UNRESPONSIVE AFFERENT NERVE FIBRES IN THE SURAL NERVE OF THE RAT

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SUMMARY

1. The proportion of primary afferent nerve fibres in a skin nerve of the rat that responded or failed to respond to mechanical or thermal stimulation of the skin in the noxious and non-noxious range was analysed.

2. Activity of afferent nerve fibres was recorded from the dorsal roots. Units projecting into the sural nerve were selected using supramaximal electrical stimulation of the nerve stem. All other hindleg nerves were cut.

3. The receptive fields were searched by carefully examining the hindleg skin with noxious and innocuous mechanical, cooling and warming stimuli. Probing of the intrinsic foot muscles and manipulation of the ankle and toe joints was employed to recruit units projecting to deeper tissues.

4. In a first series of twenty-two experiments, eighty-nine rapidly conducting myelinated $A\beta$ units, thirty slowly conducting myelinated $A\delta$ units and 101 unmyelinated C units were investigated. Most units were identified as belonging to one of the established classes of cutaneous sensory units and this was also ascertained by a collision test.

5. Two $A\beta$, eight $A\delta$ and forty-six C fibres did not respond to any one of the stimuli. Electrical thresholds and conduction velocities of the unresponsive C fibres were not significantly different from those of the units responding to natural stimulation of their receptive fields. In the $A\delta$ group unresponsive and high threshold mechanoreceptive units were preferentially found among the units with the slowest conduction velocities.

6. In a second series of seven experiments, one single nerve filament containing responsive and unresponsive C fibres was tested repetitively at 30 min intervals. Twenty unresponsive units and seven units responding to noxious mechanical and/or heat stimuli were studied. Ten of the twenty initially unresponsive units became activated by mechanical and/or heat stimuli after observation times of 30–150 min. Some of these units had mechanical thresholds as low as 64 mN (tested with calibrated von Frey hairs), or thermal thresholds down to 42 °C.

7. Two of the ten C units which became responsive in the course of an experiment later lost their responsiveness again. On the other hand, two of the C units which were initially responsive to noxious heat and/or noxious mechanical stimuli became completely unresponsive after repetitive stimulation, whereas one unit initially only

responding to noxious heat became responsive to mechanical stimuli, suggesting that mechanical and heat responsiveness may be separately gained or lost by sensory C fibres.

8. We conclude that a large proportion of afferent C and Aδ units innervating the hairy skin of rat are insensitive to acute physical stimuli under physiological conditions. At least some of these units may be sensitized under more long-lasting perturbations of the tissue, notably in inflammation, and thus may contribute to inflammatory pain.

INTRODUCTION

The receptive properties of primary afferent units in the skin of mammals have been analysed in numerous classical studies (for review see Burgess & Perl, 1973; Iggo, 1977). From these studies the cutaneous afferents have been categorized into well defined receptor or 'sensor' classes which are remarkably similar in different species including man (Vallbo, Hagbarth, Torebjörk & Wallin, 1979). A large sub-population of the afferent nerve fibres, mainly characterized by slow conduction velocities, have been regarded as nociceptors, since their responses obviously code the intensity of painful stimuli. For example, evidence was provided that the discharge rates of mechano-heat-sensitive unmyelinated nociceptors (CMH units) may code the intensity of pain induced by skin heating (Gybels, Handwerker & van Hees, 1979), and also to some extent the hyperalgesia following heat trauma (LaMotte, Thalhammer, Torebjörk & Robinson, 1982; LaMotte, Thalhammer & Robinson, 1983; Torebjörk, LaMotte & Robinson, 1984). Hence, CMH units and their myelinated counterparts, the AMH units (Meyer & Campbell, 1981), were considered to constitute the peripheral nociceptive elements mediating cutaneous pain and hyperalgesia. However, recent studies on the afferent innervation of the cat's knee joint (Schaible & Schmidt, 1988a, b) and urinary bladder (Häbler, Jänig & Koltzenburg, 1990) provided evidence that a hithertho unknown class of small fibre afferents may contribute to pain and hyperalgesia, namely those which under physiological conditions are not responsive even to noxious stimulation, but become activated in inflammation. This raises the question whether such afferents may also be present amongst the cutaneous afferents. In the past those units may have been missed due to the restricted range of search stimuli used. Recently, unresponsive cutaneous afferent units have been identified in the primate among the slowly conducting myelinated (Aδ) fibres by employing transcutaneous electrical search stimuli (Davies, Meyer, Cohen & Campbell, 1989).

