

Acetylcholine Induces Different Cutaneous Sensations in Atopic and Non-atopic Subjects

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The mediators eliciting pruritus in atopic eczema are a matter of discussion, since several substances may be involved and histamine is unlikely to be the main agent. Hence, in this study we examined the cutaneous sensations and vascular reactions in 15 patients with atopic eczema and in 15 non-atopic subjects after i.c. injection of acetylcholine (Ach, 0.5 M, 20 µl) or buffered saline, respectively. The sensory perceptions were rated by a visual analogue scale (VAS) as to quality and intensity, and the vascular reactions were monitored by laser Doppler flowmetry and evaluated planimetrically as to flare and wheal extension. The flares and wheals in both groups were similar; yet the cutaneous sensations significantly differed, since all patients with atopic eczema complained of "pure" itching after Ach-injection, whereas the controls reported a burning pain. The patients with atopic eczema started their ratings significantly earlier and rated significantly longer than the controls. Our results provide evidence that Ach may play an important role in the etiology of pruritus in atopic eczema patients. **Key words:** skin atopy; itch sensation; burning pain; vascular reactivity.

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Generalized and often chronic pruritus is a basic feature in patients suffering from atopic eczema (AE) (1–3); yet, its pathophysiologic conditions are by no means clear to date. Since increased histamine levels have been found in both the skin and blood of patients with AE, in particular during exacerbation (4–8), this substance was supposed to be the main pruritogenic mediator for some time. However, Wahlgren et al. (9), who studied the effects of H1-antagonist and a placebo in relief of pruritus in atopics in a double-blind, cross-over trial, found no difference between the substances. Moreover Uehara (10) and Heyer et al. (11, 12) found a diminished itch sensation to both intracutaneously injected and iontophoretically applied histamine as compared to non-atopic healthy controls. Thus histamine does not seem to play the leading role in eliciting pruritus in AE.

As patients with AE usually complain of general itching during or after sweating, release of the cholinergic transmitter substance acetylcholine (Ach) may be involved in pruritic sensations. Former studies concerning cutaneous response to Ach in atopics revealed either abnormal vascular reactions (13, 14) or sweating disturbances, in particular reduced sweat production (15).

However, the role of Ach in causing pruritus has not been investigated systematically. So we performed a clinico-physiological study aimed at examining the cutaneous sensations after i.c. injection of Ach in atopic and non-atopic subjects.

MATERIALS AND METHODS

Fifteen healthy non-atopic volunteers (10 females and 5 males, aged 24–38 years) and 15 patients with AE (9 females and 6 males, aged 17–36 years) participated in the study after giving their informed consent. All subjects were examined by the same physician for any signs or symptoms of atopic diathesis or eczema, using the criteria of Hanifin & Rajka (1). The study was approved by the local ethics committee. The collected data were evaluated according to an atopy score previously published by Diepgen et al. (2), and only patients with clearly defined skin atopy (score level >10–19 or more) were included in the study. The healthy control subjects (atopy score level 0–3) had no history of skin disease, atopic eczema, allergic rhinitis or asthma. The patients with AE were recruited from all patients who had attended our department between 9/1993–9/1994. None of the participants had used any medication (including topical corticosteroids) 3 weeks prior to the study. Constancy of environmental conditions (room temperature 21–22°C, and air humidity 60–65%) was provided. All examinations were performed between noon and 4 p.m.

Acetylcholinum ophthalmicum Dispersa® (Ach) was dissolved in normal saline to a 0.5 M-solution for intracutaneous injection (0.02 ml) with a 1-ml tuberculin syringe into the right or left volar forearm of the participants in randomized order.

We have chosen the concentration of 0.5 M Ach as the dose effect curves indicated that this concentration was the lowest one inducing sensations in all subjects tested. Additionally, we used the same Ach concentration in former studies focusing on Ach-induced vasoreactions in AE (16).

Each subject received one i.c. injection of the freshly prepared solution. Buffered saline injection (0.02 ml) served as control.

Test areas were always normal or eczema-free appearing skin, respectively.

Skin blood flux was measured continuously by laser Doppler flowmetry (LDF) using MBF 3D (Moore Instruments Ltd., UK) following Ach-injection. Baseline skin blood flux was determined 1 min prior to the injection. Measurements were taken 1 cm proximal the site of Ach-injection.

