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Bradykinin is a potent pruritogen in atopic dermatitis: A switch from pain to itch

Miwa Hosogi [a], Martin Schmelz [b], Yoshiki Miyachi [a], Akihiko Ikoma [a,*]

[a] Department of Dermatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[b] Department of Anesthesiology and Intensive Operative Medicine, Clinical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

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Abstract

Histamine, substance P, serotonin and bradykinin were applied by iontophoresis to lesional and visually non-lesional skin of 14 patients with atopic dermatitis, and normal skin of 15 healthy volunteers. Itch could be evoked by light stroking of skin with a cotton swab (alloknesis) in all lesional skin sites, but not in non-lesional or normal skin. Substances were applied in the same skin area before and 3 h after administration of placebo or antihistamine (olopatadine hydrochloride: H1-receptor-blocker). Intensities of itch and pain sensation and areas of flare and wheal were measured. All the substances induced significantly more intense itch in lesional skin than in non-lesional skin of patients. Even bradykinin, which evoked only weak itch and pain of similar intensities in non-lesional skin of patients and in healthy volunteers, induced intense itch in lesional skin, while the simultaneously increased pain did not suppress the itch sensation, indicating central sensitization. Histamine- and substance P-induced itch was almost completely suppressed by antihistamines, whereas bradykinin- and serotonin-induced itch was not. This suggests that substance P is a histamine-dependent pruritogen also in lesional skin under sensitized conditions but that bradykinin and serotonin are histamine-independent pruritogens in lesional skin. It is concluded that serotonin and bradykinin, classic endogenous algogens, can turn into potent histamine-independent pruritogens in lesional skin of atopic dermatitis.

Keywords: Pruritus; Sensitization; Histamine; Nociception

1. Introduction

Itch is a common symptom accompanying various skin diseases such as atopic dermatitis. Anti-pruritic treatments play an important role for such diseases, not only since itch is annoying and impedes quality of life, but also since itch-induced scratching worsens skin conditions and leads to a vicious itch-scratching cycle (Yosipovitch et al., 2005). Neurons conveying itch had not been identified for decades, until single-nerve-fiber recordings have shown that histamine-induced itch is transmitted by a selective slowly conducting and mechano-insensitive subpopulation of unmyelinated C-neurons (Schmelz et al., 1997; Andrew and Craig, 2001). Although explaining histamine-induced itch, this discovery did not clarify itch in most clinical itch conditions presumed to be histamine-independent.

Histamine, released from mast cells in an early phase of inflammation, is the best-known pruritogen in humans and the involvement of mast cells in producing inflammation of atopic dermatitis has often been demonstrated (Leung, 1998). There have been, however, reports showing desensitization of patients with atopic dermatitis to histamine and suggesting that histamine only plays a minor role as a pruritogen in atopic dermatitis (Uehara, 1982; Heyer et al., 1989). Moreover, antihistamines are frequently used but do not satisfactorily relieve pruritus in atopic dermatitis. Although other

* Corresponding author. Tel.: +81 75 751 3310; fax: +81 75 751 4949.
E-mail address: akiikoma@kuhp.kyoto-u.ac.jp (A. Ikoma).

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mediators than histamine have been reported to induce itch in atopic dermatitis (Greaves, 2000), the main pruritogens are still to be identified.

It has been suggested that neuronal sensitization is involved in itch of atopic dermatitis. Strong pruriceptive input leads to sensitization of spinal neurons so that itch can be evoked by light touch (alloknesis). Moreover, even painful stimuli, that would normally suppress itch, can elicit itch when applied in sensitized skin areas (Ikoma et al., 2004). Yet, only exogenous painful stimuli such as electrical stimulation, heat and low pH solution have been so far used to induce itch in sensitized patients. We therefore set out to investigate whether the application of endogenous pain mediators could also elicit itch when applied under sensitized conditions in patients with atopic dermatitis. We chose bradykinin and serotonin, main endogenous algogens (Nojima et al., 2003), and compared their reactions with those of histamine. Additionally, the role of substance P, which has been demonstrated to be a histamine-independent pruritogen in rodents (Kuraishi et al., 1995; Andoh et al., 1998; Ohmura et al., 2004) but not yet clearly in humans, was investigated under sensitized conditions. The compounds were applied before and after systemic treatment with antihistamine in a regular therapeutic dose or placebo to assess possible histamine-dependent effects.

