INVESTIGATIVE REPORT

Mechano-insensitive Nociceptors are Sufficient to Induce Histamine-induced Itch

Marcus SCHLEY1, Roman RUKWIED1, James BLUNK1, Christian MENZER1, Christoph KONRAD2, Martin DUSCH1, Martin SCHMELZ1 and Justus BENRATH1

1Department of Anesthesiology and Operative Intensive Care Mannheim, Medical Faculty Mannheim, University Heidelberg, Heidelberg, Germany, and 2Department of Anesthesiology, Kantonsspital, Luzern, Switzerland

The nerve fibres underlying histamine-induced itch have not been fully elucidated. We blocked the lateral femoral cutaneous nerve and mapped the skin area unresponsive to mechanical stimulation, but still sensitive to electrically induced pain. Nerve block induced significantly larger anaesthetic areas to mechanical (100 mN pin-prick, 402 ± 61 cm²; brush, 393 ± 63 cm²) and heat pain stimuli (401 ± 53 cm²) compared with electrical stimulation (352 ± 62 cm², p<0.05), whereas the anaesthetic area tested with 260 mN (374 ± 57 cm²) did not differ significantly. Histamine was applied by iontophoresis (7.5 mC) at skin sites in which mechanical sensitivity was blocked, but electrical stimulation was still perceived 30 min after the nerve block (n = 9). In these areas iontophoresis of histamine provoked itching in 8/9 subjects with a mean maximum of 4.6 ± 1 (on an 11-point rating scale). Histamine-induced itch can thus be perceived at skin sites where input from mechano-sensitive polymodal nociceptors is blocked. In conclusion, input from mechano-insensitive nociceptors is sufficient to generate histamine-induced itch. Key words: pruritus; peripheral nerve block; axon reflex erythema; heat pain; mechanical pain.

Accepted Sep 18, 2012; Epub ahead of print Feb 14, 2013

Acta Derm Venereol 2013; 93: 394–399.

Martin Schmelz, Department of Anesthesiology, University of Heidelberg, DE-68167 Mannheim, Germany. E-mail: martin.schmelz@medma.uni-heidelberg.de

The neuronal pathways mediating histaminergic itch in humans have been discussed controversially for decades. However, the question appeared to be resolved after a class of mechano-insensitive C-fibres were characterized that responded to histamine application with a time course that matches the accompanying itch sensation (1). The role of mechano-insensitive afferents in histamine-induced itch was supported by experiments in the cat, reporting mechano-insensitive spinothalamic projection neurones that responded to histamine iontophoresis with a similar time course to that of itch sensation in humans (2). Moreover, recordings from a patient with chronic itch, showing spontaneously active histamine-sensitive “itch-fibres” confirmed a specific class of afferent nerve fibres for the sensation of itch (3). However, recent results from histamine stimulation in monkeys have suggested that “polymodal” (heat- and mechano-sensitive) nociceptors may also be involved in histamine-induced itch (4, 5). Mechano-insensitive C-nociceptors (CMi) cannot be activated even by strong mechanical stimuli (750 mN v.Frey) and are characterized by high electrical and thermal thresholds (6). Due to their high activation thresholds, the CMi fibres cannot be selectively stimulated for differential functional analysis. The innervation territories of CMi fibres are approximately twice as large (7) as those of polymodal nociceptors (8). Approximately 10% of the mechano-insensitive nociceptors are histamine-responsive (9). The innervation territories of these histamine-sensitive mechano-insensitive fibres cover large areas (diameters up to 8 cm (1)). In a given cutaneous peripheral nerve, these larger innervation territories of CMi-fibres may extend further than that from mechano-sensitive afferents, and thus may allow for their selective stimulation: following a complete nerve block the sensory capacity in the border zone of anaesthesia will be determined by the unblocked neighbouring skin nerves that partially overlap the innervation territories of the anaesthetized fibres. Obviously, a sensation is still provided in this zone by the unblocked skin nerves. It can be hypothesized that the larger innervation territories of mechano-insensitive nociceptors reach farther into the anaesthetized skin compared with the mechano-sensitive units with smaller innervation territories. Thus, under these conditions, one would predict that after a nerve block there is an area of skin adjacent to the completely anaesthetized skin in which mechano-insensitive fibres from unblocked neighbouring skin nerves can be excited, whereas this skin area does not contain mechano-sensitive nociceptors from the unblocked neighbouring skin nerves. In this particular zone of differential overlap, the excitation of polymodal mechano-sensitive nociceptors by, for example, pin-prick stimulation, would not be perceived, whereas high-intensity electrical stimulation, also recruiting mechano-insensitive units, should still evoke pain.

