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Correlations between histamine-induced wheal, flare and itch

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Abstract **Correlations between the skin reactions wheal and flare and the subjectively reported degree of itch were investigated in response to 1% histamine, intradermally applied by standardized skin prick and by iontophoresis. Experiments were performed with 15 male volunteers using a threefold repeated measures design (skin prick, and iontophoresis with 0.13 mA for 10 s and with 2.0 mA for 10 s). Skin reactions (perpendicular diameters) were determined at the time of their maximum (10 min). Itch was rated on a computerized visual analogue scale which was anchored upon the individual scratch threshold. Most effective in producing itch was the skin prick which caused strong sensations markedly above the scratch threshold during the entire period of measurement (30 min), whereas iontophoresis induced only transient itch sensations. On the other hand, the largest wheals were generated by iontophoresis of both intensities (mean 10 or 14 mm vs 6 mm with skin prick). The higher current induced higher itch, wheal and flare responses, but after eliminating this effect of stimulus intensity, no correlations were found. In contrast, skin prick-induced flare reactions varied with the degree of itch above the scratch threshold ($r = 0.56$; $P < 0.01$). Repeated measurements showed a higher stability for the itch reaction with skin prick compared with iontophoresis. It is hypothesized that in iontophoresis the brief (10-s) histamine bolus passed the most superficial pruritoceptive C fibres too quickly to induce long-lasting itch sensations, whereas the skin prick caused a deposit at the dermal-epidermal junction releasing histamine during the entire time of measurement. Consequently, both the C fibre-mediated itch and the axon reflex flare were more pronounced with the skin prick, and the wheal resulting from a permeability increase in the postcapillary venule walls was an independent phenomenon.**

Key words Histamine itch · Wheal · Flare · Skin prick · Iontophoresis

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Introduction

The neurophysiological mechanisms of itch are far less understood than those of pain. Itch has been regarded as subliminal pain for many years since both sensations are obviously connected by common features of sensor detection via thin myelinated A delta and unmyelinated C fibres (see, for example, reference 20). In recent years a subgroup of pain-related C fibre polymodal nociceptors has been proposed as itch-related [6]. Human physiological investigations, however, using electrical intraneural microstimulation have provided evidence that itch can be conveyed by a population of nerve fibres different from those propagating pain [19]. Moreover, itch sensation seems to be bound to the functional integrity of the epidermis since the removal of the epidermis has been reported to impair the capacity for itch sensation [20], also in clear contrast to pain.

Many experimental pain models have been reported with precisely controlled chemical, mechanical, electrical or heat stimuli (for review see reference 1). However, investigations concerning the mechanisms of itch have been hampered by the lack of a specific, reliable and potent itch-inducing stimulus. Histamine is regarded as the main natural mediator of itch, and to date all described models of experimental pruritus are at least partially histamine-mediated (for review see reference 5). Since histamine is unable to penetrate the intact skin barrier, intracutaneous injections of defined quantities of histamine or histamine-releasing compounds have frequently been used [5, 8, 21, 22]. Another histamine application mode is the skin prick which is established as a control stimulus in routine allergy

diagnosis [14], although the quantity of substance delivered into the skin can only be estimated (about 10^{-6} ml [16]). A third method to achieve transepidermal histamine transport is the application of the compound by iontophoresis using an electric current, which has been used in some drug evaluation studies in the past decades [7, 17]. Recently, this method has been used by Magerl et al. [13] investigating dose-dependent histamine effects in volunteers and patients.

Another reason for the deficits in the neurophysiological understanding of itch is the lack of a reliable method for objective quantification of this subjective parameter. Whereas phasic pain can, for example, be correlated with evoked brain potentials [2], clinical itch studies usually rely on subjective ratings, registered via visual analogue scales (for review see reference 5). Measurement of nocturnal scratch movements to quantify itch in patients has been hampered by the influence of sleep stages (for review see reference 25). Consequently, high inter- and intraindividual variations are obtained. Skin reactions to histamine injections are known as triple response [10]: transient and faint localized erythema (i.e. initial local vasodilatation), wheal (i.e. oedema due to an increase in small vessel permeability), and flare (circumscribed erythema due to vasodilatation following an axon reflex). H1 and H2 receptors on skin vessels are involved in the proinflammatory effect of histamine (for review on histamine see reference 18). Wheal and flare can easily be measured by their diameters at different times after histamine injection and attempts to employ them as physiological covariates of itch have been made over many decades [7, 12, 17, 22].

