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Research article

Capsaicin-sensitive cutaneous primary afferents convey electrically induced itch in humans

Hjalte H. Andersen[a], Antoinette I.M. van Laarhoven[a,b,c,d], Frederik D. Justesen[a], Jacob B. Pedersen[a], Laurits L. Sørensen[a], Line P. Jensen[a], Lars Arendt-Nielsen[a,*]

[a] Laboratory for Experimental Cutaneous Pain Research, SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark
[b] Health, Medical and Neuropsychology Unit, Faculty of Social and Behavioral Sciences, Leiden University, Netherlands
[c] Leiden Institute for Brain and Cognition (LIBC), Leiden University, Netherlands
[d] Department of Psychiatry, Leiden University Medical Center, Leiden, Netherlands

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ABSTRACT

Specially designed transcutaneous electrical stimulation paradigms can be used to provoke experimental itch. However, it is unclear which primary afferent fibers are activated and whether they represent pathophysiologically relevant, C-fiber mediated itch. Since low-threshold mechano-receptors have recently been implicated in pruriception we aimed to characterize the peripheral primary afferent subpopulation conveying electrically evoked itch in humans (50 Hz stimulation, 100 μs square pulses, stimulus-response function to graded stimulus intensity). In 10 healthy male volunteers a placebo-controlled, 24-h 8% topical capsaicin-induced defunctionalization of capsaicin-sensitive (transient receptor potential V1-positive, 'TRPV1'$^{+}$) cutaneous fibers was performed. Histaminergic itch (1% solution introduced by a prick test lancet) was provoked as a positive control condition. Capsaicin pretreatment induced profound loss of warmth and heat pain sensitivity (pain threshold and supra-threshold ratings) as assessed by quantitative sensory testing, indicative of efficient TRPV1-fiber defunctionalization (all outcomes: $P < 0.0001$). The topical capsaicin robustly, and with similar efficaciousness, inhibited itch intensity evoked by electrical stimulation and histamine ($-89 \pm 4.1\%$ and $-78 \pm 4.9\%$, respectively, both: $P < 0.0001$ compared to the placebo patch area). The predominant primary afferent substrate for electrically evoked itch in humans, using the presently applied stimulation paradigm, is concluded to be capsaicin-sensitive polymodal C-fibers.

1. Introduction

Itch in both experimental and clinical itch studies is frequently elicited by various transcutaneous electrical stimulation paradigms [1–6]. These methods have been used to document increased itch sensitivity in chronic itch patients [5–7] and allow for temporal control and customization of the stimulation intensity, opposite to the more thoroughly investigated chemical itch models [8]. On the other hand electrical stimulation is non-selective, unphysiological, and it is unclear which primary afferent fibers that are involved in conveying electrically elicited itch [1,2]. Notably, electrical stimulation often produces co-sensations such as tapping, buzzing or tingling, generally associated with activity of large myelinated primary afferents [1,9,10]. Recently, low-threshold mechano-receptors (LTMRs), i.e. C-tactile and Aβ-fibers, have been implicated in mechanically evoked itch in response to stimuli that are probably below the threshold of pruriceptive nociceptors [11,12]. This prompts reconsideration as to whether electrically evoked itch paradigms actually probes the afferent units that are spontaneously active and sensitized in patients with chronic itch or whether an entirely different pathway is activated. In humans prolonged topical application of high-concentration capsaicin profoundly defunctionalize dermo-epidermal nociceptive fibers expressing transient receptor potential V1 channel (TRPV1) [13], while leaving e.g. LTMRs intact, and has previously been shown to almost entirely inhibit warmth/heat pain sensations as well as itch evoked by activation of both C-mechano-insensitive (histaminergic) and polymodal C-fibers (cowhage-induced) [14]. This desensitization pretreatment allows the investigation of, e.g., sensory responsiveness, in a skin area where TRPV1-expressing fibers have been robustly defunctionalized. This study aimed to elucidate the primary afferent class responsible for conveying electrically evoked itch. In a double-blinded, placebo-controlled, cross-over design, an ablation of capsaicin-sensitive dermo-epidermal C- and Aδ-fibers in human skin was performed, validated by psychophysical warmth/heat pain assessments. Subsequently, electrically evoked itch sensitivity was

* Corresponding author at: Fredrik Bajers Vej 7, Bld. D3, DK-9220 Aalborg E, Denmark.
E-mail address: LAN@hst.aau.dk (L. Arendt-Nielsen).

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tested in the capsaicin/placebo-pretreated areas and histaminergic itch was used as a positive control.

2. Materials and methods

Ten healthy male volunteers were enrolled (23.6 years, range 21–25). The local ethics committee approved the protocol (N-20160026). Subjects were informed about the procedures involved in the study and gave their written informed consent prior to the experiment. The study consisted of three sessions and lasted approximately 2.5 h in total. The first session was a screening where non-responders to the electrical paradigm were excluded; defined as a peak itch of < 20 ($VAS_{0\text{-}100}$) [2], prompting $N = 3$ exclusions. Then capsaicin and placebo patches were then applied and removed after 24 h (session two). In the third session, 24 h after patch removal the psychophysical tests were conducted in both the placebo and capsaicin-treated areas.

