Separate Peripheral Pathways for Pruritus in Man

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1Department of Physiology and Pathophysiology, University of Erlangen/Nürnberg, Germany; 2Department of Neurosurgery, Johns Hopkins University, Baltimore, Maryland; and 3Department of Anesthesiology Mannheim, University Heidelberg, Germany

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Namer B, Carr R, Johanek LM, Schmelz M, Handwerker HO, Ringkamp M. Separate peripheral pathways for pruritus in man. J Neurophysiol 100: 2062–2069, 2008. First published June 18, 2008; doi:10.1152/jn.90482.2008. Recent findings suggest that itch produced by intradermal insertion of cowhage spicules in human is histamine independent. Neuronal mechanisms underlying nonhistaminergic itch are poorly understood. To investigate which nerve fibers mediate cowhage induced itch in man, action potentials were recorded from cutaneous C-fibers of the peroneal nerve in healthy volunteers using microneurography. Mechano-responsive and -insensitive C-nociceptors were tested for their responsiveness to cowhage spicules, histamine, and capsaicin. Cowhage spicules induced itching and activated all tested mechano-responsive C-units (24/24, but no mechano-insensitive C-fibers (0/17). Histamine also induced itch, but in contrast to cowhage, it caused lasting activation only in mechano-insensitive units (8/12). In mechano-responsive C-units, histamine caused no or only short and weak responses unrelated to the time course of itching. Capsaicin injections activated four of six mechano-responsive fibers and three of four mechano-insensitive C-fibers. Cowhage and histamine activate distinctly different nonoverlapping populations of C-fibers while inducing similar sensations of itch. We hypothesize that cowhage activates a pathway for itch that originates peripherally from superficial mechano-responsive (polymodal) C-fibers and perhaps other afferent units. It is distinct from the pathway for histamine-mediated pruritus and does not involve the histamine-sensitive mechano-insensitive fibers.

INTRODUCTION

The primary afferent pathway mediating non histaminergic itch in human is unknown. Previously, a class of C-fiber neurons has been characterized that responds to intracutaneous histamine application with a time course that matches the accompanying itch sensation (Schmelz et al. 1997b). Furthermore, in a patient suffering from chronic itch (prurigo nodulosa) spontaneously active histamine-sensitive "itch-fibers" were found (Schmelz et al. 2003a). Mechano-insensitive C-fibers (CMI), of which histamine-responsive fibers are a subgroup, are also responsible for the axon-reflex erythema (Schmelz et al. 2000a). In agreement with the presumed role of this fiber class in histamine-induced itch, skin reddening around the histamine application site, i.e., an axon reflex flare, is a regular epiphenomenon. Recent experimental findings suggest, however, that the sensation of itch is not exclusively served by histamine-sensitive, mechanoinensitive afferents. Thus itch can be produced in the absence of an erythema (Ikoma et al. 2005; Johanek et al. 2007), and mechanical and heat stimuli unlikely to activate mechano-insensitive afferents can produce the sensation of itch. Therefore primary afferent nerve fibers other than histamine-sensitive, mechanoinensitive afferents must be able to mediate the sensation of itch.

Also under clinical conditions, histamine-independent mechanisms are involved in chronic pruritus. While itch induced by insect bites, in urticaria or allergic reactions can often be treated effectively by H1-receptor antagonists (Twycross et al. 2003) in many systemic diseases such as kidney failure or cholestasis H1-receptor antagonists are ineffective in blocking the pruritus (Cheigh 2003; Greaves 2001; Hoare et al. 2000; Krajnik and Zyllicz 2001a,b; Twycross et al. 2003).

