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Research Article

Antipruritic Effects of Doxepin Cream on Experimentally Induced Histaminergic and Nonhistaminergic Itch

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Background. Itch can be transmitted by two parallel pathways, histaminergic and nonhistaminergic. Histaminergic itch is transmitted by a subgroup of mechano-insensitive C-fibers, while nonhistaminergic itch by a subgroup of polymodal C-fibers. Experimental models are used to study pruritus: histamine for the histaminergic itch by antagonizing the histamine H1-receptors, and BAM8-22 and cowhage for the nonhistaminergic by activating Mas-related G protein-coupled receptors and protease-activated receptors, respectively. This study aims to evaluate the antipruritic effects of topical doxepin (H1-receptor antagonistic effect) on histaminergic and nonhistaminergic itch induced by histamine, BAM8-22, and cowhage. *Methods*. This study was conducted on 22 healthy subjects. Histamine, BAM8-22, cowhage, and vehicle were applied to 4 areas on the forearms. After 7 days, the same substances were applied after 11/2 hour-pretreatment with doxepin. After the application of pruritogens, itch and pain intensities were assessed for 9 minutes, followed by the measurement of superficial blood perfusion (SBP), mechanical and thermal sensitivities. *Results*. Application of histamine, BAM8-22, and cowhage all induced itch as compared to a vehicle. The pretreatment with doxepin almost abolished the histamine-induced itch and modestly reduced BAM8-22- and cowhage-induced itch. Histamine induced a higher SBP compared to the other conditions. Doxepin reduced SBP induced by each pruritogen, even though SBP of histamine remains the highest. *Conclusion*. Doxepin cream abolished histaminergic itch by antagonizing the peripheral H1-histamine receptors. Moreover, doxepin reduced nonhistaminergic itch and related neurogenic inflammation. Further studies are needed to elucidate the molecular mechanisms underlying this peripheral modulation of nonhistaminergic itch by a topically applied H1-antagonist. This trial is registered with NCT04588532.

1. Introduction

Itch, the unpleasant somatosensation that evokes the desire to scratch [1], is a common symptom of several skin conditions and systemic diseases [2], e.g., psoriasis, atopic dermatitis, drug-induced itch, chronic kidney disease, and brachioradial pruritus [3]. In these conditions, itch usually persists for more than 6 weeks and it is defined as chronic itch [3]. Chronic itch causes a reduction in the life quality for the patients by affecting functions such as attention, sleep, and sexual activity [4]. Up to now, knowledge in the itch field should be improved, and this could explain why no completely efficient treatments are available yet, particularly for the nonhistaminergic itch [5, 6].

In research practice, different human experimental models for histaminergic and nonhistaminergic itch are used, even though none of them can mimic chronic conditions [7]. For histaminergic itch, histamine is used, and it binds histamine receptors 1 (H1R) present on the surface of a subgroup of mechano-insensitive C-fibers (CMi-fibers), also expressing transient receptor potential vanilloid 1 (TRPV1) [8–10]. For nonhistaminergic itch, cowhage and bovine adrenal medulla (BAM)8-22 are used. Cowhage, which contains mucunain, induces itch by the biding to the protease-activated receptors (PAR2 and PAR4) [11–13], while BAM8-22 to Mas-related G protein-coupled receptors (Mrgprs) [14], in particular MrgprX1 [15]. In both cases, the itch transmission occurs through a subgroup of polymodal C-fibers (PmC-fibers) [7, 14] and a coactivation of transient receptor potential ankyrin 1 (TRPA1) [10–13].

Another difference between these models concerns the induced neurogenic inflammation. In fact, histamine induces an axon-reflex flare in the area surrounding the application site, while no or only minimal flare is observed for cowhage or BAM8-22 [16–18]. This could be explained by the theory that PmC-fibers reside more superficially in the skin than the CMi-fibers [19–21] and histamine acts into the vascularized dermis [18, 22].

Considering that, this study aimed to (1) compare the itch and pain induced by histamine, cowage, and BAM8-22; (2) assess if these three pruritogens induce different neurogenic inflammation and alteration in mechanical and thermal sensitivities; and (3) compare the effect of the pretreatment with doxepin cream (a tricyclic antidepressant drug with H1R-antagonistic properties) on histaminergic and nonhistaminergic itch.

2. Methods

2.1. Subjects and Study Design. For the present experiment, 22 healthy subjects were recruited (age: from 20 to 33; 8 females and and 14 males). Subjects could not take part in the experiment if pregnant or in lactation; have skin diseases; use of medications (e.g., painkillers or antihistamines); previous or current neurologic, musculoskeletal, or mental illnesses; acute or chronic itch or pain; and allergic to antihistaminic drugs or tricyclic antidepressant. The regional Ethics Committee approved the protocol (N-20190062), and all subjects signed an informed consent form in accordance with the Helsinki Declaration. The data were collected at Aalborg University in 2021. The study (randomized, single-blinded, and controlled trial) included two sessions over a period of 7 days. During the first session, four squared areas (4 × 4 cm, 4 cm apart) were selected on the forearms (two areas in each forearm) of each subject. The four areas were randomly treated with BAM8-22, cowhage, histamine, or vehicle for 9 minutes during which itch and pain intensities were assessed followed by the measurements of superficial blood perfusion (SBP), and mechanical and thermal sensitivities. Each area was treated and tested separately and the pruritogens application and the tests on a single area took approximately 30 minutes. During the second session (after 7 days), doxepin cream was applied under occlusion to the same four areas for 11/2 hours. After the removal of the cream, the first session was repeated.

2.2. Induction Parameters. BAM8-22: BAM8-22 (Sigma-Aldrich, SML0729) was dissolved in distilled sterile water and 1 mg/ml solution was obtained. BAM8-22 was delivered by using heat-inactivated cowhage spicules. Cowhage spicules (inactivated by autoclaving, 120°C for 50 minutes) were soaked overnight in the BAM8-22 solution. 25-30 spicules (counted by forceps using a stereo-microscope) were gently rubbed in the middle of the skin area (∅ 1 cm) reaching approximately the keratinous layer of the skin (0.05–0.15 mm) [23, 24], and 9 minutes after the application, they were rapidly removed by a tape [11].

Cowhage: 25–30 cowhage spicules were applied by gently rubbing in the middle of the skin area (∅ 1 cm), and 9 minutes after the application, they were removed by a tape [11]. Mucinain (the active substance of the spicules) has been calculated to be delivered in the nanogram range [25].

Histamine: Histamine dihydrochloride (1%, in saline, Diagenic, England) was applied through standard allergy skin prick test (SPT) lancets (1 mm shouldered tip adapt to reach approximately the dermoepidermal junction [26, 27]). A small drop of histamine was placed in the center of the selected area and 1 prick was performed by applying 120 g of pressure to a weight-controlled SPT used to decrease the variability of the application method.

Vehicle: Distilled sterile water was used as vehicle and it was applied by SPT lancet with the same procedure used for histamine.

