjörk, 1974a), a phenomenon not observed for the responses of the afferent C units reported here, when stimuli were applied at intervals exceeding 30 sec.

Not surprisingly, the majority of all C-fibers (about 45%) were classified as CMH, that is, the conventional polymodal nociceptor type. Their mechanical thresholds, as measured during electrical stimulation, were in the same range as in other studies using conventional estimations (Torebjörk, 1974; Van Hees and Gybels, 1981; Adriaensen et al., 1983), suggesting that the electrical stimulation per se did not influence thresholds to any considerable degree. With the electrical search strategy, another 13% responded to mechanical but not to heat stimuli. Their mechanical thresholds spanned over a wide range and were not significantly different from the thresholds of CMH units. Furthermore, most of the CM units responded in a graded fashion to suprathreshold stimuli in the noxious intensity range, supporting the conclusion that many of them could be regarded as nociceptors. Little if any attention has been paid to this class of human nociceptors in the past. Yet, we cannot exclude that some CM units were actually low-threshold C mechanoreceptors of the type commonly encountered in hairy skin in the cat (Iggo, 1960) and less commonly found only in proximal parts of the extremities in the monkey (Kumazawa and Perl, 1977) and in the human forehead (Nordin, 1990). A typical feature of these low-threshold C mechanoreceptors is their responsiveness to cooling. As judged from the acoustically monitored discharges, no low-threshold cold responsive units were encountered in the present sample. However, responsiveness to this stimulus modality was not systematically studied, since lowering of temperature by itself and the repetitive firing caused by cooling both would have resulted in slowing of impulse conduction, and hence we had difficulties in differentiating these effects with our technique.

Of the remaining units identified with electrical search stimuli, 6% responded to heating, but not to mechanical stimulation. This type of CH unit has been reported before in animals, albeit rarely (Georgopoulos, 1976; Welk et al., 1984; Baumann et al., 1991), but it was not yet known that CH nociceptors also exist in human skin.

Almost 24% of the units found with the electrical search strategy, and still about 14% of the units encountered with the mechanical search technique, were insensitive to mechanical and heating stimuli. They were labeled CM_iH_i as suggested in a recent review (Handwerker and Kobal, 1993). Even though there are differences in search techniques and classification criteria this proportion is in a similar range as the 30% insensitive C units that has been found in the hairy skin in monkey (Meyer et al., 1991) and the 26% recorded *in vivo* and the 15% found *in vitro* in the hairy skin in rat (Kress et al., 1992). These units may have been either efferent or afferent. However, silent and unresponsive postganglionic sympathetic units probably constitute at best a minor part of the CM_iH_i population, since the proportion of efferent C-fibers is generally much lower than that of afferent ones in nerves supplying hairy skin in mammals (Baron et al., 1988) and the piloerector and vasodilator supply is less numerous than the sudomotor and vasoconstrictor supply (Jänig, 1984). It is also conceivable that the needle electrodes in the skin excited some afferent axons at a distance from their receptive endings, and if the receptive area was not properly tested, then these units would remain "unresponsive." However, we tried to reduce this possibility by always testing large regions of skin around the stimulating electrodes when insensitive units were encountered.

The most striking argument in favor of the afferent nature of most CM_iH_i units is provided by the fairly high percentage of them that were activated by irritant substances. About one-third were excited by mustard oil, and these units therefore can be regarded as chemonociceptors. However, chemonociception is probably mediated also by other unit types, since about one-third of CM and CH units and almost 60% of CMH units were sensitive to mustard oil. Anecdotal observations on insensitive C units activated by capsaicin have been made in humans (LaMotte et al., 1992), but this is the first documentation that chemosensitive CM_iH_i nociceptors constitute a substantial proportion of afferent C units in human skin.

It may be of interest that excitation of C-fibers by chemical irritants and sensitization to subsequent heating or mechanical stimulation were not always corresponding. A simple explanation would be that excitation needs a more profound depolarization of the receptive nerve membrane than sensitization. However, occasionally CM_iH_i units were found to be excited but not sensitized. This might hint that excitation and sensitization of afferent C-fibers by chemical irritants could depend on different membrane processes.

Mustard oil and capsaicin are known to induce marked primary hyperalgesia to heating and also to mechanical pressure in the treated skin area (Jancsó et al., 1967; Simone et al., 1987; Szolcsanyi, 1988; Culp et al., 1989; Koltzenburg et al., 1992). Capsaicin-induced hyperalgesia to heat correlates with sensitization of CMH nociceptors to heating in human skin (Konietzny and Hensel, 1983; Handwerker et al., 1991a; LaMotte et al., 1992). Evidently, sensitization to heat can occur in CM and CM_iH_i units as well, implying that these units can also contribute to primary heat hyperalgesia after chemical irritation of the skin.

The evidence for a contribution of C-fibers to experimentally induced primary mechanical hyperalgesia in humans has been mainly derived indirectly from psychophysical experiments utilizing differential nerve blocks (Koltzenburg et al., 1992), whereas direct proof of C-fiber sensitization to von Frey filament stimulation in microneurography experiments was either absent or unconvincing (Handwerker et al., 1991a; LaMotte et al., 1992). However, previous studies have focused on the increased number of spikes in CMH units to a given mechanical stimulus. Only anecdotal observations on insensitive C units becoming sensitized to mechanical stimuli after capsaicin treatment have been reported in human (LaMotte et al., 1992). As shown here, some of the mechanically insensitive CH and CM_iH_i units did indeed become mechanoresponsive after chemical irritation, as has also been observed in the skin of rat (Kress et al., 1992) and monkey (Davis et al., 1993). Such sensitization may contribute to primary hyperalgesia to static pressure after topical application of capsaicin and mustard oil (Culp et al., 1989; Koltzenburg et al., 1992; Kilo et al., 1994).

Thus, our study contributes the proof of recruitment of further, previously silent units into the sensitized state in humans. While sensitization of previously responsive units leads to temporal summation of the nociceptive input on central neurons, the recruitment of previously silent units will add a component of spatial summation (Handwerker and Reeh, 1991; Kress et al., 1992). Our results indicate that spatial summation from newly recruited sensitized C nociceptors can contribute to primary heat and mechanical hyperalgesia due to chemical irritants. Furthermore, it is conceivable that different classes of nociceptive units may have different central connections and may release different types and amounts of neurotransmitters. For instance, it has been shown that the neuropeptide release in the spinal cord differs in normal versus arthritic cats (Schaible

et al., 1990). Thus, it is possible that the recruitment of previously unresponsive nociceptors may cause or enhance central sensitization that may express itself as various forms of secondary hyperalgesia (LaMotte et al., 1991). Our demonstration of the existence of chemonociceptors in human skin adds support for this theory.

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