The short barbed hairs (trichomes, spicules) that cover the seed pods of the tropical plant Macuna pruriens produce intense itch without the extended erythema that is typical for histamine induced itch (Shelley and Arthur 1955b, 1957; Johanek et al., 2007). M. pruriens induced itch in human is nonhistaminergic as it is not abolished by topical antihistamine treatment (Johanek et al. 2007). Therefore M. pruriens, which is commonly called “cowhage” (originating from the old Hindu name “kiwach” meaning “bad rubbing”), is a suitable tool to explore the neuronal mechanisms of nonhistaminergic itch. While the lack of a flare response in cowhage-induced itch provides indirect evidence against the activation of CMI, the responsiveness of unmyelinated nerve fibers in human to cowhage has not been investigated. The results of such studies could provide new insights into the neuronal mechanisms underlying the sensation of itch. Using microneurography we therefore tested if histamine and cowhage spicules activate the same types of cutaneous C-fibers in human.

METHODS

Subjects

Fourteen healthy subjects took part in the main microneurography study and two in two additional experiments. None of the subjects suffered from neurological, dermatological, or other forms of chronic disease. They did not take any medication prior to the experiments. Subjects were familiarized with the experimental procedures and gave their written informed consent according to the declaration of Helsinki. Experiments were performed at the University of Erlangen-Nuremberg, and the study was approved by the local ethics committee.

Microneurography

The method of microneurography has been described elsewhere (Torebjork and Hallin 1974; Vallbo and Hagbarth 1968); we used the
experimental procedure as described in detail in previous papers of our group (Schmelz et al. 1995). The recording electrode was inserted into the common peroneal nerve at the level of the fibular head. When the needle was close to a C-fiber bundle, neuronal activity characteristic for unmyelinated afferents could be induced by light scratch stimuli applied to the dorsum of the foot. Innervation territories of individual C-fibers were then located with transcutaneous electrical stimulation with a pointed electrode (10–30 mA, 0.5 ms). C-fibers were identified by their low conduction velocity (<2 m/s). A pair of thin needles (0.15 mm diam) was intracutaneously inserted into the innervation territory and used to stimulate the C-fibers under observation continuously at a low repetition rate (0.25 Hz; 0.5 ms; 1–30 mA) via a constant current stimulator (Digitimer DS7, Digitimer, Hertfordshire, UK). Following repetitive electrical stimulation at a fixed frequency from the skin, action potentials of individual C-fibers can be registered with the recording electrode at stable conduction latencies. An increase in conduction latency is observed when the stimulation frequency at the skin is increased or after the afferent fiber has been additionally activated, e.g., by natural stimuli (“marking”) (Torebjork and Hallin 1974). Marking is due to activity-dependent slowing of conduction in C-fibers. For example, conduction of an action potential renders the axonal membrane of afferent C-fibers less excitable for tens of seconds and thus slows down conduction velocity of subsequent action potentials (Schmelz et al. 1995).

During repetitive electrical stimulation at increasing low frequencies (0.125, 0.25, 0.5 Hz), we investigated conduction latency changes. These activity-dependent changes of conduction velocity form characteristic slowing patterns that segregate mechano-insensitive afferents (marked slowing), mechano-sensitive fibers (intermediate slowing) (Weidner et al. 1999), and cold fibers (little slowing) (Serra et al. 1999).

After characterizing the frequency-dependent slowing of a unit, we investigated the responsiveness to natural (mechanical, thermal, and pruritic) stimuli. Marking induced by these stimuli was observed during electrical stimulation at the skin with a frequency of 0.25 Hz (see also Fig. 1).

To locate and to map the mechanoreceptive fields, the skin was probed repetitively with a stiff von Frey filament (750 mN). It was shown previously that fibers, which were unresponsive to a 750 mN von Frey filament, were also unresponsive to forces much higher and even to needle insertion into the skin (Schmidt et al. 1997). Thus a cutoff force of 750 mN was chosen for all further experiments. Responsive fibers were classified as mechano-sensitive and fibers unresponsive as mechano-insensitive (CMI). Mechanical thresholds of the mechano-responsive fibers were determined with a calibrated set of von Frey filaments. The receptive fields of CMi were mapped characteristic for unmyelinated afferents could be induced by light scratch stimuli applied to the dorsum of the foot. Innervation territories of individual C-fibers were then located with transcutaneous electrical stimulation with a pointed electrode (10–30 mA, 0.5 ms). C-fibers were identified by their low conduction velocity (<2 m/s). A pair of thin needles (0.15 mm diam) was intracutaneously inserted into the innervation territory and used to stimulate the C-fibers under observation continuously at a low repetition rate (0.25 Hz; 0.5 ms; 1–30 mA) via a constant current stimulator (Digitimer DS7, Digitimer, Hertfordshire, UK). Following repetitive electrical stimulation at a fixed frequency from the skin, action potentials of individual C-fibers can be registered with the recording electrode at stable conduction latencies. An increase in conduction latency is observed when the stimulation frequency at the skin is increased or after the afferent fiber has been additionally activated, e.g., by natural stimuli (“marking”) (Torebjork and Hallin 1974). Marking is due to activity-dependent slowing of conduction in C-fibers. For example, conduction of an action potential renders the axonal membrane of afferent C-fibers less excitable for tens of seconds and thus slows down conduction velocity of subsequent action potentials (Schmelz et al. 1995).