We therefore blocked the lateral femoral cutaneous nerve (LFCN) in healthy volunteers and mapped the anaesthetic areas for touch, pin-prick, heat, and electrical stimulation. When areas were identified in which...
Mechano-insensitive nociceptors and histamine-induced itch

Mechanical stimuli were not perceived, but electrical current still evoked pain, histamine was applied in this zone. Activation of histamine-sensitive fibres was assessed subjectively by psychometric itch recording and objectively by measurement of the axon reflex flare.

METHODS

Subjects
Ten healthy Caucasian male volunteers were recruited from the university and local community. Participants were 30 ± 6.3 years old (mean ± standard deviation (SD); age range 22–36 years), height 181 ± 1.9 cm, weight 74.6 ± 8.4 kg. All participants provided written informed consent in accordance with the Declaration of Helsinki prior to participation, as approved by the local ethics committee. Participants were informed about health risks of the study and instructed about risks or side-effects of a nerve block of the LFCN. In addition, written information was provided about the study procedures. Participants with atopic disposition and who were on antihistaminic medical treatment, or who had had such treatment in the past, were excluded.

Peripheral nerve block
Ultrasoundography (Sonoline G40, Siemens, Germany) was used to identify the anatomical structure of the LFCN. With the ultrasound probe (11.4 MHz) scanning transversely approximately 1 cm inferior to the anterior superior iliac spine (ASIS), the sartorius muscle was identified. The LFCN runs superficially along the sartorius muscle in a tissue plane deep to the fascia lata, but superficially to the fascia iliaca. A G27-needle was inserted in line with the ultrasound probe in between the 2 fasciae and the location of the LFCN confirmed by the patient experiencing an electric sensation (DSTA Digitimer Ltd, Welwyn Garden City, UK). After identification of the LFCN, a total volume of 1 ml mepivacaine 1% was injected, and 30 min thereafter the sensory capacity areas of the LFCN tested.

Histamine iontophoresis
Histamine (histamine dihydrochloride, 1% in aqua dest.) was applied by iontophoresis (0.5 mA, 30 s) (WPI A360 Stimulus Isolator, World Precision Instruments, New Haven, CT, USA) via a plastic applicator (diameter 5 mm, volume 50 µl) equipped with a silver chloride electrode connected to the anode of the stimulator. A surface electrode (10 cm²) was attached distally on the subject’s leg as reference cathode.

The stimulation was applied after the complete mapping procedure, approximately 50 min after the injection of 1 ml mepivacaine 1%. The exact site of histamine administration was chosen according to the mapped mechano-sensitive and electro-receptive fields. The maximum distance between the larger area insensitive for mechanical stimuli, and the smaller area insensitive for electrical stimuli, was located. Within this field, the iontophoresis applicator was placed inside the mechano-negative, but electro-positive, area. In particular, it was carefully located at the border of electrical sensitivity and towards the completely anaesthetized zone, in order to minimize the stimulation of neighbouring mechano-sensitive fibres. After the final itch rating, the mechano-insensitivity of the application site was tested with a 260 mN v. Frey filament.

Activation of histamine-responsive mechano-insensitive fibres was assessed by measuring the area of the axon reflex by laser Doppler imaging (Moor LDI, Axminster, UK). Two images were registered as baseline followed by histamine iontophoresis (0.5 mA, 30 s), and immediately afterwards 2 laser Doppler imagin scans of blood flow were captured to assess the histamine-induced axon reflex. Axon reflex areas were defined as pixels that increased their flux values >2 SD in the baseline scan (10). Subjects were asked to rate the intensity of the itch sensation on an 11-point numerical rating scale (NRS), from 0 (“no itch”) to 10 (“maximum itch imaginable”) and maximum itch-ratings were recorded.

