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INVESTIGATIVE REPORT

Innovative Neurophysiological Methods in Itch Research: Long-latency Evoked Potentials after Electrical and Thermal Stimulation in Patients with Atopic Dermatitis

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The aim of this study was to examine the findings of innovative neurophysiological methods of itch research. Short-latency and pain-related somatosensory-evoked potentials after electrical stimulation, as well as long-latency evoked potentials after thermal stimulation were studied in 38 patients with atopic dermatitis (AD) and 26 healthy volunteers. Quantitative Sensory Testing of thermal perception was performed in 22 patients with AD from the main AD group and in 15 healthy volunteers. Brain hyperactivity to electrical stimuli, delayed thermal-evoked potentials and elevated thermal thresholds were revealed in patients with AD compared with healthy controls. The data indicate small nerve fibre dysfunction in patients with AD, which may contribute to the pathogenesis of AD and chronic itch. The study demonstrates objective approaches to assess the function of small nerve fibres in patients with chronic itch. ***Key words: pruritus; atopic dermatitis; somatosensory evoked potentials; long-latency evoked potentials to thermal stimulation; CHEPS; thin sensory fibres.***

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Pruritus or itch is defined as a cutaneous sensation that provokes a desire to scratch (1, 2). Pruritus is one of the most common symptoms of skin diseases, and is thus of increasing interest. Central mechanisms of itch and its neuronal pathways are being investigated using different approaches, but they are not yet fully understood. The itch sensation is thought to be conducted along unmyelinated nerve fibres with very low conduction velocity (0.5–1 m/s) and to activate different areas of the cerebral cortex (3–7). The neurophysiological investigation of the activity of unmyelinated thin fibres has always been difficult. Existing laser technologies allow only the selective stimulation of A-delta fibres with rather a high conduction velocity (15–30 m/s). An Israeli company, Medoc Ltd, has developed a device that allows the selective stimulation of small fibres; a Contact Heat-Evoked Potentials Stimulator (CHEPS). CHEPS has been used in a number of studies and has been proved a reliable method of objective evaluation of functioning of thin fibres (3–11). However, CHEPS has not been used in studies of pruritus.

Our previous study on central mechanisms of itch revealed central sensitization in atopic dermatitis (AD) patients with chronic itch, with the help of short- and long-latency somatosensory evoked potentials (SEP) (12). In the present paper we continued our research by investigating long-latency evoked potentials to electrical and selective thin fibre stimulation in patients with AD who were experiencing severe pruritus.

MATERIALS AND METHODS

A total of 38 patients with AD (29 females, 9 males; mean age 23.5 ± 10.0 years) and 26 healthy volunteers (20 females, 6 males; mean age 25 ± 6.5 years) were included in the study. All participants were informed about the goal of the study and the procedures involved. The subjects gave written consent and were free to withdraw from the experiment at any time. All patients with AD were under exacerbation and reported severe pruritus of 5–10 points (mean 6 ± 1.5) according to a 10-graded visual analogue scale (VAS). SCORAD index (Score of Atopic Dermatitis) (13) varied from 22 to 83 points (mean 55 ± 15), corresponding to the severe degree of AD. Skin eruptions, as well as itch intensity, were observed mostly in the forearm flexion regions, popliteal and radiocarpal areas, lesions that presented with erythematosquamous patches 1.5–5 cm in diameter, erythematous papules, excoriations, crusts and lichenification. Most patients had slight erythema and peeling in the perioral and periorbital areas.

All electrophysiological recordings were performed by means of the Nicolet System® with "Bravo" software (Nicolet®, 1998 Madison, Wisconsin, USA). Subjects were seated comfortably in a quiet room with an ambient temperature of approximately 23°C throughout the study.

Short-latency SEP were registered to electrical square wave stimuli of 0.2 ms duration applied at a rate of 5 Hz to the median nerve at the wrist. Their intensity was adjusted to elicit a slight thumb twitch (motor threshold). The recording electrodes were placed according to the 10–20 system in positions C3' and C4'. Linked earlobe electrodes served as reference. The impedance from all electrodes was below 3 KΩ. The electroencephalogram (EEG) was filtered with a bandpass of 1–2000 Hz and sampled at 500 Hz for 100 ms. At least two separate averages of 300–500 trials each were obtained. The reproducibility of responses served as verification criteria.

Long-latency SEP were registered after short-latency SEP without the replacement of the stimulating and recording elec-

trodes. The subjects were given two stimulus levels: (*i*) motor threshold (non-painful); and (*ii*) subject's pain threshold. The inter-stimulus interval was approximately 1 s in random order. Two separate recordings blocks consisted of 20–30 trials per level. The EEG was filtered within a 1−1,000 Hz bandpass. The recording epoch included the first 500 ms post-stimulus.