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A feedback-controlled halogen lamp was used to assess the heat responsiveness of the fiber by increasing skin temperature from 32 to 50°C at a rate of 0.25°C/s. The subject was given a remote control and instructed to turn off the stimulus when the heat became too painful.

Pruritic and algogenic stimuli

Active and inactive (control) cowhage spicules were used in this study. Inactivation of spicules was produced by heating within an autoclave. These spicules do not produce itch sensations. Active and control cowhage spicules were applied to the skin by pressing the head of a cotton swab (Q-tip) applicator loaded with spicules against the skin. To mount the spicules, the head of the applicator was coated with a small drop of nail polish. Spicules were stuck with their dull ends into the slightly drying polish such that they were protruding perpendicularly from it. Cowhage application was done under a dissecting microscope to ensure that more than five spicules were inserted at the previously marked spot in the receptive field. At the end of the observation time (for details see Experimental protocol), spicules were removed from the skin with adhesive tape that was repetitively applied at the application site. The application site was checked under a microscope to ensure that all spicules had been removed.

A fresh solution of histamine (1%) in distilled water was prepared shortly before the experiment. A cotton disk was soaked with this solution and mounted into the application chamber of an iontophoresis applicator. As described previously (Magerl et al. 1990), anodal current and a charge of 20 mA (1 mA, 20 s) were used to deliver histamine iontophoretically into the skin at the marked spot where cowhage had been applied before.

Some of the afferents were also tested for their responsiveness to capsaicin. A volume ≤10 μl saline containing 0.1% capsaicin in Tween 80 was injected slowly into the skin until the subject rated the pain associated to “5” on a 10-point numeric scale, or until the full volume of 10 μl (maximal dose of 10 μg) had been injected (Schmelz et al. 2000b).

In a second set of experiments, histamine was applied not by iontophoresis, but by inactive cowhage spicules that had been dipped into histamine solution (1% in distilled water) and subsequently had been air dried for 5 min. By applying histamine-coated but heat-inactivated spicules to the receptive field via the Q-tip (see preceding text), we controlled for the different application modes. In this set of experiments, inactivated cowhage was applied first, followed by histamine-coated spicules, and finally by active cowhage spicules.

Psychophysics

Subjects were asked to rate the magnitude of their itch sensations on an open numeric scale. They were instructed to compare the perceived itch to the magnitude of itch of an imagined mosquito bite, which should be rated as “10.” An itch sensation half as strong as that of a mosquito bite was to be rated as “5,” and an itch sensation of double strength as “20.”

While concentrating on the itch sensations, the subjects were asked to ignore the electrical stimulation (1 pulse every 4 s, see preceding text). Furthermore, subjects were trained to rate the intensity of itch during ongoing intracutaneous stimulation. For this, cowhage was applied to the contralateral leg while the electrical stimulation protocol was running to characterize the C-fibers on the ipsilateral leg. At the end of the experiment, another cowhage stimulus was applied to the contralateral leg in the absence of electrical stimulation to evaluate the effect of continuous painful electrical stimulation on the sensation of itch.

In the first experiments, which were not included into the analyses of the psychophysics, a numerical rating scale from 0 (0 = no itch) to 10 (10 = maximal imaginable itch) was used (Fig. 1).