Evaluation of the mapped areas
Skin markings were transferred to a transparency and scanned at 300 dpi. In addition, a photograph was taken with a digital camera for documentation. Areas were assessed off-line by a Digital Imaging and Communications in Medicine computer program (OsirisX, Apple).

Acta Derm Venereol 93
Statistics
All data were analysed using the software package Statistica 6.0 (StatSoft, Tulsa, OK, USA). Two-way analysis of variance (ANOVA) and repeated measures with “anaesthesia area” as categorical factor were used. Differences between areas anaesthetic to electrical stimulation and those anaesthetic to the other stimuli were calculated by Fisher’s least significant difference test. Correlations between itch ratings and flare area were analysed by Spearman’s rank correlation, with values of $p < 0.05$ considered significant.

RESULTS
Differential local anaesthetic effect
The injection of local anaesthetic provoked an anaesthetic area in the thigh in each of the 10 subjects. In 1 out of 10 subjects, however, no areas of differential innervation territories could be identified. It was notable that in this subject the anaesthetized zones were relatively small, $82 \pm 24$ cm$^2$ (mean ± standard error of the mean (SEM)), thus indicating failure of a successful nerve block, and thus this subject was omitted from the analysis. The areas anaesthetic ($n = 9$) to electrical stimulation were the smallest ($352 \pm 62$ cm$^2$), whereas those anaesthetic to brush ($393 \pm 63$ cm$^2$), punctate mechanical stimuli of 100 mN ($402 \pm 61$ cm$^2$) and heat ($401 \pm 53$ cm$^2$) were the largest ($p < 0.05$). The area anaesthetic to pin-prick stimulation of 260 mN ($374 \pm 57$ cm$^2$) was not significantly larger than the one anaesthetic to electrical stimulation. It is noteworthy that heat stimulation was negative in 4 of the subjects and only weak heat pain was evoked when the 45ºC heat stimulus was kept on the skin for more than 10 s. Fig. 1 shows a specimen of the anaesthetic zones mapped with mechanical, heat and electrical stimuli. The largest distance between the electrically sensitive field and the pin-prick negative mechano-receptive field was 5.5 cm. The minimum distance between positive transcutaneous electrical stimulation and negative pin-prick sensation was 2 cm (mean maximum distance $4.8 \pm 1.2$ cm).

Histamine iontophoresis and itch intensity
Histamine administered iontophoretically at the border of differential sensitivity to mechanical and electrical stimuli (Figs 1 and 2) evoked a large axon reflex erythema of mean $28.9 \pm 1.6$ cm$^2$ in 9 subjects. In 8 out of the 9 tested subjects, an itch sensation was perceived, which gradually increased and reached peak levels after 1–2 min (Fig. 3). A mean maximum itch intensity of $4.6 \pm 1.2$ (NRS) was recorded (Fig. 3). The subject who did not perceive any itch (S1 in Fig. 3), however, responded with a moderate histamine-evoked erythema. No correlation between the area of axon reflex erythema and maximum itch ratings was found (Spearman’s rank correlation coefficient 0.22, n.s.).

DISCUSSION
Local regional block of the LFCN provoked a large anaesthetic skin area at the lateral thigh, the borders of which were determined by the innervation territories of the neighbouring non-blocked skin nerves. The areas anaesthetic to mechanical stimuli were larger than those anaesthetic to strong electrical stimulation. Thus, the outer part of the anaesthetic skin area was anaesthetic to mechanical stimulation, but electrical stimuli were still perceived as painful. This suggests that mechano-insensitive fibres from neighbouring, non-anaesthetized skin nerves were reaching farther into the innervation...
Mechano-insensitive nociceptors and histamine-induced itch

...territory of the blocked LFCN compared with mechanically sensitive nociceptors. Hence, after the LFCN block, we identified skin areas in which input from mechanically sensitive nociceptors was blocked, whereas input from electrically stimulated mechano-insensitive nociceptors was still perceived. Histamine application in these mechano-insensitive but electrically sensitive areas provoked an itch sensation in 8 out of 9 subjects, indicating that activation of mechano-insensitive nerve fibres was sufficient to provoke histamine-induced itch.