The skin depth at which histamine is delivered has a decisive role in the induction of itch. For example, injection of histamine into deep skin layers induces pain instead of itch [11, 25]. In transfer experiments investigating late phase allergic reactions, allergens were injected intradermally under a suction blister (dermal-epidermal dissection) developing on the skin of ten sensitized human volunteers. Reported sensations following the injection comprised mild burning or burning pain, but no itch [4]. Subcutaneous histamine injection has also recently been reported to provoke itch, though with a significant delay [22]. Similarly, the different skin reactions are expected to depend on the depth of cutaneous histamine injection.

In this study, skin prick and iontophoretic histamine application were evaluated with respect to their abilities to induce itch rated subjectively, as well as wheal and flare reactions measured objectively. These parameters were also compared with regard to their reproducibility. Iontophoresis was investigated with mild and strong electrical stimulus intensities in order to evaluate the influence of current on reactions and dose-response relationships.

Material and methods

Subjects

Included in the study were 15 healthy male volunteers aged 25 to 35 years with no history of skin disease. Use of antihistamines or a history of urticaria were exclusion criteria. Four of the volunteers had a history of allergic rhinoconjunctivitis without having symptoms at the time the study was performed, and three volunteers showed a white dermographism. Informed consent was obtained from every subject, according to the requirements of the Declaration of Helsinki.

Study design

Without previous training the volunteers were randomly exposed to each of the three itch-provoking methods. Before histamine application the effect of the vehicle alone was tested for a 3-min period. Room temperature was stable at between 20° C and 22° C, and care was taken to ensure non-distracting surroundings. All experiments were carried out in the same season (winter) to decrease the confounding influence of differences in thickness of the stratum corneum due to UV irradiation. The area of stimulation was the hairy skin on the dorsum of the left lower arm. Subjects participated in repeat sessions (not on the same day) to test the reliability of the results.

Skin prick test

The technique was performed as described by Pepys [16] using conventional blood lancets, as usual in allergy diagnosis. After the application of one drop of histamine gel (1% histamine dihydrochloride in 2.5% methyl cellulose) the skin was punctured superficially. If bleeding occurred, the session was ended and repeated later to ensure essentially epidermal histamine delivery. All skin pricks were done by the same investigator to minimize variability in the application technique.

Iontophoresis

Histamine iontophoresis was performed with 1% histamine dihydrochloride in the same vehicle, following the method described by Magerl and Handwerker [12]. The anode (glass tube, 5 mm diameter) was filled with the gel and was placed upon the skin area to be stimulated. The cathode of larger size (30 mm diameter), placed in a sponge soaked with tyrode solution, was held in the palm by the subject. The duration of the stimulus was 10 s. Two intensities were chosen: 0.13 mA (following the suggestion of Magerl et al. [13] and 2.0 mA, resulting in charges of $0.13 \times 10 = 1.3$ mC and 20 mC, respectively. Higher current intensities produced pain (see Results). Currents were administered using a commercial optoelectric isolator coupled to a constant current generator.

Skin reactions

The wheal and flare diameters of each volunteer were continuously observed. Regardless of the application mode, their maxima were reached between 5 and 10 min after histamine application; these maxima remained approximately constant for a further 10 min. For quantification the diameters at 10 min after application were determined as averages of four perpendicular measurements.

Itch rating

Itch intensity was rated on a visual analogue scale (VAS) at 20-s intervals for a total of 30 min. The scale was displayed on the monitor of a personal computer (PC) and the rating was performed by moving the cursor using a PC mouse along the 20-cm scale between the end points ‘no itch’ and ‘unbearable itch’. At one-third of the scale the intervention point ‘scratch threshold’ was installed as suggested by other investigators in this field [12]; above this threshold each individual had the impulse to scratch (which was, of course, not allowed). Itch causing clinical problems is in most cases strong enough to cause a desire to scratch.