2. Materials and methods

2.1. Subjects

Fourteen patients with atopic dermatitis (AD) (10 men and 4 women; age 24.5 ± 4.8 years, mean $\pm$ SD) and 15 healthy volunteers (8 men and 7 women; age 28.2 ± 4.7 years) participated in this study. The diagnosis of AD was verified by a dermatologist according to the criteria by Hanifin and Rajka (1980). All the patients with AD had chronic pruritic eczema with a typical distribution. None of the participants had received any oral or topical medication at least for a week prior to the experiment. The healthy volunteers had neither skin lesions nor a history of atopic diseases including allergic rhinitis and asthma, both personally and in their family. The local Ethic Committee approved this study and informed consent was obtained in a written form from all the participants before participation.

2.2. Study design

This study was performed in a single-blinded and placebo-controlled cross over manner. Responses to the test stimuli were assessed before and 3 h after medication. Olopatadine hydrochloride 5 mg (Allelock®, H1-receptor-blocker, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan; the regular dosage in Japan is 5 mg twice daily) or placebo was administered orally to each participant in a randomized order. A washout period of at least one week was kept between each experiment to prevent any carry-over effect. Tests were performed at least 2 h after meal. The room temperature was kept constantly at 23 °C.

Iontophoresis as well as detection of alloknesis was performed, as described below, in lesional (AD lesion) and visually non-lesional skin area (AD non-lesion) of the AD patients in the antecubital fossa. Tests in lesional and non-lesional skin were performed in contralateral arms. In healthy volunteers, tests were performed at the antecubital fossa (control).

2.3. Detection of alloknesis

Before performing iontophoresis, the skin areas chosen for iontophoresis were stroked smoothly and lightly by a cotton swab at a rate of 1 Hz. The participants reported the evoked sensation.

2.4. Iontophoresis

Histamine dihydrochloride (Wako Pure Chemical Industries Ltd., Tokyo, Japan), substance P acetate salt, serotonin hydrochloride and bradykinin triacetate salt (Sigma–Aldrich Co., MO, USA) were dissolved in distilled water at concentrations of 1, 2, 17 and 1 mg/ml, respectively.

The compounds were administered to the skin of participants by an iontophoreser (Nihon-Koden Ltd., Tokyo, Japan) through a 0.5 cm^2 cotton applicator at constant current of 0.1 mA for 10 s (histamine), 30 s (serotonin and bradykinin) or 60 s (substance P). The flare and wheal response was assessed 5 min after the end of iontophoresis. Their maximum diameter ($r1$) and orthogonal diameter ($r2$) were visually measured and the flare and wheal area was calculated as $r1/2 \times r2/2 \times 3.14$. Distilled water was applied as control at constant current of 0.1 mA for 60 s to AD lesion and non-lesion at antecubital fossa.

The tests were repeated before and 3 h after placebo or antihistamine administration on the same skin spots.

2.5. Psychophysics

The participants were asked to report the intensity of the evoked sensation on a numerical scale of 0 (no sensation) to 10 (the maximum sensation imaginable) at 10-s intervals for 10 min after the iontophoresis, giving separate ratings for itch and pain. The area under curve (AUC) of ratings for 10 min (min 0, max 610) was calculated for analysis.

2.6. Statistics

The statistical analysis for multiple comparisons among three groups (control, AD non-lesion and AD lesion) was performed using Kruskal–Wallis test. For comparison of reactions 'before' and 'after' administrations as well as itch intensities after 'placebo' and 'H1-blocker' administrations, Wilcoxon's matched-pairs sign test was used. P-values less than 0.05 were regarded to be significant.

3. Results

3.1. Alloknesis

All the patients found the stroking of the lesional skin so itchy that they wanted to scratch, both before and

after application of antihistamines. On the other hand, no itch sensation was evoked in non-lesional skin of the patients or healthy controls.

3.2. Water iontophoresis

About half of the participants described very weak prickling or burning sensation during iontophoresis. This sensation disappeared immediately after the termination of iontophoresis.

