Acta Derm Venereol (Stockh) 1996; 76: 129–132

Effects of Topical Application of Capsaicin to Human Skin: A Comparison of Effects Evaluated by Visual Assessment, Sensation Registration, Skin Blood Flow and Cutaneous Impedance Measurements

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A new non-invasive device, which enables local measurements of electrical impedance, has been used to evaluate the degree of irritation in human skin. The results have been compared with visual scoring, sensations and laser Doppler flowmetry. Capsaicin (50 µl 1% solution) and control solutions (50 µl 50% ethanol) were applied in a chamber for 30 min on the volar forearm of 7 volunteers. Values were recorded before application and during the total test period of 4.5 h. Sensations like sting/prick, burn and pain were produced by this treatment, and the flare response was observed. Using the non-invasive laser Doppler flow technique to measure blood flow in human skin, we have shown that topical application of capsaicin abolishes the vasodilator response to local heat provocation (40°C). There was close agreement among values obtained using visual assessments, sensations and laser Doppler flowmetry. Results obtained using electrical impedance measurements were not consistent with the other three methods. ***Key words: hot pepper; peptides; skin irritation; electrical impedance.***

(Accepted October 13, 1995.)

Acta Derm Venereol (Stockh) 1996; 76: 129–132.

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Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent compound of chilli pepper and other spices, is used as an experimental tool because of its rather selective actions on afferent nerve fibres. The effects appear to be principally mediated by type C sensory neurons (1). In humans, topical skin application of capsaicin produces itch, sting, burning pain and flare due to neurogenic vasodilatation spreading out from the area of application (2). The initial excitation induced by the chemical is followed by desensitization of the treated skin to heat stimuli. Capsaicin has been proven to first induce the neuronal release of neuropeptides, such as substance P (SP), and then to block further synthesis and transport of the transmitter within the neuron (3). The effect may be similar to cutting a nerve or ligating it, which also depletes the SP content. Capsaicin is clinically used as an external analgesic for temporary relief of neuralgia and is also widely used as a research tool to study peripheral pain (4).

The purpose of the present study was to compare the effects of topical capsaicin treatment, using several methods, and to evaluate whether some of the methods are more sensitive than others in detecting skin irritation. Of special interest was a device for skin impedance measurement, claimed to be much more sensitive in detecting skin irritation than other methods. In the present study, we examined: (i) the flare area of the neurovascular response to topical application of capsaicin, (ii) occurrence of cutaneous sensations during the treatment, (iii) cutaneous blood flow changes and the effect on the vasodilatation produced by heat provocation, and (iv) electrical impedance changes in the skin.

MATERIAL AND METHODS

Test subjects

Seven Caucasian volunteers (3 females and 4 males), between the ages of 22 and 45 years, participated in the study. The experiments were carried out in a quiet room at a temperature of 21–23°C. The subjects were comfortably clothed and seated. No medication had been taken by any of the subjects prior to the test. All had given informed consent and the study was approved by the Ethics Committee of the University of Umeå.

Test substance

A 0.03 M (1%) capsaicin solution (Sigma, lot.no.22H7815, MO, USA) was prepared by dissolving 0.1 g of capsaicin in 10.0 ml of 50% ethanol in water. The solution was prepared prior to use and kept in a refrigerator. The ventral aspect of the forearm, exactly 35 cm from the 3:rd digit tip, was exposed to 50 µl capsaicin solution in a chamber. The plastic chamber (diameter 12 mm) with a filter paper disk prevents evaporation, and this chamber was attached to the skin with harmless tape for 30 min. As a reference, the corresponding skin area of the contralateral arm was used. Fifty microlitre control solution of 50% ethanol in water was applied. Subjects were blinded as to treatment and control sides. Ink marks on the skin around the chamber permitted correct placement of the solutions and the instrument probes. All experiments were performed identically on both capsaicin-treated and reference sides.

Evaluation methods

Visual assessment

Each test site was examined visually before application. After the removal of the test chamber and the evaporation of the solvent, the flare area was measured every 10 min during the first hour, followed by measurements every 30 min. The area of flare was outlined and traced on transparent plastic film. The measurement was continued until the flare disappeared.

