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In vitro percutaneous penetration of topically applied capsaicin in relation to in vivo sensation responses

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Abstract

Capsaicin, the primary pungent element in several spices, elicits a variety of physiological effects which are due to neurogenic responses. The aim of the study was to explore the in vivo sensation responses of capsaicin and to compare the results with the in vitro percutaneous absorption of the substance. The overall objectives were to determining an in vitro–in vivo correlation for capsaicin. Capsaicin was applied in a chamber on the volar forearm of twelve volunteers and in a flow-through diffusion chamber on excised human epidermal membranes. Topical administration of capsaicin produced a complex cutaneous sensation that changed in intensity and quality as a function of time and was characterized by sting, prick, burn and pain. Percutaneous steady-state penetrations of capsaicin with a receptor fluid consisting either of 4% bovine serum albumin in phosphate buffered saline or 50% ethanol in water were 28.2 ± 2.7 and 29.6 ± 2.9 μg/cm^2 per h, respectively. The corresponding cumulative penetrated amounts of capsaicin after 30 min were 14.7 ± 1.7 and 19.2 ± 2.1 μg/cm^2, respectively. The present investigation indicates that there is a good correlation between in vivo physiological responses and in vitro percutaneous penetration of topically applied capsaicin.

Keywords: Capsaicin; Transdermal delivery; Sensation responses; Diffusion chamber

1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the pungent principle constituent in hot pepper, produces marked alterations in the function of unmyelinated sensory afferent fibers, which are believed to signal pain and to initiate inflammatory responses (Lynn, 1992). The substance first excites and then desensitizes these nerves to subsequent stimulation both by itself and by a variety of physicochemical stimuli (Fuller, 1990). Initial application of capsaicin to skin produces irritation and hyperesthesia. This reaction is thought to be due to capsaicin-induced release of substance P from peripheral sensory C fibers. After the initial

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exposure, capsaicin produces a long-lasting desensitization to burn and pain (Bernstein, 1988).

Topical administration of capsaicin to human skin induces a neurogenic response characterized by erythema at the site of application accompanied by sensations such as sting, prick, burn, and pain (Magnusson and Koskinen, 1996). While capsaicin cream appears to be a promising treatment for a variety of dermatological conditions including psoriasis, pruritus, postmastectomy pain syndrome, apocrine chromhidrosis, and contact allergy, its major use has been in the treatment of superficial sensory disorders, especially postherpetic neuralgia. In postherpetic neuralgia, capsaicin appears to be equally effective in relieving painful as well as pruritic dysethesias, and has proved to be safer and more effective than previously employed systemic remedies (Carter, 1991; Rumsfield and West, 1991).

The principal resistance to penetration of drugs through human skin resides within the outermost layer of the human skin, the stratum corneum. Its structure has been depicted in the brick and mortar model by Elias (1981), in which anucleate keratinised cells are embedded in a lipid mortar. This densely packed cellular and lipid apparatus forms an effective barrier to transepidermal water loss and to external chemical access. In an earlier in vivo study in humans, we found that topically applied capsaicin caused sensory and vascular effects after a few minutes (Magnusson and Koskinen, 1996). The results showed a close correlation between visual assessments, sensation registration, and laser Doppler flowmetry, when used for monitoring capsaicin effects.

The purpose of the present study was to investigate the percutaneous penetration of capsaicin by using human epidermis in a flow-through diffusion chamber, and to correlate the in vitro results with the results of sensation registration after topical application of capsaicin in human volunteers. The aim was to quantitatively predict the penetration of topically applied substance, in vivo, and to gain insight into the absorption of the compound through the skin.

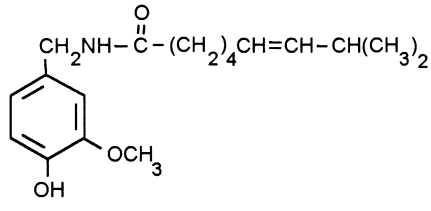

Fig. 1. Structural formula of capsaicin (8-methyl-*N*-vanillyl-6-nonenamide).

