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VASCULAR AND SENSORY RESPONSES OF HUMAN SKIN TO MILD INJURY AFTER TOPICAL TREATMENT WITH CAPSAICIN

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1 Immediately after several topical applications of capsaicin at 2-hourly intervals, human forearm skin would no longer develop flare (vasodilatation) around a small injury. At the same time heat pain thresholds were reduced on average by 3.5°C. These results are consistent with block by capsaicin of the effector side of the axon reflex, perhaps by depleting nerve terminals of substance P.

2 Over a period from several days to several weeks after treatment, flare was diminished and heat pain thresholds were slightly elevated. These changes may be due to long-lasting damage of cutaneous nerve terminals by capsaicin.

Introduction

Substance P produces widespread vasodilatation (flare) on injection into human skin (Hagermark, Hökfelt & Pernow, 1978) and there is evidence for its release from the central terminals of nociceptive afferent fibres (Nicoll, Schenker & Leeman, 1980). These findings are consistent with the proposal that flare around regions of skin injury may involve the release of substance P from nerve terminals (Burnstock, 1977; Henry, 1977; Hagermark, *et al.*, 1978).

Treatment of rats with capsaicin reduces levels of substance P in skin (Gamse, Holzer & Lembeck, 1980; Hayes & Tyers, 1980) and the ability of certain inflammatory agents and of antidromic nerve stimulation to increase vascular permeability (Jancso, 1960; Arvier, Chahl & Ladd, 1977; Gamse *et al.*, 1980). In view of this, we wondered whether the axon reflex flare in human skin would be diminished by pretreatment with capsaicin. We have used topical application of capsaicin, which Jancso (1960) found produced a local desensitization of skin to 'neurogenic' irritants (e.g. xylene, mustard oil), and have examined both flare responses and pain thresholds in treated areas.

A preliminary account of some of our results has already been published (Carpenter & Lynn, 1981).

Methods

Subjects were the authors and 6 colleagues from the departments of Physiology and Pharmacology who gave their informed consent. Areas on the flexor apsect of the forearm 6 to 28 cm^2 in extent were lightly abraded and painted with a solution of capsaicin (1% in 85% ethanol). After approximately 10 min the capsaicin was washed off. On the first occasion, considerable burning and stinging sensations were evoked and a widespread flare was produced. Painting was repeated 2–6 times at approximately 2-hourly intervals on the first day and further applications were made on subsequent days until no local reaction occurred. On average a total of 7 applications were made. Control areas were treated with 85% ethanol only.

To test flare reactions in treated skin it was heated by contact with a thermode consisting of a copper disc (diameter 7 mm) that was heated under feedback control from a thermocouple on the disc. The thermode rested on the skin with a pressure of 5 KPa and its temperature was raised at 1°C/s to 55°C at which point it was lifted off the skin. This level of heating produced a good flare but no irreversible skin damage. In one subject flare was produced by crushing small skin folds. Flare areas were calculated from measurements of the diameter of the response in the longitudinal and the transverse directions.

Heat pain thresholds were also determined with the contact thermode. The rate of heating during threshold determinations was 0.5, 0.75 or 1.0°C/s, the rate being varied randomly from trial to trial to reduce time cues. Sensitivity to mechanical 'pricking' pain was determined using von Frey bristles with forces ranging from 3 to 140 mN and diameters of 0.23 to 0.42 mm.

Intradermal injections of synthetic substance P were made in three subjects (the authors and Dr John Foreman). Substance P was diluted with sterile saline (0.9% w/v NaCl solution) and volumes of 0.02–0.03 ml were injected with 0.4 or 0.5 mm diameter needles.

Drugs used were: capsaicin (or 8-methyl-*N*-

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vanillyl-6-nonenamide) (gift from Dr A. Jancso-Gabor); synthetic substance P (Beckman).

Results

Flare reactions

In skin that had been untreated or had only received applications of 85% ethanol, heating the thermode to 55°C produced a pale, well-defined flare within 30 s. The reaction was maximum in area and in depth of colour 30 s to 2 min after heating and then slowly shrank and faded over 5 to 60 min. In 6 subjects, average flare diameters 1 to 2 min after heating ranged from 2.4 to 5.0 cm. Responses to crushing a skin fold were similar in the one subject tested.

Heating or crushing in the centre of areas of skin pretreated topically with capsaicin caused no flare in 4 subjects and a small, faint flare of brief duration (<25% of control values) in 2 subjects when carried out 0.4–10 h after the last treatment. Recovery was gradual. One to ten days after treatment the flare responses were still reduced in duration (<40% of control values) and were paler than in control skin, although the average maximum area was close to normal (82% ± 3%, s.e. mean, n=4). After 14 to 20 days the intensity and extent of flares were in the normal range but the flares were still a little shorter in duration than normal. A typical set of curves for flare area during recovery from capsaicin treatment is shown in Figure 1, together with control data from untreated skin.

The small disc of skin that was directly heated always became red after a delay of a few minutes and stayed red for an hour or more. These local responses occurred in both treated and untreated skin and were of similar intensity and duration in both.

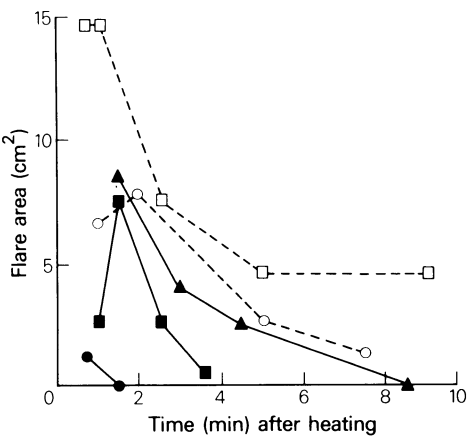

Figure 1 Area of flare response at different times after heating a 0.4 cm² patch of skin at 55°C in a typical subject. Dashed lines, open symbols (○, □): responses after 2 heat stimuli in control skin areas treated with 85% alcohol. This degree of variability in baseline responses was not unusual. Continuous lines, solid symbols: responses in skin treated with 7 applications of 1% capsaicin over 24 h. Time from last capsaicin application: (●) 2.3 h; (■) 125 h; (▲) 312 h.

