Separate peripheral pathways for pruritus in man

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Abstract
Recent findings suggest that itch produced by intradermal insertion of cowhage spicules in human is histamine independent. Neuronal mechanisms underlying non-histaminergic 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 mechano-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 non-overlapping 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.

(196 words)
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 1997b). Furthermore, in a patient suffering from chronic itch (prurigo nodularis) spontaneously active histamine sensitive "itch-fibers" were found (Schmelz 2003a). Mechano-insensitive C-fibers (CMi), of which histamine-responsive fibers are a subgroup, are also responsible for the axon-reflex erythema (Schmelz 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 mechanoinensitive 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 2003) in many systemic diseases such as kidney failure or cholestasis H1-receptor antagonists are ineffective in blocking the pruritus (Krajnik and Zylicz 2001a; Krajnik and Zylicz 2001b; Twycross 2003; Greaves 2001; Hoare 2000; Cheigh 2003).

The short barbed hairs (trichomes, spicules) that cover the seed pods of the tropical plant *Mucuna pruriens* produce intense itch without the extended erythema that is typical for histamine induced itch (SHELLEY and ARTHUR 1955b; SHELLEY
Mucuna pruriens induced itch in human is thought to be non histaminergic, since the axon reflex flare response after application of cowhage spicules is lacking (Johanek et al., 2007). Therefore, mucuna 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 non histaminergic 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.

415 words

Methods

Subjects

Fourteen healthy subjects took part in the main microneurography study and two in two additional experiments. None of the subjects suffered of 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 (Vallbo and Hagbarth 1968; Torebjork and Hallin 1974); we used the experimental procedure as described in detail in previous papers of our group (Schmelz 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 (10-30mA, 0.5ms). C-fibers were identified by their low conduction velocity (< 2 m/s). A pair of thin needles (0.15 mm diameter) 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 Ltd. 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. E.g. 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 1995).

During repetitive electrical stimulation at increasing low frequencies (0.125, 0.25, 0.5 Hz) we investigated conduction latencies changes. These activity dependent changes of conduction velocity form characteristic slowing patterns that segregate
mechano-insensitive afferents (marked slowing), mechanosensitive fibers (intermediate slowing) (Weidner 1999) and cold fibers (little slowing) (Serra 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 1997). Thus a cutoff force of 750mN was chosen for all further experiments. Responsive fibers were classified as mechanosensitive and fibers unresponsive as mechanoinsensitive (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 electrically by applying additional electrical pulses transcutaneously with a pointed steel probe (1 mm diameter; 0.5 ms). The receptive field was defined as skin sites from which additional pulses produced marking in the unit. Substances were applied within the previously mapped innervation territories of the nociceptors at a spot suitable for the application of cowhage and histamine (e.g. far enough from the stimulation needles to apply the iontophoresis chamber).

A feed-back 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 per second. 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 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 5 spicules were inserted at the previously marked spot in the receptive field. At the end of the observation time (for details see Experimental protocol, below) 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 mC (1mA, 20 s) was 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. Up to 10 µl of saline containing 0.1% capsaicin in Tween 80 was injected slowly into the skin until the subject rated the pain as "5" on a 10-point numeric scale, or until the full volume of 10 µl (maximal dose of 10 µg) had been injected (Schmelz 2000b).

In a second set of experiments histamine was applied not by iontophoresis, but by inactive cowhage spicules which had been dipped into histamine solution (1% in distilled water) and subsequently had been air dried for 5 minutes. By applying histamine coated, but heat inactivated spicules to the receptive field via the Q-tip (see
above) we controlled for the different application modes. In this set of experiments inactivated cowhage was applied first, followed by histamine coated spicules and in the end 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 seconds, see above). Furthermore, subjects were trained to rate the intensity of itch during ongoing intracutaneous stimulation. For this, cowhage was applied to the contralateral lower leg while the electrical 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 upon repetitive intracutaneous electrical stimulation was studied. At the same time subjects were trained to rate the intensity of itch under ongoing electrical stimulation (see above). 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 minutes. 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 minutes. Then histamine iontophoresis was applied in the same part of the receptive field. Finally, capsaicin was injected at the same spot as described above. The interval between applications of cowhage, histamine and capsaicin were about 15 minutes, ensuring that at least two minutes had passed after the end of the last fiber activation and that the sensation of itch had subsided. After termination of the fiber recording one active cowhage application was repeated on the contralateral lower leg (see above).

Data-analysis and statistics

Microneurography signals were amplified, processed online 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 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 more than 1.5 minutes following 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 mean (±SEM). Statistical significance was tested with ANOVA. A p-value <= 0.05 was estimated as significant.