Pruriceptors vs. polymodal nociceptors

Previous studies in cat (2) and man (1, 9) suggested that histamine-induced itch is mediated by a specific subtype of mechano-insensitive C-fibres. Recently, activation of polymodal nociceptors has been described in monkeys after the injection of histamine (4, 5). It is possible that the different mode of histamine application, i.e. injection compared with iontophoresis, leads to higher peak concentrations, and thereby contributes to these differences. Our results do not rule out that activation of polymodal nociceptors by histamine injection plays a role in the ensuing itch sensation. Thus, we can conclude that activation of mechano-insensitive nociceptors is sufficient to provoke histamine-induced itch. However, our study cannot prove that the activation of mechano-insensitive nociceptors is necessary to provoke histamine-induced itch.

A similar time course of itch and pain was generated by applying cowhage spicules, native or inactivated, covered with either histamine or capsaicin (11). As no qualitative difference in the itch reaction of these mediators was found, a common neurophysiological pathway of itch was proposed (11). This common pathway may not include mechano-insensitive fibres, as their activation is functionally linked to the generation of an axon reflex flare erythema (12), and this was not observed following cowhage application (13). Thus, as the results in monkeys show (4, 5), polymodal nociceptors could also be candidates to mediate histamine-induced itch.

Overlapping innervation territories of nociceptors

Our results demonstrate that a peripheral nerve block induced very similar anaesthetic areas for touch and pin-prick. The borders of the anaesthetic areas are determined by the non-blocked skin nerves adjacent to the LFCN. The receptive field sizes of human polymodal nociceptors are approximately 1–2 cm² in the lower leg (8), with a mean maximal diameter of approximately 20 mm. This extent matches that measured for mechano-sensitive A fibres in the human arm (14). Thus, similar anaesthetic areas for touch and pin-prick are in line with the similar size of innervation territories of the primary afferents. In contrast, mechano-insensitive C nociceptors have larger receptive fields with median diameters of 46 mm (7). Thus, it might be predicted that mechano-insensitive units of neighbouring (not anaesthetized) skin nerves would extend further into the skin innervated by the blocked LFCN according to the larger spatial extent of the innervation territory of mechano-insensitive units. In addition, there is evidence that very strong mechanical stimulation (260 mN) can produce some activation of C-nociceptors normally classified as mechano-insensitive (15, 16); this might explain the rather small anaesthetic areas found for stimulation with a 260 mN filament.

Skin areas anaesthetic to heat could not be tested with the same spatial resolution as for mechanical stimuli. We would expect them to match those observed for pin-prick stimulation, based on the role of polymodal nociceptors for mechanically and heat-induced pain (17). However, there is also some heat responsiveness in mechano-insensitive nociceptors, albeit their activation thresholds are higher (42°C to > 50°C) (7, 9, 18). Given that our test stimulus for heat was only 45°C, it may have activated just a few of the mechano-insensitive nociceptors, which can explain the delayed response and the lower intensity of heat pain in some of the investigated subjects.
Histamine-induced itch in skin sites anaesthetic for mechanical stimulation

Histamine iontophoresis in skin areas that were anaesthetic for mechanical stimulation provoked itch in 8 of the 9 subjects. This observation suggests that the activation of mechano-insensitive C-fibres is sufficient to cause histamine-induced itch. As pin-prick stimulation was not perceived at the histamine application site, it can be assumed that polymodal nociceptors were either completely blocked or that non-blocked polymodal fibres of neighbouring nerves were not sufficient to convey the pin-prick sensation. As activation of mechano-sensitive fibres at the suprathreshold level for 1 s did not provoke an overt sensation in the partially anaesthetized skin, we would conclude that these mechano-sensitive fibres did not crucially contribute to the itch sensation elicited from the same skin site. However, we cannot completely exclude that tonic discharge of polymodal nociceptors might still contribute to the histamine-induced itch. Although we cannot exclude a role of mechano-sensitive nociceptors, we conclude that histamine can induce itch without overt input from mechano-sensitive nociceptors. Our results therefore support the idea that activation of specific mechano-insensitive histamine-sensitive primary afferents is sufficient to provoke histamine-induced itch.