The intensity and duration of Ach-induced local sensations (itch and/or burning pain, respectively) were monitored continuously every 10 s for up to 15 min after the injection, using a linear potentiometer, equipped with a 100-mm visual analogue scale (VAS) (17). Both endpoints of the VAS were marked as no and maximal sensations, respectively. For differentiation between itching or burning pain, each subject had to rate the grade of either sensation by using separate VAS for itching or burning, at 30-s intervals.

The skin flare and wheal areas were outlined with a marking pen 10 min after Ach-application and drawn onto transparent paper for planimetric evaluation.

Statistical evaluation

Data were organized in a spreadsheet (EXCEL®, Microsoft), and the STATISTICA® software package (Statsoft, Tulsa, OK, USA) was used for statistical evaluation. $p < 0.05$ was regarded as statistically significant.

RESULTS

Laser Doppler flowmetry

A significant mean increase of cutaneous blood-flow after local Ach-injection was measured in the atopics as compared to

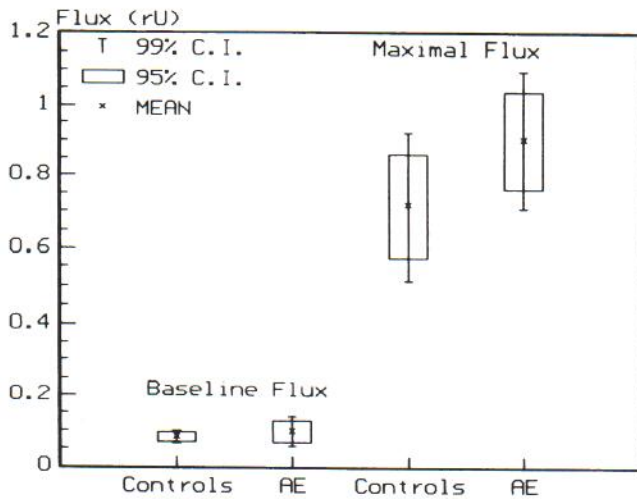


Fig. 1. Baseline flux in atopic eczema (AE) and controls compared to the maximal flux after acetylcholine injection ($p < 0.03$).

controls ($p < 0.03$), whereas buffered saline only produced a minimal and very short-lived rise of blood flow in both groups (Fig. 1).

In both AE patients and controls, a slight erythema was observed immediately after the Ach-injection. In 10/15 patients a delayed blanching phenomenon occurred gradually, reaching its maximum after 5 to 10 min, after which it subsided.

Ratings

The quality and duration, yet not the intensity, of the perceived sensations significantly differed within the test groups. The control subjects reported only a burning pain lasting 4-5 min after the Ach-injection, without any itching sensations. AE patients started their ratings significantly earlier (14 s versus 20 s in controls, $p < 0.003$) and perceived sensations significantly longer (7.1 min) than did the controls (3.9 min), $p < 0.001$.

In the patients, Ach initially induced a "mixed" sensation of burning and itching that changed into continuous "pure" itching within 4-5 min. (Fig. 2)

Wheal and flare

The planimetric evaluation of both phenomena yielded no differences between the groups. In the controls, the mean \pm SD of the flares was $13.04 \text{ cm}^2 \pm 5.49$, and that of the wheals $0.59 \text{ cm}^2 \pm 0.35$; the values in the patients were $13.38 \text{ cm}^2 \pm 6.66$ and $0.63 \text{ cm}^2 \pm 0.42$, respectively.

DISCUSSION

Acetylcholine acts as a well-known transmitter substance between the pre- and postganglionic neurons in both the sympathetic and parasympathetic autonomic system as well as between postganglionic parasympathetic neurons and effector cells (18). Since the first description of the phenomenon of delayed skin blanching in patients with AE (13), many authors re-examined this "paradox" reaction, showing paleness around the Ach-induced wheal with slow spreading after 3-5 min. The interpretation of this abnormal vascular phenomenon is still a matter of discussion. Whether it is due to vasoconstriction

