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INVESTIGATIVE REPORT

Impact of Scratching on Itch and Sympathetic Reflexes Induced by Cowhage (*Mucuna pruriens*) and Histamine

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Cowhage and histamine, both applied via spicules, were used to induce itch. The quality and intensity of the sensations, axon reflex flare, sympathetic skin vasoconstrictions and the interference of scratching with itch processing were studied. Axon reflex flare reactions were measured by laser Doppler imaging and reflex vasoconstrictions in the finger were recorded by laser Doppler flowmetry. Magnitude of itch sensations was assessed on an electronic visual analogue scale while the skin was intermittently scratched proximal to the application site. The quality of itch was assessed with a questionnaire. Only histamine produced an axon reflex flare. Histamine itch increased faster, but recovered more slowly after scratching, by which it was more effectively suppressed. Cowhage induced a sharper itch sensation and stronger vasoconstrictor reflexes. These findings support the notion that both agents activate different pathways. The differences in sympathetic reflex induction and in the modulation by scratching indicate differential central nervous processing. ***Key words: cowhage; histamine; pruritus; itching; vasoconstrictor reflexes; axon reflex flare.***

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For decades histamine released from mast cells in the skin has been regarded as the main pruritogenic agent. It has been shown to excite a subgroup of mechano-insensitive cutaneous C-fibres with widely distributed receptive territories (1). Excitation of these nerve terminals through H1 receptors leads to the well-known axon reflex reaction, since the axon collaterals release the vasodilatory substance calcitonin gene-related peptide (CGRP) (2). Flare reactions are regarded as characteristic of histamine-mediated pruritus. In clinical dermatology this type of pruritus has been treated successfully with antihistaminic H1-blocking drugs. However, many forms of itching diseases are insensitive to H1 blockers, leading to the assumption that non-histaminergic forms of itching must exist (3).

This provoked a renewed interest in experimental stimuli that reliably induce itching without a flare reaction and therefore seem not to involve histamine. An example of such stimuli are the spicules of the tropical plant *Mucuna pruriens* (cowhage), which were used extensively for itch research half a century ago (4–6). Meanwhile, the active agent in the cowhage spicules has been identified and synthesized, the protease "mucunain" a ligand for protease-activated receptors (PAR) 2 and 4 (7). In microneurography experiments we have recently shown that cowhage spicules excite a different class of C-fibres, the mechano-sensitive C-nociceptors (8).

From these findings the question arose: how similar are the sensations and neuronal reactions provoked by cowhage and histamine? To answer this question the two agents had to be introduced to the skin using a method which cannot be distinguished by the subjects. Indeed, Shelley & Arthur (4–6) have suggested such an experimental procedure: if cowhage spicules are autoclaved at >80°C they lose their itching potency, since the protein mucunain is denatured. Inactivated spicules have been proven as reliable control stimuli in previous psychological and microneurography studies (8, 9). However, when these inactivated spicules are soaked in a histamine solution, they induce histaminergic itching. The purpose of this study was to apply these two stimuli to healthy volunteers in a randomized fashion, the subjects being blind with respect to the type of stimulus. For control, the axon reflex flare was assessed with a laser Doppler scanner. In a first experiment the quality of the itch sensations was assessed. In a second experiment the effect on the quantity and time-course of the itching of scratching the skin in the surrounding of the stimuli was tested. Furthermore, we studied sympathetic vasoconstrictor reflexes, which are induced by strong itching stimuli (10).

MATERIALS AND METHODS

Subjects

Twenty-five healthy young volunteers (16 women, 9 men; age range 20–38 years) participated in the study. All of the volunteers were free of allergies, atopic eczema and/or other dermatological diseases. To exclude subjects with an atopic disposition, the atopic score of Diepgen et al. (11) was applied. Only subjects

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with an assessment "no atopic skin diathesis" and "atopic skin diathesis improbable" were included. The local ethics committee had approved the study. The subjects were informed about the aim of the experiments and the procedures involved. They received financial compensation for their time.

