The actions of capsaicin applied topically to the skin of the rat on C-fibre afferents, antidromic vasodilatation and substance P levels

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1 Single applications of solutions of capsaicin were made to the intact skin of anaesthetized rats and the effects on cutaneous blood flow and the firing of C-nociceptor afferents determined. Blood flow was measured by laser-Doppler flowmetry. C-fibre activity was recorded from filaments dissected from the saphenous nerve.

2 Following the application of a capsaicin solution (concentration ≥ 1 mM) to rat saphenous skin, low frequency firing occurred in C-polymodal nociceptors that sometimes continued for >10 min. At the same time, large increases in skin blood flow occurred exceeding 300% in some instances.

3 After the initial excitation, some C-polymodal nociceptors lost their sensitivity to pressure whilst their sensitivity to heat was lost or enhanced depending on the vehicle used.

4 Sensitivity of C-polymodal nociceptors to heat recovered in <1 day following a single application of 33 mM capsaicin. Thresholds to mechanical pressure, however, were still significantly elevated by 123% on day 1, but had recovered on day 2.

5 Vasodilatation in response to saphenous nerve stimulation ('antidromic vasodilatation') was significantly reduced by 35%, 2 days after a single application of 33 mM capsaicin, but was normal at 4 days.

6 Following a single application of 33 mM capsaicin, skin substance P levels fell to only half the normal value at day 1 and remained at this level throughout the 4 day period examined.

7 It is suggested that the ability of relatively low concentrations of capsaicin to desensitize C-fibre nociceptors may underlie the analgesic action of topical capsaicin in man.

Keywords: Capsaicin; C-fibre; neurogenic inflammation; nociceptor; substance P; laser-Doppler flowmeter; skin blood flow

Introduction

Topical application of capsaicin to human skin causes, initially, vasodilatation and a hot painful sensation. On repeated treatment, these effects diminish or disappear, and there is also loss or reduction of the vasodilator flare around localized skin injuries (Bernstein *et al.*, 1981; Carpenter & Lynn, 1981). Changes also occur in sensitivity to noxious stimuli. Initially the skin is markedly hyperalgesic to heat, but over 24–48 h this changes to a slight hypo-algesia (Carpenter & Lynn, 1981). Treatment of painful skin areas in patients, for example in post-herpetic neuralgia, with topical capsaicin creams has also been claimed to produce useful analgesia (Bernstein *et al.*, 1989).

On rat, rabbit and primate skin, local application of capsaicin selectively excites C-polymodal nociceptors (C-PMNs) (Kenins, 1982; Konietzny & Hensel, 1983; Szolcsanyi, 1987; Baumann *et al.*, 1991). This *in vivo* action presumably operates via the receptor-cation channel complex defined by *in vitro* studies on cells from the dorsal root ganglion of the rat (Baccaglioni & Hogan, 1983; Wood *et al.*, 1988; Bevan & Szolcsanyi, 1990). Topical application of high concentrations of capsaicin in dimethylsulphoxide (DMSO) has also been shown to desensitize C-PMNs (Kenins, 1982). Overall the picture from experimental studies is of a dual action of capsaicin on nociceptor terminals, an initial excitation followed by a period of desensitization.

The present study set out to examine in more detail the effect of topically applied capsaicin on afferent and 'efferent'

functions of C-PMNs in the same preparation and for this purpose the skin of the anaesthetized rat was used. The dose-dependence of the initial excitation of C-PMNs and of the simultaneous increase in local blood flow was assessed and the changes in C-PMN heat and pressure thresholds were compared with the reduction in antidromic vasodilatation over a period from immediately after treatment to 4 days later. This study also examined whether treatment of the skin with capsaicin could lead to long-term loss of C-PMNs, as has been observed after treatment of nerve trunks (Pini & Lynn, 1991). Finally, we have examined the levels in the skin of substance P, a peptide that may play a role in neurogenic inflammation (Holzer, 1988), for 1–4 days following a single topical treatment with capsaicin.

Two preliminary accounts of parts of this work have appeared (Lynn *et al.*, 1991; Lynn & Cottrell, 1992a).

Methods

Experiments were carried out on albino rats (SD or Wistar) anaesthetized with urethane (i.p. 1.5–1.8 g kg⁻¹) or sodium pentobarbitone (Sagatal, May and Baker; initial i.p. injection, 50–80 mg kg⁻¹; maintained by additional 20 mg kg⁻¹ h⁻¹ given i.v. or i.a.). Results were similar with both strains. Unit data were similar for either anaesthetic, but antidromic vasodilatation was greater with pentobarbitone anaesthesia. All antidromic vasodilatation data reported here have been taken from pentobarbitone-anaesthetized preparations. Cannulae were inserted in the trachea, one common carotid artery and one jugular vein. Blood pressure was measured via the carotid cannula. Rectal temperature was measured with a thermistor and was maintained at 36–38°C by automatic

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control of a heating pad.