The aim of this study was to search for afferent units which do not belong to one of the established sensor classes in the hairy skin of the rat. To recruit an unbiased sample out of all afferent nerve fibres in the sural nerve, single-unit recordings were performed from dorsal roots and the conduction velocity of these units was assessed from the conduction delay after electrical stimulation of this nerve. When a single unit was identified we carefully searched for its receptive field with mechanical, heat and cold stimuli which have previously been established to excite all known sensor classes, such as mechanoreceptors, warm or cold sensors, and different types of nociceptors.

Some of these data have recently been published in abstract form (Handwerker, Kilo & Reeh, 1989).

<div align="center">METHODS</div>

Thirty adult Wistar rats weighing 300–500 g were used for this study. The animals were anaesthetized with a barbiturate injected intraperitoneally (ten animals with 120 mg kg^{-1} thiobutabarbitone, fifteen with 100 mg kg^{-1} thiopentone and five with 60 mg/kg pentobarbitone). To reduce mucus secretion the animals received 20 mg N-butylscopolamine. The trachea and the left jugular vein were cannulated for artificial respiration and for injection of all further drugs. Additional doses of the barbiturate were given, if needed, to keep the animal in an areflexive state.

The left sural nerve was separated from the connective tissue in the popliteal fossa and exposed in a pool filled with warm paraffin oil. All other branches of the sciatic nerve and the femoral nerve innervating skin and muscles of the hindlimb were cut. A laminectomy was performed between T12 and L2. The dorsal roots L2–L6 were exposed and covered with warm paraffin oil. The animal's left hind paw was fixed to keep the lateral and plantar sides upwards.

Throughout the experiment electrocardiogram (ECG), ventilation pressure and end-tidal CO_2 were monitored. The body core temperature was measured with a rectal thermometer and kept between 36·5 and 37·5 °C by feedback-controlled infrared radiation. After completion of the surgery the animals were paralysed with pancuronium bromide (2 mg, given I.V.) and artificially ventilated keeping the end-tidal CO_2 between 35 and 45 mmHg. Supplementary doses of the anaesthetics (1/8 of the initial dose) were given I.V. at 2 h intervals, or when the animal reacted with heart rate acceleration to noxious stimulation. Pancuronium was supplemented as needed (0·5 mg, given I.V.).

The sural nerve was electrically stimulated with constant voltage pulses of 0·2 ms duration delivered from an isolation unit (Digitimer, Herts) via bipolar platinum-wire electrodes. Stimuli of 0·05–3 V were applied for searching for myelinated (A) fibres and 20 V stimuli for unmyelinated (C) units or slowly conducting myelinated (Aδ) units with high electrical thresholds. To prevent current spread to the adjacent stumps of the cut hindlimb nerves, a ground electrode was attached to the sural nerve proximal to the stimulating electrodes.

The dorsal root in which the largest compound action potential was elicited by sural nerve stimulation, and its neighbouring roots, were chosen for single unit dissection using a microscope (25 ×). Fine strands were dissected from the roots with sharpened watchmaker forceps and placed on a platinum-wire electrode for monopolar recording with the reference electrode positioned nearby. Unitary action potentials were recorded with an AC-coupled amplifier and monitored on an oscilloscope screen and by a loudspeaker. For further analysis of the spike form and the conduction delay of the responses to nerve simulation the recordings were digitized (12 bit AD converter, 25 kHz sampling rate) and processed in an AT type computer. The 'SPIKE/SPIDI' software package was used which was developed in our department and previously described in detail (Forster & Handwerker, 1990).

Generally, filaments containing not more than five single units having at least an action potential size of four times the average noise level were included in this study, but occasionally the conduction delays of up to seven C units were so clearly different as to allow inclusion of these larger filaments. The conduction velocities (CVs) computed from the conduction delay and the distance between stimulating electrode and recording site at the dorsal root were in the same range as those found in a previous study on afferent units in a rat peripheral nerve (Handwerker, Anton & Reeh, 1987). In agreement with this and other previous studies in the rat, units with CVs greater than 19·9 m s^{-1} were considered to be Aβ units, those with 2·2–18·3 ms s^{-1} Aδ units, and those with 0·4–1·9 m s^{-1} C units.