Pruritic stimuli

Cowhage spicules (Zandu Pharmaceuticals, Mumbai, India) were inserted into the skin of the volar aspect of the lower arm. Active spicules induced a perception of itch in all subjects. For control, inactivated spicules were obtained by autoclaving. After this procedure the spicules do not produce any sensation of itch, because the proteins responsible for itching are denatured.

The spicules were fixed to a cotton applicator coated with a small drop of glue. Approximately 30 spicules were stuck with their blunt ends into the slightly drying glue, such that the spicules protruded from it perpendicularly (9). Histamine was applied with inactivated spicules, which had been charged by dipping them into a histamine solution (1%) and followed by drying for 20 min (8).

Active or histamine-loaded spicules were applied to the skin by pressing the head of the cotton stick applicator carrying the spicules against the skin. Approximately 15–20 spicules with a mean length of 1.5 mm were thus inserted superficially into the skin on an area of approximately 10 mm^2. Since the applicators looked identical, the subjects were unable to determine the itching agent from the form of the application. At the end of the observation time all spicules were removed from the skin with an adhesive tape that was repeatedly applied to and removed from the skin. In all experiments active cowhage spicules and histamine-coated spicules were applied in randomized order. The subjects were blind to the type of stimulus and were prevented from seeing the application site and the erythema that might have been developing. None of the subjects had been exposed to cowhage stimulation previously. Twenty-three subjects had participated in another experiment in which histamine was applied iontophoretically.

Experimental protocol

Two experiments were performed. In both experiments cowhage and histamine-coated spicules were consecutively applied to the skin of the volar side of the forearms. In the first experiment itching stimuli were applied approximately 4 cm proximal to the wrist. One substance per arm was deployed and arms were assigned randomly to substances. In the second experiment both substances were applied to the left forearm approximately 4 cm and 10 cm proximal to the wrist, in a balanced, randomized sequence, to avoid effects of lateralization in reflex measurements and in planned functional magnetic resonance imaging studies. The subjects used their right hand to manipulate the visual analogue scale (VAS) for rating itch intensity.

All subjects participating in the second experiment had also participated in the first. The second experiment was performed at least 2 months later.

First experiment: qualitative assessment of cowhage- and histamine-induced itch. Seven minutes after application of one of the itch stimuli the subjects completed a questionnaire with 24 items describing sensory qualities. They had to tick each item on a "0" to "4" point scale, "0" meaning "not appropriate" and "4" "absolutely appropriate". The questionnaire was a shortened version of the "Eppendorfer Juckreiz-Fragebogen" (12). Since this list of adjectives was originally developed for clinical use on patients with chronic itch, some items were not appropriate in our experiment and therefore were deleted after preliminary trials. Finally, 24 of the 80 items in the questionnaire were selected. These 24 items are shown in Table I.

When the itch sensation had completely subsided the next test stimulus was applied to the other arm.

Table I. *Twenty-four German items from the "Eppendorfer Juckreiz-Fragebogen" (12) and their English translations, which were used to describe the sensory qualities of the itch sensation*

Items in the itch questionnaire	
German	English translation
schmerzend	painful
scharf	sharp
pulsierend	intermittent
kribbelnd	crawly
wellenförmig	undulating
spitz	prickly
pochend	pulsatile
hell	lightish
pieksend	pricking
nadelstichartig	like needles
bohrend	drilling
juckend	itching
beissend	biting
prickelnd	tingly
stechend	stinging
mückenstichartig	like a mosquito bite
warm	warm
einnehmend	attention-capturing
brennend	burning
unangenehm	unpleasant
eindringend	penetrating
unerträglich	unbearable
oberflächlich	superficial
störend	disturbing

After completion of the experiment, when both itch agents had been applied and the sensations had vanished, subjects were asked to describe the difference between the stimuli in their own words. Thereafter, they were permitted to look at the flare reaction on their arms.