Sensations

During the time of application, the subject was asked to report cutaneous sensations. Sensation qualities were based on four descriptors: itch, sting/prick, burn and pain. *Itch* was described as 'the sensation associated with a desire to scratch'; *sting/prick* was defined as 'sharp sensation similar to that produced by extreme temperature or by a chemical irritant, which may or may not be associated with a thermal sensation'; and *burn* and *pain* was explained as 'an appropriated level for sensation that hurts'. These terms have been used in earlier studies by Green (5). The subject was encouraged to use as few or as many of the terms as necessary to describe the sensations.

 ISSN 0001-5555

Laser Doppler flowmetry

Basal skin blood flow was measured by the laser Doppler flow technique (6,7) (Periflux, PF3, Perimed, Sweden). The stability of the flowmeter was determined by measuring the relative flow value in a test vial containing a colloidal suspension of microscopic latex particles in random Brownian motion. Flow is expressed as an arbitrary perfusion unit (PU) and is described as being equivalent to the number of red blood cells contained in the volume of blood through which the laser light is passing and at the speed at which these cells are moving. Capsaicin has been reported to induce an increase in skin temperature (8). In order to correct for deviations in basal blood flow due to variations in core temperature, the temperature of the skin was stabilized at 34°C with a probe heater (PF206, Perimed, Sweden). Skin microcirculation is affected by its level relative to the heart. Therefore the arm was positioned at the heart level. The probe (PF308, Perimed, Sweden, fibre separation 0.25 mm) was placed at the skin without pressure, and the blood flow was measured at 0, 30, 40, 50, 60, 90, 120, 150, 180 and 210 min. Heat provocation of the skin from 34°C to 40°C was performed at 0, 30 and 210 min. The laser Doppler signal was transferred on-line to a computer and analyzed with the Perisoft software (Perimed, Sweden).

Electrical skin impedance

Electrical impedance of the skin has been reported to be very sensitive to skin irritation (9). Therefore, changes in electrical impedance are detected before any visual effect is observed. Electrical skin impedance was measured with a new device (10) (Servo Med AB, Kinna, Sweden). The instrument consists of an impedance monitor for characterization of skin, measuring complex electrical impedance, i.e. both magnitude and phase, at 31 frequencies in the range of 1kHz to 1MHz. Five depths are available. Using the present probe design we registrated the electrical impedance at all five depths (10,11). Electrical impedance was measured and converted to four electrical impedance indexes, MIX = magnitude impedance index, PIX = phase impedance index, RIX = real impedance index, and IMIX = imaginary impedance index, and considered as irritation indexes (Ollmar personal communication). The indexes are defined as: $MIX = abs(Z_{20kHz})/abs(Z_{500kHz})$; $PIX = \varphi_{20kHz} - \varphi_{500kHz}$; $RIX = Re(Z_{20kHz})/Re(Z_{500kHz})$ and $IMIX = Im(Z_{20kHz})/Im(Z_{500kHz})$. $Abs(Z_{xkHz})$ is the electrical impedance modulus at the frequencies x kHz, φ_{xkHz} is the phase angle value at the indicated frequency. $Re(Z_{xkHz})$ represents the real impedance modulus and $Im(Z_{xkHz})$ the imaginary impedance modulus. Before application of the probe, the skin was soaked with physiological saline for 1 min. Stratum corneum, which consists of dead cells, would otherwise dominate the contribution to total electrical impedance. The probe lay on the skin with its own weight and a holder ensured stable positioning. Registration of the electrical impedance was performed at four sites, which enabled total observation of the skin reaction. C1 was the capsaicin-exposed site, as described above; C2, 2 cm beside the exposed site; E1, the control-exposed site and E2, 2 cm beside the control site. Measurements were performed every 10 min during the first hour and then every 30 min for a total of 4.5 h.

Statistics

The results were statistically analysed by Student's *t*-test for paired observations or by analysis of variance (ANOVA) with repeated measurements. Statistical comparisons were performed between the exposed site vs. the nominal value, i.e. the value before exposure at $t = 0$ min. Values are reported as means ± SEM.

RESULTS

Visual assessment and sensations

Topical application of capsaicin induced a flare response and local warming. The maximal flare area was 1,250–2,700 mm^2 with a mean of 1,830 mm^2 (Fig. 1). Visually detectable effects disappeared after 3 h. No effect was registered at the control site. The perceived modality of the chemical stimulus during the 30-min application started with sting/prick at 3.1 ± 0.6 min. This was followed by burn at 5.3 ± 0.6 min. The last sensation to occur was pain, which started at 23.3 ± 1.6 min. The perceived sensations ended at 14.6 ± 1.0, 30.3 ± 0.5 and 34.0 ± 1.3 min, respectively. The data show that the sensations started for the majority of the subjects with sting/prick, followed by burn and pain. Itch was absent during the experiment. No abnormal sensations were elicited at the control side.