Doxepin: 2 grams of doxepin cream (5 g of doxepin-hydrochloride in 100 g basis cream) were applied in each area during the second session under topical occlusion (TegaDerm) to maximize the absorption of the cream. After 1 hour and a half, residual cream was removed.

2.3. Itch and Pain Intensity Assessments. To assess the intensity and duration of itch and pain, a visual analogue scale (VAS) with two bars (one for itch and one for pain) was used after the application of each pruritogen and vehicle. Itch and pain intensities (sampled at 0.2 Hz) were continuously rated by participants for 9 minutes on two computerized 100 mm VASs from 0 to 100 (eVAS Software, Aalborg University, Denmark) installed on a tablet. On the VAS, 0 indicated "no itch" or "no pain" and 100 indicated "worst imaginable itch" or "worst imaginable pain." Subsequently, peak, AUC, and temporal profiles were extracted.

2.4. Neurogenic Inflammation. To assess cutaneous neurogenic inflammation (quantified by the superficial blood perfusion, SBP), full-field laser perfusion imaging (FLPI, Moor Instruments, Axminster, Devon, UK) was used. FLPI is a technique that uses a laser light pattern (wavelength around 750 nm) to illuminate a skin area [22]. The reflection of the laser light from the skin produces a contrast laser pattern; a lower contrast corresponds to an increased

SBP [28]. In the present experiment, the device was placed approx. 25 cm above the skin area and 5 Hz display rate, 8.3 ms exposure time, and 160 units of gain were used. By a region of interest (ROI, equivalent to the predefined cream application area) approach, the obtained data were analyzed, and mean and peak perfusion values were extracted.

2.5. Measurement of Mechanical Sensitivity: Mechanically Evoked Itch (MEI), Mechanical Pain Thresholds (MPT), and Mechanical Pain Sensitivity (MPS). Three von Frey filaments (size: 4.08, 4.16, and 4.31; North Coast Medical, Gilroy, CA) were used to assess MEI. 3 stimulations (each stimulation was composed of 3 pricks in short succession) with each filament were performed and participants were instructed to rate the itch sensation on a numerical rating scale (NRS) from 0 to 10 (0 = "no itch"; 10 = "worst imaginable itch"), and a total average was calculated.

To assess MPT and MPS, a pin-prick set (MRC Systems GmbH, Germany) composed of 8 needles (diameter: 0.6 mm; force applications: 8, 16, 32, 64, 128, 256, and 512 mN) was used. For the MPT, each needle, starting with the lightest, was applied (rate 2 s on, 2 s off) in ascending order until the participant reports a sharpness or pain perception. Five thresholds in total were determined by the "methods of limits" in series of ascending and descending stimuli. The geometric mean was calculated by the five thresholds. For the MPS, each stimulator was applied in ascending order from the lightest. For each stimulus, the subjects were instructed to rate the pain on an NRS from 0 to 10 (0 = "no pain"; 10 = "worst imaginable pain"). This procedure was performed twice.

2.6. Measurement of Thermal Sensitivity: Cold Detection Threshold (CDT), Warm Detection Threshold (WDT), Cold Pain Threshold (CPT), Heat Pain Threshold (HPT), and SupraThreshold Heat Sensitivity (STHS). For the assessment of thermal sensitivity, a thermal stimulator Medoc Pathway (Medoc Ltd, Ramat Yishay, Israel) was used: a probe (3 × 3 cm) was placed on each application area. Staring from the baseline temperature (32°C), an ascending or descending ramp stimulus (1°C/s) was delivered until the subject identified the relevant threshold and pressed the button on a mouse (after the click, the temperature returned to the baseline at a rate of 5°C/s). It was the perception of a temperature change, cold and warm sensation, for CDT and WCT, respectively; for CPT and HPT, it was the detection of a painful sensation induced by cold and heat, respectively. Cut-off temperatures of 0 and 50°C were used. All the thresholds were assessed three times and the final outcome was their arithmetic mean. For the STHS, two suprathreshold heat pain stimuli (starting from the baseline, 32°C, an increasing ramp of 5°C/s, 3 s plateau at 50°C and decreasing at 5°C/s to the baseline) were provoked, and the subject was instructed to rate the pain on an NRS from 0 to 10. The final result was the average of the two values obtained.

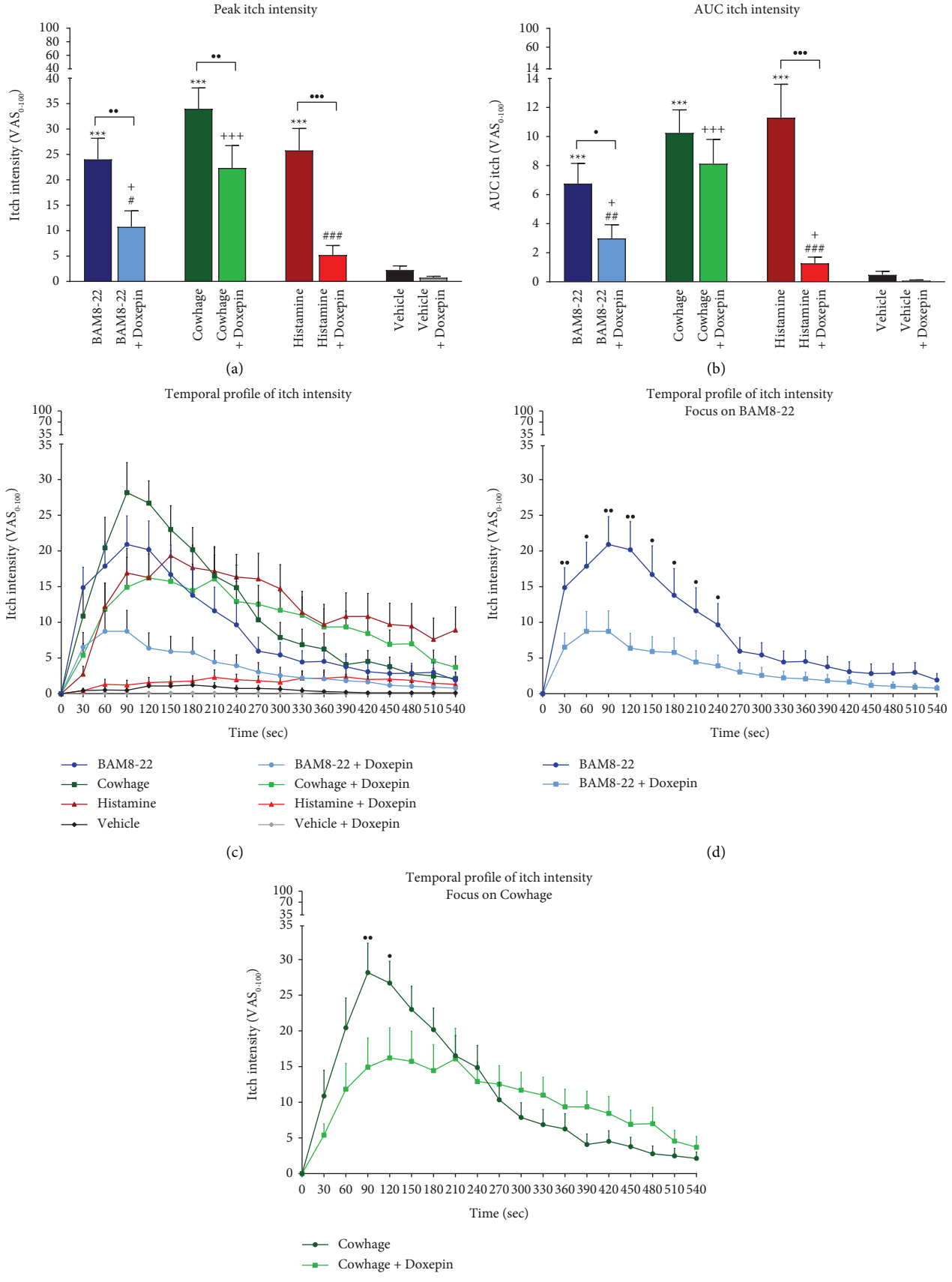
2.7. Statistical Analysis. The sample size was calculated in G∗Power 3.1.9.4, Universität Düsseldorf. For the calculation, type I error was set to 5% (alpha = 0.05), type II error was set to 20% (80% power), and the effect size was set to 0.3 (small-to-moderate effect). Statistical analysis was performed on SPSS software (v26, IBM Corporation, NY, USA). The Shapiro–Wilk normality test was used on the data from all assessments to check the normality. Repeated measure analysis of variance (RM-ANOVA) followed by the Sidak post hoc test was performed to analyze the data. A significance value of $p \leq 0.05$ was considered statistically significant. RM-ANOVAs were constructed using the following factors: treatment (doxepin or not) and pruritogen (BAM8-22, cowhage, histamine, and vehicle). For the analysis of the temporal profile, time (every 30 seconds of 9 minutes of VAS) was added as a factor. Graph plotting was realized in GraphPad Prism 6 (GraphPad Software Inc., CA, USA).