Itch intensity is encoded by the discharge frequency of the action potentials. Interestingly, the axon reflex flare, mediated by mechano-insensitive nociceptors (12), is already maximum at frequencies of approximately 2–5 Hz (19). Bursts of action potentials at higher frequencies are therefore expected to cause intense itching, but not necessarily a further increase in the axon reflex erythema. Even though higher discharge frequencies increase the release of vasodilatory neuropeptides, such as calcitonin gene-related peptide (CGRP) (20), due to the very steep dose-response curve for CGRP-induced vasodilation (21), CGRP levels might readily become supra-maximum, which explains the homogenous level of vasodilation inside the axon reflex flare (22).

The present study does not exclude a role for polymodal nociceptors in histamine-induced itch, as the activation of polymodal nociceptors by a histamine injection might contribute to the ensuing itch sensation. Moreover, activity of polymodal nociceptors stimulated by cowhage (23) exemplifies the potential of this nociceptor class for the induction of itch. However, previous work suggested that mechanically induced pain accompanying a histamine injection would reduce rather than increase the histamine-induced itch (24). Regardless of a possible contribution of polymodal nociceptors to the histamine-induced itch it should be pointed out that the existence of a histamine-specific pathway of primary afferents and spinal cord neurones for itch does not provide evidence against other, non-specific, pathways for itch (25) that may be activated by cowhage (4, 5) or even capsaicin (11). On the other hand, clinically, histamine-independent pathways of itch are considered crucial in the pathogenesis of chronic itch (26).

In summary, this study demonstrates that blocking the LFCN results in differential areas of anaesthesia to mechanical and electrical stimuli. In skin sites negative to mechanical stimulation, but positive to electrical stimulation, histamine application evokes a typical itch and flare response. We conclude that mechano-insensitive nociceptors are sufficient to provoke histamine-induced itch.

ACKNOWLEDGEMENTS

This study was supported by the “Kompetenzzentrum Schmerz” Baden-Württemberg, Germany.

The authors declare no conflicts of interest.

REFERENCES

1. Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjörk HE. Specific C-receptors for itch in human skin. J Neurosci 1997; 17: 8003–8008.
2. Andrew D, Craig AD. Spinothalamic lamina 1 neurons selectively sensitive to histamine: a central neural pathway for itch. Nat Neurosci 2001; 4: 72–77.
3. Schmelz M, Hilliges M, Schmidt R, Örstavik K, Vahlquist C, Weidner C, et al. Active “itch fibers” in chronic pruritus. Neurology 2003; 61: 564–566.
4. Davidson S, Zhang X, Yoon CH, Khasabov SG, Simone DA, Giesler G Jr. The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. J Neurosci 2007; 27: 10007–10014.
5. Johaneck LM, Meyer RA, Friedman RM, Greenquist KW, Shim B, Borzan J, et al. A role for polymodal C-fiber afferents in nonhistaminergic itch. J Neurosci 2008; 28: 7659–7669.
6. Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjörk HE. Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. J Neurosci 1999; 19: 10184–10190.
7. Schmidt R, Schmelz M, Weidner C, Handwerker HO, Torebjörk HE. Innervation territories of mechano-insensitive C nociceptors in human skin. J Neurophysiol 2002; 88: 1859–1866.
8. Schmidt R, Schmelz M, Ringkamp M, Handwerker HO, Torebjörk HE. Innervation territories of mechanically activated C nociceptor units in human skin. J Neurophysiol 1997; 78: 2641–2648.
9. Schmelz M, Schmidt R, Weidner C, Hilliges M, Torebjörk HE, Handwerker HO. Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. J Neurophysiol 2003; 89: 2441–2448.
10. Dusch M, Schley M, Obreja O, Forsch E, Schmelz M, Rukwied R. Comparison of electrically induced flare response patterns in human and pig skin. Inflamm Res 2009; 58: 639–648.
11. Sikand P, Shimada SG, Green BG, LaMotte RH. Similar itch and nociceptive sensations evoked by punctate cutaneous application of capsaicin, histamine and cowhage. Pain 2009; 144: 66–75.
12. Schmelz M, Michael K, Weidner C, Schmidt R, Torebjörk HE, Handwerker HO. Which nerve fibers mediate the axon reflex flare in human skin? Neuroreport 2000; 11: 645–648.
Mechano-insensitive nociceptors and histamine-induced itch