Qualitative assessment of itch

A questionnaire with 67 items on the quantitative and qualitative properties of itch (Eppendorf Itch Questionnaire (Darsow et al.; in preparation) was used to assess intra- and interindividual differences in the perceptual dimensions of histamine-induced itch. This questionnaire was completed by the subjects 5 and 30 min after stimulus application. It also contained questions concerning painful sensations.

Statistics

Descriptive statistics of wheal and erythema diameters, as well as of the itch parameter maximum ratings, mean and cumulative (area under curve) ratings and total itch duration were calculated. Itch parameters were also referred to the intervention point (desire to scratch = 33% of scale). The 50% duration value (half-life time) of the itch sensation was calculated by fitting a biexponential function to the time course of the individual itch intensity ratings, and computing the time when the maxima of the mean itch sensation had decreased to 50%. The number of indicated items in the Eppendorf Itch Questionnaire was used to describe the quality of the itch sensation induced by the three methods. After having successfully checked all parameters as normally distributed by means of the Kolmogorov-Smirnov one-sample test, differences between treatments were evaluated by a one-way analysis of variance with repeated measures using a Greenhouse-Geisser correction for unequal variances. When a significant main effect was present, differences between the histamine application procedures were compared by Bonferroni-corrected *t*-tests. The similarity between the different parameters was quantified by Pearson's product moment correlations. Correlation analysis was also used to determine intraindividual reproducibility of subjective and objective parameters. The analyses were performed using SPSS software [15].

Table 1 Mean correlation between subjective parameters. Pearson product-moment correlations were computed for each histamine application mode separately and then averaged, using Fisher's *Z*-transformation for normalization. Significant correlation coefficients are shown in bold type ($P < 0.01$)

		(1)	(2)	(3)	(4)
Mean itch intensity	(1)	1.00	**0.82**	**0.90**	**0.57**
Maximal itch intensity	(2)		1.00	**0.76**	**0.54**
Itch half-life	(3)			1.00	**0.58**
Number of different Adjectives (questionnaire)	(4)				1.00

Results

Itch perception described by questionnaire

Histamine-induced itch was described predominantly as tickling, pricking, warm, radiating but localizable, tiresome and often perceived in waveforms if histamine was applied by skin prick or by low-current iontophoresis (0.13 mA). In contrast, 2.0 mA iontophoresis produced clear cut painful sensations: 13 volunteers reported localized pain when the current was switched on and off, and during the 10-s application time they perceived dysaesthesia due to the electric flux. One subject reported coexisting burning pain and increasing itch over the first 90 s of rating. The interference of pain raised some doubt as to the quality of the sensation elicited by high-charge histamine iontophoresis. The number of chosen adjectives on the questionnaire was significantly correlated with several quantitative itch parameters (Table 1).

Itch perception described by visual analogue scale

Figure 1 demonstrates the mean itch amplitudes as a function of time for the three histamine application procedures.