3. Results

Twenty-two participants took part in the present study. None of them showed adverse reactions during and/or after the experimental procedure. The mean and SD of all tests are reported in supplementary materials (S. Table 1).

3.1. Itch and Pain Analysis. Itch peak intensity and AUC are shown in Figures 1(a) and 1(b). Cowhage, BAM 8-22, and histamine induced itch compared to vehicle (peak: $F_{3,63} = 6.60$, Sidak, $p < 0.001$; AUC: $F_{2,46} = 8.82$, Sidak, $p < 0.001$). After the treatment with doxepin, itch induced by pruritogens decreased in intensity. Histamine-induced itch decreased significantly after the doxepin treatment ($p < 0.001$ for both peak and AUC) and differentiated from the vehicle (AUC ($p < 0.05$)). BAM8-22- and cowhage-induced itch intensities were decreased after the doxepin: BAM8-22 + doxepin was significantly lower than BAM8-22 in both peak intensity ($p < 0.01$) and AUC ($p < 0.05$) and cowhage + doxepin was significantly lower than cowhage for peak intensity ($p < 0.01$). The itch intensity for both BAM8-22 + doxepin and cowhage + doxepin areas were significantly higher than the vehicle for both peak intensity and AUC (BAM8-22 $p < 0.05$; cowhage $p < 0.001$). Moreover, histamine + doxepin resulted in significantly lower peak intensity and AUC ($p < 0.001$) than cowhage + doxepin.

The results of peak and AUC were confirmed by the temporal profile of itch intensity (Figures 1(c)–1(f)). In particular, BAM8-22 + doxepin-induced itch was significantly lower than BAM8-22 from 30 to 240 seconds ($F_{6,137} = 3.51$, Sidak, 30, 90, and 120 sec $p < 0.01$, 60 and from 150 to 240 sec $p < 0.05$; Figure 1(d)). Cowhage was significantly higher than Cowhage + doxepin only at 90 sec ($p < 0.01$; Figure 1(e)), where cowhage-induced itch peaked, and at 120 sec ($p < 0.05$). For histamine doxepin almost abolished histamine-induced itch for all 9 minutes of application (from 60 to 300 sec $p < 0.001$, 330 and 390 sec $p < 0.01$, 360 and from 420 to 540 sec $p < 0.05$; Figure 1(f)).



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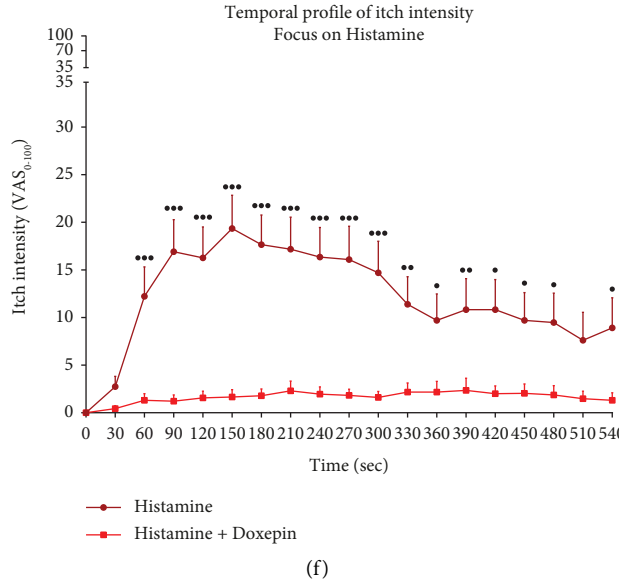


(f)

Figure 1: Itch induced by histamine, cowhage, and BAM8-22 and the effect of doxepin: (a) peak itch intensity, (b) AUC itch, (c) temporal profile of itch intensity, (d) temporal profile of itch intensity-focus on BAM8-22, (e) temporal profile of itch intensity-focus on cowhage, and (f) temporal profile of itch intensity-focus on histamine. Significance indicators: any condition vs. vehicle (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$; any condition + doxepin vs. vehicle + doxepin (+) $p < 0.05$, (+++) $p < 0.001$; BAM8-22/histamine + doxepin vs. cowhage + doxepin (#) $p < 0.05$, (##) $p < 0.01$, and (###) $p < 0.001$; any condition vs. any condition + doxepin (•) $p < 0.05$, (••) $p < 0.01$, and (•••) $p < 0.001$. BAM8-22 = blue, BAM8-22 + doxepin = light blue, cowhage = green, cowhage + doxepin = light green, histamine = dark red, histamine + doxepin red, vehicle = black, and vehicle + doxepin = grey. Values are reported as mean + SEM.

An overall significant difference was detected for both peak intensity and AUC pain (peak: $\chi^2 = 20.45$ $p < 0.01$; AUC $\chi^2 = 21.80$ $p < 0.01$; Figures 2(a) and 2(b)). After a post hoc analysis, no specific differences were found.