The saphenous nerve was exposed for recording and stimulation in the thigh and was cut proximally. A pool of mineral oil was formed around the nerve and a pair of platinum electrodes was placed under the nerve for electrical stimulation. When recordings were made, the stimulating electrodes were positioned just above the knee and the proximal cut end of the nerve was placed on a small mirrored platform. Recordings were made from fine filaments dissected from the proximal cut end of the nerve. Units were identified by maximal electrical stimulation of the nerve. Receptive fields were located by searching the skin of the anterior and medial aspects of the lower leg and foot with mechanical and thermal stimuli. C-polymodal nociceptor (C-PMN) units were identified by their slow conduction velocity ($0.5\text{--}1.5\text{ m s}^{-1}$) and ability to respond both to strong pressure and to heating to $40\text{--}65^\circ\text{C}$.

When antidromic vasodilatation was studied, stimulating electrodes were placed on the cut end of the nerve. In some experiments, compound action potentials were recorded from the nerve between the stimulating site and the skin. Stimuli were adjusted to be maximal for the C-fibre component of the compound action potential or to give a maximum blood flow response. Typically, maximal antidromic vasodilatation and compound action potentials occurred with stimuli of $1\text{--}3\text{ mA}$ (pulse width 0.5 ms).

Capsaicin (Merck or Sigma) solutions were applied to the skin with a small paint brush or a cotton wool bud. To examine the recovery from local application, preliminary treatment was given to skin in the saphenous area under general anaesthesia (sodium pentobarbitone or halothane ($2\text{--}4\%$) in 67% nitrous oxide: 33% oxygen), with the skin being washed with ethanol after 15 min . Three vehicles were used for experiments on the immediate effects on C-fibre units. These were (1) ethanol, (2) 50% DMSO + 50% ethanol, and (3) a vehicle ('vehicle 3') comprising 48% ethanol, 25% propylene glycol, 25% water and 2% methyl laurate (Carter & Francis, 1991). Only vehicle 3 was used for the blood flow experiments, both those on immediate effects and on recovery over 4 days, and for the experiments on recovery of function by C-fibres.

To examine the immediate effects of topical capsaicin, controlled heat and mechanical stimulators were mounted so that identical repeated stimuli could be applied to the receptive field of a unit. Mechanical stimuli were forces of $10\text{--}100\text{ mN}$ applied for 15 s to a fine ($0.24\text{--}0.8\text{ mm}$ diameter) probe. Heat stimuli were 1°C s^{-1} ramps starting from a hold temperature of 35°C and stopping at $45\text{--}62^\circ\text{C}$. Stimuli were generated with a focused projector bulb and were monitored by a fine thermocouple pressing on the skin. Alternate heat and mechanical stimuli were given at $2\text{--}3\text{ min}$ intervals to minimize fatigue. Once a stable baseline was achieved the test solution was applied to the receptive field with a small paintbrush. In most cases the heat and mechanical stimuli were stopped for 15 min so that any firing could be observed continuously. In two instances stimulation continued throughout the application period. No difference was observed between these two protocols. After 15 min , the skin was washed several times with alcohol and mechanical and thermal testing continued. Excitability was followed for around 1 h after a single application.

To ascertain the proportions of different classes of C-fibre after pretreatment, only filaments with a small number ($1\text{--}5$) of electrically driven C-units were examined. The saphenous skin was tested systematically with gentle mechanical stimulation, strong pressure, cooling with ice, and heating to 52°C . Units were classified into the major classes of mechanoreceptors, thermoreceptors and nociceptors found in earlier studies on the saphenous nerve (Lynn & Carpenter, 1982). Heat thresholds were measured with 1°C s^{-1} 'ramp' stimuli; pressure thresholds were determined with calibrated von Frey bristles. As described previously (Lynn & Carpenter, 1982), the distribution of pressure thresholds is positively skewed.

However, the distribution of log thresholds is approximately normal, so the statistical analysis used log pressure thresholds in units of log mN.

