Specific C-Receptors for Itch in Human Skin

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In microneurography experiments 56 unmyelinated nerve fibers were studied in the cutaneous branch of the peroneal nerve of healthy volunteers. Units were identified with the “marking” technique as mechanically and heat-responsive (CMH; n = 30), heat-responsive (CH; n = 13), or unresponsive to mechanical and heat stimulation (CMH; n = 13). None of the units showed spontaneous activity.

These units were tested for responsiveness to iontophoresis of histamine (1 mA, 20 sec) from a small probe (diameter, 6 mm), which induced itch sensations lasting several minutes. Twenty-three units were unresponsive to histamine, and 25 units responded weakly with a few spike discharges after iontophoresis.

Eight units, however, responded with sustained discharges to histamine, and their discharge patterns were matching the time course of the itch sensations. All C-units in this group were mechanically insensitive, and five of them were heat-responsive. They had very low conduction velocities of only 0.5 m/sec, on average, which is significantly lower than conduction velocities of the “polymodal” CMH units. This slow conduction velocities attributable to small axon diameters may be one reason why these units have not been encountered in previous studies. Histamine-sensitive C-units had very large innervation territories extending up to a diameter of 85 mm on the lower leg.

We conclude that these C-fibers represent a new class of afferent nerve fibers with particularly thin axons but excessive terminal branching. This type of C-fiber probably represents the afferent units long searched for mediating itch sensations.

Key words: microneurography; human; nociceptors; C-fibers; pruritus; histamine

Although the perception of itch poses major problems in medical praxis, its neuronal substrate in the skin has not yet been identified. This is even more surprising because indirect knowledge has accumulated during the last decades about the initiation of itch by external stimuli. It has been proven that most experimental itch stimuli act via histamine release from mast cells, and that application of different concentrations of histamine into the skin causes different degrees of itching (Handwerker et al., 1987; Simone et al., 1987; Magerl et al., 1990). From these observations nerve fibers sensitive to histamine have been postulated (LaMotte et al., 1988; LaMotte, 1992). These presumed “itch units” are probably unmyelinated, because differential blocking of the myelinated fibers in skin nerves does not abolish histamine-induced itching (Handwerker et al., 1987). Pretreatment of the skin with capsaicin, which temporarily destroys unmyelinated nerve endings in the skin (Simone et al., 1996), also abolishes histamine-induced itching (Handwerker et al., 1987).

These should be sufficient data for finding and characterizing the respective units in human skin nerves by means of microneurography, and indeed there are anecdotal reports on responses of cutaneous C-fibers to itching stimuli (Van Hees and Gybels, 1972, 1981; Torebjörk, 1974). It has also been reported that occasionally itch is induced during microstimulation in skin nerves at points from which C-fibers were recorded (Torebjörk and Ochoa, 1981). However, the most common type of C-fibers, mechanical and heat nociceptors (CMH or “polymodal nociceptors”), which have been extensively characterized in animal (Bessou and Perl, 1969; Beck et al., 1974; LaMotte and Campbell, 1978; Meyer and Campbell, 1981; Lynn and Carpenter, 1982; Fleischer et al., 1983) and human (Torebjörk, 1974; Gybels et al., 1979; Hallin et al., 1982) skin, are either insensitive to histamine or very weakly activated (Tuckett and Wei, 1987; Handwerker et al., 1991). By no means does the population response of CMH units match the prolonged itching sensations induced by well defined histamine stimulation, e.g., by intracutaneous injection (Simone et al., 1987) or iontophoretic application from a small probe (Handwerker et al., 1987; Magerl et al., 1990). Hence, CMH units are probably not mediating itch sensations.

Recently we were able to demonstrate new types of C-fibers in human skin by using a microneurography technique, which does not rely on mechanical excitation of nerve endings and allows characterization even of small units having spikes of poor signal-to-noise ratio (Schmidt et al., 1995; Torebjörk et al., 1996). C-units are detected by electrical search stimuli, and their activation by histamine, heat, mechanical, or other types of stimulation is documented by transient slowing of impulse conduction velocity (cv) after the response (Schmelz et al., 1995).