2. Materials and methods

2.1. In vivo sensation responses of capsaicin

The permeant was capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), supplied by Sigma Chemical Company (lot. no. 26H7812, MO, USA). The chemical structure of the substance is illustrated in Fig. 1. Fifty microlitres of a solution consisting of 1% capsaicin in 50% ethanol was applied in a plastic chamber on the ventral aspect of the forearm of twelve Caucasian volunteers (six females and six males, ages 22–52 years), exactly 35 cm from the 3rd digit tip. The plastic chamber with a filter paper disk (0.50 cm^2) prevents evaporation of the substance and the chamber was attached to the skin with hypoallergenic tape for 30 min. As a reference, the corresponding skin area of the contralateral arm was used. Fifty microlitres control solution of 50% ethanol in water was applied. The optimal volume of the applied solutions was established in pilot testing as the amount of solution adequate to saturate the filter paper without producing run-off onto adjacent skin. The solutions were equilibrated at 32°C for 24 h prior the experiments. All subjects had normal healthy-appearing skin. Instructions were given to avoid use of moisturizing creams or lotions for 24 h prior to testing. Subjects were blinded as to treatment and control sides. All experiments were performed identically on both capsaicin-treated and reference sides. 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.

During the period of application, the subject was asked to report cutaneous sensations at one minute intervals for a total of 35 min. The subject was required to (1) state on which arm the sensation was felt, (2) describe its quality or qualities, and (3) state the quality with the most intensive sensation at the specific time. Sensation qualities were based on four descriptors: itch, sting/prick, burn and pain as described previously (Green and Flammer, 1988; Magnusson and Koskinen, 1996). The descriptors were considered self-explanatory, but the subjects were given written definitions. The subject was encouraged to use as few or as many of the terms as necessary to describe the sensations.

2.2. In vitro percutaneous penetration of capsaicin

In vitro diffusion studies were performed on a automated system using miniature diffusion cells with flow-through receptor compartments. The design has been previously described by Magnusson et al. (1997a,b). Caucasian breast skin was obtained from plastic surgery. In addition, skin from the forearm of one individual was obtained. Epidermal membranes were prepared by the heat separation technique of Kligman and Christophers (1963). Specimens rich in appendages ($\geq$ six appendages per piece) were discarded to reduce the risk of damage to the membrane during preparation, and to avoid excessive transappendageal penetration. The epidermal membrane samples were hydrated for 18 h in phosphate-buffered saline (PBS, pH 7.4) at 4°C before mounting in the diffusion chamber. The cell has a nominal diffusion area of 0.50 cm^2. All parts in contact with the epidermal membrane, donor fluid, and perfusion fluid were made of stainless steel, teflon, or polyethene. The donor compartments were covered by a teflon plug to prevent evaporation. The receptor compartments were maintained at 32.0 ± 0.1°C and placed directly above a Fractomin® autosampler.

Prior to all experiments, the integrity of the barrier layer was verified for each epidermal membrane by the determination of water permeability. This was done as a control procedure to identify defect epidermal membranes. One hundred microlitres of a [^{3}H]-H_2O solution (9.25 MBq/ml) was applied to the donor compartments. Sink conditions were maintained by pumping through a degassed PBS with a flow of 4.0 ml/h. Samples were collected in 15 min intervals for 1.5 h. The amount of radio-labelled penetrant was determined by liquid scintillation counting (Beckman LS 5000 CE scintillation counter). The [^{3}H]H_2O solution was washed from the membrane with PBS solution for a minimum of 3 h, while continuously replacing the donor solution. This step reduces the residual [^{3}H] activity to background levels. Membranes with a water permeability of < 5.0 μl/cm^2 per h were accepted for further experiments (Magnusson et al., 1997a). The epidermal membranes in the present study were prepared from five different subjects. Penetration of capsaicin was determined using a donor solution containing 1% of capsaicin in 50% ethanol/water. The solutions were equilibrated at 32°C for 24 h prior the experiments. Since capsaicin is freely soluble in ethanol but practically insoluble in cold water, either bovine serum albumin (BSA), 4% in PBS, or 50% ethanol in water were used as receptor fluid. At the start of the study, 50 μl of the donor solution was applied to the donor compartment. In order to compensate for changes in capsaicin concentration 25 μl of donor solution was exchanged every 30 min. Perfusion fluid, with a flow of 1ml/min, was sampled with 1-min or 15-min intervals over a 3 h period.