Skin blood flow was determined by use of 2 laser-Doppler flow meters, one with a single channel (Moor Instruments MBF2) and another with two channels (Moor Instruments MBF3D). The single channel flowmeter used optic fibre light guides of 1 mm diameter, with the collecting and transmitting fibres separated by 1.2 mm . The two channel meter used 0.2 mm diameter fibres separated by $0.4\text{--}0.65\text{ mm}$. Both meters used near infra red (approx 800 nm) laser diodes. Probes were standardized by immersing them in a 0.5% solution of $0.48\text{ }\mu\text{m}$ diameter polystyrene microspheres and the Brownian motion of the microspheres was used as a fixed velocity source. Where appropriate, flux data are expressed as '% standard' units. However, data are normally expressed as % initial baseline level since we found that, on average, effects such as antidromic vasodilatation or the peak flow following capsaicin treatment were proportional to the baseline flow. We found it necessary to avoid locations immediately over the saphenous neurovascular bundle. At these locations a large flow signal was measured that changed little when the nerve was stimulated antidromically. We concluded that the flowmeters were detecting a signal from the deep vessels that only to a small degree reflected fluctuations in local skin blood flow.

To determine their substance P content, skin samples (weight $58\text{--}102\text{ mg}$) from the area innervated by the saphenous nerve were removed at the end of experiments and were dropped into approximately 10 volumes of boiling 0.5 M acetic acid for 10 min . Extracts were stored frozen and radioimmunoassay (RIA) was performed on 0.2 ml aliquots (Lynn & Shakhaneh, 1988a). Skin from untreated or vehicle-treated rats contained $3\text{ to }10\text{ pmol g}^{-1}$ wet wt. substance P. To allow for the substantial rat-to-rat variation, data have usually been expressed as % contralateral (untreated control) levels.

Variability of measurements is given as ± 1 standard error (s.e.); t tests were used to compare 2 means, except where distributions were very non-normal or variances differed markedly when the Mann-Whitney U-test (unpaired data) or the Wilcoxon signed rank test (paired data) were used. In some cases, comparisons of >2 means was by 1-way analysis of variance and either t tests for specified trends or the Newman-Keuls test for comparing all differences between group means were used.

Results

Immediate effects of capsaicin application on C-polymodal nociceptors (C-PMNs)

Afferent units of the C-PMN class were isolated from the saphenous nerve and their properties examined during, and for up to 1 h after, topical administration of capsaicin. Painting 1% capsaicin on to the receptive fields of 29 C-PMN units caused irregular low frequency firing for many minutes (Figure 1a,b). Firing was usually greatest $1\text{--}4\text{ min}$ after application, although occasionally firing built up only slowly or, on 2 occasions, after a delay of several minutes (e.g. see Figure 1b). The degree of firing varied between the 3 vehicles used for dissolving capsaicin (Figure 1c). Capsaicin in 50% DMSO caused most firing, with an average of 79 spikes (± 30 , $\pm$ s.e., $n = 11$) recorded during the period from 0.5 to 2.5 min after application. In contrast, capsaicin in ethanol caused firing of only 24 (± 13 , $n = 10$) spikes in the same period. Capsaicin in vehicle 3 (48% ethanol, 25% propylene glycol, 25% water and 2% methyl laurate) produced intermediate levels of firing, with 36 (± 13 , $n = 8$) spikes recorded over the $0.5\text{--}2.5\text{ min}$ period after application. Capsaicin in vehicle 3 appeared to be the most effective in generating firing at times greater than 6 min after application (see

Figure 1 Responses of C-polymodal nociceptor (C-PMN) units recorded from the rat saphenous nerve after topical application of capsaicin. (a) Action potentials (upper trace) and instantaneous frequency (lower trace, on logarithmic scale) recorded in response to topical application of 33 mM capsaicin in 50% dimethyl sulphoxide (DMSO)-ethanol. (b) Action potentials from another unit in response to capsaicin dissolved in vehicle 3 (48% ethanol, 25% propylene glycol, 25% water and 2% methyl laurate), plotted as in (a). Note that the delay before firing started was unusually long in this unit. (c) Average firing of 8–11 units after topical 1% capsaicin in 3 different vehicles. Dashed line, capsaicin in ethanol; $\blacklozenge$, in vehicle 3; solid line, in 50% DMSO/ethanol. (d) Dose-dependence of firing for capsaicin in vehicle 3. Ordinate scale, total number of spikes fired from 0.5 to 5.5 min after application. Note great variability between units in amount of firing.

Figure 1c). Firing following application of vehicle alone was negligible for ethanol and for 6 out of 7 units tested with vehicle 3. However, 50% DMSO did produce significant firing in 4 out of 11 units tested, with an average (over all 11 units) of $15 (\pm 9)$ spikes fired from 0.5 to 2.5 min after application.