This “marking” technique has been used for studying the responsiveness of afferent C-fibers in human skin to controlled histamine application and for comparing these responses with the magnitude and time course of itching. In this paper we present evidence that the putative receptors for itching are C-fibers with exceptionally low conduction velocities and an insensitivity to mechanical stimuli.
MATERIALS AND METHODS

Recordings were obtained from 53 subjects (38 male, 13 female; age, 22–32 years) in the microneurography laboratories at Uppsala and Erlangen. In an additional psychophysical study 21 male subjects (age, 21–32 years) were tested. The sensations produced by histamine stimuli in the absence of nerve recordings were obtained at stable latencies, the marking technique was approved by the local ethics committees.

Methods of microneurography used in this study have been described in detail elsewhere (Schmelz et al., 1994; Schmidt et al., 1995). Micro-electrodes were inserted at the level of the fibular head into the superficial branch of the peroneal nerve. When a stable recording position in a nerve fascicle was obtained, the skin field innervated by this fascicle was identified by stroking the skin and listening to the characteristic sound from the returning afferents. This was done for all C-fibers and is procedurally similar to that used in mechanically insensitive A-fibers.

The ensuing search for single C-units was by electrical stimuli to avoid a bias toward mechanically receptive C-units. To this purpose a steel electrode with a blunt tip of 1 mm² was gently pressed to various points within the respective skin area, and electrical pulses of 0.2 msec duration were delivered from an insulated constant voltage stimulator (Grass S48 or S8). Electrode gel was used to reduce the impedance. Stimulus strength was adjusted until stable C-fiber responses were obtained to iterative stimulation (0.2 msec, 10–30 V, 4 sec interstimulus interval). It has to be pointed out that part of the units encountered by intracutaneous stimulation had not been excited before by the electrical search stimuli from the surface electrode.

When responses of one or several C-fibers to the intracutaneous stimulation were obtained at stable latencies, the marking technique was used for characterizing the unit(s). This technique is based on the slowing of conduction velocity in a C-fiber when it is activated by an additional stimulus (Torebjörk and Hallin, 1974). Pronounced slowing is used for characterizing the unit(s). This technique is based on the fact that any C-unit, especially those innervating the skin, has been excited before by the electrical search stimuli from the surface electrode.

According to their responsiveness to mechanical, heat, and electrical stimuli, C-units were classified as mechanically and radiant heat stimuli (CMH), only heat-responsive (CH), or insensitive to physical stimuli (CMiHi) (Schmidt et al., 1995). For testing mechanical excitability, calibrated von Frey bristles (Stoelting Co., Chicago, IL) were used. Units responding to mechanical forces up to 1.2 newtons (N) were regarded as mechanically insensitive. For thermal stimulation, a radiant heat stimulus was produced by a halogen bulb, and feedback was controlled from a thermocouple attached to the skin as described previously (Beck et al., 1974). Heating started from a baseline of 32°C. The temperature was raised at 0.25°C/sec. The subjects stopped the stimulus when their tolerance level was reached (usually at 50°C). Cutoff temperature was 52°C.

CMH units were electrically excited (1 mA applied for 20 sec; see Materials and Methods), and the ensuing itch sensations were rated by the subjects. An additional psychophysical study was performed on another population of subjects to get more precise itch ratings undisturbed by the microneurography situation (see Materials and Methods). Time courses of itching sensations were roughly identical in both studies and also comparable with those in previous studies using the same method (Handwerker et al., 1987; Magerl et al., 1990). Itching started within 30 sec after iontophoresis, usually after termination of the current. It always increased after termination of the current and reached a maximum during the following 2–3 min. Thereafter itch intensity slowly declined, but in most subjects it was still clearly perceived after 10 min. Figure 1, bottom, shows the average time course of itching in 21 subjects.