Water iontophoresis in AD lesion induced very weak itch sensation in 4 of 14 participants (14 ± 8, AUC, mean $\pm$ SEM) and small flare in 3 of 14 participants ($0.3 \pm 0.2\ cm^2$). Wheal was induced in 1 of 14 participants. None of itch, flare or wheal was evoked in AD non-lesion.

3.3. Itch and pain by iontophoresis

The four mediators provoked itch with different intensities, among which histamine was the most pruritic substance followed by serotonin, substance P and bradykinin, while they provoked only faint pain sensation (Fig. 1). Itch was evoked with a similar time course, i.e. it started at 10 s and the peak intensity was found at around 60 s (Fig. 2a and b).

Histamine-induced itch was of similar intensity when applied in control (225 ± 17, AUC) and in AD lesion (218 ± 17, AUC), but of much lower intensity in AD non-lesion (102 ± 16, AUC; $P < 0.001$) (Fig. 1). Repetition of histamine stimulation at an interval of 3 h under placebo conditions led to a slight reduction of itch ratings, which was significant in AD lesion (226 ± 26 to 189 ± 23, AUC, $P < 0.05$, mean $\pm$ SEM). Antihistamine almost completely suppressed histamine-induced itch for each stimulation site (6 ± 2 in control, 0 in AD non-lesion, 4 ± 3 in AD lesion, AUC) (Fig. 3), significantly more than placebo in all groups including AD lesion ($85 \pm 6\%$ vs. $2 \pm 2\%$, placebo vs. antihistamine; $P < 0.001$) (Fig. 4).

Substance P also provoked itch, though less than histamine (57 ± 12 in control, 27 ± 7 in AD non-lesion, 79 ± 10 in AD lesion, AUC). Intensities of itch and pain were significantly higher in AD lesion than in AD non-lesion (itch; $P < 0.001$, pain; $P < 0.01$), although itch was much more intense than pain (Fig. 1). Repetition of substance P stimulation under placebo conditions led to a slight reduction of itch ratings, which was significant in AD lesion (76 ± 13 to 46 ± 11, AUC, $P < 0.05$, mean $\pm$ SEM). Itch was suppressed almost completely by antihistamine (18 ± 9 in control, 2 ± 2 in AD non-lesion, 6 ± 3 in AD lesion, AUC) (Fig. 3), significantly more than by placebo in all groups including AD lesion ($106 \pm 53\%$ vs. $9 \pm 5\%$, placebo vs. antihistamine; $P < 0.05$) (Fig. 4).

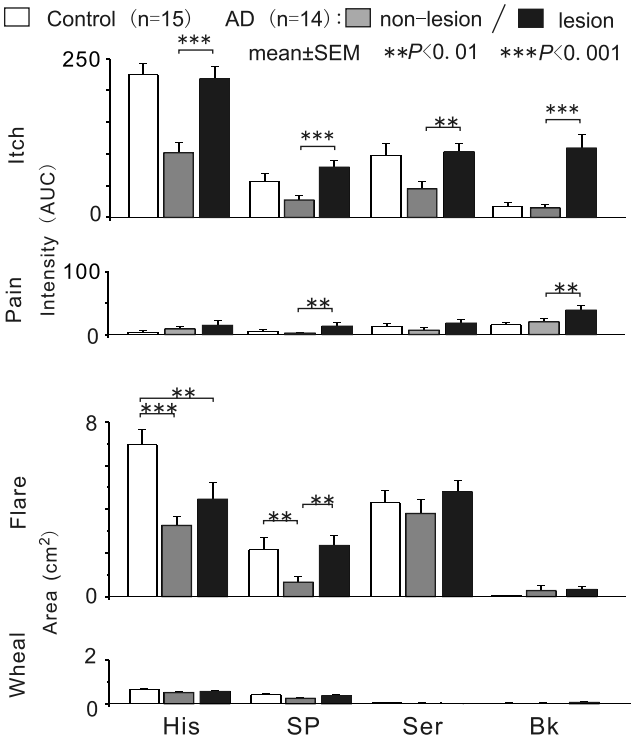

Fig. 1. The AUC of itch and pain ratings and the size of flare and wheal induced by histamine (His), substance P (SP), serotonin (Ser) and bradykinin (Bk) in healthy volunteers (white columns) and in lesional skin (grey columns) and non-lesional skin (black columns) of AD patients before administration of placebo or antihistamine. Histamine-, substance P-, serotonin- and bradykinin-induced itch as well as substance P- and bradykinin-induced pain was significantly more intense in AD lesion than in AD non-lesion. Histamine-induced flare in AD lesion and AD non-lesion was significantly smaller than in control. Substance P-induced flare in AD non-lesion was significantly smaller than in AD lesion and control.