Stimulation of thin fibres was performed by means of CHEPS (Medoc Ltd, Ramat Yishai, Israel). The stimulating thermode, 27 mm in diameter, is comprised of a heating thermofoil (external layer) with two thermocouples (electronic thermal sensors) and a peltier element (lower layer) with two thermistors (electronic thermal sensors). The rapid heating rate, up to 70°C/s, is created by the external foil, while the peltier element allows a cooling rate of 40°C/s. Cooling begins immediately following attainment of the target heat pulse temperature of 54°C. The basic temperature was 33°C for all stimuli, as recommended by the manufacturer. Constant-intensity stimuli were trigged manually at random inter-stimulus intervals of approximately 4–8 s to the ventral surfaces of the forearms near the elbow flexion and to the cheek area on both sides. The stimulus was perceived subjectively as a moderate burning sensation, by both healthy individuals and patients with AD. The thermode was removed from the skin after each stimulus and placed again nearby in order to avoid the adaptation effect. The total area of stimulation was approximately 6 × 6 cm. Several stimuli were applied before beginning evoked potential recording in order to avoid anticipation and to reduce the novelty effects. The procedure consisted of two separate blocks of 8–12 stimuli given to each area. The subjects were given a 3–5 min break after each stimulation block. The recording electrodes were placed on the vertex (active position) and on the linked earlobe. EEG segments of 1,000 ms duration following each stimulus were recorded within a 1−1,000 Hz bandpass. The data was stored and averaged manually; segments contaminated by artefacts were not included in averaging. At the final stage four obtained responses from symmetrical sites of stimulation (2+2) were averaged into the resulting curve of thermal evoked potentials (thermal EP).

The last part of the study included quantitative measurement of thresholds of warm and cold sensations as well as heat and cold-induced pain in 22 patients with AD from the main AD group (25 ± 10.0 mean years, SCORAD 52 ± 14.0 points, itch intensity 6.5 ± 1.2 points according to VAS) and in 15 healthy volunteers (26.7 ± 6.5 mean years) by means of Quantitative Sensory Testing (QST) tool (Medoc Ltd). The active thermode, with a base temperature of 32°C was fixed by a belt to the skin of the internal surface of the wrist, and its temperature changed according to the test protocol: four ascending and descending trials with heating and cooling rates of 1ºC/s for warm and cold thresholds, and three trials with heating and cooling rates of 1.5ºC/s for heat and cold-induced pain. Patients and healthy subjects responded to the temperature stimuli by pushing a button. The sensory thresholds were recorded and then averaged automatically; test result reports were generated in graphical format.

All procedures were the same for all subjects.

Statistical analyses were performed with Microsoft Office (Excel). Significance was determined using a standardized Student's *t*-test for samples with different distributions.

RESULTS

Patients with AD showed lowered motor and pain thresholds to electrical stimulation, compared with healthy individuals (Table I). Thus SEP registration in the AD group was performed to very weak electrical stimulation. In AD patients such stimuli evoked the primary cortical response (component P1) with increased amplitude (Fig. 1b). In general, long-latency pain-related SEP were relatively normal (Fig. 1d). Taking into account the low intensity of the stimulation, normal long-latency pain-related SEP in patients with AD can also be considered as enhanced brain responses. The data were compared with the results of our previous study (12) of patients with more severe AD (SCORAD 77 ± 5, itch 9 ± 1 points). These patients demonstrated long-latency SEP of increased amplitude both to weak (at motor threshold) and algesic stimulation (Fig. 1a, c). Thus, all AD patients demonstrated brain hyperexcitability to electrical stimuli, which correlated with the severity of the disease. The most significant changes were observed in the late components of SEP in AD patients with very high SCORAD levels.

Table I. *Motor and pain thresholds to electrical stimulation in* n = *38 patients with atopic dermatitis (AD) and* n = *26 healthy subjects (mA, mean ± standard deviation)*

Thresholds	Patients with AD	Healthy subjects	*p*
Motor	7.2 ± 3.1	8.8 ± 3.4	0.009
Pain threshold	16.1 ± 8.1	21.4 ± 7.8	0.002

Thermal EP were obtained in the control group in the form of negative-positive complex (Fig. 2) with a latency of the most prominent positive peak (P2): 453 ± 44 ms (mean ± SD) after forearm stimulation and 399 ± 38 ms after cheek stimulation. The conduction velocity of the afferent impulse was calculated by dividing the tape-measured distance between the sites of stimulation to the latency differences of thermal EP in each healthy volunteer. It was 8.5 ± 2.6 m/s, which corresponded to the conduction velocity along thin fibres. This data agreed with the similar results of previous studies with CHEPS (10, 11, 14, 15).