3.2. Superficial Blood Perfusion. Neurogenic inflammation was assessed by superficial blood perfusion (SBP) (Figure 3). The SBP peak after histamine was significantly higher than the vehicle, BAM8-22, and cowhage regardless of doxepin pretreatment (main effect of itch: $F_{2,\ 40} = 145{,}62$; Sidak, $p < 0.001$, Figure 3(a)). The SBP peak induced by cowhage was significantly higher than BAM8-22 (Sidak; $p < 0.01$). A decrease in SBP peaks was found after the pretreatment of doxepin for all pruritogens (main effect of treatment: $F_{1,21} = 4.72$; Sidak, $p < 0.05$).

The mean SBP (Figure 3(b)) of histamine resulted in a significantly higher value than the vehicle, BAM8-22, and cowhage (itch × treatment: $F_{1,25} = 20.75$; Sidak, $p < 0.001$). SBP in the histamine + doxepin area was significantly higher than BAM8-22 + doxepin, cowhage + doxepin, and vehicle + doxepin (itch × treatment: $F_{1,25} = 20.75$; Sidak, $p < 0.001$). The peak, the mean SBP of histamine, BAM8-22, and cowhage were significantly higher than histamine + doxepin, BAM8-22 + doxepin, and cowhage + doxepin, respectively (Sidak, histamine vs histamine + doxepin $p < 0.001$, BAM8-22/cowhage vs. BAM8-22 + doxepin/cowhage + doxepin $p < 0.01$).

For the flares (Figure 3(b)), histamine induced a significantly larger flare than BAM8-22, cowhage, and vehicle (itch x treatment $F_{1,22} = 54.28$; Sidak, $p < 0.001$). The same result was obtained comparing histamine + doxepin vs. BAM8-22 + doxepin, cowhage + doxepin, and vehicle + doxepin (Sidak, $p < 0.01$). Of note, the pretreatment with doxepin reduced the flare induced by histamine (Sidak, $p < 0.001$), without affecting the flare induced by BAM8-22, cowhage, and vehicle.

3.3. Mechanical and Thermal Sensitivities. Mechanically evoked itch (MEI), mechanical pain threshold (MPT), and mechanical pain sensitivity (MPS) were measured and analyzed to assess the mechanical sensitivity (Figures 4(a)–4(c)).

For mechanically evoked itch (Figure 4(a)), several differences were detected (itch x treatment: $F_{3,63} = 6.44$). Histamine MEI was significantly higher than cowhage and vehicle (Sidak, $p < 0.05$), histamine + doxepin was higher than BAM-822+ doxepin and vehicle + doxepin (Sidak, $p < 0.05$), and cowhage + doxepin was significantly higher than vehicle + doxepin (Sidak, $p < 0.05$). Moreover, it was detected a significant decrease in the response induced by BAM8-22 and histamine after the pretreatment of doxepin (Sidak, BAM8-22 vs BAM8-22 + doxepin $p < 0.01$, histamine vs. histamine + doxepin $p < 0.001$).

For the mechanical pain threshold (Figure 4(b)), no differences between groups were detected ($\chi^2 = 8.75$, $df = 7$, and $p = 0.27$).

Regarding MPS (Figure 4(c)), there was a significant difference between histamine and vehicle (main effect of itch: $F_{3,63} = 3.22$; Sidak, $p < 0.05$). Moreover, an overall main effect of treatment was found, and the MPS of any condition was higher before the treatment with doxepin ($F_{1,21} = 10.79$; $p < 0.01$).

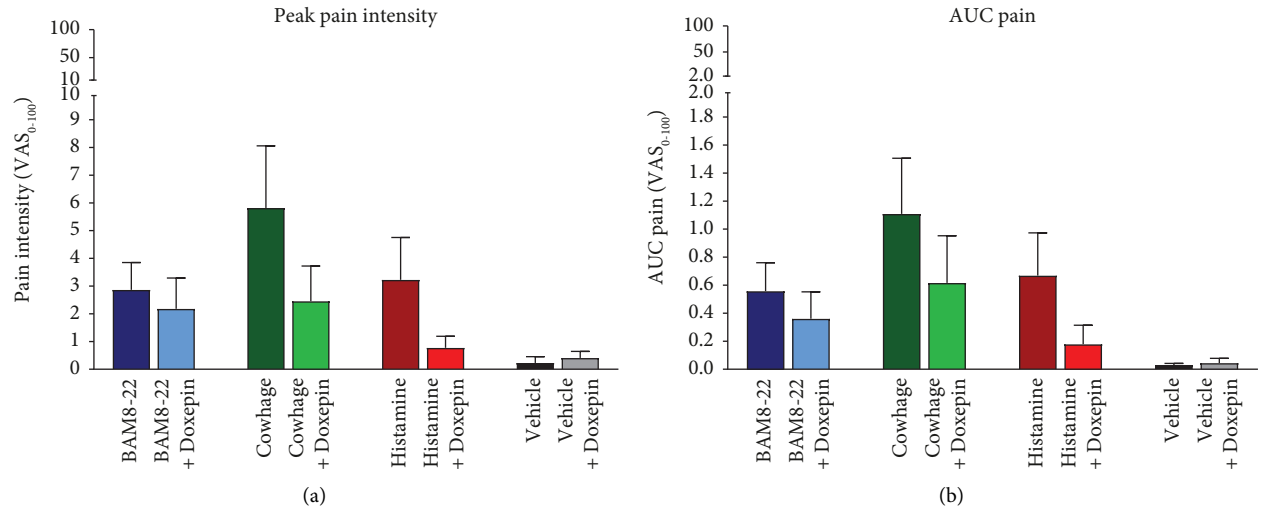


Figure 2: Pain induced by histamine, cowhage, and BAM8-22 and the effect of doxepin: (a) peak pain intensity and (b) AUC pain. BAM8-22 = blue, BAM8-22 + doxepin = light blue, cowhage = green, cowhage + doxepin = light green, histamine = dark red, histamine + doxepin red, vehicle = black, and vehicle + doxepin = grey. Values are reported as mean + SEM.

Cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), heat pain threshold (HPT), and suprathreshold heat sensitivity (STHS) were used to assess thermal sensitivity (Figures 4(d)–4(h)). The treatment with doxepin reduced the cold sensitivity; in fact, there was a significant difference between any condition and any condition + doxepin for the CDT (main effect of treatment: $F_{1,21} = 9{,}67$, $p < 0.01$, Figure 4(d)). For the CPT, there was only a tendency for reduction (main effect of treatment: $F_{1,21} = 4.30$, $p = 0.051$, Figure 4(f)).

The other tests to assess the thermal sensitivity showed no differences for WDT ($p = 0.71$, Figure 4(e)), HPT ($p = 0.52$, Figure 4(g)), nor STHS ($p = 0.07$, Figure 4(h)).