Experimental protocol

First, conduction velocity slowing on repetitive intracutaneous electrical stimulation was studied. At the same time, subjects were trained to rate the intensity of itch under ongoing electrical stimulation (see preceding text). After mapping the receptive field and completing the characterization of the fiber with mechanical and thermal stimuli, one to three consecutive applications of inactivated cowhage were administered in a previously marked spot of the receptive field at an interval of 5–8 min. Sometimes several C-fibers with overlapping receptive fields were accessible from one spot. Up to three consecutive active cowhage stimuli were then applied at an interval of 15 min. Then histamine iontophoresis was applied in the same part of the receptive field. Finally, capsaicin was injected at the same spot as described in the preceding text. The interval between applications of cowhage, histamine, and capsaicin were ~15 min, ensuring that ≥2 min had passed after the end of the last fiber activation and that the sensation of itch had subsided. After termination of the fiber recording
were recorded during stimulation with increasing frequencies (see preceding text). One active cowhage application was repeated on the contralateral lower leg (see preceding text).

Data analysis and statistics

Microneurography signals were amplified, processed on-line, and stored on disk using a micro1401 DAC card and custom-written software in Spike2 (CED, Cambridge, UK). For semi-quantitative analyses of the responses of the fibers to chemical stimulation, the marking method was used. The amount of the induced conduction delay is a function of the number of additional action potentials (Schmelz et al. 1995). Both the number of “activation periods” (= total number of traces in which the conduction latency of the electrically induced action potential is delayed relative to its preceding electrical stimulation) and “cumulative increase of latency” (= the amount of the delay is measured in ms for each activation period and added up) were assessed as a semi-quantitative measure of the activation pattern. Responses to cowhage and histamine stimulation were defined as positive when activation was observed for >1.5 min after stimulus application and the total number of activation periods was >23.

Because intensity of the induced itch sensation was rated relative to the itch of a common mosquito bite (set as “10”), no further normalization was required for the comparisons between pruritic stimuli or subjects.

Statistical analyses were performed with the 6.0 STATISTICA software package (StatSoft, Tulsa, UK). All values are given as means ± SE. Statistical significance was tested with ANOVA. A P value < 0.5 was regarded as significant.

RESULTS

Classification of C-fibers

We recorded 41 afferent C-units from 14 healthy subjects for the main microneurography study.

According to their receptive and electrophysiological properties (Weidner et al. 1999), we classified 24 of them as mechano-responsive and 17 as mechano-insensitive nociceptors (CMi). Nineteen of 22 mechano-sensitive and 5/8 CMi units responded to heat stimuli (mean heat threshold CM: 42.4 ± 0.8°, CMi: 43.3 ± 1°). Mechano-sensitive C-fibers conducted significantly faster than CMi fibers (0.92 ± 0.05 vs. 0.69 ± 0.05 m/s, P < 0.001 ANOVA). As also evident in the specimen recording in Fig. 1, mechano-sensitive fibers showed a significantly smaller activity-dependent slowing than CMi fibers during repetitive electrical stimulation with increasing frequency (2 ± 0.03% of initial latency vs. 6.9 ± 0.06% of initial latency, P < 0.0001 ANOVA). These findings are in agreement with previous studies (Weidner et al. 1999).

Cowhage activates mechano-sensitive but not CMi nociceptors

Figure 1 shows a representative specimen in which fibers of both nociceptor classes were tested in the same recording. Receptive fields of the different fibers overlapped, and therefore their responsiveness could be tested with one stimulus application. Application of inactive spicules briefly activated the mechano-sensitive afferent but not the CMi units. While the mechano-sensitive unit is repetitively excited by active cowhage and unresponsive to histamine, both CMi fibers responded to histamine but not to cowhage. All tested mechano-sensitive nociceptors, but none of the CMi fibers, responded to cowhage (Table 1). Compared with active cowhage, heat-inactivated cowhage only produced a short-lasting, mechanically induced activation in the mechano-sensitive units.