Receptive fields were searched by systematically probing the skin innervated by the sural nerve (Swett & Woolf, 1985) and adjacent skin areas. Brushing and tapping stimuli were used for identifying the Aβ mechanoreceptor units. Vibration units (Pacinian corpuscles) were identified by applying vibration with a tuning fork. The thresholds and the location of the receptive fields of mechanoreceptors were determined with calibrated von Frey hairs. Search stimuli for high threshold Aδ and C fibre sensors consisted of pinching skin folds with blunt forceps. When a unit was excited by this kind of stimulus its receptive field was further determined with calibrated von Frey bristles exerting forces up to 362 mN or with pointed glass rods.

Thermal search stimuli were only used if the unit under study did not respond to these

mechanical stimuli. For this purpose small pieces of ice were placed on the skin for periods up to 30 s. Heat ramp stimuli controlled via a thermocouple (Beck, Handwerker & Zimmermann, 1974) were systematically applied to all parts of the skin innervated by the sural nerve and to adjacent skin fields. Each such heat stimulus started from an adaptation temperature of 32 °C linearly
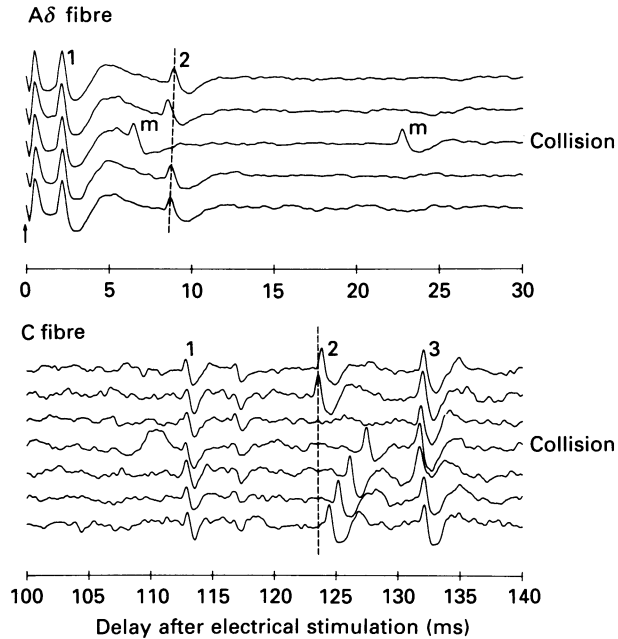

Fig. 1. Identification of units with receptive fields for mechanical stimulation with the 'collision' method. In the upper part of the figure spike responses of two myelinated units are shown. The traces represent successive recordings during electrical stimulation of the nerve trunk at 3 s intervals. The two units are labelled '1' and '2' in the first trace. The stimulus artifact is marked by an arrow. In the third trace marked 'collision', the electrically evoked action potential (AP) of unit 2 is occluded by a preceding AP marked 'm' which was evoked by mechanical stimulation of the receptive field. The conduction delay of unit 2 is indicated by a dashed line. In the lower part of the figure a similar occlusion is shown in the case of an unmyelinated unit. In this filament three C units were studied which are labelled in the first trace. One C unit with a conduction delay of 117 ms was considered too small for an analysis. The electrically evoked AP of unit 2 is occluded in the third trace by preceding activity induced by mechanical stimulation of the receptive field which is not shown in the figure (marked 'collision'). Since the relative refractory period of C fibres lasts many seconds, the conduction velocity of unit 2 is slowed in the following traces. It returns slowly to the initial value marked by a dashed line.

raising the skin temperature to 52 °C at a rate of 1 °C s^{-1}. The same heat stimulus was used to test C and Aδ units for heat sensitivity when their receptive fields had been found with mechanical search stimuli.

Since the sural nerve of the rat contains a few motoraxons and presumably also some muscle afferents from musculi lumbricales, flexor digiti quinti brevis and abductor digiti quinti (Nakanishi & Norris, 1970; Harrison, 1975), we tried to identify those units by deeply probing the intrinsic foot muscles and by twisting and by extreme extension of the ankle joint and of the joints of the toes.

To prove the identity of the units driven by electrical and 'natural' stimulation the electrically induced action potentials were conditioned with those induced by stimulation of the respective receptive field. Whenever an electrical stimulus happened to be delivered during the absolute or relative refractory period of a nerve fibre, its electrically induced action potential was occluded or delayed (Iggo, 1958). Figure 1 shows examples of this 'collision' in an $A\delta$ and in a C unit which were found responsive to mechanical stimuli.