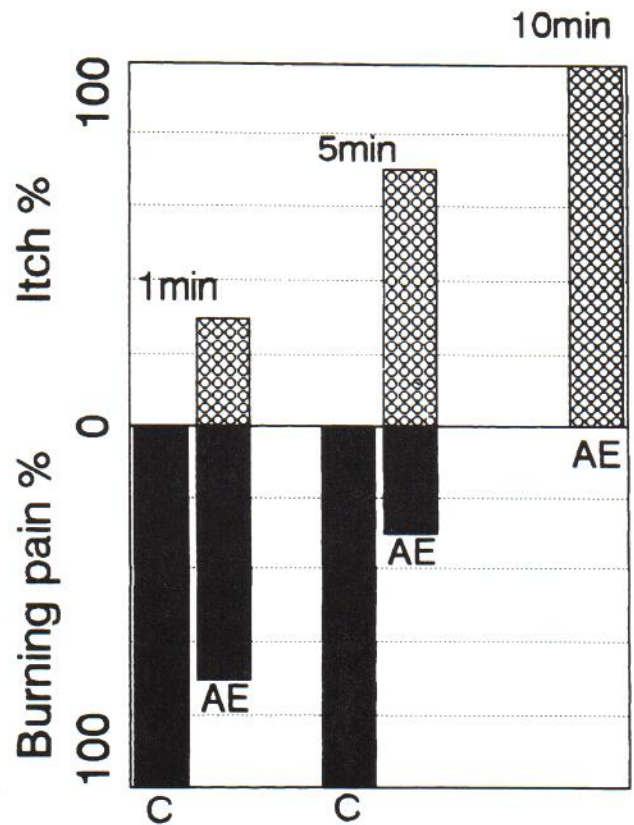


Fig. 2. An overview of the quality of cutaneous sensations after acetylcholine injection. Controls (C) reported a short-lasting burning pain. In contrast, atopic eczema (AE) patients initially reported a "mixed" sensation of burning pain and itch, turning into "pure" and long-lasting itch after 4-5 min.

tion (13) or vasodilation with concomitant oedema (19) is controversial. In any case, the response is considered to reflect a general cholinergic hyperreactivity of cutaneous vessels in patients with AE (20). Some authors observed the highest frequency of Ach blanching in lichenified skin (75%) whereas in dry/rough or normal-appearing skin of AE the figures were 58% and 4%, respectively (14, 21). We found corresponding erythema and blanching after Ach in approximately the same percentages of all our AE patients as reported by Juhlin (5). All controls showed a clear erythematous reaction to Ach. No differences concerning the wheal and flare were seen in either group, but in AE the maximum of skin blood flux was significantly higher. On the other hand, reduced wheal and flare responses to applied histamine have been found by other authors (10, 11). Since increased histamine levels (1, 5, 22) were not only observed in the skin of patients with AE but also with other eczematous conditions (5, 17), histamine may be of minor importance for contributing to the occurrence of blanching in AE.

Notably, Ach caused itch in all of our patients with AE, but in none of the controls. Scott (23), who found strikingly high Ach levels in skin biopsies from patients with AE, suggested an overproduction or excess release of Ach to be the basic fault in atopic skin.

According to Grandó et al. (24), human keratinocytes that express *in vitro* muscarinic receptors can be induced to attach each other by Ach. In another study he showed that cultured

human keratinocytes can synthesize, secrete and degrade Ach (25). Therefore, in his opinion keratinocyte-derived Ach may act in the epidermis like a local hormone.

Recently, an interesting hint for a direct effect of Ach on cutaneous nociceptive C-fibers was given by Steen & Reeh (26), who studied in vitro adult rat skin saphenous nerve preparations. They could demonstrate that carbachol, an Ach-analogue, selectively excites cutaneous nociceptive C-fibers in rat skin. None of the A_β-fibers responded, even to high levels of carbachol, or developed tachyphylaxia.

Whether sweating is directly involved in the pathophysiology or enhancement of itch is not known so far, as sweat gland function in AE has been a matter of contradictory reports. Early statements suggested an impaired sweat delivery (27); yet in later experiments an increased local sweating following cholinergic stimulation was reported both in patients with eczematous or respiratory atopy (28). According to Szentivanyi's hypothesis, this hyperactivity was considered to result from β-adrenergic receptor hyporeactivity (29); yet Murphy et al. (30) could not find any remarkable differences in the sweating of healthy controls and patients with AE. In extended recent studies, however (31, 32), a significantly decreased sweat release after Ach-injections in atopics was shown.

Except in aquagenic pruritus that is challenged by water contact without visible skin changes, the pruritogenic role of Ach has not been discussed. Topical application of a 3% solution of Hyoscine, an anticholinergic agent, abolished the water-induced itching. However, intracutaneous application of methacholine did not provoke aquagenic pruritus in concerned patients (33, 34).

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