CMi nociceptors can be separated into a histamine responsive and unresponsive group. Larger receptive fields and lower conduction velocities were previously found in the histamine responsive fibers (Schmelz et al. 1997b). However, regarding the responsiveness to cowhage a similar separation of mechano-responsive nociceptors into responsive and unresponsive units.
is not apparent (Fig. 2). Furthermore, the magnitude of the cowhage response (activation periods and cumulative increase of latency) did not correlate with mechanical threshold, conduction velocity, amount of activity-dependent slowing, or histamine or capsaicin responsiveness. In particular, no correlation between heat thresholds and activation periods \( r^2 = 0.00 \) or cumulative latency \( r^2 = 0.16 \) was found. Taken together, these data do not allow for a separation of fibers into cowhage pruriceptors and other types of nociceptors (Fig. 2).

Responses to cowhage are variable

Responses to active cowhage varied considerably between the three repetitive applications even within the same mechanosensitive fiber. For the unit shown in Fig. 3, the smallest cowhage response (left) had only a few activation periods and a cumulative latency increase of 87 ms, whereas the largest response (middle) showed 47 activation periods and a cumulative latency increase of 360 ms. Similarly, responses to cowhage varied widely across the population of mechanoreceptive C-fibers (Fig. 4). The mean responses of three successive cowhage applications showed no tachyphylaxis or sensitization [activation periods 1–3: 29.5 ± 6.2, 35.1 ± 7.8, and 42.9 ± 6.7; cumulative latency 1–3: 91 ± 23, 155 ± 33, and 163 ± 25 ms (Fig. 4)].

Histamine strongly activates CMi fibers

Seventeen mechano-responsive nociceptors and 12 CMi fibers were tested with both histamine and cowhage. Eight of 12 CMi

<table>
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<tr>
<th>Mechano-Sensitive Nociceptors</th>
<th>Mechano-Insensitive Nociceptors</th>
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<tbody>
<tr>
<td>First cowhage 8 16 17 0</td>
<td>&lt;1.5 Min or Absent &gt;1.5 Min</td>
</tr>
<tr>
<td>Second cowhage 3 20 17 0</td>
<td></td>
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<tr>
<td>Third cowhage 2 13 11 0</td>
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<tr>
<td>Fourth cowhage 0 2 0</td>
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<tr>
<td>Cowhage cumul 0 24 17 0</td>
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<tr>
<td>Histamine 17 0 4 8</td>
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Up to four successive cowhage applications were performed. Units responding for 1.5 min following stimulus application and with a total number of activation periods 23 in at least one cowhage were classified as responders. The same criteria were applied to classify responses to histamine.

![Histogram of the number of activation periods induced by cowhage (top) and histamine stimulation (bottom) in mechano-responsive (○) and mechano-insensitive nociceptors (■).](image)

![Specimen recordings from a mechano-responsive nociceptor during three successive cowhage stimulations (arrows 1, 2, 3). An original trace with the action potential of the fiber is plotted above the 1st stimulation. Note the bursting component of the response in repetition 2 and 3 and the weaker and somewhat delayed nonbursting pattern following the 1st application of cowhage.](image)

![Responses of mechano-responsive nociceptors to 3 successive cowhage applications as measured by number of activation periods (top) and cumulative "marking" (bottom). Responses of the single units are linked, and median responses are marked with •. Note the considerable variation of the responses between and within the fibers; however, the median responses remain virtually constant.](image)
units tested with histamine were strongly activated (median activation periods: 68; range: 51–131; median cumulative latency: 312 ms; range: 100–1,455 ms) and match the criteria of previously reported histamine sensitive units (Schmelz et al. 1997b). Four CMi fibers did not respond to histamine or were very weakly activated only for a short while (<1.5 min). In contrast, of 17 mechano-responsive fibers tested with histamine, 8 were unresponsive and 9 were weakly excited. None of the mechano-responsive nociceptors showed a histamine activation fulfilling our criterion of a positive response (criteria: activation >1.5 min. or >23 activation periods: here: maximal cumulative increase of latency 36 ms; median of activation periods: 10.5; range: 2–23; median cumulative latency: 8.4 ms range: 2–36 ms; Fig. 2). As can be seen from Fig. 2, there was no overlap between fibers showing sustained responses to histamine and cowhage (Fig. 2).