13. Johanek LM, Meyer RA, Hartke T, Hobelmann JG, Maine DN, LaMotte RH, Ringkamp M. Psychophysical and physiological evidence for parallel afferent pathways mediating the sensation of itch. J Neurosci 2007; 27: 7490–7497.

14. Vallbo AB, Olausson H, Wessberg J, Kakuda N. Receptive field characteristics of tactile units with myelinated afferents in hairy skin of human subjects. J Physiol 1995; 483: 783–795.

15. Obreja O, Ringkamp M, Namer B, Forsch E, Klusch A, Rukwied R, et al. Patterns of activity-dependent conduction velocity changes differentiate classes of unmyelinated mechano-insensitive afferents including cold nociceptors, in pig and in human. Pain 2010; 148: 59–69.

16. Meyer RA, Davis KD, Cohen RH, Treede RD, Campbell JN. Mechanically insensitive afferents (Mias) in cutaneous nerves of monkey. Brain Res 1991; 561: 252–261.

17. Torebjörk HE, LaMotte RH, Robinson CJ. Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: simultaneous recordings in humans of sensory judgments of pain and evoked responses in nociceptors with C-fibers. J Neurophysiol 1984; 51: 325–339.

18. Schmidt R, Schmelz M, Forster C, Ringkamp M, Torebjörk E, Handwerker H. Novel classes of responsive and unresponsive C nociceptors in human skin. J Neurosci 1995; 15: 333–341.

19. Dusch M, Schley M, Rukwied R, Schmelz M. Rapid flare development evoked by current frequency-dependent stimulation analyzed by full-field laser perfusion imaging. Neureport 2007; 18: 1101–1105.

20. Sauerstein K, Klede M, Hilliges M, Schmelz M. Electrically evoked neuropeptide release and neurogenic inflammation differ between rat and human skin. J Physiol 2000; 529: 803–810.

21. Klede M, Weidner C, Rukwied R, Lischetzki G, Petersen LJ, Schmelz M. Effects of SP and CGRP in human skin – a microdialysis study. Seattle: IASP Press, 1999.

22. Bickel A, Kramer HH, Hilz MJ, Birklein F, Neundorfer B, Schmelz M. Assessment of the neurogenic flare reaction in small-fiber neuropathies. Neurology 2002; 59: 917–919.

23. Namer B, Carr R, Johanek LM, Schmelz M, Handwerker HO, Ringkamp M. Separate peripheral pathways for pruritus in man. J Neurophysiol 2008; 100: 2062–2069.

24. Atanassoff PG, Brull SJ, Zhang J, Greenquist K, Silverman DG, LaMotte RH. Enhancement of experimental pruritus and mechanically evoked dysesthesiae with local anesthesia. Somatosens Mot Res 1999; 16: 291–298.

25. Davidson S, Giesler GJ. The multiple pathways for itch and their interactions with pain. Trends Neurosci 2010; 33: 550–558.

26. Weisshaar E, Szepietowski JC, Darsow U, Misery L, Wålengren J, Mettang T, et al. European guideline on chronic pruritus. Acta Derm Venereol 2012; 92: 563–581.