In the course of long-lasting experiments sometimes all afferent units suddenly lost their responsiveness to natural stimuli while their axons were still excited by electrical stimulation of the nerve, probably due to a deterioration of the blood circulation in the skin. Since under normal conditions virtually all $A\beta$ fibres were easily identified as low threshold mechanoreceptor units (see Results), in such case of a suspected loss of excitability we prepared somewhat larger filaments containing more than one A fibre unit. The experiment was terminated when filaments with more than one unresponsive $A\beta$ fibre were encountered.

In twenty-two experiments we tried to assess the proportion of responsive and unresponsive afferent units in the sural nerve. In a further seven experiments, only one filament containing several responsive and unresponsive units was observed over an extended period while the skin was probed at 30 min intervals, to find out whether the units would change their responsiveness in the course of several hours. In two of these experiments the skin was painted with 50 mg arachidonic acid dissolved in 25 μl xylol after the initial assessment of the filament.

RESULTS

Sample of units and receptive fields

In the first series of twenty-two experiments 89 $A\beta$, 30 $A\delta$ and 101 C fibres projecting into the sural nerve were studied in dorsal root filaments. Receptive fields were found for 87 $A\beta$, 22 $A\delta$, and 54 C units (Fig. 2). Generally, the distribution of the receptive fields corresponded to the skin area innervated by the sural nerve as described previously (Swett & Woolf, 1985; Fig. 2).

However, even among the low threshold mechanoreceptors we found some units with receptive fields on the calf medial to the mid-line and in the plantar glabrous skin, i.e. in regions which are generally not considered to be innervated by the sural nerve (Swett & Woolf, 1985). In addition, three of the $A\beta$ units apparently did not respond to skin stimulation but to probing of deeper tissues.

The proportion of the units in different sensor classes is shown in Table 1.

Among the $A\beta$ units the rapidly adapting hair follicle mechanoreceptors were most frequent, in agreement with a previous study on the hairy skin of rats (Lynn & Carpenter, 1982). About half of the $A\delta$ units also had hair follicle sensors; the remaining units were high threshold mechanoreceptors (HTM). One HTM $A\delta$ unit also responded to cooling with ice at temperatures below 10 °C.

The most frequent type of unmyelinated unit only responded to mechanical stimuli (CM). This is at variance with previous studies on C fibre sensors in the saphenous nerve of the rat in which the mechano-heat (CMH) or 'polymodal' nociceptors were most frequently found (Lynn & Carpenter, 1982; Fleischer, Handwerker & Joukhadar, 1983; Fig. 3). This difference may be explained by the fact that units with very high mechanical thresholds and subcutaneous units are included in the present sample. Three CMH units and six CM units had mechanical thresholds exceeding the 362 mN of our strongest von Frey hair (see Fig. 3).

Figure 4 shows the distribution of the mechanical thresholds of $A\delta$ and C fibres. In both fibre classes we found units with low mechanical thresholds which were in the case of the $A\delta$ units of the 'down hair' (D hair) type (Brown & Iggo, 1967; Lynn &
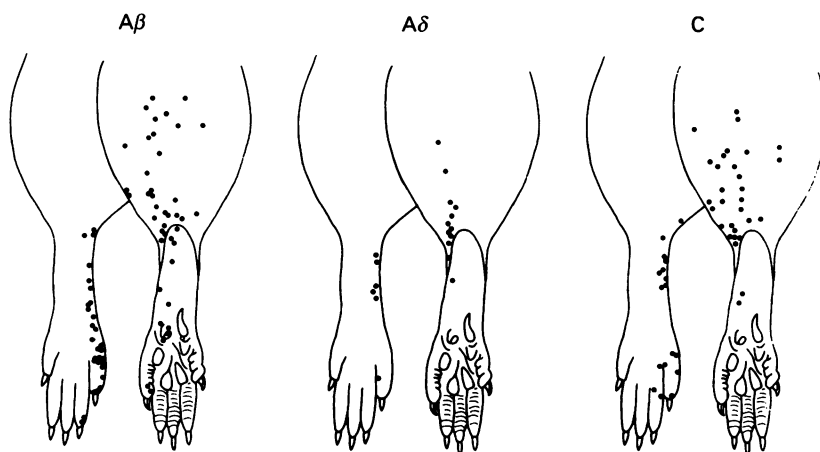
Aβ   Aδ   C

Fig. 2. Distribution of the centres of the receptive fields of Aβ, Aδ and C units.