Patients with AD showed thermal EP of the same configuration; however, larger variations of the latencies were observed. When compared with normal data, a significant latency prolongation of the main positive peak P2 in patients with AD was revealed: 487 ± 57 ms vs. 453 ± 44 ms ($p = 0.004$) to forearm stimulation and 436 ± 48 ms vs. 399 ± 38 ms ($p = 0.0007$) to cheek stimulation (Fig. 3). No differences in amplitude of thermal EP were observed between patients with AD and healthy subjects: peak-to-peak amplitude of P2 was 34.5 ± 18.0 vs. 38.1 ± 24.1 μV to forearm stimulation and 34.8 ± 16.7 vs. 35.9 ± 17.0 μV to cheek stimulation.

In the last part of the study, patients with AD showed significant elevation of cold and warm thresholds, as well as cold-induced pain measured by QST, compared with healthy individuals (Table II). They also demonstrated greater distributions of these parameters. No differences in heat-induced pain thresholds were revealed.

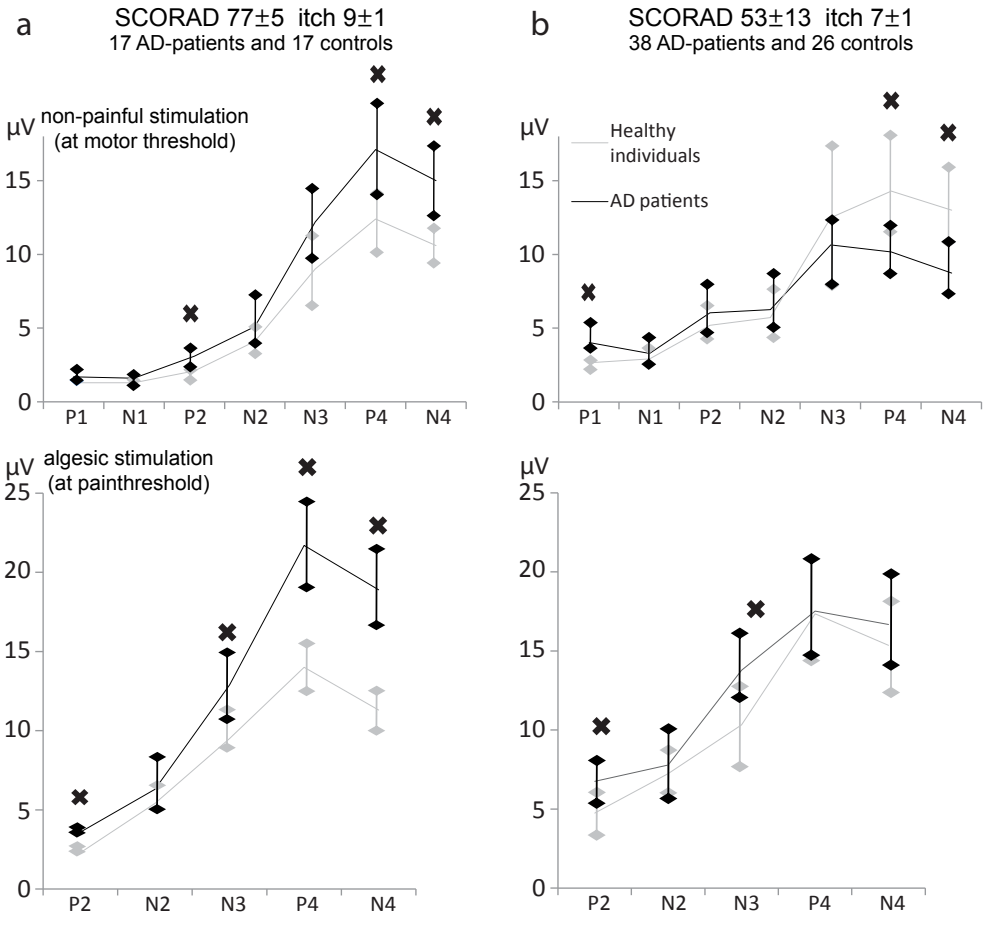

Fig. 1. Amplitude of somatosensory evoked potential (SEP) to non-painful (at motor threshold) and algesic (at pain threshold) stimulation in two groups of patients with atopic dermatitis (AD) with different severity of the disease. (a) Seventeen patients with AD with Score of Atopic Dermatitis (SCORAD) 77 ± 5 vs. $n = 17$ healthy individuals; (b) $n =$ Thirty-eight patients with AD with SCORAD 53 ± 13 vs. 26 healthy controls. Statistically significant differences between the healthy controls and AD groups ($p < 0.05$) are marked with ×.

DISCUSSION

Our SEP findings showed brain hyperexcitability in patients with AD. The present results confirm the data obtained in our previous SEP research (12) and demonstrate correlation between the degree of brain hyperexcitability and the severity of the disease. The most significant changes were observed in the late components of SEP in AD patients with a very high SCORAD levels.