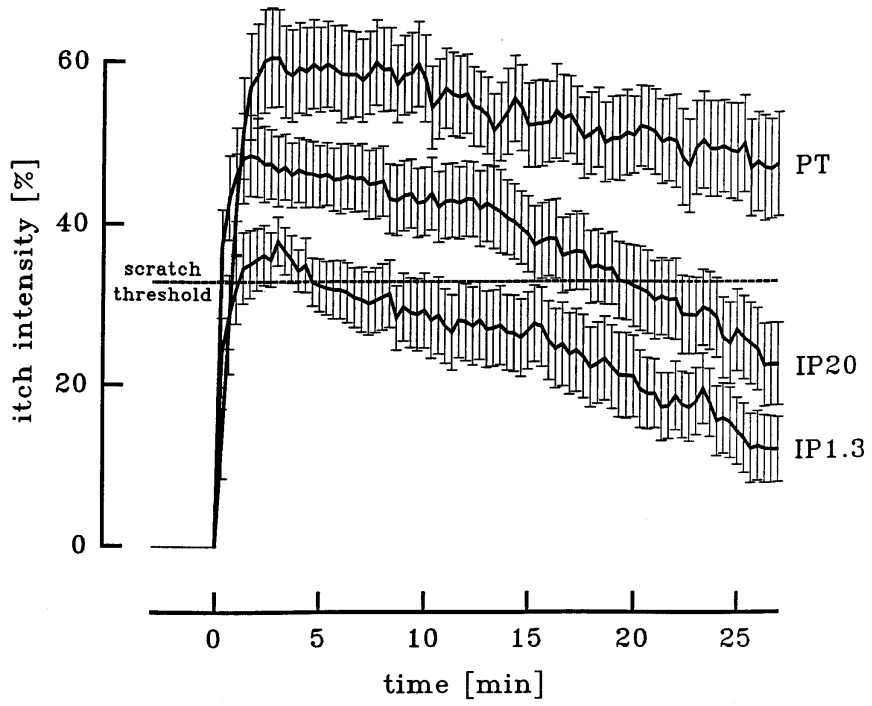

Fig. 1 Mean visual analogue scale ratings (± SEM, $n = 15$) of histamine itch induced by the skin prick test *(PT)* and iontophoresis at 1.3 mC *(IP 1.3)* and 20 mC *(IP 20)*. Stimuli were given after 3 min of baseline control. The scratch threshold is the point at which subjects had the impulse to scratch

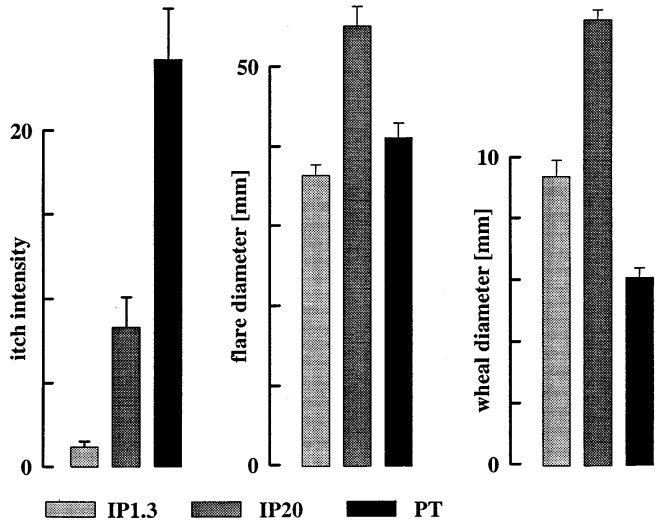

Fig. 2 Comparison of wheal, flare and histamine itch intensity induced by the skin prick test (PT) and iontophoresis at 1.3 mC *(IP 1.3)* and 20 mC *(IP 20)*. Intensity was calculated as mean area under the rating curve above the scratch threshold. IP values show dose dependency. Values are means ± SEM (n = 15); all differences are significant at the 1% level

Although there was a marked variability in individual itch perception in all trial parts, the mean itch ratings showed clear differences with respect to the histamine application mode. In general, there was a rapid onset of itch after histamine application with latencies between 5 and 60 s, for all three application modes. The maximum itch sensation lasted for 2–5 min. In contrast, the strength and the duration of histamine-induced itch was considerably different between the three application modes, especially with regard to the scratch threshold.

All itch parameters, mean intensity, maximal and cumulative ratings and suprathreshold duration calculated from the VAS were largest with the histamine skin prick test compared with both iontophoresis charges. These differences reached significance ($P < 0.01$) when only skin prick and iontophoresis at 1.3 mC were compared. Figure 2 shows the itch intensities calculated as area under the curves above the scratch threshold. Not surprisingly, itch elicited by the stronger iontophoresis was rated as significantly longer and stronger ($P < 0.01$) than itch induced using the lower current. However, even the 20 mC iontophoresis generally led to lower ratings than the skin prick.

Wheal reaction and surrounding flare

A developing flare became visible approximately 1 min (mean) after histamine application; wheal formation occurred in the third minute, no matter which application mode was chosen. Both skin responses had developed to full size by 5–10 min after stimulation in all subjects; in this state their diameters were repeatedly measured for averaging.