All units were tested by iontophoresis of histamine in the vicinity of the intracutaneous needles from which the terminals were electrically excited (1 mA applied for 20 sec; see Materials and Methods), and the ensuing itch sensations were rated by the subjects. An additional psychophysical study was performed on another population of subjects to get more precise itch ratings undisturbed by the microneurography situation (see Materials and Methods). Time courses of itching sensations were roughly identical in both studies and also comparable with those in previous studies using the same method (Handwerker et al., 1987; Magerl et al., 1990). Itching started within 30 sec after iontophoresis, usually after termination of the current. It always increased after termination of the current and reached a maximum during the following 2–3 min. Thereafter itch intensity slowly declined, but in most subjects it was still clearly perceived after 10 min. Figure 1, bottom, shows the average time course of itching in 21 subjects.

All polymodal CMH units but none of the mechanically insensitive CH and CMiHi units were activated by the current delivered for iontophoresis, indicating a lower electrical activation threshold of CMHs (also see Fig. 2). A histamine response was only assumed when activation was observed during the first 2 min after termination of the current when the itching was most intense. Of the 56 units studied, 23 were not excited by histamine (10 CMH, 6 CH, and 7 CMiHi). Twenty CMH units and 5 mechanically insensitive units (CH and CMiHi) were only weakly activated, and their discharges did not match the time course of the itch sensations of the subjects.

The remaining eight units, five CH and three CMiHi, however, showed long-lasting responses to histamine. The five CH units had thermal thresholds of 41–46°C. Four of them were also tested...
by intracutaneous injection of capsaicin (20 μl of a 0.1% solution) and were found to be responsive to this agent.

In one recording of a CMH_H unit the signal-to-noise ratio was so good that the unit could be analyzed without taking recourse to the marking technique. Figure 1, top, shows the discharge pattern of this unit after histamine iontophoresis compared with the average itch sensations shown in Figure 1, bottom. During the first 4 min after histamine iontophoresis the unit fired at fairly regular intervals of ~1 sec. Interestingly, during the following minute interspike intervals did not become much longer, but silent periods interrupted activation periods occurring approximately once per minute.

Figure 2 compares responses of different units with the marking technique (Torebjörk, 1974; Schmidt et al., 1995). In the left panel the histamine response of another CMH_H unit is shown. Successive spike responses to regular electrical stimulation of the terminals in the skin applied at 4 sec intervals are represented on subsequent sweeps from top to bottom. Each electrical stimulus initiated a sweep, and the conduction delay of the unit can be seen from the abscissa. Histamine activation of the unit is indicated by an increase in conduction delay after electrical test stimuli and hence by a deviation to the right of the trail formed by the successive spike responses of the unit under study. The magnitude of the deviation is closely correlated with the number of additional action potentials elicited in the period before the conditioned spike was induced, and every trace in which the delay was further increased indicates activation in the preceding 4 sec period (Schmelz et al., 1995). Hence, the jagged contour of the trace reflects the irregular bursting of the respective unit. The mechanically and heat-insensitive unit (CMH_H) shown in Figure 2, left panel, was not excited by the iontophoresis current (marked at the upper left corner) but responded thereafter for more than 10 min, strongly during the first 3 min, and then with a decreasing number of activation periods. For comparison the discharges of two CMH units are shown (Fig. 2, right panel), which were simultaneously recorded in another experiment. Both units were excited by the iontophoresis current, indicating a lower threshold to activation by electrical stimuli (see above). However, only the unit with the shorter conduction delay (i.e., the faster conduction velocity) was activated during the following minute. Although this was the CMH unit in our sample with the most prominent histamine response observed, the activation is apparently too weak to match the itch sensations induced by histamine iontophoresis (see Fig. 1). All other CMH units that responded at all after histamine iontophoresis showed even weaker responses, usually confined to the first 3 min after current termination.

Figure 3 summarizes the histamine responses of all units in our sample. Because they were characterized with the marking method, the measure of responsiveness was “number of activation periods,” i.e., number of transient increases in latency that occurred in the 15 min after histamine delivery. There is a distinct population of units showing sustained activation by histamine, and this population consists only of mechanically insensitive CH and CMH_H units. This population is unlikely to be a chance selection of afferent C-fibers, because CH and CMH_H units constitute only about 20% in an unselected population of afferent C-fibers (Torebjörk et al., 1996).