TABLE 1. Numbers and properties of units of different sensor classes

No. of units	Sensor type	Conduction velocity (m s^{-1})	Mechanical threshold (mN)
Aβ units (n = 89)			
43	HF/RA	20·0–58·8	1·0–8·0
30	SA	26·0–55·6	1·0–45·3
2	PC	44·7/52·8	1·0/22·0
12	**	25·0–80·0	**
2	—	36·4/39·9	—
Aδ units (n = 30)			
9	HF	10·7–17·0	1·0–5·7
10	HTM	3·2–18·3	32·0–128·0
1	MC	13·2	*
2	**	4·0–8·0	**
8	—	2·2–13·19	—
C units (n = 101)			
18	M	0·6–1·55	1·0–362·0
6	M	0·53–0·95	*
10	MH	0·6–0·9	45·0–362·0
3	MH	0·64–1·10	*
2	heat	0·64/0·68	—
8	cold	0·72–1·06	—
1	MC	1·32	*
7	**	0·57–1·08	**
46	—	0·44–1·9	—

Fibre types: HF/RA, hair follicle or rapidly adapting units; SA, slowly adapting units; PC, Pacinian units; HTM, high threshold mechanoreceptive units; M, mechanoreceptive units; MH, mechano-heat units; MC, mechano-cold units.

** Units with receptive fields in positions where the threshold could not be specified correctly for technical reasons (see Methods section).   * Units which did not respond to stimulation with the calibrated von Frey hairs (up to 362 mN) but to squeezing of the skin with a blunt forceps or to strong pressure with a pointed glass rod.
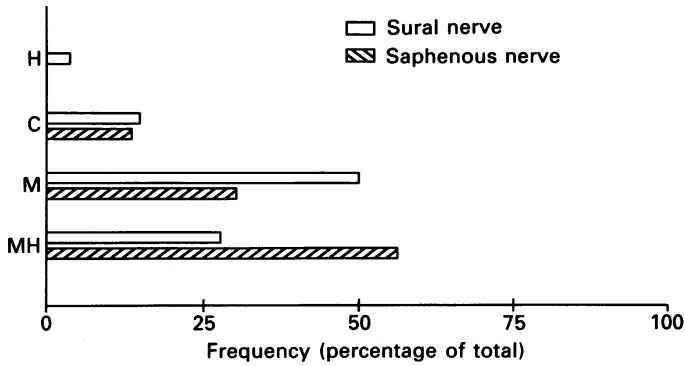
Fig. 3. Frequency of C fibre sensor types in the present study (open bar) and in a previous study in which the units were recorded from the saphenous nerve (hatched bar; Fleischer *et al.* 1983). H, purely heat sensitive units; C, cold units; M, purely mechanoreceptive units (CM); MH, mechano-heat sensitive units (CMH) or polymodal nociceptors.
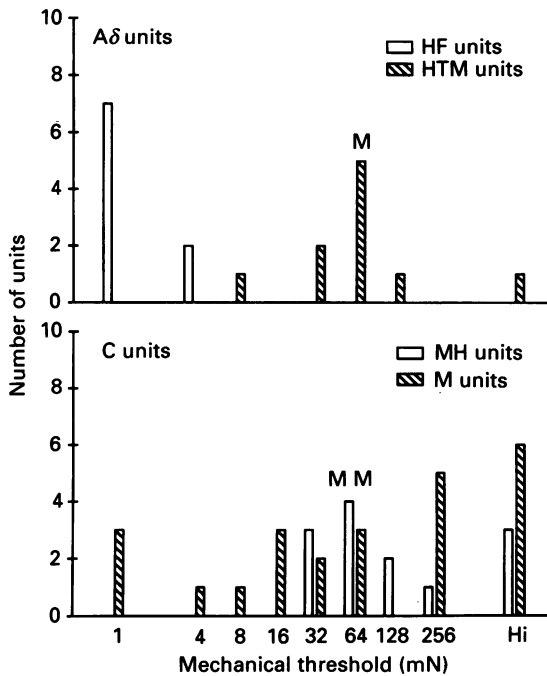

