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Reactions to Intradermally Injected Substance P and Topically Applied Mustard Oil in Atopic Dermatitis Patients

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Skin reactions and itch or burning pain sensations following intradermal injection of the neuropeptide substance P and topical application of the substance P releasing agent mustard oil were studied in 20 atopic dermatitis patients and 20 healthy controls. Changes in skin blood flow were measured with a Laser Doppler flowmeter. Areas of wheal and flare reactions were evaluated planimetrically. Simultaneous with Laser Doppler flowmeter measurements, subjective itch and burning pain ratings were verbally reported on a category partitioning scale at 10-second intervals. Substance P evoked dose-dependent wheal, flare, and itch reactions in both patients and controls. However, substance P doses of 10^{-9}–10^{-11} mol elicited smaller flares in patients than in the controls whereas the wheal sizes were similar in both groups. Substance P-induced itch ratings were lower in patients at a dose of 10^{-10} mol, and the onset of itching was delayed at all substance P levels applied. Mustard oil elicited similar neurogenic inflammatory reactions in both groups, although pain sensations were significantly delayed in atopic dermatitis patients at two mustard oil concentrations, which is further indication of a desensitization of afferent nerve endings contributing to the neurogenic inflammatory reactions in the skin of these patients. ***Key word: Neurogenic inflammation.***

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Several studies on atopic dermatitis (AD) patients have reported that i.d. histamine injection causes only mild wheal and flare reactions (1, 2). Recently we reported a reduced sensitivity of AD patients to iontophoretically applied histamine and a lack of dose-dependent itch response, indicating impaired sensitivity of unmyelinated cutaneous nerve fibres mediating itch sensations (3, 4).

These nerve fibres probably contain neuropeptides, in particular substance P (SP), which act as mediators of axon reflex vasodilation and neurogenic inflammation (5). On the other hand, intradermally injected SP induces itch accompanied by wheal and flare reactions probably mediated by histamine released from cutaneous mast cells (6).

The objective of this study was to elucidate possible abnormalities in AD patients' itch responses and skin reactivity to SP, compared with healthy controls. To study the effect of SP on cutaneous nerve endings and vasculature, we injected it intradermally. Mustard oil (MU) is known to excite unmyelinated polymodal nociceptors and to release SP (7). MU was used to obtain additional information about SP release from afferent nerve endings.

MATERIAL AND METHODS

Patients and control subjects

The patient group consisted of 6 males and 14 females suffering from acute exacerbation of atopic dermatitis (AD) (ages: 15–59 yrs, mean 26.4) admitted to the Department of Dermatology between October 1988 and April 1989. Antihistamine or other medication influencing skin blood flow was stopped 3 days prior to the investigation. Patients who had undergone systemic or topical corticoid or ACTH therapy in the previous 3 months were excluded.

Twenty healthy volunteers with no family history of atopy, 11 males and 9 females (ages: 24–59, mean: 31.8) served as controls. The drug exclusions of the patient group also applied to the controls.

Investigations were performed between noon and 3 p.m. at constant room temperature (21–22°C) and air humidity (60–65%). The right or left forearm was placed on a temperature controlled arm rest at 34°C. All patients and controls gave informed consent to participate in the study, and were told before the experiment that they were free to withdraw at any time. The study was approved by the Medical Ethics Committee at the Medical Faculty of the University of Erlangen.

Substance P injection

0.01 ml of substance P (SP, Serva) in four different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} M) in sterile physiological saline and one control stimulus (sterile physiological saline) was intradermally injected in alternating sequence in the