One additional finding supports the notion that the eight histamine-sensitive CH and CMH_H units in our sample represent a particular class of C-fibers, namely their exceptional slow conduction velocities (Fig. 4). Mean cvs of CMHs in the present sample were ~0.9 m/sec, in agreement with previous studies.
Schmidt et al. (1995), regardless of whether the units were unresponsive or weakly responsive to histamine. CH and CM$_{iHi}$ units with sustained histamine responses had mean cvs of 0.52 (SD, 0.15) m/sec. These units with sustained responses to histamine have significantly lower cvs compared with CMHs (Mann–Whitney U test, $p < 0.0001$) and also to mechanically insensitive CH and CM$_{iHi}$ units with or without weak histamine sensitivity (Mann–Whitney U test, $p = 0.02$ and 0.001, respectively). Within the population of units without and with weak sensitivity to histamine, the cvs of mechanically insensitive (CH and CM$_{iHi}$) units were significantly lower than those of CMHs (Mann–Whitney U test, $p = 0.01$).

The slow cvs of the histamine sensitive units indicate rather small axon diameters, and this may explain why we were not able to determine the innervation territories of these units by transcutaneous electrical stimulation as in previous studies on mechanically responsive and mechanically insensitive C-units (Schmelz et al., 1994). Probably the activation thresholds of the terminal branches were so high or the terminals were deep in the skin, so that transcutaneous activation would have required stimulus currents that would have been intolerable to the subjects. Instead, we tried to determine the innervation territories of these units by iontophoresis of histamine to various spots in the vicinity of the intracutaneous needles from which the units were electrically excited. The diameter of the probe for delivery of histamine was 6 mm (see Materials and Methods), and these tests could be performed only at intervals of a minimum of 15 min, restricting, of course, the resolution of this assessment of innervation territories. However, lateral spread of the histamine can probably be excluded, because the histamine-induced weal was always confined to the area under the iontophoresis probe. Despite the limited spatial resolution of the method for assessment of innervation territories, the results shown in Figure 5 are remarkable. One of the units had an innervation territory with a longest extension of at least 85 mm; another one had a territory of at least 45 mm diameter. In comparison, in a large sample of CMH units the median diameter of the longest axis of the innervation territories was 24.4 mm on the lower leg and 15 mm on the foot dorsum, which is smaller than average flares induced by histamine iontophoresis (Schmidt et al., 1997). This indicates that at least some of the histamine-sensitive units have exceptionally large innervation territories despite their tiny axons.

**DISCUSSION**

Because previous studies have failed to identify any particular class of primary sensory neurons that would respond preferentially to pruritogenic stimuli, itch has been hypothesized to be induced by low-frequency excitation of nociceptors also mediating pain sensation (von Frey, 1922), by a particular pattern of activation (Wall and Cronly-Dillon, 1960), or by activation of a subpopulation of polymodal C-fibers (Tuckett and Wei, 1987; Handwerker, 1992). There is no substantial evidence for any of
these hypotheses. On the contrary, results from transcutaneous (Tuckett, 1982) and intraneuronal electrical stimulation (Torebjörk and Ochoa, 1981) in humans have proven that the frequency or pattern of stimulation of nociceptive fibers does not influence the quality of itch versus pain. Furthermore, the size and intensity of the neurogenic flare induced by itch-provoking histamine are larger and more persistent than the flare induced by pain-provoking mechanical stimuli, which, in turn, excite CMH units more effectively than histamine. This seems to rule out CMHs as mediators of histamine flare and concomitant itch.

Instead, the evidence put forward in the present report favors the concept that there are histamine-sensitive receptors with unique properties that make them appropriate for signaling itch. That those receptors are supplied by C-fibers is to be expected, because itch sensitivity is not lost by peripheral nerve compression block until C-fiber conduction is impaired (Handwerker et al., 1987). Their unusually wide innervation territories in the skin fit with the exceptionally large flares observed after histamine application (Handwerker et al., 1987; Magerl et al., 1990).