In 18 of the subjects the lower arm was scanned during the experiment with a laser Doppler imager (moorLDI2-VR, Moor Instruments, Axminster, UK) for measuring blood flow increases in the surroundings of the test stimuli (axon reflex flare). A 4.2× 4.2 cm area was scanned (145 × 145 pixels resolution, 4 ms/pixel scan time). The application site was in the centre of this area. The scan lasting 120 ses was taken 9 min after application.

Image analysis. Analysis of the Doppler imaging data was performed using the Moor scanner software. To identify changes in blood flow associated with the application of the pruritic agents, the mean and standard deviation (SD) of the perfusion in an area outside the flare was computed. When the perfusion value of a pixel increased more than 2 SD above average baseline this was regarded as an increase in skin blood flow. The total number of such pixels was counted and thus the area of the flare determined.

Statistical analysis. Wilcoxon matched-pairs tests were used for statistical analysis of the questionnaires. Gender-related differences were tested using a Mann-Whitney *U* test. The area of the flares after cowhage and histamine stimulation, respectively, were analysed using an analysis of variance (ANOVA) repeated measure design with the factors "gender" and "substance" (cowhage vs. histamine as a within effect).

Second experiment: time-course of itch sensations and the effect of scratching on cowhage- and histamine-induced itch. Spicules were applied as described in the experimental protocol. Ninety seconds after the start of application, which lasted less than 3 sec, the skin was scratched 3 cm proximal to the application site for 9 sec. Care was taken not to scratch the application site itself, so that the transduction process in the itch-mediating nerve fibres was not affected. Scratching was performed by the investigator with an L-shaped bendable copper sheet (thickness: 1 mm; width: 10 mm; length: 130 mm). The buckled smaller end was 10 mm wide with rounded edges. It was used to scratch with a constant force of approximately 2.65 N, a scratch stroke of 3 cm and a velocity of scratching of 6 cm/sec. To ensure a constant force and velocity of scratching, it was always performed by the same experimenter. In total, 4 scratching cycles of 9 s were applied with an interval of 51 sec (start of one cycle to the start of the next cycle).

This procedure was repeated with the second test stimulus as soon as the effect of the first pruritic stimulus had eased, after approximately 10 min.

In this experiment itch perceptions were assessed quantitatively, and sympathetic vasoconstrictor reflexes were measured. The subjects tracked their itching sensations continuously on an electronic VAS, which they manipulated by turning a rotary switch (13).The VAS ranged from 0% (no itch sensation) to 100% (unbearable itching). The scale had a mark at 30% of its length. The subjects were instructed that this should represent an itch intensity that induces a strong urge to scratch. They were also instructed to tune the rating back to zero as soon as the scratching started and continue with the rating as soon as the scratching stopped and the itch sensation reappeared.

Skin blood flow in the pad of the ring finger was recorded by laser Doppler flowmetry (DRT4, Moor Instruments). The recording started one minute before application in order to get the baseline conditions of the blood flow. The mean value of this baseline was set to 100% and used for individual normalization. The subjects were requested not to speak and to breathe as regularly as possible to minimize accessory influences on the skin blood flow.

Statistical analysis. Time-courses of the ratings were sampled at a rate of two values per second. The rating values from the beginning of the application of histamine or cowhage to the start of the first scratching period were averaged and these mean values of cowhage and histamine were compared using a *t*-test for dependent samples. The intensity of the itch sensation within a 30 sec interval before the next scratching period was expressed by the mean rating value within this period. These values of the two stimulation groups were compared using an analys of variance (ANOVA) repeated measure design (factor "group" and "scratch period"). A *post-hoc* least significant difference (LSD) test was performed on significant factors. Gender differences were not considered since only four men participated.

For assessment of the itch latency after stimulus application two parameters were determined: (a) latency to the threshold of the first itch sensation, determined at 1% of the rating scale; and (b) latency until the "urge to scratch" intervention point was reached, set at 30% of the scale. Differences between the two substances regarding these parameters were statistically analysed using *t*-tests for dependent samples. Duration of itch-free interval, determined at below 1% of the scale, after each scratch cycle was compared using an ANOVA repeated measure design (factors "group" and "scratch period"). A *post-hoc* LSD test was performed on significant factors.