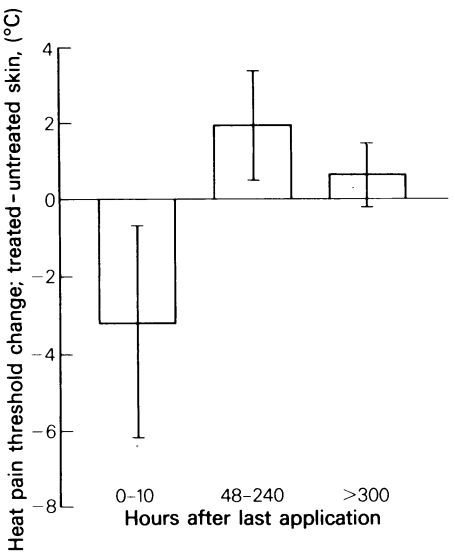

Figure 2 Heat pain threshold in skin treated topically with capsaicin relative to heat pain threshold in untreated skin at various times after the last treatment. Values are averages from 7 (0–10 h), 5 (48–240 h) or 4 (> 300 h) subjects. Error bars are 95% confidence limits calculated using the t-distribution.

Pain thresholds

Over the period from 0.4–10 h after the last treatment heat pain thresholds were reduced significantly in treated zones despite the fact that these areas were insensitive to capsaicin. The average fall in threshold was 3.5°C(± 1.1°C, s.e. mean, n=7) from 46.3 to 42.9°C (Figure 2). Thresholds for pricking pain were also slightly reduced at this time in capsaicin-desensitized skin in the two subjects examined. Two to ten days after stopping capsaicin treatment, average heat pain thresholds became significantly higher than normal by 1.9°C in the test areas; sensitivity gradually returned to normal over the next few weeks (see Figure 2).

Responses to intradermal injections of substance P

In control skin, injection of small volumes of 10–20 μM synthetic substance P into the skin produced a long-lasting flare, a localised oedema and, in one

subject, itch, as previously described by Hagermark *et al.* (1978). In skin areas previously treated with capsaicin, the oedema produced was similar to that in untreated skin. However, flare responses were reduced or absent, although a small local vasodilatation was present in an annulus 1–2 mm across at the edge of the bleb. In the one subject whose skin became itchy in response to synthetic substance P, no itch occurred in treated skin.

Discussion

Flare was shown by Lewis (1927) to be an axon reflex phenomenon and probably involves unmyelinated nociceptive afferent terminals (Celander & Folkow, 1953). The production of flare involves (a) the excitation of afferent nerve terminals by the stimulus, (b) the spread of excitation to nerve terminals over a distance of several cm, (c) the release of vasodilator agent(s) from these terminals and (d) the dilatation of arterioles. For the first 10 h after ending treatment with capsaicin, pain thresholds are reduced. After a single topical application of capsaicin a similar reduction in heat pain thresholds has been reported (Szolcsanyi, 1977). Capsaicin can directly excite nociceptors (Szolcsanyi, 1977) and the hyperalgesia following desensitization may be due to a slight persistence of this effect. From this it appears that capsaicin enhances the sensitivity of afferent nerve terminals to heat, and possibly also mechanical stimuli; it certainly does not appear to block their excitation by these stimuli. The main immediate effect of capsaicin appears, however, to be a block on the efferent side of the axon reflex. Since blood vessels in the treated area still dilate to direct heat and to injected synthetic substance P, it appears that the block is in the release of dilator agent. An obvious possibility is that topical capsaicin depletes the skin nerve terminals of substance P, as do parenteral injections (Gamse *et al.*, 1980; Hayes & Tyers, 1980), and that substance P is the agent that produces axon reflex flare, as previously suggested (Burnstock, 1977; Henry, 1977; Hagermark *et al.*, 1978). However, it should be noted that although capsaicin probably does not act by depleting mast cells (Arvier *et al.*, 1977) its effects on many vasodilator substances in the skin has yet to be examined and it has recently been shown to deplete a dilator peptide that is not substance P from the spinal cord (Chahl & Manley, 1980).

The pattern of recovery from capsaicin treatment is complex and it takes several weeks for flare responses and thresholds to become normal. During recovery, flare responses were almost normal in area, but were paler than usual and of brief duration. This pattern of recovery is consistent with a gradual reaccumulation of dilator agent in the nerve terminals. However, heat pain thresholds were slightly elevated during much of the recovery period. In fact there was a switch from a hyperalgesic to a hypoalgesic state during the 12 to 48 h following the ending of treatment. One interpretation of these observations would be that, as well as initially depleting the skin of substance P, over a longer time scale capsaicin reduces the excitability of cutaneous nociceptors, thus causing hypoalgesia and contributing to the decreased flare. Such a toxic action of capsaicin would be consistent with the effects of topical applications to the cornea which produce changes in the fine structure of sensory neurones (Szolcsanyi, Jancso-Gabor & Joo, 1975) and of parenteral injections which cause destruction of sensory neurones in neonatal animals (e.g. see Jancso, Kiraly & Jancso-Gabor, 1980a). The site of action may be on axons in the skin rather than on receptor terminals since it has been found that capsaicin applied directly to peripheral nerves can cause heat analgesia and block neurogenic inflammatory responses (Jancso, Kiraly & Jancso-Gabor, 1980b).

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