In two additional experiments, the effect of an alternative application mode for histamine was assessed in two other healthy subjects. Inactivated cowhage spicules were dipped into the histamine solution and then pricked into the receptive field of five mechano-sensitive C-fibers. All five fibers showed a mechanical response to insertion, but only showed a weak and short-lasting response to the chemical stimulation by histamine (maximal cumulative increase of latency: 11.6 ms; median of activation periods: 7.5; range: 3–12; median cumulative latency: 6 ms; range: 1–11.6 ms). After the stimulation with histamine, a wheal and an axon-reflex flare were visible at the application site. When active cowhage spicules were inserted at the same place in the receptive field, all five fibers showed vigorous and long-lasting responses (maximal cumulative increase of latency: 575.7 ms; median of activation periods: 25; range: 20–78; median cumulative latency: 516 ms; range: 92.2–575.7 ms).

Activation pattern of C-fibers by capsaicin

Four CMi fibers and six mechano-sensitive fibers were tested with capsaicin injection. Three histamine negative CMi fibers were weakly excited by capsaicin (mean activation periods: 16; mean cumulative shift of latency: 116 ms), whereas one histamine responsive CMi fiber was not activated. Four of the six tested CM fibers were excited transiently by capsaicin (mean activation periods: 4; mean cumulative shift of latency: 27 ms). Two of these showed also a weak excitation after histamine (13 and 16 activation periods) and the other two of them were not activated at all. The remaining two CM fibers did not respond to the injection of capsaicin.

Psychophysics

During the microneurography experiments ratings of the itch sensations evoked by cowhage and histamine were obtained. Time courses of itch were similar with durations of 5–10 min (Fig. 5).

The quality of itch caused by cowhage and histamine was felt differently by some subjects. Cowhage was reported to cause burning or pricking itch and could be localized very well, whereas histamine was felt as purer itch over a larger area.

There was no significant difference between the ratings of the three successive cowhage applications either in magnitude or in duration. Itch ratings of the control trial, which was performed at the end of the experiment and in the absence of the electrical stimulation did not significantly differ from the other cowhage applications with ongoing electrical stimulation. Thus simultaneous electrical stimulation did not significantly change the magnitude of itch ratings or the duration of the sensation.

Discussion

Our results provide evidence for at least two separate and nonoverlapping peripheral C-fiber pathways for the sensation of itch consisting of 1) histamine-sensitive mechano-insensitive (CMi) nociceptors and 2) histamine-insensitive, mechano-responsive (polymodal) nociceptors that are activated by cowhage. This result corroborates studies in spinthalamic tract neurons in the monkey, showing a strikingly similar pattern of two nonoverlapping populations being activated by either histamine or cowhage (Davidson et al. 2007). Interestingly, this type of dichotomous response was also found for endothelin, which provoked “burning itch” on intradermal application (Katugampola et al. 2000) and activated the majority of polymodal nociceptors but no CMi fibers in humans (Namer et al. 2007).

Role of C-fibers in pruritus

The discovery of histamine-sensitive, mechano-insensitive C-fibers (CMi) (Schmelz et al. 1997a) and projection neurons in the dorsal horn receiving selective input from CMi afferents (Andrew and Craig 2001) suggested the existence of a “labeled line” for pruritic sensations based on histamine iontophoresis data.