The blood flow in the finger pad was assessed by averaging the laser Doppler flow signal within the 30 sec period preceding each scratching period. The blood flow values were converted and expressed as a percentage to compensate for individual differences in blood flow and to make the measurements comparable. This was done by putting the individual baseline blood flow, which was measured within the 60 s period preceding the application of the spicules and averaged, on 100%. The values were compared using the same ANOVA repeated measure design, as described for the rating values above.

All data are expressed as the mean ± standard error of the mean (SEM). For all statistical analyses the software package STATISTICA (data analysis software system), version 8.0, was used (StatSoft, Inc.). A p-value <0.05 was considered to be significant.

RESULTS

Qualitative assessment of cowhage- and histamine-induced itch

Twenty-five subjects (16 women, 9 men) participated in this experiment; in 18 of them flare measurements were also performed (see Methods).

Application of both active cowhage spicules and histamine-coated inactivated spicules induced itch in all subjects. After the subjects had experienced both types of stimuli they found it difficult to name differences between the sensations induced by the stimuli. Since all subjects were not experienced in the sensations induced by cowhage it did not make sense to ask if they were able to distinguish this stimulus from histamine.

The questionnaire was completed while the stimulus-induced sensation was still present. Of the 24 attributes used for rating on a 4-point scale no significant effects were found in 21. Only three items showed significant differences between cowhage and histamine. These attributes were "stinging", "sharp" and "prickly" (Table I). On these closely related items cowhage obtained higher ratings compared with histamine. One other attribute, namely "like a mosquito bite" brought higher ratings for histamine; however, the difference did not reach statistical significance ($p>0.07$). Fig. 1 presents attributes with the greatest differences between cowhage and histamine. None of these five items showed gender-related differences.

In contrast to these small differences in the sensory quality of the two stimulus modalities the axon reflex flares were dramatically different, as reported previously (9). The vasodilatation produced by cowhage did not spread to more than 1 cm from the spicule application site in all subjects. In contrast, histamine reliably produced a substantial flare response, seen both on visual

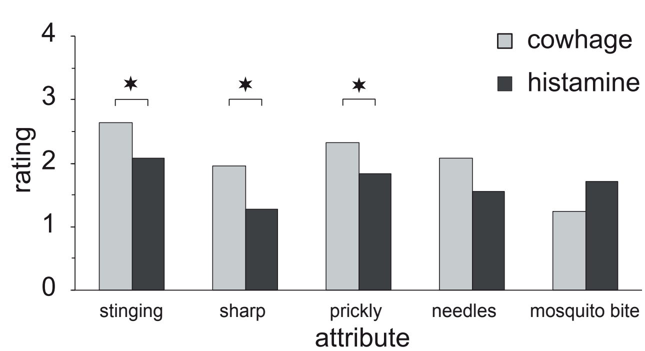

Fig. 1. Items from the Eppendorfer Juckreiz-Fragebogen (12) on which different ratings were obtained after cowhage and histamine application. Mean ratings are shown. Statistical significance was tested with the Wilcoxon matched-pairs test (*$p<0.05$, no correction for repeated measurements).

examination of the skin and in the laser Doppler scans (see Fig. 2). On average, cowhage produced a flare area of 0.58 cm^2 (women) and 0.52 cm^2 (men), respectively. After histamine the flare areas were significantly larger: 4.50 cm^2 (women) and 7.71 cm^2 (men), respectively. ANOVA revealed that histamine-induced flares were significantly larger in men than in women. A wheal was noticed after each histamine application, but not after cowhage.

Quantitative itch assessment and effect of scratching

In the second experiment 14 subjects (10 women, 4 men) took part, and in 12 of them also the vasoconstrictor response in a finger pad was measured.