Since the generation of long-latency EP is known to be linked with the activity of non-specific limbic systems and sensory input along thin fibres that conduct pain and are also supposed to conduct itch, we decided to investigate the activity of these fibres in greater detail by means of CHEPS. Taking into account the SEP data we also expected to obtain enhanced brain responses to thermal stimulation. On the contrary, long-latency thermal EP in patients with AD had normal amplitudes but delayed latencies. These findings were observed for

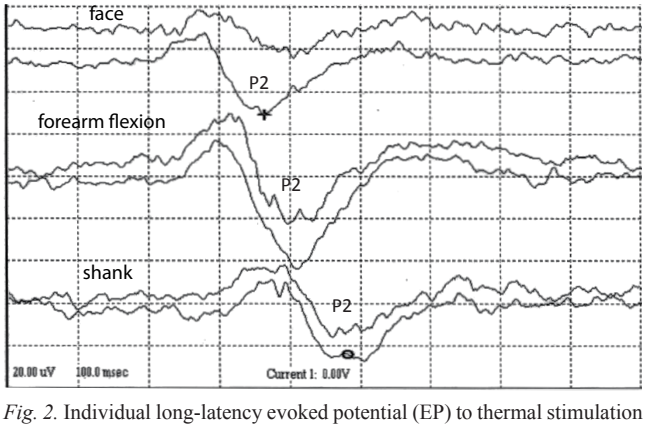

Fig. 2. Individual long-latency evoked potential (EP) to thermal stimulation of cheek, elbow and shank in a healthy volunteer. Thermal EP are presented in the form of negative-positive complex with the most prominent peak marked P2.

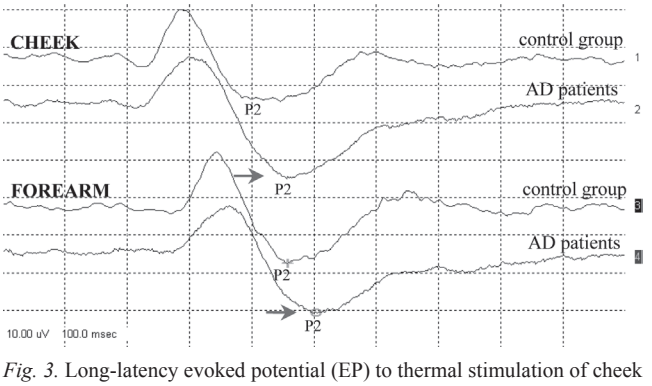

Fig. 3. Long-latency evoked potential (EP) to thermal stimulation of cheek and forearm flexion. Each curve is an overall averaged thermal EP of 38 atopic dermatitis (AD) and 26 healthy controls groups. The most prominent peak is marked P2.

Table II. *Thermal perception thresholds in patients with atopic dermatitis (AD) (*n = *22) and healthy subjects (*n = *15) measured by Quantitative Sensory Testing*

	Mean ± SD, dispersion		
Thresholds	Patients with AD	Healthy subjects	*p*
Cold (non-painful)	29.99 ± 1.32°C, 1.75	30.81 ± 0.65°C, 0.42	0.003
Warm (non-painful)	34.32 ± 0.98°C, 0.96	33.65 ± 0.48°C, 0.24	0.006
Cold-induced pain	10.31 ± 10.30°C, 105.4	18.17 ± 6.19°C, 38.4	0.002
Heat-induced pain	43.33 ± 4.87°C, 23.8	44.49 ± 2.84°C, 8.1	0.169

SD: standard deviation.

both forearm and cheek stimulation, indicating their common nature. We also found insufficient subjective perception of thermal stimuli in patients with AD by means of QST, which agrees with thermal EP data.

Thus we consider that patients with AD have thin fibre dysfunction, as the central structures seem to be safe, since electrically EP showed high or normal amplitudes of the late components. We also hypothesize that input deficit along thin nerve fibres may contribute to the sensitization of central structures, which can result in generation of enhanced brain answers to the electrical stimulation. Moreover, central sensitization may be sustained by constant scratching, which stimulates nerve endings and increases the afferent input along thin fibres, thus producing a compensatory effect. We cannot exclude that the dysfunction of thin nerve fibres might be an initial peculiarity of patients with AD, since changes in long-latency thermal EP were observed both to the stimulation of forearm and much more faintly affected cheek area. This dysfunction may determine a predisposition to AD, the severity of pruritus and the tendency to its chronic course, although further evaluation is required.

Overall, the neurophysiological data provide evidence of an alteration in the central responses to afferent inputs in patients with AD who have chronic itch. This study demonstrates an objective measure to assess the function of small nerve fibres in patients with chronic itch.

The authors declare no conflicts of interest.

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