Fig. 4. Mechanical thresholds of the different types of mechanoreceptive Aδ and C units. The figures at the abscissa indicate forces exerted by calibrated von Frey hairs. The category '256' also comprises units responding to a von Frey hair calibrated to 362 mN. Hi, units with high thresholds which were only excited by squeezing of the skin with forceps, or pressure exerted by a blunt glass rod.
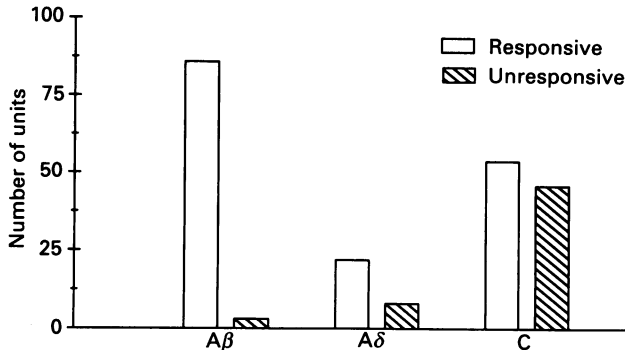
Fig. 5. Proportion of responsive and unresponsive units in the fibre classes with different conduction velocities.
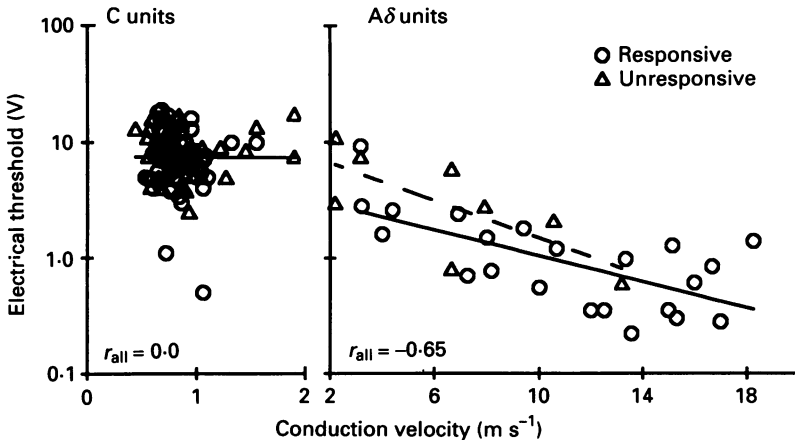

Fig. 6. Relationship between conduction velocities and electrical thresholds in the C and Aδ fibres. The continuous lines show the linear regressions for all C units and for the responsive Aδ units; the dashed line shows the respective regression line for the unresponsive Aδ units. The correlation coefficients (r_{all}) were computed for the total sample in both fibre classes.

Carpenter, 1982). The four low threshold C units apparently belonged to the group of the low threshold C-mechanoreceptor units (C-LTM) described previously (Iggo and Kornhuber, 1977; Lynn & Carpenter, 1982).

The HTM Aδ units, the mechanoreceptive C and the CMH units had median thresholds of 64 mN, similar to those found previously (Fleischer *et al.* 1983).

Proportion of unresponsive units

Figure 5 shows the proportion of unresponsive and responsive units in each fibre class. Two Aβ, eight Aδ and forty-six C fibres did not respond to any of the search

stimuli. The proportion of unresponsive units did not significantly change when only the first filament encountered in an experiment was taken into account (χ^2 test).

Current spread from the stimulating electrodes at the sural nerve to the central stumps of the other cut hindleg nerves would have resulted in falsely unresponsive
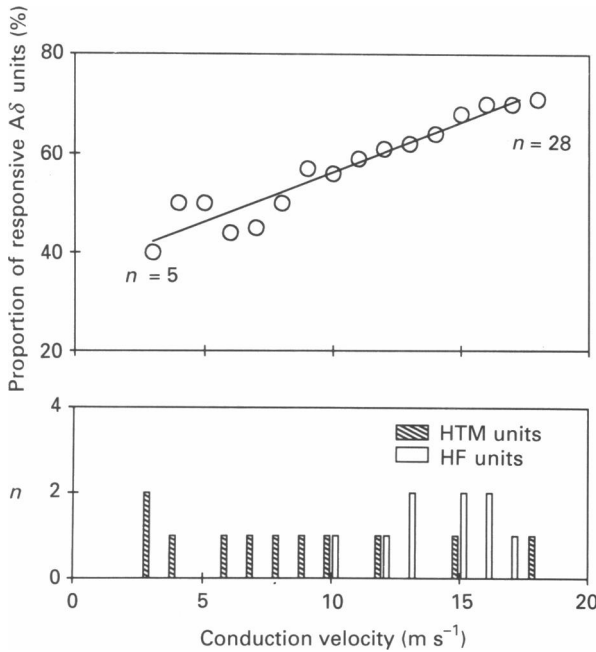