All subjects felt intense itch sensations after application of both agents, achieving at least ratings of 50% of the visual analogue scale (VAS). The mean itch ratings following application up to the first scratching period were not significantly different between cowhage and histamine (*t*-test for dependent samples), indicating that the two forms of itching stimuli were roughly equipotent. Fig. 3a shows the average time-course of the itch ratings. Itch sensations emerged and rose faster on average after histamine application compared with cowhage. Fig. 3b shows the quantitative assessment of latencies to reaching the "itch threshold" (i.e. 1% of the rating scale) and the "urge to scratch" threshold (i.e. 30% of the rating scale, see Methods). Also the peak of itch sensation was reached earlier in case of histamine application. However, after 90 sec the cowhage induced ratings had passed those obtained after histamine. Under both agents, itch had completely subsided by the end of the scratching periods and itch ratings started again with a delay of several seconds. Fig. 3c shows the mean latencies to reaching the "itch threshold" after each subsequent scratching period. In contrast to the first appearance, itch sensations reappeared later after histamine compared with cowhage (repeated measures ANOVA, $p<0.05$).

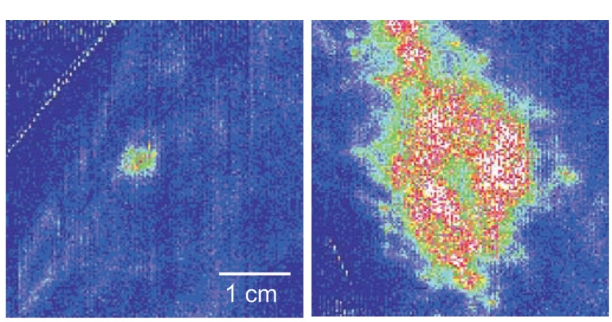

Fig. 2. Specimen record of the areas of flare produced by cowhage and histamine. Laser Doppler scanning of the volar side of the lower forearm. Stimulus application was made in the centre of the scanning area. The upper side of the scan is proximal. Scans were obtained 9 min after application of histamine and cowhage.