**Results**

**Classification of C-fibers**

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

According to their receptive and electrophysiological properties (Weidner 1999) we classified 24 of them as mechano-responsive and 17 as mechano-insensitive nociceptors (CMi). 19/22 mechanosensitive and 5/8 CMi units responded to heat stimuli (mean heat threshold CM 42.4° ± 0.8, CMi 43.3° ± 1). Mechanosensitive C fibers conducted significantly faster than CMi fibers (0.92 m/s ± 0.05 vs 0.69 m/s ± 0.05, p<0.001 ANOVA). As also evident in the specimen recording in figure 1, mechanosensitive fibers showed a significantly smaller activity-dependent slowing than CMi fibers during repetitive electrical stimulation with increasing frequency (2 % of initial latency ± 0.03 vs 6.9% of initial latency ± 0.06, p<0.0001 ANOVA). These findings are in agreement with previous studies (Weidner 1999).

**Cowhage activates mechanosensitive, 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 mechanosensitive afferent, but not the CMi units. While the mechanosensitive unit is repetitively excited by active cowhage and unresponsive to histamine, both CMi fibers responded to histamine, but not to cowhage. All tested mechanosensitive nociceptors, but none of the CMi-fibers responded to cowhage (tab. 1). Compared to active cowhage, heat-inactivated cowhage only produced a short lasting, mechanically-induced activation in the mechanosensitive 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 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, 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 figure 3, the smallest cowhage response (left panel) had only a few activation periods and a cumulative latency increase of 87 ms, whereas the largest response (central panel) 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, 2, 3: 29.5 ± 6.2, 35.1 ± 7.8, 42.9 ± 6.7; cumulative latency 1,2,3: 91 ± 23 ms, 155 ± 33 ms, 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 units tested with histamine were strongly activated (median activation periods 68; range 51-131; median cumulative latency 312 ms; range:100-1455 ms) and match the criteria of previously reported histamine sensitive units (Schmelz 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 figure 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 5 mechanosensitive C-fibers. All 5 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 5 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 mechanosensitive 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 minutes. (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, while 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 non-overlapping 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 spinothalamic tract neurons in the monkey, showing a strikingly similar pattern of two non-overlapping populations being activated by either histamine or cowhage (Davidson 2007). Interestingly, this type of dichotomous response was also found for endothelin, which provoked “burning itch” upon intradermal application (Katugampola 2000) and activated the majority of polymodal nociceptors, but no CMi fibers in humans (Namer 2007).

1. The role of C-fibers in pruritus

The discovery of histamine sensitive, mechano-insensitive C-fibers (CMi) (Schmelz 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 2007; Simone 2004) and human primary afferent histamine-sensitive fibers also responded to capsaicin (Schmelz 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 (Simone 2004; Atanassoff 1999). 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 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 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 (SHELLEY and ARTHUR 1957; Ikoma 2005), providing further evidence that the sensation of itch can be dissociated from cutaneous vasodilatation. Because of the long delay between electrical stimulation and sensation (>1 sec) Ikoma suggested that C-fibers might be the nerve fiber class involved (Ikoma 2005). Therefore, C fiber afferents with electrical thresholds lower than those of CMi (Weidner 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 (von Frey M 1922; SHELLEY and ARTHUR 1955b) 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. 2005). Polymodal C-fibers are the most frequent type of afferent C-fibers in human skin nerves (Schmidt
1995) and they are not involved in sustained axon reflex flare reactions (Schmelz 2000a). This is consistent with the observation that cowhage induced itch is not accompanied by a widespread axon reflex flare (SHELLEY and ARTHUR 1955b; SHELLEY and ARTHUR 1957; Johanek et al. 2007).

2. Mechanisms of cowhage induced itch

A recent study demonstrated that cowhage produces the sensation of itch through a non-histaminergic 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; SHELLEY and ARTHUR 1955b). 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 2003). However, PAR-2 receptors were absent or only weakly expressed on peripheral cutaneous nerve endings in healthy subjects (Steinhoff 2003) suggesting that cowhage induced itch may be mediated through a receptor other than PAR-2.

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.
3. The 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 which excites the same mechanosensitive nociceptors induces burning pain, but not itch. Furthermore, scratching, which activates mechanosensitive 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 (mechanosensitive 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 mechanosensitive 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 M 1922). Since we can not 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 fibers 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 (Koltzenburg 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; Van Hees and Gybels 1981; Robinson 1983). Anecdotal reports, (GRAHAM 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 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 5 spicules were inserted into the mechano-receptive field, one third of the fibers, however, did not respond to the first cowhage application. Thus, from a given skin site, cowhage will produce intense neuronal activity in some fibers, whereas fibres 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 central nervous system 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 central nervous system. 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 (Wahlgren 1991; Ikoma 2005).