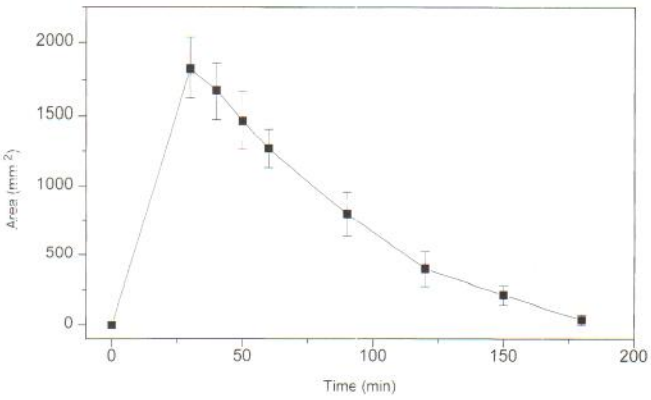

Fig. 1. The flare area after topical application of capsaicin as a function of time ($n = 7$). Values are means ± SEM.

Blood flow

Blood flow responses are shown in Fig. 2. Capsaicin elicited a statistically significant increase in skin blood flow (ANOVA, $p < 0.0001$). Ethanol induced a much smaller significant increase in flow (ANOVA, $p < 0.01$). As depicted in Fig. 2, capsaicin-induced hyperaemia was maximal at 50 min (98.8 ± 12.2 PU) and at 40 min at the ethanol-treated site (35.6 ± 12.6 PU). Statistically significant differences in effect between the capsaicin and ethanol sites were observed at 30 ($p < 0.001$), 40 ($p < 0.005$), 50 ($p < 0,005$), 60 ($p < 0.005$) and 90 min ($p < 0.05$) post-exposure, as evaluated by the paired Student's *t*-test. The first heat provocation (40°C) increased the cutaneous blood flow by 59.8 ± 10.3 PU ($p < 0.01$, paired

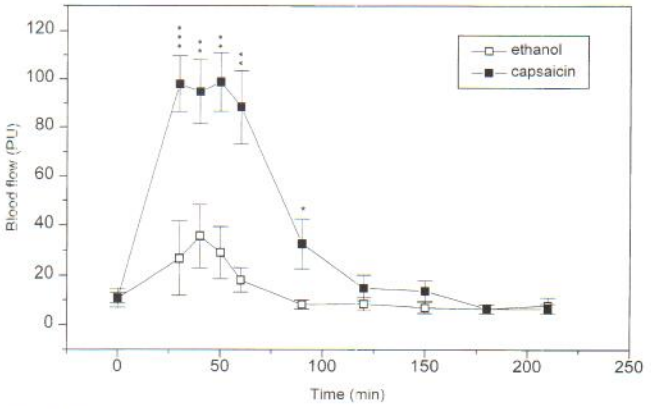

Fig. 2. Skin blood flow recorded before and after topical application of capsaicin and control (ethanol) solution as a function of time ($n = 7$). Values are means ± SEM. $*p < 0.05$, $**p < 0.005$, $***p < 0.001$ capsaicin vs. control, paired Student's *t*-test.

Student's *t*-test) at the control site and by 60.8±14.1 PU ($p<0.01$, paired Student's *t*-test) at the site later treated by capsaicin. Capsaicin markedly reduced the effect of heat provocation at 30 min after exposure. The increase was only 8.5±2.6 PU. At the control site the increase was 56.6±11.6 PU and significantly different, compared to capsaicin treatment ($p<0.01$, paired Student's *t*-test). The response to the heat stimuli after 210 min was equal for both sides, resulting in a nine-fold increase in flow.

Electrical impedance

Irritation indexes are reported for depth five, corresponding to a depth of approximately 2.0 mm (10). This depth was used due to the fact that the effect of capsaicin on the vasculature is elicited in this area. The baseline values on the C1 site were 11.5±0.5, -29.1±1.3, 4.5±0.2 and 15.6±0.3 for MIX, PIX, RIX and IMIX, respectively. There were no statistically significant differences in the baseline indexes as compared to the C2, E1 and E2 sites. The capsaicin treatment did not evoke any statistically significant changes in the irritation indexes over time. Skin blood flow and flare area were maximal at 30 min after capsaicin treatment. At this time the irritation indexes at C1 were 11.9±0.5, −26.4±1.3, 4.9±0.2 and 15.6±0.5 for MIX, PIX, RIX and IMIX. Nor were any statistically significant changes elicited at the C2, E1 nor E2 sites. We also evaluated the results at the other depths but no consistent results were observed.