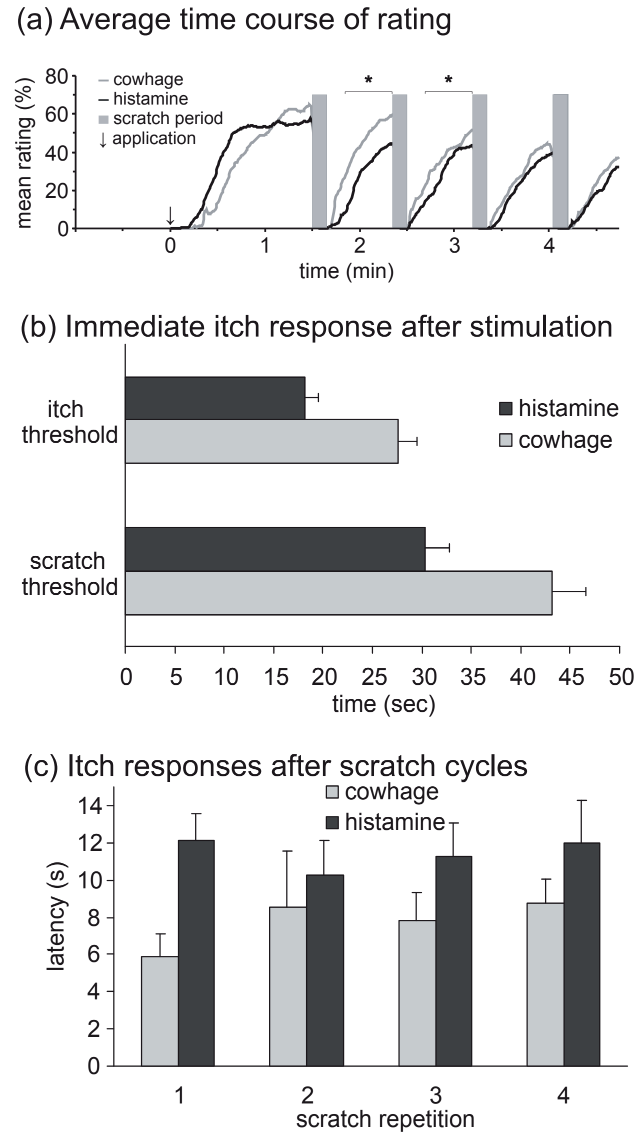

Fig. 3. Itch ratings after application of itch stimuli and intermittent scratching. (a) Time-courses of average ratings. During the scratching period ratings were interrupted and the rating scale was reset. For statistical evaluations 30 sec periods before the start of each scratch period were averaged. Itch ratings before the second and third scratch period were significantly higher under cowhage than histamine itch (analysis of variance and *post-hoc* test, $*p<0.05$). (b) Latencies from stimulus application to the start of itching ("itch threshold", defined as rating passing 1% of the rating scale, see Methods) and threshold of the "serious urge to scratch" (defined as passing 30% on the rating scale). Bars represent means and standard errors of the mean (SEM). (c) Duration of itch suppression after scratching. Latencies from the end of scratching to the reappearance of itching after each of the successive scratch periods. Mean latencies and SEM.

Autonomic reflexes

The application of itching stimuli to the volar side of the forearm elicited vasoconstriction in the finger pads of the ipsilateral hand, which started immediately after insertion of the spicules. This fast sympathetic reflex was independent of the nature of the stimulus. A sustained vasoconstrictor response became visible at approximately the time when the itch sensations also arose. After approximately one minute the difference between cowhage and histamine became manifest. During the 30 sec period before the first scratching this sustained vasoconstrictor response was significantly stronger in the cowhage compared with the histamine trials. A significant difference was also found in the period before the second scratching (ANOVA for repeated measures, *post-hoc* test, $p<0.05$) (see also Fig. 4).

Scratching itself also induced sharply delineated vasoconstrictor responses, which were not analysed further in this study.

The magnitude of vasoconstrictor responses and itch ratings were significantly correlated, as shown in Fig. 5. The correlation coefficient was –0.49 for all measures (obtained during the 30 sec analysis periods before the scratching intervals), –0.47 for the cowhage and –0.52 for the histamine trials (product moment correlations, $p<0.05$). The regression lines were not significantly different for the two agents (slope of the regression line histamine: –9.99; cowhage: –16.03).

DISCUSSION

Itch mediated by cowhage (Mucuna pruriens)

This agent has been used previously for experimental assessment of itch (4–6, 14, 15). Until recently its molecular nature was unclear, although Shelley & Arthur (6) had conjectured that the effect of the spicules may be due to a protein, which they named "mucunain". Recently this protein, a cystein protease, was isolated and it has been shown that it binds to PAR2 and PAR4 receptors (7). The renewed interest in mucunain is due to the fact that it is a histamine-independent itch mediator (9). This is interesting, since many clinically relevant itching diseases are apparently histamine independent and cannot be cured with histamine antagonists.

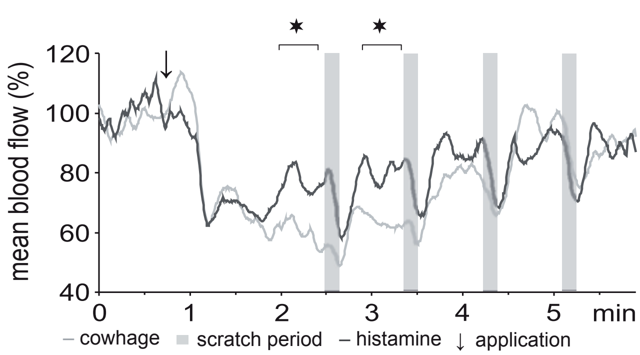

Fig. 4. Reflex changes in blood flow in the pad of the ring finger during the experiment. Averaged laser Doppler flowmetry measurements of 12 subjects are shown. To account for offsets the flux values measured 1 min before stimulus application were set to 100%. For statistical evaluations 30 sec periods before the scratching periods were averaged. The differences between cowhage and histamine before the first and second scratching period are significant. Cowhage induced a significantly stronger vasoconstriction compared with histamine (analysis of variance and *post-hoc* test, $^*p<0.05$).