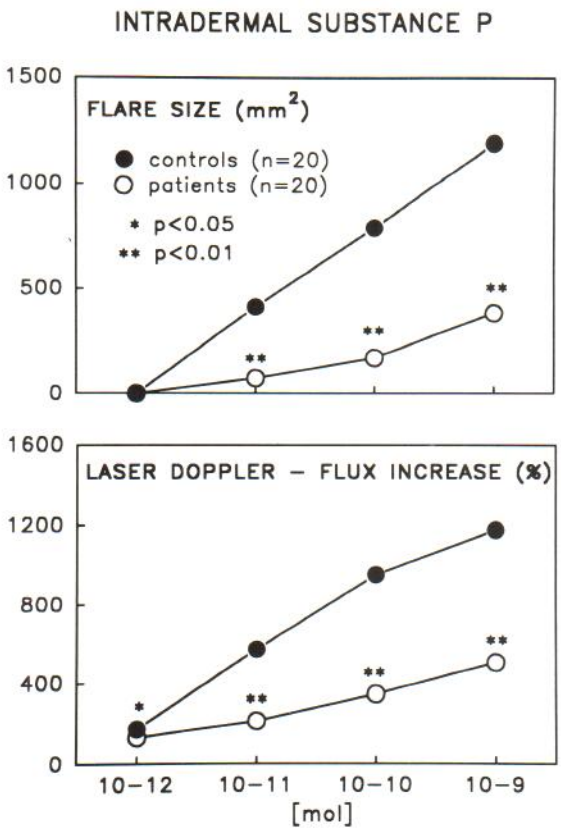

Fig. 1. Upper graph: Median areas of flare reactions 10 min after SP injection, plotted against stimulus concentrations in AD patients and controls. *Lower graph:* Comparison of blood flow increases in the erythematous area from baseline (40-s period) over an 8-min observation period after substance P *(SP)*. An increase of 100% is equivalent to an average doubling of fluxes in the 8-min observation period.

right and left volar surface of the forarm using a 27-gauge needle. Hence the SP doses were 10^{-9} to 10^{-12} mol. Stimuli were applied single-blind in randomized order. Patients and controls received the same five stimulus levels. Wheal and flare areas were outlined on translucent paper for planimetric analysis 10 min after each injection.

Mustard oil application

After cleansing the skin with alcohol, mustard oil (MU; Merck) was applied in three different concentrations on the volar surface of the left or right forearm. Standardized size filter paper (225 mm^2) dipped in 10%, 20%, or 30% MU in liquid paraffin solution was placed on the skin for 3 min. Flare areas were documented 10 min after the start of application.

Laser Doppler flowmeter

A laser Doppler flowmeter (Periflux/PFI, Sweden) was used to assess relative flow increases during flare reactions. The fiberoptic sensor was placed 10 mm from the SP injection site or filter paper edge. Laser Doppler flowmetry measures relative cutaneous blood flow as the product of velocity and number of blood cells to a depth of approximately 0.6 mm from the skin surface, or a half-sphere of about 0.45 mm^3 (8). The measure given as output voltage is termed 'flux'.

All data were read into a laboratory computer and displayed on a chart recorder. Baseline skin blood flux was determined over 40 s prior to SP or MU application. Stimulus-induced changes are expressed as a percentage of baseline averaged over an 8-min observation period. Thus, a 100% value indicates doubled average flux after stimulus.

Rating of itch and burning pain sensation

In a previous study it was found that SP regularly induced itching, whereas MU application elicited burning pain (9). In the present study, SP-induced pruritus or MU-induced burning pain was repeatedly assessed using an open-ended category partitioning scale, from 0 (no response), 1 (very low), 2 (low), 3 (medium), 4 (strong), 5 (very strong) and 6 (extreme). Each category was further subdivided in 10 parts (e.g., 3.5, 6.9). On acoustic cues, ratings were verbally reported at 10-sec intervals over an 8-min period starting at the beginning of each stimulus. Average and maximum rating values were assessed as in previous studies. Pain or itch response latency was defined as the time between stimulus application and first rating when itch was perceived.

Data processing

Data were evaluated using commercially available software: Reflex (Borland, USA) for the database, Dadisp (DSP Development Corporation, USA) for editing Laser Doppler flowmeter recordings, and CSS (Stat Soft, USA) for statistical analysis. Statistical tests used were: Mann-Whitney U-test for comparing patient and control data, and Fisher's exact probability test for comparing the proportions of patients and controls responding within a 30-s period.

RESULTS

Wheal and flare reactions to Substance P

Sterile physiological saline injection never produced wheal or flare reactions in controls or AD patients. The weakest SP dose, 10^{-12} mol, induced wheals in 14 controls and 13 patients. 10^{-11} mol and higher doses of SP always elicited wheal and flare reactions in controls. However, though 18 AD patients showed wheals, only 11 of 20, showed flares after 10^{-11} mol injection. Even at higher doses of SP some patients did not react with a flare: 4 patients at 10^{-10} mol, and 2 at 10^{-9} mol, though, the two highest SP concentrations always induced wheals.