The specificity theory of itch has been questioned lately as spinothalamic tract neurons being activated by histamine injections were found to be responsive also to noxious stimuli (Davidson et al. 2007; Simone et al. 2004), and human primary afferent histamine-sensitive fibers also responded to capsaicin (Schmelz et al. 2003b). However, the crucial issue of neuronal specificity is specificity of the algogen and pruritogen. Topical capsaicin application evokes itch in 50% of subjects (Green and Shaffer 1993), and injection of histamine is painful and activates a subpopulation of nociceptors and nociceptive dorsal horn neurons that lasted for the duration of the itch (Atanassoff et al. 1999; Simone et al. 2004). Thus obviously neither capsaicin nor histamine injections are specific enough to judge specificity of the neuronal pathway.
Histamine produces not only itch but also a wheal and a widespread axon reflex erythema. In humans, there is evidence that predominantly CMI fibers—of which the histamine-sensitive fibers are a subgroup—mediate the axon reflex flare by releasing CGRP from their cutaneous endings (Schmelz et al. 2000a). In addition, histamine-sensitive CMI afferent nerve fibers have large innervation territories that could explain the large axon reflex erythema evoked by histamine (Schmelz et al. 1997b). However, in a previous study using papain, itch was induced in the absence of an axon reflex flare (Hagermark 1973). Furthermore, itch can be elicited by weak electrical stimulation without evoking an axon reflex flare (Ikoma et al. 2005; Shelley and Arthur 1957), providing further evidence that the sensation of itch can be dissociated from cutaneous vasodilation. Because of the long delay between electrical stimulation and sensation (>1 s) Ikoma suggested that C-fibers might be the nerve fiber class involved (Ikoma et al. 2005). Therefore C fiber afferents with electrical thresholds lower than those of CMI (Weidner et al. 1999) can likely convey itch sensation, but they are not able to produce an axon reflex flare. Furthermore, the fact that itch can also be elicited mechanically (Shelley and Arthur 1955b; von Frey 1922) clearly indicates that it can also be mediated by mechano-responsive peripheral nerve fibers.

Cowhage spicules inserted into human skin produce itch comparable to that following histamine application. However, we have demonstrated here that this stimulus activates another group of C-fibers, the mechano-responsive “polymodal” C-fiber afferents. This finding is in agreement with a previous study in cat (Tuckett and Wei 1987) and a recent study in non-human primate (Johanek et al. 2008). Polymodal C-fibers are the most frequent type of afferent C-fibers in human skin nerves (Schmidt et al. 1995), and they are not involved in sustained axon reflex flare reactions (Schmelz et al. 2000a). This is consistent with the observation that cowhage induced itch is not accompanied by a widespread axon reflex flare (Johanek et al. 2007; Shelley and Arthur 1955b, 1957).

**Mechanisms of cowhage-induced itch**

A recent study demonstrated that cowhage produces the sensation of itch through a nonhistaminergic mechanism (Johanek et al. 2007). Previously, a water-soluble, heat-labile, pruritic substance (“mucunain”) with protease activity was extracted from cowhage spicules (Shelley and Arthur 1955a,b). Whereas trypsin-induced pruritus was accompanied by an axon-reflex flare and was sensitive to antihistamines (Hagermark 1973), papain provoked itch without flare (Hagermark 1973). Activation of the proteinase receptor 2 (PAR-2) by mast cell tryptase is thought to play a major role in pruritus of patients suffering from atopic dermatitis (Steinhoff et al. 2003). However, PAR-2 receptors were absent or only weakly expressed on peripheral cutaneous nerve endings in healthy subjects (Steinhoff et al. 2003), suggesting that cowhage-induced itch may be mediated through a receptor other than PAR-2. Indeed mucunain has recently been shown to be a cysteine protease that binds to PAR 2 and PAR 4 (Reddy et al., 2008).

Neuronal responses to cowhage following the superficial insertion of cowhage spicules into the skin were quite variable (see Fig. 3). If “mucunain” is a large molecule, it will diffuse slowly, and therefore its action on C-fibers will depend on the distance between the spicule and the fiber ending. As we cannot control this distance, the observed response will vary between applications and fibers. Future experiments using an extract of the pruritogenic substance in cowhage and injection of the active ingredient should minimize this response variability.