2.1. Capsaicin-induced fiber ablation

Two 4 × 4 cm areas, 3 cm apart, on the medial aspect of the dominant volar forearm were pretreated with either an 8% capsaicin patch (Qutenza®, Grüenthal, Germany) or a placebo patch for 24 h. Distal versus proximal application sites were randomized in a balanced manner. The application of additional opaque occlusion was utilized to blind the subjects (taking advantage of the poor localizability of chemonociceptive stimuli) and to blind the investigators performing the psychophysiological tests. 24 h after patch removal the test session was performed. This technique has previously been used to study the skin under capsaicin-sensitive fiber depleted conditions [13,14].

2.2. Validation of capsaicin-induced fiber ablation

A 3 × 3 cm thermal probe was attached to the 4 × 4 cm patch application skin areas. The probe was connected to a Pathway sensory stimulator (Medoc, Ramat Yishai, Israel), controlled by Medoc Main Station software. The baseline temperature was always 32° C and for warmth detection and heat pain threshold ('WDT' and 'HPT', respectively) ramping stimuli of 1° C/s were delivered until the subjects identified the associated threshold (first perception warmth and first perception heat pain) by pressing a stop button [15]. Hereafter the temperature returned to 32° C, at a rate of 1° C/s. WDT and HPT were performed in triplicates and averaged. Suprathreshold heat pain sensitivity (SHPS) was assessed by two ramps-and-hold stimuli lasting 1 s at 50° C with ascending and descending ramp rates of 5° C/s [13]. Subjects rated each evoked stimulus on a numerical rating scale from 0 = 'no pain' to 10 = 'worst imaginable pain'.

2.3. Electrically evoked itch stimulus-response function

The electrical stimuli were delivered by a constant current stimulator (DS5; Digitimer, United Kingdom), which was controlled by a laptop via a data acquisition system (NI USB-6221 or NI-DAQmx, National Instruments, TX, USA). Two surface electrodes were attached 2 cm apart within the pretreated 4 × 4 cm. Ramp stimuli were applied at 50 Hz with a pulse duration of 100 µs, using an increasing current intensity (0.05 mA/s). Current intensity started at 0.4 mA and ended at 6.4 mA (2-min duration per stimulus ramp). This stimulation paradigm is described in details elsewhere [2,4]. The stimulus-response curve was constructed by simultaneous ratings of itch intensity obtained using a 100 mm digital visual analog scale (VAS) on a tablet: 'no itch' = 0 mm, and 'worst imaginable itch' = 100 mm. Itch intensity ratings were conducted continuously and sampled every 5 s.

2.4. Histamine evoked itch

Histamine dihydrochloride 1% solution (Allergopharma, Germany) was applied using a 1-mm shouldered skin prick test (SPT) lancet. A drop was placed in the center of the placebo or capsaicin-pretreated area and pricked with the SPT lancet using a 120 g weighted device (Aalborg University, Denmark) as previously described [16]. Immediately hereafter a 10-min VAS-recording of the itch intensity was initiated using the same approach as described for electrically evoked itch.

2.5. Statistics

Analyses were performed with SPSS 25 (IBM, Armonk, USA). Residuals for all variables were normally distributed according to Shapiro-Wilk's test. Thermal validation tests were assessed using paired-samples *t*-tests. The mean and peak itch evoked electrically and by histamine following capsaicin/placebo pretreatment was assessed by two-way repeated measures ANOVAs with two factors: *treatment* (levels: capsaicin and placebo) and *provocation method* (levels: electrical and histamine-induced). Post hoc testing was adjusted with the Bonferroni procedure. $P < 0.05$ was considered significant.

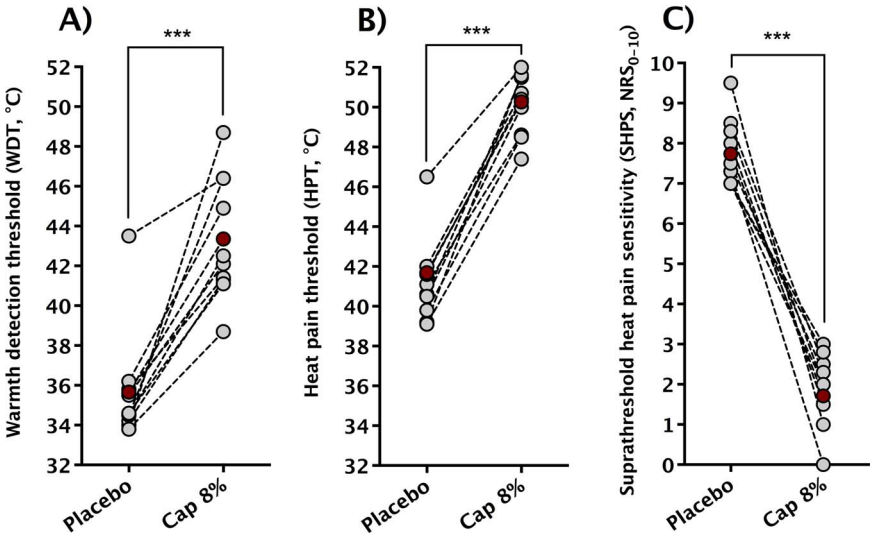

Fig. 1. A, **B** and **C**) Validation of capsaicin-induced sensory desensitization. Individual subject (grey dots) and mean (red dots / dark grey dots in print version) increase in warmth detection threshold (**A**), heat pain threshold (**B**) and suprathreshold heat pain sensitivity (**C**). Cap = capsaicin. $N = 10$ for all plots. *** = $P < 0.0001$.