A sensor for itch should react preferentially to pruritogenic stimuli, although it does not need to be entirely specific in this respect. The units described in this paper were in part CH; i.e., they were also excited by heating the skin in the range of 41–46°C and also by capsaicin application. This is interesting from a clinical point of view, because it is known that warming often enhances itch (Fruhstorfer et al., 1986). On the other hand, these histamine-sensitive CH units do not necessarily contribute to the sensation of warmth or burning caused by heat or capsaicin application, which is probably mediated by the large population of CMH and CH units with little histamine sensitivity.

There are probably four main reasons why the histamine-sensitive units have not been described before: (1) species differences and the lack of animal models for itch; (2) the insensitivity of histamine-sensitive units to mechanical stimuli; (3) the fact that histamine has not been used systematically as a search stimulus in human studies; and (4) the slow conduction of their axons.

(1) Studies on experimental animals related to itch are not easy to interpret, because their scratching behavior may be stereotypical and unspecific for itch. Furthermore, there are no flare responses in rat or cat, and mast cells in rat skin contain little histamine but instead serotonin, which is lacking in human mast cells (Wallengren, 1993).

(2) Until recently it was generally held that all afferent C-fibers in human skin nerves are polymodal, i.e., CMH. Usually, mechanical search stimuli were used, which obviously excluded silent CMH, and CH units from sampling.

The mechanical insensitivity of histamine-sensitive C-fibers seems to be at variance with the well-known observation that itch can also be induced by mechanical stimulation. However, von Frey (1922) noted the long latency of itch responses to stimulation of “pain points” in the skin and speculated about a chemical mediator. That is, mechanical excitation of histamine units may be secondary to the mechanically induced release of endogenous mediators including histamine. Furthermore, histamine-sensitive CMH, and CH units are likely to be sensitized to mechanical stimulation as are other CMH, and CH nociceptors (Schmidt et al., 1995).

(3) In early microneurography studies there were anecdotal reports of burst-like discharges in polymodal C-fibers after stimulation of the skin with nettles, which induced mixed sensations of itch and stinging pain (Van Hees and Gybels, 1972; Torebjörk, 1974). Only in one study was histamine applied systematically to CMH units, and most of them were unresponsive or responded weakly. One unit showing sustained discharges should probably be reclassified as CH on the basis of our present knowledge because of its high initial mechanical threshold (Handwerker et al., 1991).

(4) The greatest obstacle in the search for histamine units was obviously their small axon diameter, leading to a sampling bias against them attributable to the high electrical activation thresholds and low extracellular spike signals. Because of this presumed sampling bias, we cannot reasonably speculate on their frequency of occurrence.

It has been proven that afferent C-units are tapering toward the periphery. Because we measured average conduction velocities between nerve terminals and recording site at the knee level, we do not know the conduction velocities, and hence the axon diameters, at the recording site itself. Interestingly, a small group of CH units with average cvss of 0.5 m/sec has been found in one study in the monkey skin. These units were not tested with histamine, however (Baumann et al., 1991).

With the discovery of histamine-sensitive C-fibers among the slowest conducting mechanically insensitive C-fibers, the spectrum of afferent nerve fibers has been expanded, and this was achieved by the application of the computer-assisted marking technique in microneurography. This technique augments the power of microneurography mainly for two reasons: (1) it allows clear identification of units with spikes of low signal-to-noise ratio by their unique conduction velocity; this identification is supported by the long distance between stimulating electrodes at the foot and recording electrodes at the knee level, which excludes superposition of spikes from different units; and (2) the marking technique enables the study of identified single C-units over extended periods, up to several hours, although spike forms often change during the observation period. Without this novel technique, the search for the tiny histamine-sensitive C-units probably would not have led to reliable results. It will be a future task to refine our techniques further to allow routine recordings of these smallest nerve fibers in pathophysiological studies.

REFERENCES