The dose-dependency of firing to topical capsaicin has been examined with vehicle 3 and the data are presented in Figure 1d. Above 1 mM most units fired and there was a general trend for firing to increase with increasing concentration. There were marked variations in the degree of response

from unit to unit. Thus at 0.03 mM, 1 out of 3 units fired strongly but the others fired hardly or not at all. At 33 mM, most units fired strongly, but 1 out of the 8 examined did not fire at all.

Responses to mechanical pressure were depressed or abolished following topical capsaicin (e.g. see Figure 2, upper traces). Effects were seen on most units with $\geq 100 \mu\text{M}$ topical capsaicin in vehicle 3 (Figure 3c). There was again considerable variation between units in the effectiveness of capsaicin. For example, topical treatment with 3–33 mM (in vehicle 3) greatly depressed pressure-evoked firing in 7/11 units, but in 3/11 units caused no significant change (Figure 3c). The average reduction in firing to a fixed pressure stimulus was by 77% ($\pm 9\%$, $n = 11$) and this reduction was maintained for at least 45 min (Figure 3a). Again, like the effects on spontaneous firing, actions of topical capsaicin varied with the vehicle used. Topical treatment with 33 mM in 50% DMSO always abolished mechanical responses whilst the same concentration in ethanol had no significant effect.

Responses to skin heating were severely depressed or abolished by topical treatment with 33 mM capsaicin in DMSO. However, topical treatment with ≥ 1 mM capsaicin in vehicle 3 or with 33 mM in ethanol usually led to marked sensitization of heat responses as shown in the lower traces in Figure 2. Firing thresholds to a 1°C s^{-1} heat ramp fell (Figure 3b) and the number of spikes evoked by a constant heat stimulus increased. The average fall in heat threshold 5–25 min after application of 3–100 mM was $8.8^\circ\text{C} (\pm 2.5^\circ\text{C})$, $n = 11$ and there was some recovery, by $4.5^\circ\text{C} (\pm 2.0^\circ\text{C})$ over the next 20 min (Figure 3b). The dose-dependence of the fall in heat threshold is shown in Figure 3d. Note that not all units sensitized after topical capsaicin in vehicle 3, and two units actually desensitized substantially. Overall, however, most units showed simultaneous sensitization to skin heating and depression of responses to pressure following application of ≥ 1 mM topical capsaicin in vehicle 3.

Immediate effects on skin blood flow

Painting capsaicin solutions onto the skin caused a slow, dose-dependent, rise in the laser-Doppler flux signal, with maximum flux usually occurring at 10–30 min (Figure 4a). After application of a 33 mM solution, blood flow recovered to baseline in 2–4 h (4 preparations). As shown in Figure 4b, no significant effect was seen with 0.3 mM capsaicin, but 1 mM caused an average increase of 60% ($\pm 25\%$, $n = 6$), a rise that is just significant ($P < 0.05$, 1-tailed t test). The average increase with 33 mM capsaicin was 171% ($\pm 23\%$, $n = 7$) and increases of over 300% were seen at some locations.

Immediate effects on antidromic vasodilatation

Electrical stimulation of the distal cut end of the saphenous nerve caused the blood flow to increase transiently. As reported previously (Lynn, 1988; Janig & Lisney, 1989), a stimulus intensity sufficient to activate unmyelinated (C) fibres was needed to generate reliable, large antidromic vasodilatation. A typical response for 10 stimuli at 1 Hz and maximum C-fibre strength (the standard stimulus used) is shown in Figure 5a. Antidromic vasodilatation was greatly reduced or abolished following capsaicin application (Figure 5b).

Analysis of the reduction in antidromic vasodilatation after topical capsaicin treatment was complicated by the simultaneous large rise in baseline blood flow. Expressing antidromic vasodilatation as % baseline indicates a dose-dependent fall in antidromic vasodilatation over the range 1–100 mM. A significant reduction in the absolute flux increase, in standard units (see methods), was only seen for concentrations ≥ 10 mM. When there was a very high baseline flow after topical capsaicin, antidromic stimulation sometimes produced only a marked vasoconstriction. This presumably represents

Figure 2 Responses of a typical C-polymodal nociceptor (C-PMN) unit to heat and pressure stimuli after topical capsaicin treatment. Traces of action potentials fired in response to punctuate pressure (a) and skin heating (b) before (left) and 16 and 46–7 min after (middle and right traces) application of 33 mM capsaicin in vehicle 3.

an action of sympathetic efferents (Lembeck & Holzer, 1979) that can become prominent when antidromic vasodilatation is blocked and there is a high baseline blood flow.