Serotonin-induced itch in all groups (98 ± 18 in control, 45 ± 12 in AD non-lesion, 104 ± 13 in AD lesion, AUC). Itch in AD lesion was significantly more intense than in AD non-lesion ($P < 0.01$) (Fig. 1). Itch intensity became lower after antihistamine administration (Fig. 3). However, this reduction was observed also after placebo administration without any significant difference between placebo and antihistamine (Fig. 4).

Bradykinin provoked very weak itch and pain of almost identical intensity in AD non-lesion and control (18 ± 6 vs. 15 ± 4 in control, 15 ± 5 vs. 20 ± 5 in AD non-lesion, itch vs. pain, AUC). In AD lesion, on the other hand, itch intensity was remarkably increased and significantly higher than in AD non-lesion (109 ± 22 in AD lesion, 15 ± 5 in AD non-lesion, AUC, $P < 0.001$), while pain also significantly increased but only a little (39 ± 7 in AD lesion, 20 ± 5 in AD non-lesion, AUC, $P < 0.01$) (Fig. 1). The duration of itch sensation was significantly longer in AD lesion than in control (363 ± 45 s vs. 108 ± 34 s, $P < 0.05$). Treatment with antihistamine reduced bradykinin-induced itch

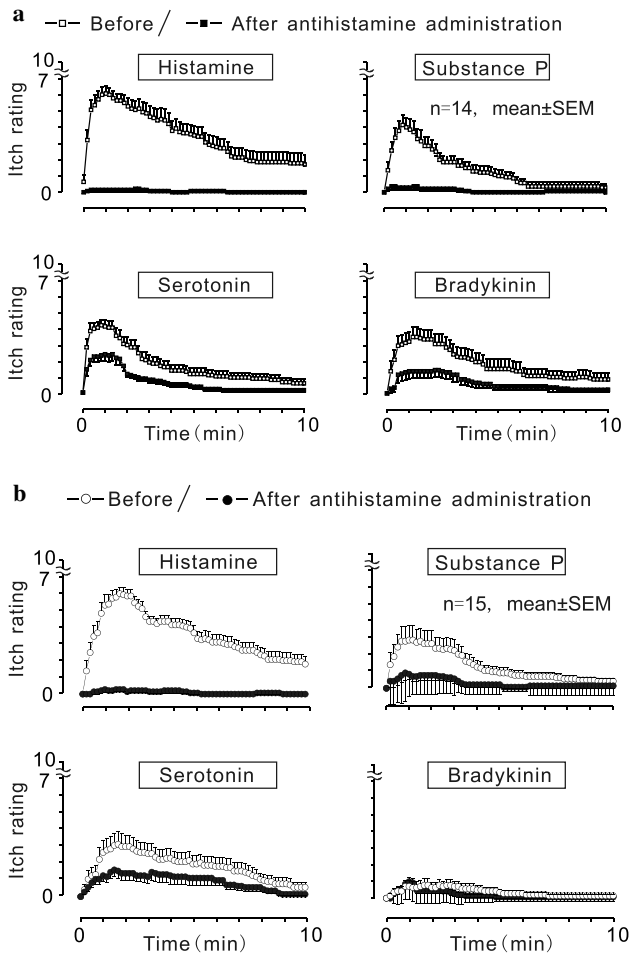

Fig. 2. The time course of itch ratings induced by histamine, substance P, serotonin and bradykinin in lesional skin of AD patients (a) and healthy controls (b) before (open squares/circles) and after (black squares/circles) the administration of antihistamine (olopatadine hydrochloride; H1-receptor-blocker).