2.3. HPLC analysis of capsaicin

The receptor fluid concentration of capsaicin was determined by HPLC-analysis. The chromatographic system consisted of a refrigerated microsampler (CMA 200), a HPLC pump (Waters 510), a sample injector (CMA 240) and a UV detector (BAS) operated at 280 nm. The samples were analysed on a Nucleosil C18 reverse phase analytical HPLC column (5 μm particle size, 20 cm length). Twenty microlitres was injected, and a mixture of methanol and water (3:2) constituted the mobile phase at a flow rate of 0.6 ml/min. Quantification was made by comparing peak areas of samples to external standards. The linear range of the calibration curve was 0.1–100 μg/ml

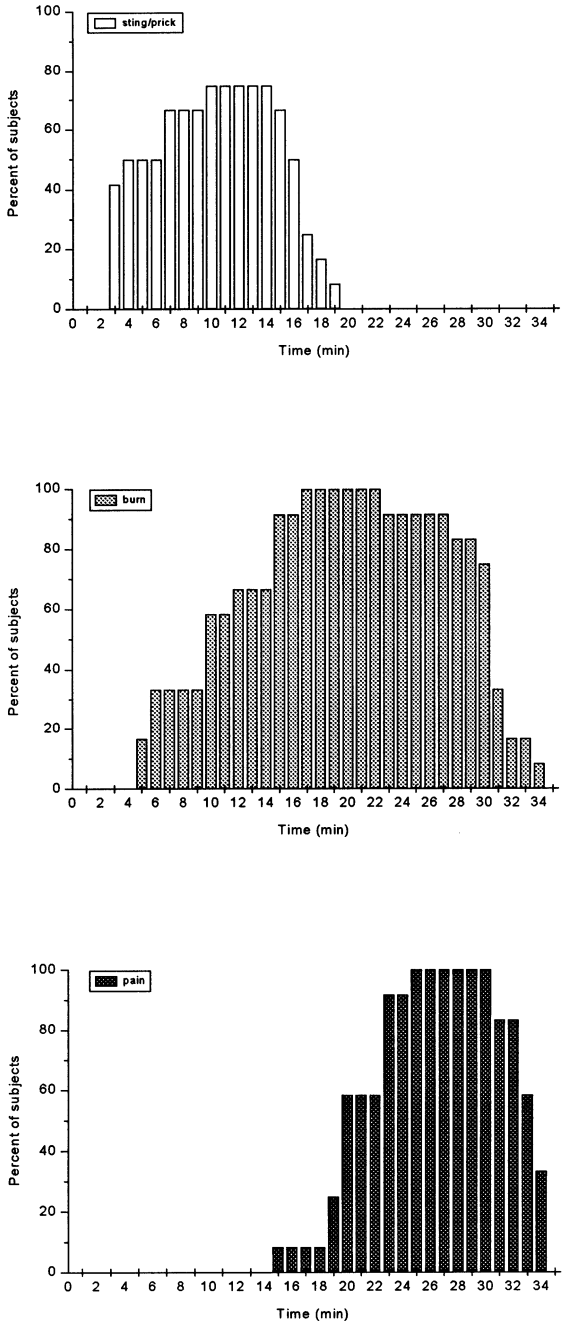

Fig. 2. Reported sensations after topically applied capsaicin. Shown as a function of time (1-min intervals) is the percentage of subjects in which particular sensation qualities as shown were reported ($n = 12$). One hundred percent corresponds to reports of a given sensation from all the subjects at the time indicated.

and the detection limit was 20 ng/ml, based on a signal to noise ratio of 3. In order to remove BSA a simple sample preparation procedure was developed. Acetonitrile, 0.8 ml, was added to 1.0 ml of sample. The denaturated protein was removed by centrifugation at 1500 rpm ($500 \times g$) during 30 min. The procedure was evaluated by analyses of samples with standard additions of capsaicin. The recovery was estimated at $80.9 \pm 2.3\%$ and this was corrected for in the final calculation. Samples without BSA were injected directly into the analytical system.

2.4. Statistics

The results were statistically analysed by Student's t-test (two-tailed) for unpaired observations. A difference was considered statistically significant if $P \leq 0.05$. Values are reported as mean $\pm$ SEM.