These objective parameters of the triple response to histamine application showed dissociated responses to the stimulus with regard to the methods used (Fig. 2). The volunteers exhibited significantly ($P < 0.001$) smaller wheals (mean 6.0 mm) in skin prick areas than in 1.3 mC iontophoresis areas (mean 9.5 mm). On the other hand, the flare diameters were significantly higher ($P = 0.01$) with the skin prick than with 1.3 mC iontophoresis. With 20 mC iontophoresis very large wheals with diameters up to 17 mm appeared and flare diameters usually exceeded those elicited by skin prick. The mean diameters were as follows: 41.0 mm (skin prick), 37.2 mm (1.3 mC iontophoresis) and 53.6 mm (20 mC iontophoresis). However, as already mentioned, these skin reactions were not accompanied by the highest degree of itch. By 2.5 h after histamine application, itch as well as wheals and flares had completely vanished; in no subject was a lasting side effect or skin alteration seen. When subjects with white dermographism or a history of atopy were compared with volunteers without these characteristics, no influence on the physiological parameters measured was seen.

Repeated measurements

Wheal diameters of two repeated measurements in the same subjects were not correlated with each other either using skin prick or iontophoresis. In contrast, there was a clearcut correlation between the flare sizes ($r = 0.68$, $P < 0.01$ in iontophoresis, $r = 0.74$, $P < 0.01$, in skin prick). Mean VAS ratings showed a weaker reproducibility ($r = 0.45$, $P < 0.05$), which was achieved only with skin prick. Iontophoresis itch showed a higher intraindividual variation resulting in a nonsignificant correlation.

Relationships between wheal, flare and itch

No significant correlations were found between itch sensation and the corresponding wheal and flare in iontophoresis. In Fig. 3, the scatter diagrams for the relationships between wheal, flare and suprathreshold mean itch intensity (area under the curve) are given for the three histamine application modes. Iontophoresis at 1.3 mC elicited small effects with flare diameters between 20 and 45 mm and mean itch suprathreshold intensities between 0 and 10. Iontophoresis at 20 mC led to flare diameters between 40 and 70 mm and itch intensities between 0 and 30. Skin prick produced large effects on itch intensity (5 to 50) and flare diameter (30 to 55 mm), which showed a moderate correlation ($r = 0.56$, $P < 0.01$). Wheal reactions clearly did not exhibit any functional relationship with itch intensities, but were essentially influenced by the histamine application mode: wheal diameters varied around 6 mm with skin prick, around 9.5 mm with 1.3 mC iontophoresis and around 14 mm with 20 mC iontophoresis.

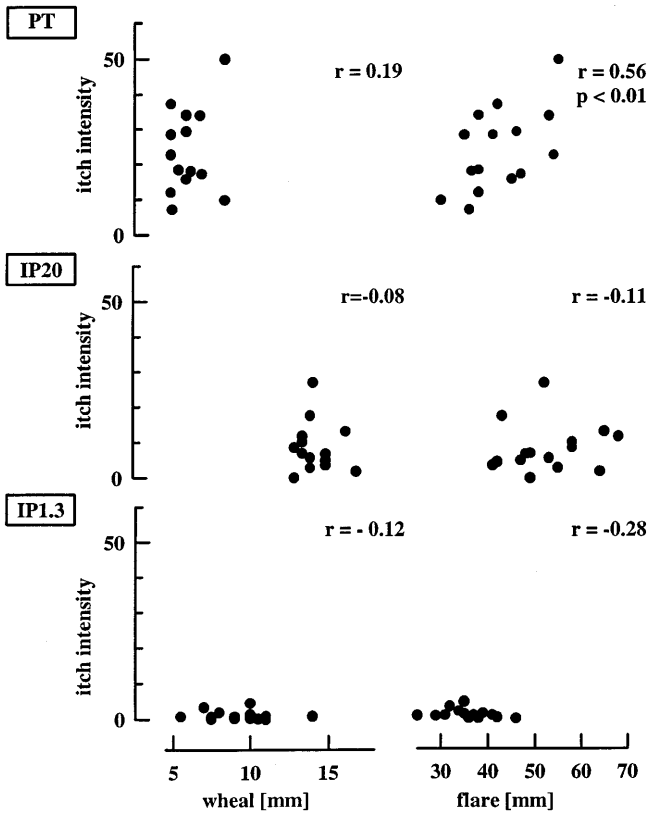

Fig. 3 Scatter diagrams showing the relationships between wheal, flare and itch intensity (area under the rating curve above the scratch threshold) elicited by the skin prick (PT) and iontophoresis at 1.3 mC *(IP 1.3)* and 20 mC *(IP 20)* with 1% histamine