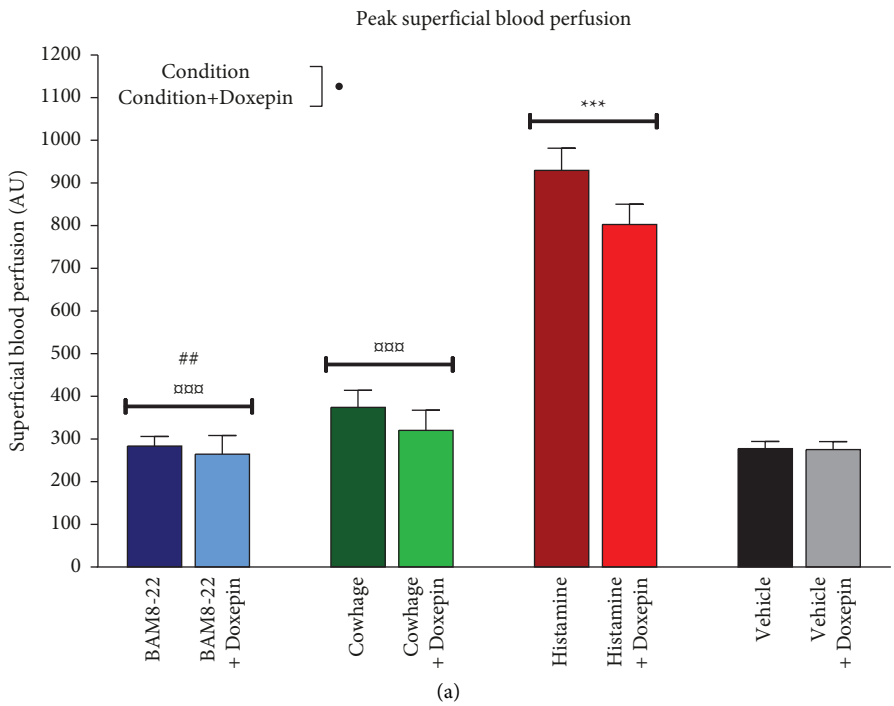
4. Discussion

Pretreatment with doxepin cream almost abolished histamine-induced itch, while BAM8-22- and cowhage-induced itch were reduced. Moreover, doxepin reduced the superficial blood flow induced by all the pruritogens. Histamine, BAM8-22, and cowhage are three valid human experimental models of itch. Histamine also induced neurogenic inflammation, hyperknesis, and higher mechanical pain sensitivity.

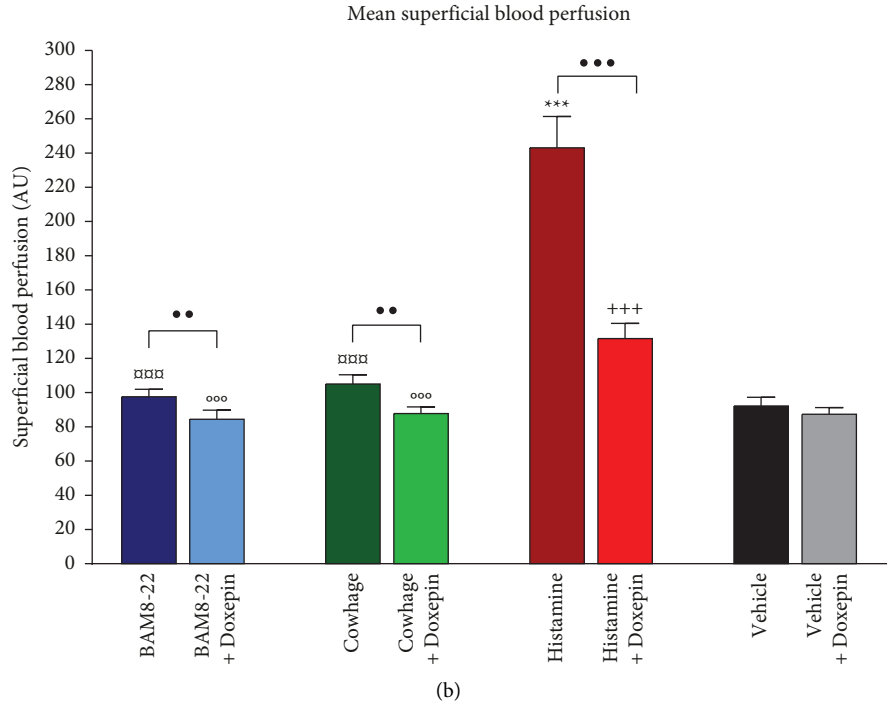
4.1. Itch and Pain Induced by Histamine, Cowhage, and BAM8-22. Histamine and cowhage are the two most used human experimental models for inducing histaminergic and nonhistaminergic itch, respectively [29]. BAM8-22 is the less investigated and most recent model and induces nonhistaminergic itch [30, 31]. The mechanisms of these three pruritogens are shown in Figure 5. In the present study, these three pruritogens were applied with the primary aim to compare, each other and with vehicle, their potentiality to induce itch. All three pruritogens induced higher itch intensities compared with the vehicle.

Histamine is an organic nitrogenous compound, and it is involved in pathological conditions such as urticaria and IgE-allergic reactions [10, 32]. It induces itch via the binding to the histamine H1 receptors (H1R) [33]. Histamine activates phospholipase Cβ3 (a downstream molecule of Gq/G11 coupled with H1R), which contributes to itch [34]. Histaminergic itch is transmitted by mechanoinsensitive C fibers (CMi-fibers) [35], that express H1R on their surface. Another receptor present on Cmi-fibers is transient receptor potential vanilloid 1 (TRPV1); in fact, these fibers are activated also by capsaicin (TRPV1 agonist) [36]. Cowhage (*Mucuna pruriens*) is a tropical legume plant, and it contains mucunain, the active and itch-inducer enzyme, in its spicules [37]. It is an exogenous agonist of PAR2/4 receptors and induces nonhistaminergic itch via polymodal C-fibers transmission (PmC-fibers) [38]. It was demonstrated that PmC-fibers are quickly activated by cowhage and less or not by histamine [20, 35]. Another nonhistaminergic itch-inducer is bovine adrenal medulla (BAM)8-22. BAM8-22, an endogenous peptide obtained after proteolytic cleavage of proenkephalin A, binds and activates Mas-related G protein-coupled receptors MrgprA3, MrgprC11 (mouse), and MrgprX1 (human) [30, 39, 40]. BAM8-22 induces itch via Gαq/11 pathway [39] and it is transmitted by PmC-fibers [7, 33]. For both nonhistaminergic itch pathways (activated by cowhage or BAM8-22), the involvement of transient receptor potential ankyrin 1 (TRPA1) is needed [41, 42].