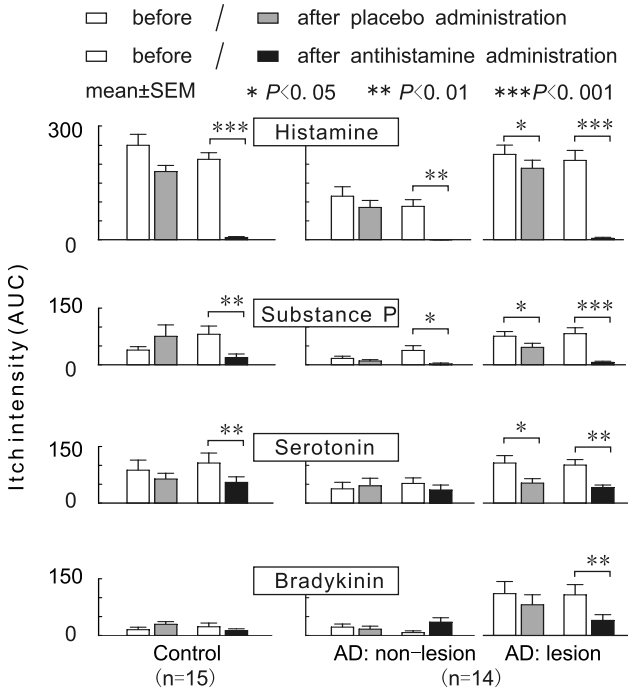

Fig. 3. The AUC of itch ratings for 10 min after iontophoresis of histamine, substance P, serotonin and bradykinin before (white columns) and after the administration of placebo (grey columns) or antihistamine (black columns). Repetition of histamine, substance P and serotonin iontophoresis after a 3-h interval under the placebo condition reduced itch intensities significantly. Histamine- and substance P-induced itch was suppressed significantly by antihistamine in all groups, while serotonin- and bradykinin-induced itch was not in all groups.

(Fig. 3), but not significantly as compared to placebo administration (Fig. 4).

3.4. Flare and wheal by iontophoresis

The four mediators differentially provoked flare and wheal reactions: flare reactions were induced by histamine > serotonin > SP, while basically not by bradykinin. Wheal responses were induced by histamine > SP, while basically not by serotonin or bradykinin (Fig. 1).

Repetition of the chemical stimulation at an interval of 3 h under placebo conditions induced almost identical wheal responses and only slightly reduced flare areas (Figs. 5 and 6).

The size of histamine-induced flare was comparable in both AD lesion and AD non-lesion, although relatively larger in AD lesion ($3.3 \pm 0.4\ cm^2$ in AD non-lesion, $4.5 \pm 0.7\ cm^2$ in AD lesion, mean ± SEM). The flare in control ($7.0 \pm 0.7\ cm^2$) was significantly larger than in AD lesion ($P < 0.01$) and AD non-lesion ($P < 0.001$) (Fig. 1). Flare was reduced to $0.5\ cm^2$, almost the same size as the probe, after systemic treatment with antihistamine in all groups (Fig. 5). There was no significant difference in the size of histamine-induced wheal among three groups (Fig. 1). The wheal development was completely blocked by antihistamine in all groups (Fig. 6).

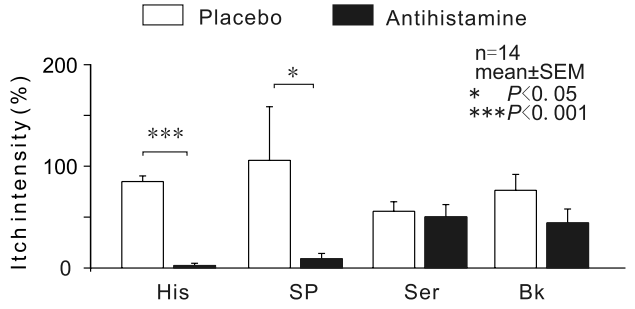

Fig. 4. The comparison of itch intensities after placebo (white columns) and antihistamine (black columns) administration. Itch AUCs after placebo and antihistamine administration were quantified as a percentage of that before administration. Histamine- and substance P-induced itch was significantly more suppressed by antihistamine compared to placebo, but not serotonin- and bradykinin-induced itch.