Discussion

This study demonstrated that the histamine-induced itch sensation and wheal and flare depend differentially on the mode of histamine application. For all techniques, the most superficial skin layers have to be overcome to deliver histamine to the tissue sites of action (for review see reference 25). Intracutaneous injections were not able to induce pruritus without pain in our preliminary trials (unpublished; see also references 16 and 25). For this reason we chose other means of histamine application intending to avoid counterirritant contamination of the itch sensation as far as possible. The skin prick method induced strong and longlasting itch sensations and large flares, but only small wheals. Thus, it can be used as an itch model with sustained itch half-life. The iontophoretic application of histamine generated larger wheals, but comparatively small itch sensations. Not surprisingly, the higher the iontophoretic current, the larger the histamine effect [12, 21].

It is common clinical experience that the alteration in the skin or the extent of the lesions do not reflect the degree of itch complained of by the patient. In an experimental itch model with iontophoretically applied histamine, correlations between wheal or flare diameter and itch sensation have been described [12]. These authors, however, used different current intensities for iontophoresis thus varying the histamine dose. Because of the dose dependency of histamine reactions, the skin responses covary with the sensation, as described by the authors. However, with an individual subject and a given histamine stimulus intensity, our results indicate that large wheals or flares were not regularly associated with a high degree of subjective itch or vice versa. We found a moderate correlation between itch and flare only with the skin prick method. It is generally agreed that the histamine effect upon vasopermeability (wheal) does not necessarily reflect the strength of the activation of pruritoceptive afferents. Instead, itch sensation and flare reaction are both assumed to be directly caused by neuronal activity, possibly of the same itch receptor (axon reflex [9, 23]). In fact, both flare and itch can be partially suppressed by depletion of substance P stores in the skin with capsaicin while wheals are maintained [24]. In a recently published itch modulation study using the histamine skin prick, we have also obtained a weak correlation between flare diameters and itch ratings [3]. Repeated measurements in the same individual showed that the flare sizes were highly reproducible with all application modes, whereas the itch ratings showed a variable degree of intraindividual variance. The peripheral activity of the C fibre afference gives rise to the axon reflex flare. Thus, central nervous components essentially influence the sensation mediated by the itch afference. As a consequence, the correlation of itch and flare depends on the stability of the experimental environment. The results of this study underline the importance of the individual scratch threshold as a 'cutoff' for the itch sensation that could most reliably be correlated with flare.

Since the small cutaneous venules (whose reaction to histamine results in increased vasopermeation [10, 18]) are situated in deeper skin layers than the most superficially ending polymodal C afferents, our findings that iontophoretic application of histamine induces larger wheals and a shorter and weaker itch sensation do not support the theoretical concept of an intracutaneous concentration gradient of histamine caused by this method. However, it cannot be excluded that a 'counterirritant'-type inhibition of itch sensation in central synapses was induced by the iontophoresis current, especially with the higher current of 2 mA. Threshold sensations to electric current may elicit pruritic sensations by themselves [20]. The smaller wheals with the skin prick compared with 1.3 mC iontophoresis could of course be due to the diameter of the application probe (5 mm) in the latter method, but this does not explain either the significantly larger flare or the duration of skin prick-induced itch exceeding even the effects of 20 mC iontophoresis. It is hypothesized that in iontophoretic application a brief (10-s) histamine bolus passed the most superficial pruritoceptive C fibres too quickly to induce longlasting itch sensations, whereas the skin prick caused a deposit at the superficial dermal-epidermal junction releasing histamine during the entire time of measurement. Consequently, both the C fibre-mediated itch and the axon reflex flare were more pronounced with the skin prick, and the wheal reaction resulting from a

permeability increase of postcapillary venule walls was an independent phenomenon.

In summary, the skin prick with histamine is able to elicit a stronger and longer-lasting reproducible itch sensation compared to iontophoretic application. A selective use of the various application modes is suggested for therapeutic and pathophysiological trials concerning pruritic skin disorders and drug-induced relief.