Generally, controls and patients had increasing wheal and flare reactions with increasing SP concentrations. Mean wheal sizes in controls and patients

Table I. *Proportion of patients perceiving itch (SP) or burning pain (MU) within 30 s after application of SP or MU agent.*

	Patients ($N = 20$)	Controls ($N = 20$)	Sign.* (p)
SP 10^{-7}M	1/20	6/20	$p < 0.05$
SP 10^{-6}M	2/20	10/20	0.007
SP 10^{-5}M	9/20	16/20	0.02
SP 10^{-4}M	9/20	20/20	0.0001
MU 10%	6/20	13/20	0.03
MU 20%	8/20	11/20	n.s.
MU 30%	10/20	17/20	0.02

* Fisher's Exact Probability Test.

were not significantly different at any given SP dose. However, healthy controls showed significantly larger flare reactions than AD patients ($p < 0.01$, U-test) except at the lowest SP dose 10^{-12}.

In 4 controls and 3 AD patients a systemic reaction to the strongest SP stimulus (10^{-9} mol) was observed. Two min after i.d. injection an upper body flush (face, neck, arms, and decollete) lasting about 20 s was observed. Subjects reported a simultaneous sensation of general warmth. Neither blood pressure nor heart rate changes were observed during flushing. One of the 4 reactive control subjects reproduced the flush reaction at a later date with 10^{-9} mol SP stimulus.

Flare reactions to mustard oil

MU application did not induce wheals, but elicited a flare in all patients and controls at the concentrations used. MU-induced flares appeared with more intense redness than those observed after SP injection. Both groups showed increasing flare sizes at higher concentrations. Differences between patients and controls were not significant.

Flux reactions to Substance P and mustard oil

Onset of SP- and MU-induced flux increases were similar in patients and controls. Generally, SP induced a steep flux increase after a short latency (about 30 s) which reached a plateau within 2 or 3 min, and stabilized at this level for the 8 min observation period, especially at higher concentrations (10^{-9} and 10^{-10} mol). In contrast, MU application induced a slower rise after a longer latency period (3–4 min). Furthermore flux often decreased 5 or 6 min after MU application, reaching baseline by the end of the 8-min observation period. Mean flux increases in AD patients and controls differed significantly at all SP stimulus levels (Fig. 1), but not after MU.

Itch ratings after Substance P injection

Itch ratings were also correlated with SP dose in both patients and controls. Patient ratings were significantly lower than control ratings at the 10^{-10} mol SP injection ($p < 0.01$, U-test), but the highest SP dose (10^{-9} mol) induced similar mean responses in both groups. At this dose, 50% (10/20) of the controls reported itching for the entire 8-min observation period, and the other 50% for 3 min or more. In contrast, 2 patients reported no itch.

Latency between stimulus application and the first positive itch rating was dose dependent. At the highest dose (10^{-9} mol), controls generally perceived itching within 20 s and invariably within 30 s. In contrast, the itch responses of AD patients generally showed a longer latency at this stimulus level. Only 2 patients reported itching during the first 20 s after SP application, 7 after 30 s, and 7 between 40 and 100 s.

Table I shows the proportions of patients and controls perceiving itch within 30 s of each of the four SP doses and their significance levels (Fisher's exact probability test).

Burning pain after mustard oil application

MU application elicited similar burning pain levels in both patients and controls. As with SP, latency period was dependent on MU concentration. Individual differences were not as large after MU application as after SP injection. Generally, both groups rated burning sensations within the first minute after application at all stimulus levels. Pain ratings reached a maximum after 2 min and then fell to a lower level. However, as shown in Table I, latency was greater in AD patients compared with the controls at two of the three MU concentrations.