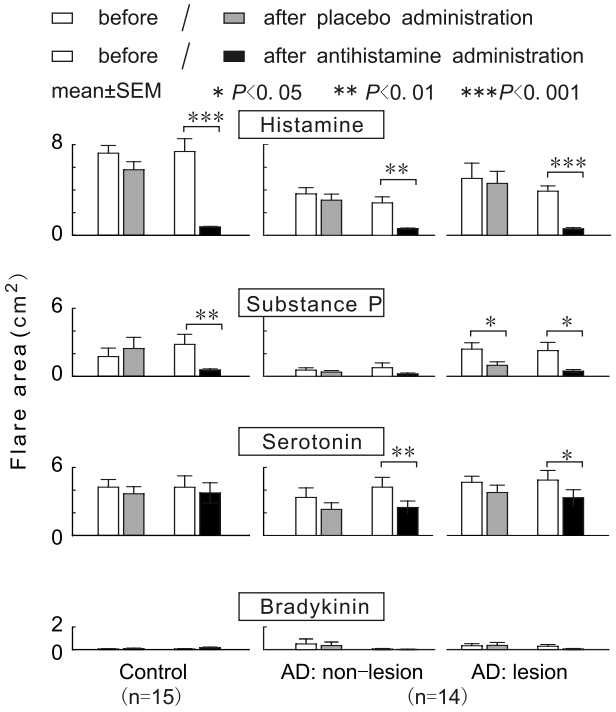

Fig. 5. The size of flare induced by histamine, substance P, serotonin and bradykinin before (white columns) and after placebo (grey columns) or antihistamine (black columns) administration. Histamine- and substance P-induced flare was reduced by antihistamine to about 0.5 cm^2, as large as the probe size, while serotonin-induced flare was not affected by antihistamine, compared to placebo.

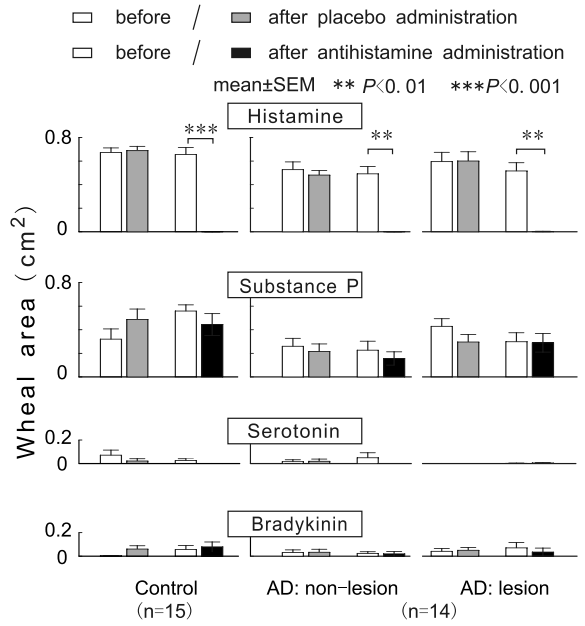

Fig. 6. The size of wheal induced by histamine, substance P, serotonin and bradykinin before (white columns) and after placebo (grey columns) or antihistamine (black columns) administration. Histamine-induced wheal was completely suppressed by antihistamine, while substance P-induced wheal was not.

Substance P-induced flare in AD non-lesion was significantly smaller than in AD lesion and in control ($0.7 \pm 0.2\ cm^2$ in AD non-lesion vs. $2.3 \pm 0.5\ cm^2$ in AD lesion and $2.1 \pm 0.6\ cm^2$ in control, both $P < 0.01$) (Fig. 1). Flare area was reduced in all groups by antihistamine and was then limited to the contact area of the probe (Fig. 5). Substance P-induced wheal response was not significantly reduced by antihistamine (Fig. 6).

Serotonin-induced flare did not differ significantly among the application sites (Fig. 1). Upon repetition, a slight reduction of flare size was observed. However, no significant difference was observed between placebo and antihistamine treatment (Fig. 5). Serotonin did not induce any wheal (Figs. 1 and 6).

Bradykinin-induced flare was restricted to the contact area of the probe in all groups, which did not change after administration of antihistamine (Figs. 1 and 5). No significant wheal was induced by bradykinin (Figs. 1 and 6).

4. Discussion

Our results confirm that non-lesional skin of patients with atopic dermatitis is rather less sensitive as compared to healthy controls (Heyer et al., 1989), but that the sensitivity of their lesional skin is enhanced compared to that of non-lesional skin.