References

1. Bromm B (1989) Laboratory animals and human volunteers in the assessment of analgesic efficacy. In: Chapman RC, Loeser H (eds) Issues in pain measurement. Raven Press, New York, pp 117–144
2. Bromm B, Treede RD (1991) Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients. Rev Neurol (Paris) 147 : 625–643
3. Bromm B, Scharein E, Darsow U, Ring J (1995) Effects of menthol and cold on histamine-induced itch and skin reactions in man. Neurosci Lett 187 : 157–160
4. Dorsch W, Ring J (1990) Exudate from human wheal and flare reactions. In: Dorsch W (ed) Late phase allergic reactions. CRC Press, Boca Raton, pp 31–34
5. Hägermark Ö, Wahlgren CF (1992) Some methods for evaluating clinical itch and their application for studying pathophysiological mechanisms. J Dermatol Sci 4 : 55–62
6. Handwerker HO, Forster C, Kirchhoff C (1991) Discharge patterns of human C-fibers induced by itching and burning stimuli. J Neurophysiol 66 : 307–315
7. Hearin DL, Mori PP (1950) A study of some anti-histamine compounds by histamine iontophoresis. J Invest Dermatol 14 : 391–394
8. Heyer G, Hornstein OP, Handwerker HO (1991) Reactions to intradermally injected substance P and topically applied mustard oil in atopic dermatitis patients. Acta Derm Venereol (Stockh) 71 : 291–295
9. Keele CA, Armstrong D (1964) Substances producing pain and itch. Edward Arnold, London
10. Lewis T (1927) The blood vessels of the human skin and their responses. Shaw & Sons, London
11. Lindahl O (1961) Experimental skin pain. Acta Physiol Scand Suppl 51 : 179
12. Magerl W, Handwerker HO (1988) A reliable model of experimental itching by iontophoresis of histamine. In: Dubner R, Gebhart GF, Bond MR (eds) Pain research and clinical management, vol 3. Proceedings Vth World Congress on Pain. Elsevier, Amsterdam, pp 536–540
13. Magerl W, Westerman RA, Möhner B, Handwerker HO (1990) Properties of transdermal histamine iontophoresis: differential effects of season, gender and body region. J Invest Dermatol 94 : 347–352
14. Malling HJ (1993) Methods of skin testing. In: Dreborg S, Frew A (eds) Allergen standardization and skin tests. Position Paper of the European Academy of Allergology and Clinical Immunology. Allergy [Suppl] 14 : 55–56
15. Norusis MJ (1992) SPSS for Windows. Advanced statistics. SPSS, Chicago
16. Pepys J (1975) Skin testing. Br J Hosp Med 14 : 412–417
17. Perry DJ, Falk MS, Pillsbury DM (1948) A comparison of the effect of antihistamine drugs in human subjects by means of histamine iontophoresis. J Invest Dermatol 2 : 461–468
18. Ring J, Sedlmeier F, Helm D von der, Mayr T, Walz U, Ibel H, Riepel H, Przybilla B, Reimann HJ, Dorsch W (1986) Histamine and allergic diseases. In: Ring J, Burg G (eds) New trends in allergy II. Springer, Berlin New York, pp 44–77
19. Schmidt R, Jörum E, Rorebjörk E (1993) Magnitude and projection of pain and itch evoked by intraneural microstimulation (abstract). Electroencephalogr Clin Neurophysiol 87 : 137
20. Shelley WB, Arthur RP (1957) The neurohistology and neurophysiology of the itch sensation in man. Arch Dermatol 76 : 296–323
21. Simone DA, Ngeow JY, Whitehouse J, Becerra-Cabal L, Putterman GJ, LaMotte RH (1987) The magnitude and duration of itch produced by intracutaneous injections of histamine. Somatosens Res 5 : 81–92
22. Simone DA, Alreja M, LaMotte RH (1991) Psychophysical studies of the itch sensation and itchy skin (“alloknesis”) produced by intracutaneous injection of histamine. Somatosens Mot Res 3 : 271–279
23. Tausk F, Christian E, Johansson O, Milgram S (1993) Neurobiology of the skin. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (eds) Dermatology in general medicine, vol 1. McGraw-Hill, New York, pp 396–403
24. Toth-Kasa I, Jancso G, Bognar A, Husz S, Obal F (1986) Capsaicin prevents histamine-induced itching. Int J Clin Pharm Res 6 : 163–169
25. Woodward DF, Conway JL, Wheeler LA (1985) Cutaneous itching models. In: Maibach HI, Lowe NJ (eds) Models in Dermatology, vol 1. Karger, Basel, pp 187–195