Fig. 7. Upper diagram, cumulative diagram of the proportion of responsive Aδ units in relation to the conduction velocity. Each point in the diagram represents this proportion with all the units up to the respective conduction velocity taken into account. Lower diagram, conduction velocities of the responsive Aδ units. Two of the thirty Aδ units from Table 1 which were responsive to mechanical stimuli but had receptive fields too close to the pool ring to be properly assessed have been omitted.

units. We tried to exclude this source of error by grounding the sural nerve proximal to the stimulating electrodes. In addition, the electrical thresholds of the unresponsive and the responsive unit populations were carefully compared. Figure 6 shows the relationship between electrical thresholds and conduction velocities in the slowly conducting fibre classes. In the C fibre group electrical thresholds were always less than 20 V and there was no correlation between electrical thresholds and CVs. No significant difference between responsive and unresponsive units was found.

Not surprisingly, in the Aδ group the electrical thresholds became lower with increasing conduction velocity and the electrical thresholds of the slowest Aδ units were in the range of those of the C fibres. Interestingly, high threshold mechanoreceptive (HTM) and unresponsive units were found preferentially among the units with the lower CVs, whereas the D hair units were predominant among the faster-conducting Aδ units. The relationship between CV and sensor type, and the

respective relationship between CV and responsiveness in the Aδ group, are shown in Fig. 7.

Changes in responsiveness observed in the course of an experiment

Most of the unresponsive afferent units in our sample were C fibres. In seven additional experiments we studied only one filament containing unresponsive and responsive C units to find out whether afferent C fibres change their responsiveness in the course of an experiment. In five of these seven experiments we just repetitively tested filaments containing four to six C units at intervals of at least 30 min for several hours. Repetitive application of the noxious search stimuli always resulted in an oedematous swelling of the skin. To augment the oedema, in two further experiments the skin was painted with a solution containing 50 mg arachidonic acid in 25 μl of xylol after the initial assessment of the C fibre responsiveness. However, from the small number of units tested in these two experiments there was no indication that the chemical treatment of the skin increased the proportion of units turning from the unresponsive to a responsive state.

During the prolonged observation periods some unitary spikes became too small to be further evaluated or new units emerged in the record. However, stable recordings were obtained from three CM units, three CMH units, one purely heat-sensitive unit, and twenty unresponsive C units for periods of up to 5 h.

Ten of the unresponsive C units remained unresponsive, i.e. half of the units tested. The remaining ten units became responsive in the course of repetitive testing. Since some of them had receptive fields at prominent sites on the toes and in the ankle region, it seems rather unlikely that they had been initially overlooked.

Five of these initially unresponsive units became sensitive to mechanical stimuli after 30–150 min. One of them was excited by von Frey hair stimulation at 90 mN, two at 128 mN and two responded only to glass rod stimulation. Two of these five units also became heat sensitive in the course of further testing and the heat threshold of one of them was as low as 42 °C.

Two other unresponsive C units became sensitive to heat and mechanical stimuli at the same time, after 60 and 45 min, respectively. Their mechanical thresholds were 64 and 90 mN (von Frey hairs), their heat thresholds 45 and 50 °C. These two units lost their heat sensitivity again after 150 and 90 minutes.

The remaining three of the initially unresponsive C units became heat sensitive, but not mechanoreceptive, after 30–120 min. One of these units had a heat threshold of 43 °C; the other two had thresholds exceeding 50 °C. Two of these units again became unresponsive in the course of further testing, but one of them kept its heat responsiveness and also became sensitive to squeezing the skin with forceps.

There was also some change in responsiveness among the initially responsive units: one CM unit became unresponsive after 30 min and one CMH unit after 60 min. Another CMH unit became unresponsive to heat stimuli after 60 min while its mechanical threshold dropped from 65 to 11 mN. One C-heat unit became responsive to mechanical stimuli 30 min after treatment of the skin with arachidonic acid and xylol. Its mechanical threshold was initially high, but dropped to 128 mN after 2 h.

DISCUSSION

Afferent nerve fibres with mechano-, thermo- and noci-receptor (or 'sensor') properties have been previously described which seem to be adequate for subserving the major sensory functions of the skin. However, it has been argued whether the established classes of sensors constitute the full spectrum of cutaneous afferent nerve fibres (McMahon & Koltzenburg, 1990).