4.1. Iontophoretic delivery of mediators

Iontophoresis of small cations like histamine and serotonin salts has been widely used in humans. Bradykinin and substance P salts, also positively charged but larger cations, have been successfully applied by iontophoresis before (Newton et al., 2001; Brown et al., 2003). For charged molecules, the iontophoretic transport is controlled by the applied current. The barrier function of the epidermis can be another main factor for non-charged or very large molecules. The barrier function is damaged in patients with atopic dermatitis, especially in their lesion. However, we observed no significant difference in bradykinin- or substance P-induced local wheal reactions among different groups, suggesting that the delivery of the mediators was not much affected by the damaged barrier function.

4.2. Role of different mediators for itch in atopic dermatitis

4.2.1. Histamine

Application by iontophoresis at a 3-h interval seemed to cause little tachyphylaxis to histamine that was reported in a previous study (Stahle-Backdahl et al., 1988). Our results confirm the reduced sensitivity to histamine in non-lesional skin, probably attributed to desensitization upon higher local histamine concentrations (Uehara,

1982; Heyer et al., 1989). However, inside the lesions, histamine-induced itch was as intense as in healthy controls, indicating that histamine can still be a very potent pruritogen in atopic dermatitis. The cutaneous concentration of histamine in this study, which caused a large wheal reaction, exceeded endogenous histamine levels in the patients by far. We can conclude that a regular clinical dose of antihistamines reliably block histamine-induced itch even under sensitized conditions. The unsatisfactory anti-pruritic effect of antihistamines in atopic dermatitis therefore suggests that histamine is not the main pruritogen of the disease.

4.2.2. Substance P

Substance P is released from nerve endings in various inflammatory conditions. The finding that the serum level of substance P in patients with atopic dermatitis correlates with its severity could suggest the involvement of substance P in atopic dermatitis (Toyoda et al., 2002). While substance P has been found to be a histamine-independent pruritogen in rodent skin (Kuraishi et al., 1995; Andoh et al., 1998; Ohmura et al., 2004), its role in humans has not been clarified yet. A previous study in healthy human skin denied its histamine-independent effect as a pruritogen (Weidner et al., 2000). As for atopic dermatitis, it has been shown that substance P-induced skin reactions in non-lesional skin of patients are even weaker than in healthy persons, suggesting desensitization to substance P in patients (Giannetti and Girolomoni, 1989). This is compatible with the significantly smaller size of flare in non-lesional skin than in healthy volunteers in this study. In the skin lesions, on the other hand, substance P provoked intense itch. However, itch and flare reactions were completely suppressed by antihistamines, indicating that the responses were provoked via histamine release. The remaining wheal, which was not suppressed by antihistamines, suggests direct effects of substance P via NK1 receptors on postcapillary venules, as shown in a previous human study (Weidner et al., 2000). Moreover, the iontophoretic challenge was obviously successful to achieve concentrations of substance P high enough to provoke protein extravasation. As the remaining wheal was not accompanied by itch, a histamine-independent pruritic effect by substance P could not be demonstrated even in lesional skin.

4.2.3. Serotonin

Serotonin is released from platelets in early phase of inflammation and could contribute to inflammation of atopic dermatitis (Dumitrascu, 1996). Serotonin has been used as an experimental pruritogen in a previous study, in which wheal was induced by application of serotonin and abolished by antihistamines (Weisshaar et al., 1997), suggesting that part of the serotonin-induced itch and flare reactions could be attributed to secondary histamine release. However, we did not observe any local wheal reaction and antihistamines did not significantly reduce serotonin-induced itch and flare reactions. The lack of mast cell activation in our study could be due to a lower dose (30%) of serotonin applied in our study (30 s, 0.1 mA vs. 10 s, 1 mA). Although serotonin has also been reported to be a weaker pruritogen than histamine (Hagermark, 1992; Thomsen et al., 2002; Schmelz et al., 2003b), serotonin-induced itch in lesional skin was of similar intensity as compared to histamine-induced itch in non-lesional skin in this study. Thus, serotonin is a potent histamine-independent pruritogen in lesional skin of patients with atopic dermatitis. Serotonin-induced itch was weaker in non-lesional skin of patients than healthy controls, which could implicate desensitization to serotonin in non-lesional skin. However, different from histamine and substance P, the size of serotonin-induced flare was almost identical, which would speak against desensitization to serotonin.