3. Results

Topical application of capsaicin to human volunteers produces a complex cutaneous sensation that changes in intensity and quality over time. Fig. 2 shows the percentages of subjects reporting a particular sensation at different time points. The data shows that the earliest sensation reported by the majority of the subjects was sting/prick. Then followed burn, and even later a sensation of pain was reported. The sensation of sting/prick occurred in 42% of the subjects after 3 min. Sting/prick showed maximal frequency of, 75% of subjects, after 10–14 min; and ended after 19 min. Burn was the most frequent sensation during the application period; it started at 5 min for 17% of subjects, and during the period 17–22 min it was reported by all individuals tested. Nearly 90% of the subjects reported burn from 15–27 min. The burn sensation ended after 34 min. The latest sensation to occur was pain, which started at 15 min for 8% of the subjects. The maximum frequency of pain was reported at 25–30 min. The sensation of pain lasted for a maximum of 34 min, at which time point the frequency was 30%. Itch did not occur during the experiment for any

of the twelve volunteers. No abnormal sensations were reported for the control side.

The barrier properties were assessed by measurement of water permeability using $[^3H]H_2O$. The water penetration of the only available sample of forearm skin was $2.8 \pm 0.1 \times 10^{-3}$ ($n = 8$) cm/h, i.e. a value very close to those obtained for breast skin (Magnusson et al., 1997a). The penetration of capsaicin as a function of time for four different subjects is illustrated in Fig. 3. The mean penetration of capsaicin was 28.2 ± 2.7 μg/cm^2 per h at steady-state with 4% BSA in PBS as receptor fluid (Fig. 3(a)). With 50% ethanol in water as receptor fluid the corresponding value was 29.6 ± 2.9 μg/cm^2 per h (Fig. 3(b)). The capsaicin flux during the first minutes was higher with