In this study, the three pruritogens did not show differences regarding the peak and AUC of itch intensities. It was possible to see some differences in the temporal profiles. BAM8-22 and cowhage showed a similar profile: the itch intensity raised very quickly, peaked around 90 seconds after the application, and gently decreased in the following few minutes. This is in line with the fast response of PmC-fibers observed previously [31, 35]. On the other hand, histamine-



(a)



(b)

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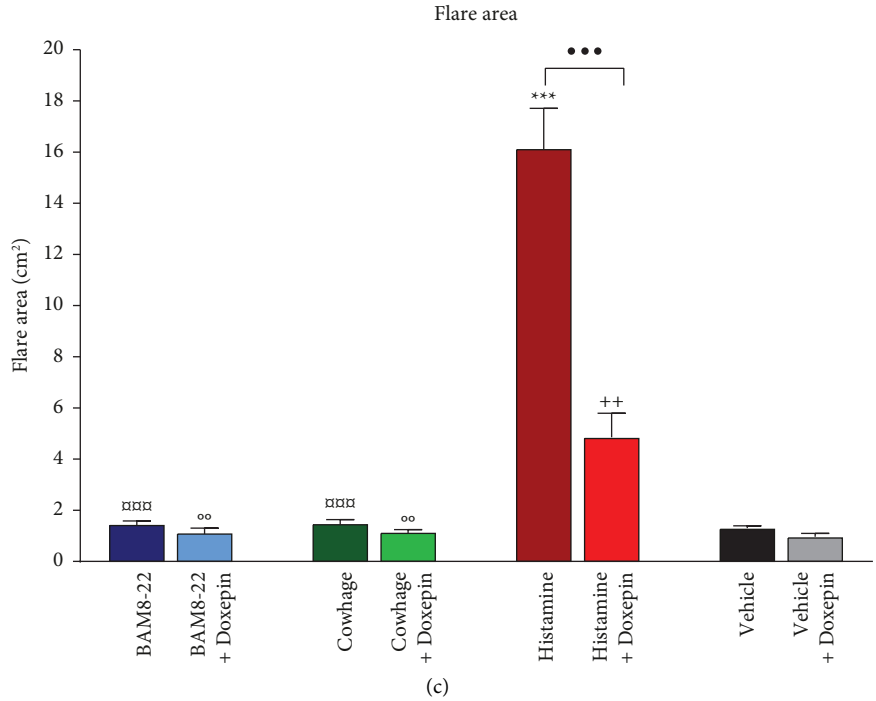

Figure 3: Superficial blood perfusion: (a) peak SBP, (b) mean SBP, and (c) flare area. Significance indicators: any condition vs. vehicle (***) $p < 0.001$; any condition + doxepin vs. vehicle + doxepin (++) $p < 0.01$ and (+++) $p < 0.001$; BAM8-22/histamine + doxepin vs. cowhage + doxepin (##) $p < 0.01$; any condition vs. any condition + doxepin (•) $p < 0.05$, (••) $p < 0.01$, and (•••) $p < 0.001$; BAM8-22/cowhage vs. histamine (¤¤¤) $p < 0.001$; BAM8-22/cowhage + doxepin vs. histamine + doxepin (°°) $p < 0.01$ and (°°°) $p < 0.001$. BAM8-22 = blue, BAM8-22 + doxepin = light blue, cowhage = green, cowhage + doxepin = light green, histamine = dark red, histamine + doxepin red, vehicle = black, and vehicle + doxepin = grey. Values are reported as mean + SEM.

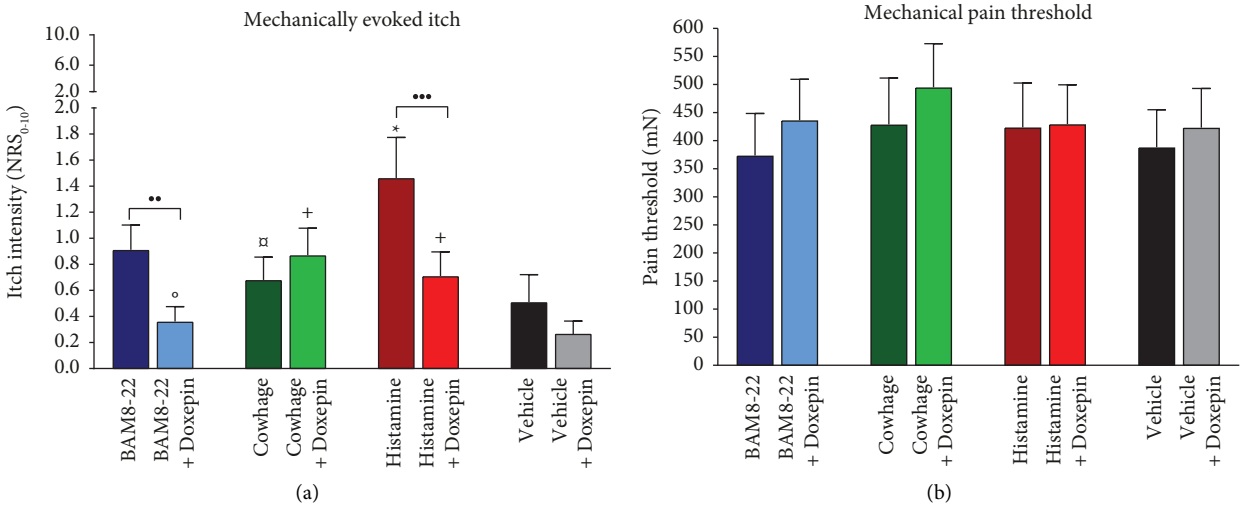

Figure 4: Continued.