A biasing factor may have been the kind of search stimuli which have been used in the past for identifying sensory nerve endings. In searching for nociceptors, traditionally mechanical stimuli and recording from the axons in a nerve stem have been used (Burgess & Perl, 1967; Perl, 1968; Bessou & Perl, 1969; Beck *et al.* 1974; Lynn & Carpenter, 1982; Fleischer *et al.* 1983). On rare occasions heat search stimuli were employed (Welk, Petsche, Fleischer & Handwerker, 1983). Close arterial injections of algogenic agents such as capsaicin were chosen to excite afferent C fibres which usually were found to be of the CMH type (Szolscanyi, Anton, Reeh & Handwerker, 1988). However, chemical search stimuli may sensitize C and Aδ units and thus change their receptive properties.

Recently transcutaneous electrical stimulation has been used for searching and a large proportion of Aδ units were found in the monkey skin which were unresponsive to mechanical stimuli (Davis, Meyer, Cohen & Campbell, 1989). In the present study on the hairy skin of the rat another approach was chosen: we recorded single units from the dorsal roots which were identified by electrical stimulation of the sural nerve and thus can be assumed to be afferent by all available evidence. With this approach we could expect to get an unbiased sample of afferent units from this nerve.

Almost half of the C fibres and more than 20% of the Aδ fibres in this sample did not respond to the mechanical and thermal stimuli which had been designed to excite almost all known cutaneous sensor types. One obvious explanation is that we may have missed the receptive fields of some of them, though we scrutinized every square millimetre of the skin innervated by the sural nerve and also searched for sensors in the surrounding skin and in deeper tissues. Indeed, we may have missed a few units with high mechanical thresholds and receptive fields at the upper thigh, close to the pool ring, or on the dorsomedial site of paw which were not easily accessible in our preparation. These fields would then have been outside of the main innervation area of the sural nerve (Swett & Woolf, 1985). Missing of those receptive fields at worst may account for a minor error in our assessment of unresponsive units.

The term 'unresponsive' as used in this paper is of course limited to the stimuli used. More units might have been recruited with stronger stimuli. Our stimuli were selected to avoid major tissue damage, but their upper limits were well in the noxious range. Repetitive application of these stimuli induced skin oedema. The known types of nociceptors were certainly recruited by these stimuli with one possible exception: the heat stimuli used in this study probably would have failed to excite part of the Aδ units with very high thresholds and long utilization times which have been described in the monkey, in particular in the glabrous skin (AMH I units) (Meyer & Campbell, 1981). These units are unknown in the rat (Handwerker *et al.* 1987). However, if they nevertheless existed in this species they probably would have been recruited by our mechanical stimuli.

One may speculate about the possible functions of the unresponsive slowly conducting afferents from the skin. Some of them might have still unknown chemoreceptor functions (LaMotte, Simone, Baumann, Shain & Alreja, 1988). According to another hypothesis a class of afferent nerve fibres might have no sensory but purely trophic functions by spontaneously releasing vasoactive agents from their peripheral nerve endings.

However, we found that part of the unresponsive C units became responsive to mechanical and/or thermal stimuli when we applied these stimuli repetitively while some of the responsive units turned unresponsive. Furthermore, some units may gain or lose their responsiveness to one stimulus modality while that to the other one is unchanged. These modality specific changes of responsiveness are of particular interest since they indicate that independent membrane processes control the sensitivity to different stimulus modalities. We have previously seen this kind of sensor plasticity in C units after blocking the axoplasmatic transport of neuro-peptides by treatment of a skin nerve with capsaicin (Welk *et al.* 1983). In the present study changes in responsiveness probably were induced by the repetitive noxious stimulations, since noxious stimuli are known to induce either sensitization or desensitization of nociceptors (Torebjörk *et al.* 1984).

The present findings are in agreement with the hypothesis that inflammatory processes sensitize at least part of the unresponsive units. These units then become responsive to mechanical and/or heat stimuli similarly to 'classical' CM or CMH units. In this case inflammatory pain and hyperalgesia may be more a function of the recruitment of a larger population of thin afferent units than of the sensitization of CMHs, and spatial summation at central synapses will be more important than temporal summation (Handwerker & Reeh, 1990). Such recruitment of 'sleeping' nociceptors in inflammation has already been demonstrated in the cat's knee joint (Schaible & Schmidt, 1988*a, b*) and urinary bladder (Häbler *et al.* 1990).

The exact nature of this recruitment has to be analysed in future studies.

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