DISCUSSION

Capsaicin has been used extensively as a probe for sensory nerve stimulation and neuropeptide release in vivo and in vitro and is also a useful tool in dermatological research (3). The effects of capsaicin on cutaneous sensation are known to differ depending on dose and duration of application. Initially an excitatory phase is elicited, during which capsaicin produces flare, burning pain and cutaneous vasodilatation (2). The present results provide direct evidence that topical application of capsaicin gives a local flare with strong sensations and increased blood flow. Flare has been shown to be an axon reflex phenomenon. The production of flare involves (i) the excitation of afferent nerve terminals by the stimulus, (ii) the spread of excitation to nerve terminals over a distance of several cm, (iii) the release of vasodilator agent(s) from these terminals, and (iv) the dilatation of arterioles (12).

Individual variations in the sensitivity to stimuli occurred. It was obvious that the first perception was sting/prick, followed by burn and pain. Burn was most frequently used to describe the effect elicited by capsaicin, with sting and prick being approximately as common as pain. It has been reported that capsaicin elicits a sensation of itch in humans (2,5). None of the subjects in our study had a sensation of itch after capsaicin treatment. In previous studies rather large areas have been exposed to capsaicin by using an arrangement of gauze pads, filter paper disks or single drops of the solution on the skin (2,5). We used a more exact application method. The plastic chamber and a filter paper disk fix the solutions in a distinct place and prevent evaporation. Thus, the previously reported succession of symptoms is not a fixed phenomenon.

The acute application of capsaicin caused marked vasodilatation and is thus in good agreement with previously reported results (12). This effect has been suggested to be due to the release of potent vasodilatory neuropeptides such as SP and calcitonin gene-related peptide (CGRP) from primary afferent C-fibres (13). To our knowledge no experimental data are available on the effect of capsaicin during heat provocation with simultaneous registration of blood flow. The effect of the second provocation was almost abolished by the capsaicin treatment, while it was unaffected on the contralateral side. This effect of capsaicin is probably due to a near maximal vasodilatation, already produced by the application of capsaicin. During the heat provocations all subjects reported hyperalgesia after the capsaicin treatment. The hyperalgesic response was very intense for about 10 s and then stabilized at a more tolerable level. This effect was not abolished during the test period.

Changes in cellular ultrastructure can occur in the skin without creating visible signs on the skin surface (14). In this respect, minor cutaneous changes can be detected using quantitative methods, such as the bioengineering tools used in diagnostic dermatology. One of these is an electrical method for studying the hydration of stratum corneum (15). Electrical impedance measurement is another method that can monitor the status of the skin and would be a fruitful parameter in monitoring skin reactions. Ollmar et al. (9,10) have shown that an electrical impedance technique can register changes in the irritation index after application of weak concentrations of sodium lauryl sulfate, which gives no visually detectable effect. Capsaicin elicited strong skin reactions. There were no detectable and reliable inter-individual changes in the skin electrical impedance. This could be due to the fact that the effect elicited by capsaicin develops in the dermis where the nerve endings are located. It has been assumed, however, that the electrical impedance device registers changes to a depth of 2.0 mm (10), which should correspond to the dermis.

In summary, our results suggest that electrical impedance registration is not suitable as an objective tool for measuring the response to substances like capsaicin. There was close agreement among the effects obtained using visual assessments, sensations and laser Doppler flowmetry. Capsaicin abolished the effect of heat provocation. Interestingly, no itch was elicited by capsaicin. This is probably due to our application method.

ACKNOWLEDGEMENTS

This study was supported by the National Defence Research Establishment, Umeå, Sweden. The authors thank Per Runn, Ph.D., and Rune Berglind, Ph.D., for fruitful discussions, Per-Åke Ohlsson, Techn.lic., for data assistance and Mrs. Marlene Lundström for technical assistance. We also wish to thank Stig Ollmar, Ph.D., Center for Dental Technology and Biomaterials, Karolinska Institute, Huddinge, Sweden, for providing us with the electrical impedance measurement device.

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