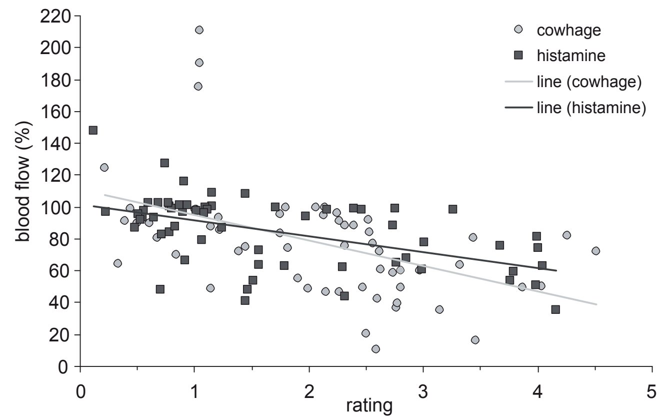

Fig. 5. Correlations of itch ratings and blood flow. Averaged ratings and fluxes during 30 sec time-segments before the scratch periods (product moment correlations, $p<0.05$).

Physiologically, an indication of independent action is the lack of the marked axon reflex flare reaction after cowhage application (9), which is typical for histamine (16), while both agents produce at least equipotent itch. In microneurography experiments on human skin nerves we have shown recently that cowhage excites the common type of polymodal mechano-heat responsive C-fibres (CMH units) and not the mechano-insensitive C-fibres (8), which are the mediators of histaminergic itch and flare (17). This dichotomy of the neuronal itch processing is apparently not restricted to the peripheral nervous system, but extends up the somatosensory nucleus of the thalamus in the monkey, where different neuronal populations are responsive to cowhage and histamine (18).

Shelley & Arthur (6), who thoroughly studied itch induced by cowhage spicules, reported the observation that autoclaved spicules no longer induced itch. This, together with the observation that itch never started immediately after insertion of a spicule, but with a latency of many seconds, led them to the conclusion that the itch-mediating factor must be chemical, i.e. the hypothetical protease mucunain. In this study we have confirmed these results and with a more precise definition of "itch threshold" found an average latency of 27.6 ± 1.9 sec (see Fig. 3).

Histaminergic itch provoked by spicule insertion

Shelley & Arthur (6) have also reported that inactivated cowhage spicules re-gain their itch-producing capacity when soaked with histamine. We have shown in the present study that such a stimulus, in which histamine was conveyed by inactive spicules indeed induces itch comparable with that provoked by active cowhage spicules, and that this itch is accompanied by the typical histamine effects, a prominent wheal (due to the plasma-extravasation effect of histamine on blood vessels) and flare reaction (see Fig. 2). The flare areas obtained with this method (5.6 ± 1.3 cm^2)

were of the same order of magnitude as those obtained by iontophoresis of histamine with 10 mC (16). This, together with the delay of 18.2 ± 1.3 sec between spicule insertion and itch initiation, shows clearly that histamine was indeed the active agent. Spicules containing active mucunain never induced respective flare responses (see Fig. 2 and Results).

Since the two itch-producing agents are introduced into the skin by the same carrier, tiny spicules with a diameter of no more than 5–10 μm, we considered these stimuli ideal for qualitative and quantitative comparison of itch sensations with different underlying neuronal mechanisms, especially as the insertion of the spicules by the cotton applicator was not painful. Johanek (9) also compared cowhage with histamine in psychophysical experiments in which histamine was applied by intracutaneous injections necessarily accompanied by a pricking sensation, which precluded a blind comparison of the ensuing sensations.