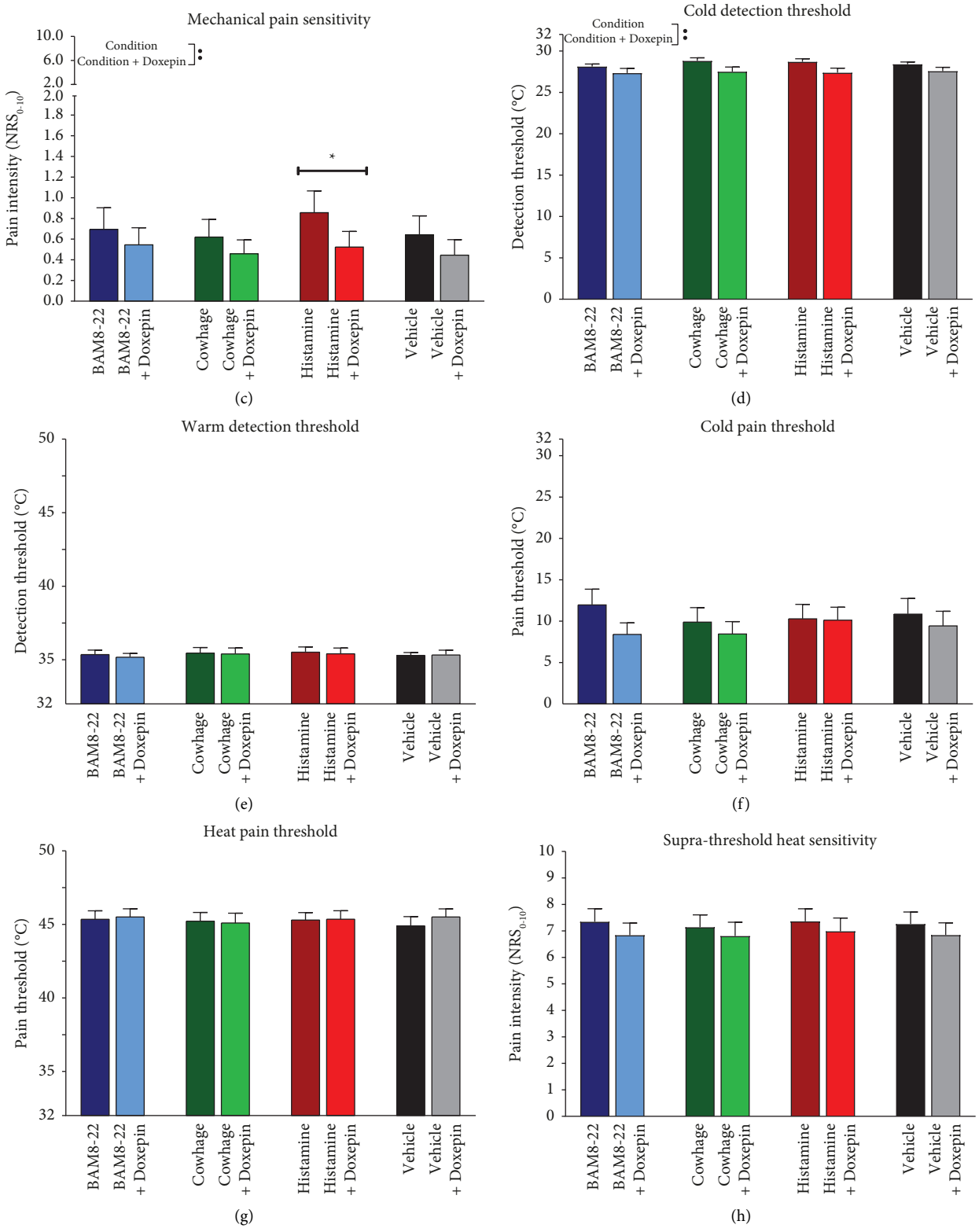

FIGURE 4: Mechanical and thermal sensitivities: (a) mechanically induced itch (MEI), (b) mechanical pain threshold (MPT), (c) mechanical pain sensitivity (MPS), (d) cold detection threshold (CDT), (e) warm detection threshold (WDT), (f) cold pain threshold (CPT), (g) heat pain threshold (HPT), and (h) suprathreshold heat sensitivity (STHS). Significance indicators: any condition vs. vehicle (*) $p < 0.05$; any condition + doxepin vs. vehicle + doxepin (+) $p < 0.05$; any condition vs. any condition + doxepin (●●) $p < 0.01$ and (●●●) $p < 0.001$; cowhage vs. histamine (¤) $p < 0.05$; BAM8-22 + doxepin vs. histamine + doxepin (◦) $p < 0.05$. BAM8-22 = blue, BAM8-22 + doxepin = light blue, cowhage = green, cowhage + doxepin = light green, histamine = dark red, histamine + doxepin red, vehicle = black, and vehicle + doxepin = grey. Values are reported as mean + SEM.

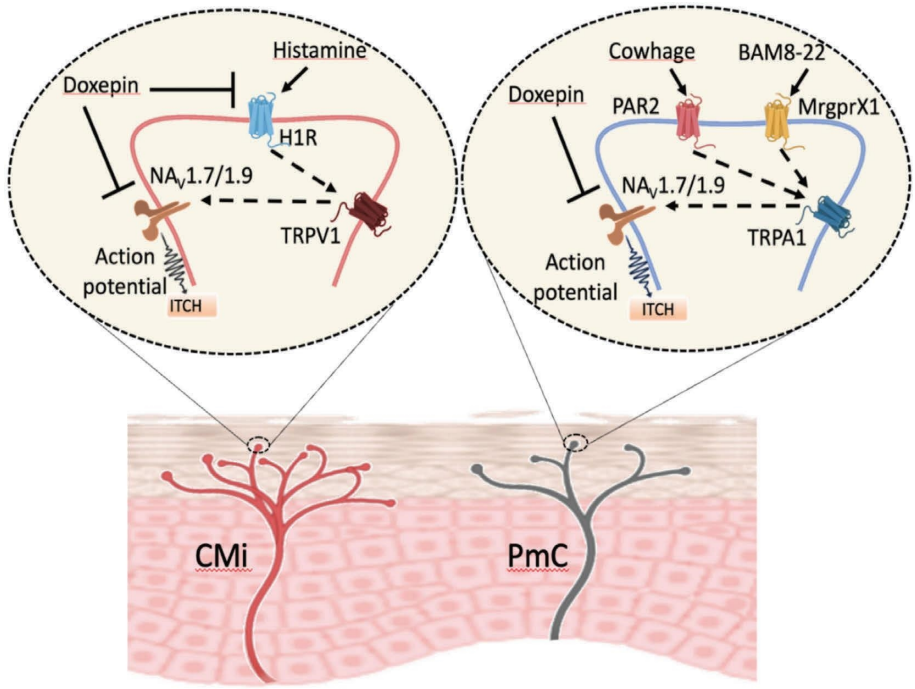

Figure 5: Neuronal receptors for the transduction of histaminergic and nonhistaminergic itch on C-fibers and mechanism of action of doxepin. Histaminergic itch is transmitted through the CMi-fibers after the activation of histamine receptors. The downstream activation of TRPV1 leads the opening of sodium channels and so the action potential. Nonhistaminergic itch, transmitted through the PmC-fibers, occurs after the activation of PAR2/4 or MrgprX1. In both cases, the downstream activation of TRPA1 leads the opening of sodium channels and so the action potential. Doxepin, a tricyclic antidepressant, bocks both the histamine receptors and sodium channels. BAM8-22 (bovine adrenal medulla 8-22), H1R (histamine receptor 1), PAR (protease-activated receptors), MrgprX1 (Mas-related G protein-coupled receptor X1), TRPV1 (transient receptor potential channel vanilloid 1), TRPA1 (transient receptor potential channel ankyrin 1), NaV (voltage-gated sodium channel), CMi-fibers (mechano-insensitive C-fibers), and PmC-fibers (polymodal C-fibers).

induced itch started to rise few seconds later (from 30 seconds), did not show a clear peak (around 90 and 150 seconds), and gradually decrease without reaching zero in the following 7 minutes.