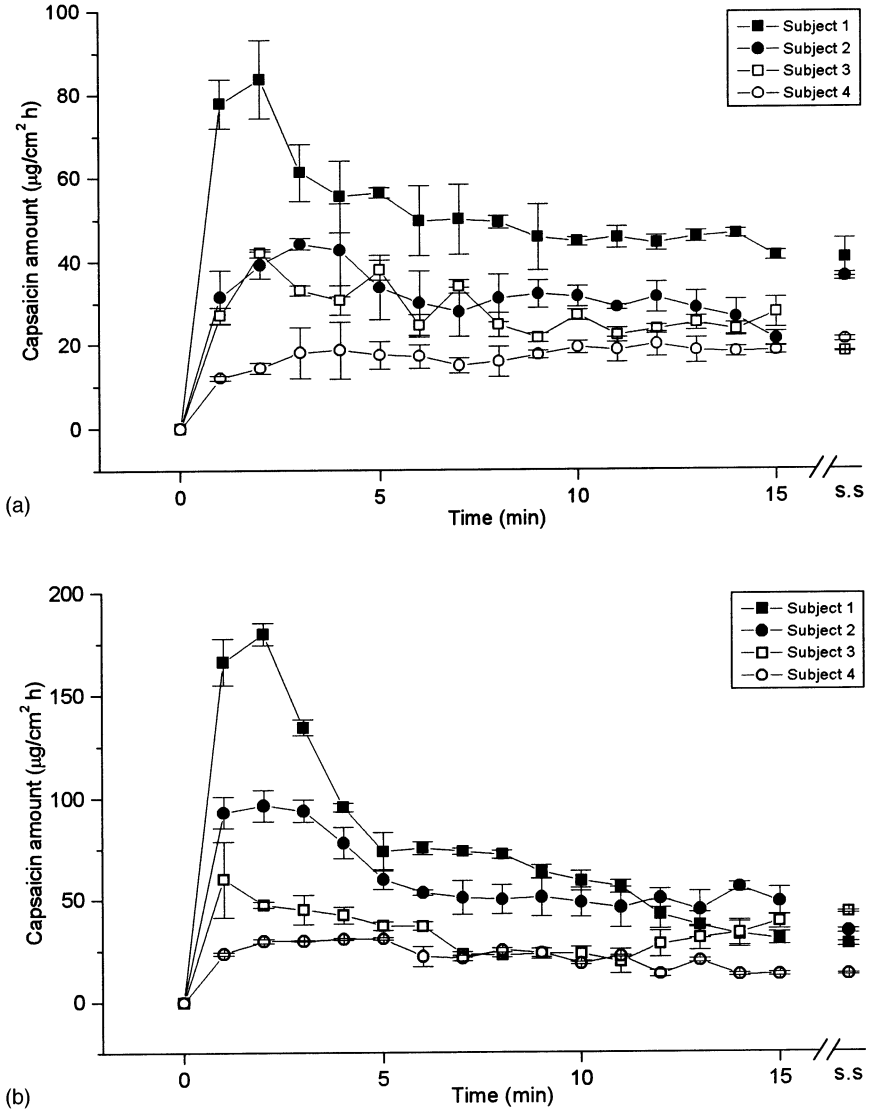

Fig. 3. Penetration of capsaicin in vitro. Experiments were carried out with human epidermal membranes prepared from four different individuals. (a) Receptor fluid = 4% BSA in PBS. (b) Receptor fluid = 50% ethanol in water. Given values are means ± SEM for four epidermal membranes.

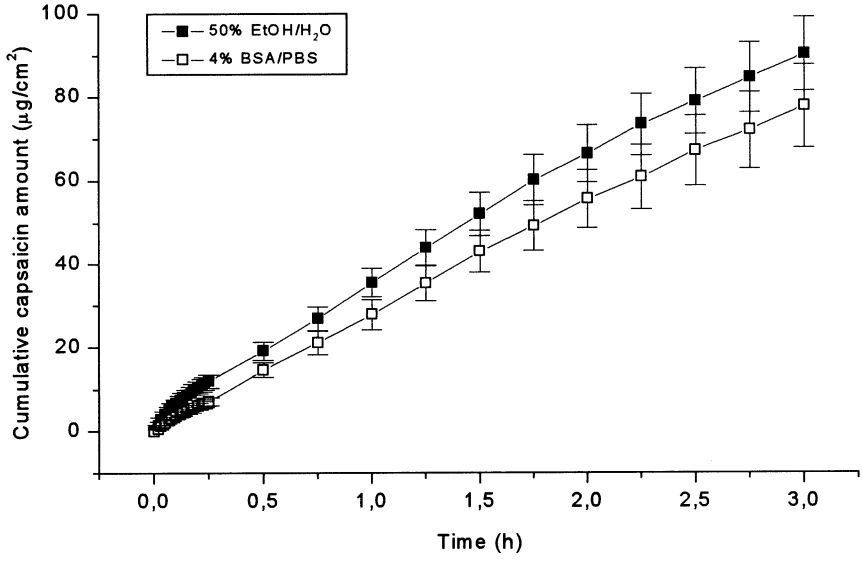

Fig. 4. Cumulative plot of capsaicin penetration through human epidermal membranes in vitro as a function of time. Filled squares represent the mean values for flux measured with a receptor fluid of 50% ethanol in water. Open squares represent the mean values with a receptor fluid containing 4% BSA in PBS. Values are mean ± SEM for four individuals; four experiments/individual.

50% ethanol than with 4% BSA as receptor fluid. No significant differences were seen during steady-state conditions. In the presence of PBS alone as receptor fluid, the penetration of capsaicin was below the detection limit of 2.4 μg/cm^2 per h. The total amount of penetrated capsaicin as a function of time is illustrated for the four subjects in the cumulative plots in Fig. 4. The average amount of penetrated capsaicin with 4% BSA as receptor fluid of was 77.8 ± 10.0 μg/cm^2 after 3 h. The corresponding value after 30 min was 14.7 ± 1.7 μg/cm^2. The presence of 50% ethanol in the receptor fluid slightly increased the amount of penetrated capsaicin to 90.3 ± 8.8 μg/cm^2 after 3 h. The corresponding value after 30 min was 19.2 ± 2.1 μg/cm^2. The differences in cumulative amounts of penetrated capsaicin between experiments with the two types of receptor fluids used were not statistically significant.