DISCUSSION

Substance P is localized in small unmyelinated primary afferent nerve fibres and is known to be a mediator of neurogenic vasodilation and plasma-extravasation (5, 10). Former studies have reported vasodilation and skin temperature increase following i.v. injection of large doses of SP in humans (11). We observed vasodilation in some cases in the upper half of the body after intradermal injection of 10^{-9} mol

SP. Probably, in some cases at this dose sufficient SP reached the systemic circulation to induce a generalized vascular reaction.

Local intradermal SP injection results in *(a)* plasma extravasation (wheal), *(b)* vasodilation (flare), and *(c)* itch sensations (6, 12). Besides being a potent vasodilator by itself, SP releases histamine in human skin (6, 13). Hence, some of the effects of i.d. injected SP are likely to be mediated by histamine which excites 'polymodal' afferent units, some of which again may release SP upon stimulation (4). Indeed, pretreatment with an H_1 histamine antagonist or histamine depletion with compound 48/80 reduced wheal and flare responses to i.d. SP (6). SP elicited smaller flares and flux increases in AD patients than in controls (Fig. 1), whereas wheal sizes did not differ significantly.

The magnitude of itch ratings was also diminished in patients, but the difference was significant only at a dose of 10^{-10} mol. However, the longer latency periods observed at all doses of SP (Table I) are an indication of an impairment of SP-induced itching responses. Recently we demonstrated diminished reactivity of AD patients to iontophoretically applied histamine (3). Hence, the differential effects of SP described in this paper may be a consequence of weaker histamine actions. However, several studies have reported increased levels of histamine in the plasma and skin of AD patients, even in unaffected skin areas (15, 16). It has further been shown that AD patients have an increased number of mast cells in their skin connected with an enhanced releasability of histamine from mast cells and basophils (17). Hence, diminished histamine-mediated SP actions observed in this study are probably a consequence of a down regulation of histamine receptors at target structures rather than being due to diminished histamine release.

Wheal reactions were apparently unimpaired by AD. They are caused by the action of SP on the small blood vessels which is probably histamine mediated, to some degree. In another study, using higher doses of SP, even larger wheals were found in AD patients than in controls (18). Hence, the responsiveness of vascular smooth muscle to SP and histamine seems to be unimpaired in AD.

However, in that previous study (18) the lowest dose of SP (10^{-8} mol) elicited larger flares in controls than in AD patients, similar to our results where a smaller flare and itch response in AD was prominent at doses of 10^{-9}–10^{-10} mol. SP-induced flares and itching – in contrast to wheal responses – were abolished by pretreatment of the skin with capsaicin which is known to inactivate nerve endings of slowly conducting chemosensitive afferents (13, 14). Thus, wheal, flare, and itching elicited by i.d. SP are mediated in part by histamine, but only flare and itch responses require intact afferent nerve endings.

Since flare and itching responses depend on the function of thin afferent nerve fibres, our results indicate their desensitization. This hypothesis has to be brought in line with the finding that the responses to MU were not much affected in AD. Application of MU to healthy skin provokes a neurogenic inflammatory response, observed as an erythema and burning pain at the site of contact without visible wheal eruption. Apparently MU also needs to act on intact peptidergic sensory nerve fibres in order to induce a flare, since it failed to act in herpes zoster or capsaicin pretreated skin (19, 20).

One possible explanation of the better preserved MU reactions in AD could be different populations of afferent fibres excited by SP and MU. Another explanation might be an increased release of SP induced by MU in patients which would compensate for the diminished responsiveness of the nerves.

Chronic – sometimes severe – itching is a leading symptom of AD. Our findings on the SP and histamine induced flare and itch responses indicate that this spontaneous itching is combined with a desensitization of small, peptidergic afferent nerve fibres. Only at first glance does spontaneous itching due to elevated histamine levels in the skin and a relative insensitivity of cutaneous nerve fibres to histamine in these patients appear to be contradictory. Probably, the down regulation of histamine receptors at nerve endings partly compensates for the elevated histamine levels in AD. The implication of this down regulation is, however, that the axon reflex mechanism in the skin of AD patients is impaired and this may also impair the protective function of the skin. The impact of this finding remains to be studied in the future.

In summary, our new findings confirm the hypothesis of our earlier investigation, that the functions of unmyelinated afferent skin nerve fibres are affected by the pathophysiological mechanism of AD.

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