**Conclusions**

Our results suggest the existence of an ‘itch’ pathway that is distinct from the pathway activated by histamine. It consists of mechanosensitive C-fiber afferent neurons which are histamine-insensitive, but chemosensitive - since they react to a pruritogenic substance released from cowhage spicules.

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

(Discussion: 1588 words)
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Legends

Table 1

Numbers of mechano-responsive and mechano-insensitive fibers responsive to cowhage and histamine. Up to four successive cowhage applications were performed. Units responding for more than 1.5 minutes following stimulus application and with a total number of activation periods above 23 in at least one cowhage trial were classified as responders. The same criteria were applied to classify responses to histamine.

Figure 1

Specimen of a multifiber recording from one mechano-responsive (CM) and two mechano-insensitive nociceptors (CMi). A trace of the raw signal containing the C-fiber action potentials is shown on top. Conduction latencies of these three marked fibers (filled square, open triangles) in response to successive electrical stimulation at the receptive field are plotted from top to bottom. Top traces were recorded during stimulation with increasing frequencies (see open rectangle on right side), followed by traces recorded during stimulation with mechanical stimuli (v. Frey filament), inactive (inact.) and active (act.) cowhage spicules, histamine iontophoresis (histamine), and heat (black triangle).

When activated by mechanical, chemical or heat test stimuli, C-fibers exhibit activity dependent increase of response latency followed by a gradual normalization ("marking"). The mechano-responsive nociceptor is characterized by its moderate slowing to the initial repetitive electrical stimulation with increasing frequencies (0.125 Hz, 0.25 Hz, 0.5Hz; open rectangle) and the slowing in response to mechanical stimulation. The mechano-insensitive units are characterized my marked slowing during repetitive electrical stimulation and the lack of increase in latency during
mechanical stimulation. The third fiber shows some “flip flopping” (marked with a cross hatch) after the first mechanical stimulation resulting from action potential initiation in a different peripheral branches inside the receptive field. “Flip flopping’ does not reflect activation.

Note that the mechano-responsive fiber is activated during mechanical stimulation with the v.Frey filament and during application of inactive cowhage, but lasting activation is only seen after application of active cowhage. In contrast, the mechano-insensitive fibers do not respond to cowhage stimulation, but are active following histamine ionotophoresis.

At the right side of the panel the itch ratings of the subject, which were assessed during this experiment, are depicted. Ratings are given on a numerical rating scale from 0 (0 = no itch) to 10 (10 = maximal imaginable itch). Inactive cowhage does not evoke any itch, whereas active cowhage and histamine evoke itch similar in time course and maximum mirroring nicely the activation pattern of the fibers.

Figure 2

Histogram of the number of activation periods induced by cowhage (upper panel) and histamine stimulation (lower panel) in mechano-responsive (black) and mechano-insensitive nociceptors (open). For fibers with multiple cowhage applications, the maximum response was plotted. The number of activation periods induced by cowhage in mechano-responsive fibers shows a nearly Gaussian distribution. In contrast, mechano-insensitive fibers were unresponsive to cowhage application. The distribution of histamine responses in mechano-insensitive nociceptors reveals histamine responsive and unresponsive fibers. Meanosensitive afferents were largely unresponsive to histamine.
Figure 3
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 first stimulation. Note the bursting component of the response in repetition 2 and 3 and the weaker and somewhat delayed non-bursting pattern following the first application of cowhage.

Figure 4
Responses of mechano-responsive nociceptors to three successive cowhage applications as measured by number of activation periods (upper panel) and cumulative “marking” (lower panel). Responses of the single units are linked and median responses are marked with grey squares. Note the considerable variation of the responses between and within the fibers; however, the median responses remain virtually constant.
Figure 5

Ratings (mean +/- SEM) of cowhage- and histamine induced itch sensations during microneurography. An open scale was used with the itch intensity of a mosquito bite arbitrarily rated as “10”, and itch sensation of double intensity to be rated as “20” and half the intensity as “5”.

A) The three successive cowhage applications produced similar itch intensities with similar time course (cowhage 1, 2, 3).

B) Histamine iontophoreses produced an itch sensation of similar intensity and duration (open circles).


33. **SHELLEY WB and ARTHUR RP.** Studies on cowhage (Mucuna pruriens) and its pruritogenic proteinase, mucunain. *AMA Arch Derm* 72: 399-406, 1955b.


figure 4

activation periods

cumulative latency

1.cowhage  2.cowhage  3.cowhage
Figure 5

Part A: Graph showing mean itch rating over time for different cowhage treatments (n = 18, 17, and 14) and histamine (n = 14).

Part B: Graph showing a different context or data set compared to Part A.