4. Discussion

Capsaicin is used as therapy for temporary relief of neuralgia, and has been studied because of its properties as a stimulant of sensory nerves and neuropeptide release in vivo and in vitro. The in vivo effects of capsaicin on cutaneous sensation are known to differ depending on dose and duration of application (Green and Flammer, 1988). An important finding of the present experiment is that, on intact skin, capsaicin produced a relatively complex cutaneous sensation that changes in intensity and quality as a function of time. In an early stage of development the sensation was characterized mainly by the qualities of stinging and pricking. Somewhat later a burning quality appeared and became a dominating sensation, first in combination with sting/prick, and later along with pain. Pain occurred latest among the sensations reported during the application period. These time-dependent differences in types of sensation reported may have been due to the recruitment of different types of afferents (Guyton and Hall, 1996). Another explanation may be that increased stimulation of the same afferents at higher concentrations of capsaicin gives rise to a change in the perceived sensation. Individual variations in the sensitivity to capsaicin occurred. Most subjects reported more than one of the four

sensory qualities during the experiments. Interestingly, the order in which the sensations were reported was the same for all subjects. The subjects were encouraged to state the quality with the most intensive sensation at the specific time. In this respect the sensation of burn dominated the early time period, and the sensation of pain the later part of the application period.

Blood flow responses measured by laser Doppler flow technique after topical application of capsaicin have been reported earlier by Magnusson and Koskinen (1996). Capsaicin-induced hyperaemia reached its highest level after 30 min with an 90% increase in blood flow, and decreased after 60 min. In the present study, the maximum frequency of pain was reported at 25–30 min after application. Thus, there seemed to be a correlation between maximum observed increase in blood flow and the induction of pain by capsaicin.

The pharmacological effects of capsaicin are well documented (Fuller, 1990), but, to our knowledge, no experimental data are available on the percutaneous absorption of capsaicin through intact human skin. We used an in vitro system to quantitatively predict the penetration of topically applied substance in vivo. The flow-through diffusion system chosen for the studies presented here is relevant to the situation where a substance is transdermally administrated to elicit a biological effect. The system is designed to predict the penetration of a test substance through the stratum corneum barrier. The validity of each membrane was controlled prior to the onset of the experiment by measurement of water permeability, and the values obtained were in agreement with previously reported data (Magnusson et al., 1997a,b). Since capsaicin is practically insoluble in water, addition of bovine serum albumin or ethanol to the receptor fluid was essential. The receptor fluid containing 4% BSA is closer to physiological conditions than 50% ethanol as receptor fluid. The use of ethanol–water (1:1) in the receptor compartment may overpredict the absorption of capsaicin, giving a faster increase in penetration during the first minutes after application.

The in vitro study showed that the hydrophobic capsaicin rapidly penetrated the epidermal membrane. The penetration reached steady-state levels after 3–5 min. The earliest sensory effect after topical application of capsaicin was reported after 3 min. If it is assumed that the penetration of capsaicin in vitro is similar to the amount of absorbed capsaicin needed to elicit sting/prick, burn, and pain, the corresponding amounts would be approximately 6, 10 and 14 $\mu g/cm^2$, respectively. The neurophysiological mechanisms of the apparently concentration-dependent differences as regards sensory responses to capsaicin remain to be elucidated.

It has been reported by many investigators that capsaicin elicits a sensation of itch in humans (Green and Flammer, 1988). Therefore the absence in our results of reported itch after topical application of capsaicin was surprising. None of the twelve subjects in this study and none of seven in our previous study reported any pruritic feeling (Magnusson and Koskinen, 1996). One explanation may be the high concentration of capsaicin (1%) applied. This may have produced early sensations of burn and pain of sufficient intensity to mask itching. Since pain blocks itch (Shelley and Arthur, 1957; Keele and Armstrong, 1964; Tuckett, 1982), the earlier capsaicin provokes pain, the less is the chance for itch to appear. Our results showed a sensation of stinging and pricking as soon as three minutes after application. This may have blocked other types of sensation qualities. Keele and Armstrong (1964) reviewed the convincing evidence that painful stimulation could either relieve ongoing itch or prevent its development following application of a pruritic substance. This masking or inhibition might be expected to be greatest when capsaicin is delivered rapidly and in high concentrations.

In conclusion, we have developed methods which allowed us to study the penetration of capsaicin through human skin in vitro, and to correlate in vitro findings to findings in human in vivo experiments with topically applied capsaicin. Our results may give a basis for further studies on the neurophysiological effects of capsaicin.

Acknowledgements

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