4.2.4. Bradykinin

Bradykinin is released in a wide range of inflammatory conditions (Hargreaves and Costello, 1990; Dray and Perkins, 1993). Although bradykinin is generally known to be a potent pain mediator (Dray and Perkins, 1993), itch was mainly evoked when applied inside lesion in this study. Though bradykinin-induced itch was reported to be histamine-mediated in humans (Hagermark, 1974) and, moreover, anti-bradykinin effects of antihistamines have often been reported (Church, 1999; Leurs et al., 2002), there was no statistically significant difference between antihistamines and placebo in this study. A previous microneurographical study showed that bradykinin injection activates also histamine-sensitive C-pruriceptors, though much weaker than histamine (Schmelz et al., 2003b). It was suggested in another previous study that bradykinin might contribute to itch in inflammatory conditions by sensitizing peripheral nerves (Koppert et al., 1993). However, there are no reports demonstrating itch directly evoked by bradykinin in humans. Bradykinin-induced itch in this study did not provoke any flare or wheal and was not suppressed by antihistamines, suggesting that bradykinin elicits itch independently of histamine or histamine-responsive pruriceptors. The existence of a new class of pruriceptors with low electrical thresholds that is not linked to the generation of an axon reflex has recently been proposed (Ikoma et al., 2005). The pattern of itch without flare reaction as described for papain before (Hagermark, 1973) could be attributed to activation of such a class of pruriceptors. Alternatively, bradykinin could activate primary afferent fibers that are normally involved in pain processing, but provoke itch in the sensitized patients. In this case, central sensitization of itch and ineffectivity of itch-inhibition by pain would be hypothesized as described below.

4.3. Peripheral and central sensitization

Although the mechanism of sensitization for itch is not yet clarified, increased expression of neurotrophic factors such as nerve growth factor and neurotrophin-4 has been found in patients with AD (Grewe et al., 2000; Kinkelin et al., 2000; Toyoda et al., 2002) and could underlie peripheral sensitization. Increased densities of epidermal nerve fibers found in atopic dermatitis (Urashima and Mihara, 1998) indicate a possible pathophysiological role of neurotrophic factors. They lead not only to nerve fiber sprouting but also to enhancement of neuronal sensitivity (Shu and Mendell, 1999).

In addition to peripheral sensitization, it is now widely accepted by several lines of evidence that central sensitization in the spinal level caused by ongoing activation of peripheral nerves plays a major role in neuronal sensitization (Treede and Magerl, 2000). Although much less studied in comparison to sensitization for pain, it has been suggested in previous studies that central sensitization can contribute also to sensitization for itch (Ikoma et al., 2003, 2004). Alloknesis and punctate hyperknesis, central sensitization phenomena for itch, have been reported after application of histamine to healthy human skin which corresponds to allodynia and hyperalgesia observed in neuropathic pain (Simone et al., 1991; Atanassoff et al., 1999; Brull et al., 1999). Ongoing activation of itch C-nerves in patients with chronic pruritus, shown in a previous microneurography study, also supports the idea of central sensitization for itch (Schmelz et al., 2003a). Itch can be elicited in lesional skin of atopic dermatitis by mechanical, electrical, thermal and proton stimuli that are normally painful and suppress itch (Ikoma et al., 2004). This attenuated suppression of itch by pain in atopic dermatitis cannot be explained by peripheral sensitization alone. In this study, the presence of alloknesis in lesional skin clearly indicates central sensitization. Moreover, although bradykinin-induced both itch and pain more intensely in lesional skin than non-lesional skin, the enhanced pain did not suppress itch. Therefore, besides sensitization of itch-signaling neurons, we cannot exclude the possibility that pain-signaling neurons have switched modality to signal itch.

It can be concluded that classic endogenous algogens, serotonin and bradykinin, can turn into potent pruritogens in lesional skin of patients with atopic dermatitis. Especially, the role of bradykinin as a histamine-independent pruritogen was demonstrated in our study. Sensitization of spinal processing as well as sensitization of local nerve endings in lesion is assumed to underlie the enhanced itch responses. As already known in pain research, the apparent redundancy among the inflammatory mediators hampers therapeutic anti-pruritic approaches based on specific blockers of single mediators such as antihistamines; identification of essential targets in the peripheral and central sensitization processes is therefore required for more successful anti-pruritic therapy.

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