**Role of polymodal C-fibers in itch**

A problem arises from our findings that all the polymodal C-fibers were activated by the pruritic cowhage. However, contact heat that excites the same mechano-sensitive nociceptors induces burning pain but not itch. Furthermore, scratching, which activates mechano-sensitive C-nociceptors, does effectively inhibit itch. A solution to this obvious problem would be the existence of a subgroup of particularly cowhage-sensitive polymodal nociceptors (mechano-sensitive C-nociceptors). Our present study gives no clear indication of such a subgroup. Furthermore, the semi-quantitative analysis of the markings in our experiments did not reveal a particular discharge pattern in mechano-sensitive C-nociceptors afferents induced by cowhage.

Our semi-quantitative analysis of the markings suggests that cowhage-induced considerable activation, similar in intensity to heating (see Fig. 1), which causes pain. Therefore we discard an “intensity hypothesis” as proposed by Max von Frey on the basis of psychophysically studying “itch points” (von Frey 1922). Because we cannot support the hypothesis of the intensity and pattern models with our results obtained with the semi-quantitative marking method, alternative hypotheses should be considered.

**Co-activation of different fiber classes**

Cowhage-induced itch may be mediated through co-activation of polymodal C- and A-fibers. It has long been known that co-activation of different fiber classes determines the sensory quality. Thus noxious cold stimuli induced more pain and burning sensation when A-fibers were blocked (Fruhstorfer 1984; Yarnitsky and Ochoa 1990). Similarly, the sensations induced by pin pricks during an A-fiber block changed to burning and, sometimes, to pure heat (Kolzenburg et al. 1993), suggesting that coactivation of low-threshold mechanoreceptors reduces mechanically induced pain. Indeed, high discharge frequencies in unmyelinated nociceptors are painful when induced by heating but not when provoked by mechanical stimulation (LaMotte and Campbell 1978; Robinson et al. 1983; Van Hees and Gybels 1981). Anecdotal reports (Graham et al. 1951) found that the fast, pricking itch sensation produced by cowhage was eliminated during an A-fiber block and the slow, burning itch sensation produced by cowhage was eliminated during a C-fiber block. Recent systematic studies confirm the contribution of A-fiber input to cowhage-induced itch sensation (Shimada et al. 2007). Indeed, in non-human primates, cutaneous A-delta fibers unresponsive to histamine can be excited by cowhage (Johanek, personal communication).

**Spatial activation pattern**

Lack of consistent graded peripheral activation pattern might lead to central misinterpretation. Our results suggest that the spatial effect of cowhage spicules is very restricted. In a given
fiber, cowhage will produce substantial activation when inserted close enough to the sensory endings. Even when more than five spicules were inserted into the mecano-receptive field, one-third of the fibers, did not respond to the first cowhage application. Thus from a given skin site, cowhage will produce intense neuronal activity in some fibers, whereas fibers in the immediate proximity remain silent, even if their heat and mechanical threshold were lower. In contrast, less focal standard mechanical or heat stimuli induce a more graded response across fibers innervating the application site, and information about intensity and location of the stimulus can be extracted by the CNS from the afferent input. While cowhage-induced activation in primary afferents may perfectly encode the location of the stimulus, the interpretation of this unusual signal could be problematic for the CNS. A sensory quality that is inconsistent with the input from primary afferents has previously been shown for the thermal grill illusion (Craig and Bushnell 1994) in which the simultaneous presentation of spatially alternating non painful warm and cold stimuli is perceived as painful. Similarly, the incoherent neuronal activity induced by cowhage might be interpreted as itch. Importantly, such a model may also explain the sensation of itch produced by small, punctate mechanical and heat stimuli.

Alternatively, focal activation of a small population of afferents may lead to a reduced surround inhibition, which already has been proposed as an explanation for pruritus following very localized stimulation (Greaves and Wall 1996). Reduced surround inhibition may also explain the observation that punctuate mechanical, heat, and electrical stimuli can cause the sensation of itch (Ikoma et al. 2005; Wahlgren et al. 1991).

Conclusions

Our results suggest the existence of an itch pathway that is distinct from the pathway activated by histamine. It consists of mechano-sensitive C-fiber afferent neurons that are histamine insensitive but chemosensitive because they react to a prurito-genic substance released from cowhage spicules.

Future studies will have to test if activity in polymodal nociceptors is crucial for clinically relevant pruritic diseases.

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