Regarding the associated pain induction by the three pruritogens, the study confirmed that the itch sensation elicited by histamine and cowhage is accompanied by a pain sensation, especially for cowhage [17, 31]. The pain sensation was described in that study as "pricking/stinging" and "warm/hot/burning" [17]. Also in this study, the subjects reported pain in concomitance with itch, more pronounced after the cowhage stimulation. Interestingly, BAM8-22 did not induce the same pain intensity of cowhage even though they both induce nonhistaminergic itch. A possible explanation could be that in general, although not in a significant manner, cowhage provocation was higher than BAM8-22's (itch and pain induced by cowhage were higher than BAM8-22); this could indicate a stronger activation induced by cowhage. Another explanation could be linked to the specific receptors bound by the pruritogens (PAR2/4 activated by cowhage, and MrgprX1 by BAM8-22), that once activated may contribute differently to pain perception.

4.2. The Effect of Doxepin on Itch and Pain Induced by the Pruritogens. Doxepin is a tricyclic antidepressant (TCA). It has an effect on different pathways in different areas and functions in humans. First at all, at brain level, it increases the concentration of serotonin and norepinephrine by preventing their reuptake in the presynaptic terminals [43]. In cardiomyocytes, doxepin acts as inhibitor of sodium and potassium channels [44]. This action on the sodium channels was also proved at peripheral levels by testing the activity of the channels in bovine adrenal chromaffin cells [45]. Moreover, doxepin blocks histamine (H1), alpha-1 adrenergic, and muscarinic receptors in the central nervous system [43]. Its action on H1R explains the antipruritic properties of doxepin [46] (Figure 5).

Doxepin cream almost abolished histamine-induced itch; however, the pretreatment with doxepin cream decreased also the itch perceived by the subject after BAM8-22 and cowhage application. In particular, the mode-of-action caused a reduction of the itch intensity without affecting the duration of nonhistaminergic itch.

A possible explanation could be that BAM8-22 and cowhage itch pathways share some mechanisms with the histaminergic pathway. In fact, the effect of doxepin on histaminergic itch could be explained by its block of H1R. Likely, doxepin decreases nonhistaminergic itch by partly blocking the voltage-gated sodium channels and in this way decreases the action potentials [31]. Further studies are necessary to better clarify this hypothesis and to assess if the itch intensity reduction of doxepin is due to its effect on H1R or sodium channels.

4.3. Changes in Neurogenic Inflammation. An increased superficial blood (SBP) perfusion is an indicator of neurogenic inflammation (or neurogenic flare), and it is caused by retrograde signaling from activated dermoepidermal peptidergic nerve fibers [23, 47, 48]. It can be observed in chemical irritant application site and the close surrounding area. In some circumstances, also the unprovoked surrounding area can show an increase in SBP, and this phenomenon is called axon-reflex flare [18, 49, 50]. It was proposed that CGRP and substance P (the primary mediators of vasodilatation) are involved in neurogenic inflammation [16, 48, 51]. In general, CMi-fibers largely contribute to neurogenic inflammation and axon-reflex flare, while PmC-fibers involvement may only induce a homotopic flare, without axon-reflex flare [16–18, 40, 52]. The present study confirms this knowledge. Histamine induced an increase in superficial blood perfusion and a bigger flare area compared to vehicle, cowhage, and BAM8-22. Cowhage and BAM8-22 did not show any differences compared to the vehicle. It can be hypothesized that also the little flare present in these areas is due to the provocation modality instead of the pruritogens' action. The pretreatment with doxepin diminished the superficial blood perfusion and the flare size, and this effect was predominant in the histamine area. To notice, the peak SBP of histamine was decreased by doxepin, but still present and very different from the vehicle area indicating only a slight effect on the homotopic flare. In contrast, doxepin heavily reduced the flare area of histamine (no more different from vehicle) indicating a predominant effect of doxepin on axon-reflex flare. This finding is in line with previous knowledge indicating a positive correlation between itch intensity and axon-reflex flare size mediated by CMi-fibers [17, 24, 31, 53, 54].

4.4. Changes in Mechanical and Thermal Sensitivities. It was verified that stimulation with von Frey filaments could evoke a modest mechanically evoked itch intensity [17]. In certain circumstances, itch sensation provoked by the same stimulus could be enhanced [55]. It was proposed that type-I Aδ-fibers mediate hyperknesis through a central mechanism [22]. The present research results show that only histamine induced a significantly higher itch intensity compared to the vehicle in response to von Frey filament stimulation. Doxepin cream clearly affected the hyperknesis, and so probably acts also on the Aδ-fibers. In fact, there was a reduction of mechanically evoked itch intensity on both histamine and BAM8-22 areas, but no effect was observed in cowhage and vehicle areas. These results could mean that doxepin prevents the central mechanisms that cause hyperknesis, maybe by decreasing the first and peripheral itch transmission. This is a known mechanism for the parallel phenomenon of allodynia in the field of pain [56].

The mechanical pain sensitivity (MPS) was assessed by using pinprick stimuli. In this case, C-, Aβ-, and Aδ-fibers could be mainly involved [57]. Also for this test, only histamine showed an increase in sensitivity compared to the vehicle, as well doxepin decreased the MPS, confirming an effect of doxepin on these fibers.

For the thermal sensitivity, histamine, cowhage, and BAM8-22 did not show any changes in line with previous data [58]. The pretreatment with doxepin did not change the thermal sensitivity. The only exception is for the cold detection threshold, decreased by doxepin. Further studies are needed to assess if this change is due to an effect of doxepin on TRPM8 or just to test habituation of the subjects.

4.5. Limitations. One limitation is the different delivery methods of histamine, cowhage, and BAM8-22. In this way resulted impossible to have complete blinding for the investigator and the participants. Moreover, it is almost impossible to be sure about the exact number of spicules inserted in the skin (used for cowhage and BAM8-22). Another limitation in this study could be the absence of the effect of a vehicle cream that would possibly have allowed a better understanding of the doxepin effect. Further studies will be conducted in order to overcome this limitation.

5. Conclusions

Histamine, cowhage, and BAM8-22 are three good and well-established models of acute itch in humans. Cowhage-induced itch comes with a modest pain sensation, not present for BAM8-22 and histamine. Histamine caused an increase in neurogenic inflammation and mechanical sensitivity.

The pretreatment with doxepin cream almost abolishes the histaminergic itch, while it only reduces non-histaminergic itch. Doxepin also reduces the axon-reflex flare and the increased mechanical sensitivity induced by histamine.

Further studies are necessary to confirm if the mechanism of doxepin-induced itch reduction involves H1R or sodium channels.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

Ethical Approval

The regional (Region Nordjylland) Ethics Committee approved the protocol (N-20190043).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

LAN, SLV, and GEA designed the study. GEA collected, analyzed the data, and drafted the manuscript. JE created

Figure 5 with https://BioRender.com. All authors discussed the results and commented on and approved the manuscript.

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Supplementary Materials

In the supplementary materials, it is possible to find a supplementary table (S. Table 1) presenting mean and SD of all conducted tests. (*Supplementary Materials*)

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