Sympathetic reflexes

Besides testing sensory differences we also studied sympathetic vasoconstrictor reflexes induced by the two different pruritic agents. Sympathetic reflexes to itching stimuli have rarely been studied, although it has been known that excitation of unmyelinated cutaneous nerve fibres generates sympathetic reflex vasoconstriction in the skin and our group demonstrated that the itch-provoking histamine is similarly effective as the pain-producing mustard oil. These sympathetic reflexes can be distinguished from transient arousal reactions, which are triggered by all forms of suddenly appearing and unexpected stimuli, since they mirror the duration of the pain and itch sensations and may last many minutes under strong stimulation. In contrast to arousal stimuli they are lateralized to the side of stimulation (10). In the present experiments we were able to demonstrate that histamine and cowhage provoke this kind of prolonged stimulus-specific reflex vasoconstriction following an unspecific arousal response during the first 30 sec after application of the spiculi. The cutaneous sympathetic vascular responses involve a rapidly fading non-specific on-component (vasoconstriction), probably reflecting arousal, and a tonic slowly adapting somato-sympathetic reflex component, which probably reflects the pruritic input. The component relating to the arousal is independent of the type of itch; it is induced only by the mechanical scratch event. In contrast, the slow recovery after this initial vasoconstriction is related to the type of itch. Very similar vascular responses during different types of stimuli were reported by Magerl et al. (19), who applied short noxious stimuli to the skin.

Effects of scratching on cowhage and histamine actions

Scratching was always performed by one experimenter (FK) rather than by the subjects themselves. We never scratched at the stimulus application site, but 3 cm proximal to it. This precluded a direct interaction with the itch-mediating nerve terminals. In the past, itch suppression by scratching has often been attributed to a temporary damaging effect on the itch-mediating terminals. In our experiments this was excluded and hence the scratch effects must be due to central nervous suppression of the itch processing. This finding is in agreement with a previous psychophysical study (20). We did not assess the time-course of the itch suppression during the scratching periods, since our subjects found it difficult to rate the itching on the background of the scratch perception. Interestingly, we found that the itching sensation had completely subsided by the end of each scratching period and we were able to test the latency to reappearance of itch.

Differential effects of cowhage and histamine

The main purpose of this study was to look for differential effects of the two itching agents, which are thought to activate different peripheral and central nervous pathways. In the first experiment we found that the cowhage-induced itching was described to be more stinging, sharp and prickly. Previously, Wolff's group had assumed that "pricking itch" is mediated by A-fibres, whereas "burning itch" is due to excitation of C-fibres (21). From the results of human microneurography it is clear, however, that both cowhage and histamine excite C-fibres, albeit different populations (8). CMH units excited by cowhage form a larger C-fibre population with slightly higher conduction velocities compared with the mechano-insensitive, histamine-sensitive C-fibres (1). It is still not known to what extent both agents excite A-delta units, but in monkey experiments it was again found that different populations are activated by the two agents (M. Ringkamp, John Hopkins University, Baltimore, USA: personal communication). Hence, one may conclude that the small qualitative sensory differences found in the questionnaire are probably due to the different nerve fibre populations and their differential central processing. Another recent study showed that PARs can induce pain; therefore, it has to be considered that cowhage as ligand for PAR can also induce nociceptive responses (22). Cowhage and histamine differed in the time-course of itch induction; histaminergic itch started significantly earlier and increased faster (Figs 3a and b). This may be due to a faster diffusion of histamine from the spicules compared with mucunain and/or to a faster binding to membrane receptors on the receptive nerve terminals. Other explanations could be different histamine and PAR-receptor densities or varying ligand-affinities for the receptors. Cowhage-induced itch recovered significantly faster after the scratching periods. This differential effect is particularly interesting since it can only be explained by a differential time constant of the scratch-induced inhibitory mechanisms in the central nervous system (Figs 3a and c).

Discriminative central processing is also indicated by differences in the sympathetic reflex vasoconstriction, which was stronger under cowhage than histamine, in the period before and after the first scratching. These findings together point to a more efficient central processing and a lower susceptibility of cowhage itch to inhibition.

CONCLUSION

By using identical means of application we have shown commonalities and differences in the processing of pruritus provoked by cowhage and histamine. When introduced into the superficial layers of the skin cowhage induces qualitatively similar itching sensations to those of histamine, although of a sharper character. Both substances induce sympathetic vasoconstrictor reflexes, although cowhage reflexes are more pronounced. Scratching has a more profound inhibitory action on both itch sensations and reflexes in the case of histamine.

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