The time course of antidromic vasodilatation was not significantly altered in the first hour after topical treatment. Time for half recovery towards baseline ($t_{1/2}$) was 3.2 min before treatment and 2.8 min over the 15–60 min after treatment with 10–33 mM capsaicin. In 4 experiments antidromic vasodilatation was examined for 2–4 h after a single topical application of capsaicin. Peak antidromic vasodilatation showed some recovery over this period, but was still significantly reduced at 2–4 h by 41% ($\pm 9\%$). Recovery of antidromic vasodilatation was therefore slower than the return of baseline blood flow to pre-application levels since at 2–4 h baseline blood flow was back to pretreatment levels in the same preparations.

Effects of C-fibre afferents 1–2 days after a single application of 33 mM capsaicin in vehicle 3

The types of afferent fibre present and the properties of C-fibre PMNs have been examined 'blind' in 13 rats treated 1–2 days previously with either capsaicin (33 mM in vehicle 3) or vehicle alone. The numbers and proportions of the major classes of afferent found after vehicle or capsaicin treatment were similar and are also similar to those reported previously for the rat saphenous nerve (Lynn & Carpenter, 1982; Pini *et al.*, 1990; Pini & Lynn, 1991). Note in particular that the proportion of C-PMN units in treated skin (63% of 40 C-units excited from the skin on day 1; 78% of 36 on days 2) was close to the value in vehicle-treated skin (69% out of 49 units). A proportion of afferents cannot be driven from the skin, and this group is usually around 25% for the C-fibre sample (Pini & Lynn, 1991; Koltzenberg *et al.*, 1991). This proportion was also unchanged following topical capsaicin treatment, the proportions of inexcitable units being 23% ($\pm 3\%$, $n = 4$ preparations) for vehicle treatment, 27% ($\pm 6\%$, $n = 5$) at 1 day after capsaicin treatment, 10% ($\pm 4\%$, $n = 3$) at 2 days.

Heat thresholds of C-PMN units were similar for vehicle and for capsaicin treatment (Figure 6a). However, there was

a significant rise in pressure thresholds 1 day after capsaicin treatment, with full recovery at 2 days (Figure 6b). Average thresholds after vehicle treatment were 0.88 ± 0.08 log mN ($n = 38$), similar to previously published values. At 2 days after capsaicin treatment thresholds were again similar at 0.95 ± 0.07 log mN ($n = 28$), but at 1 day thresholds were significantly raised ($P < 0.01$, Newman-Keuls test) at 1.23 ± 0.07 log mN ($n = 36$). This rise of 0.35 log units is equivalent to an average elevation of threshold by 123%.

Antidromic vasodilatation 4 h to 4 days after a single application of 33 mM capsaicin

Baseline blood flow and antidromic vasodilatation were measured at 12–18 sites on each leg in rats pretreated 4–9 h ($n = 2$) or 1 ($n = 5$), 2 ($n = 5$) or 4 ($n = 4$) days previously by a topical application of 33 mM capsaicin in vehicle 3 for 15 min. No differences in baseline blood flow were present between vehicle and capsaicin-treated legs at any time from 4–9 h to 4 days. This is consistent with the finding from acute experiments that the blood flow had returned to normal by around 3 h. Antidromic vasodilatation was, however, significantly depressed until 4 days (Figure 7). Peak flow was $51 \pm 3\%$ of control at 4–9 h, $86 \pm 11\%$ at 1–2 days and $99 \pm 14\%$ at 4 days. Similarly, responses were of shorter duration with $t_{1/2}$ (the time for half recovery) at $64 \pm 3\%$ of vehicle at 4–9 h, $78 \pm 7\%$ at 1–2 days and $105 \pm 7\%$ at 4 days. Figure 7 uses a response 'area' measure (the product of peak [% baseline] times $t_{1/2}$) and this shows a highly significant time trend ($P < 0.01$, 1-way analysis of variance) from 4–9 h to 4 days.

Substance P levels 1–4 days after a single application of 33 mM capsaicin

Substance P levels were determined by RIA in samples taken 1–4 days after a 15 min treatment with 33 mM capsaicin in vehicle 3. Substance P levels were significantly reduced throughout this period. Levels in vehicle-treated contralateral legs averaged 5.4 pmol g⁻¹ wet wt (± 0.4 pmol g⁻¹, $n = 14$). Levels in treated skin fell to 41% ($\pm 8\%$, $n = 4$) of control

Figure 3 Average trends in (a,b) and dose-dependence of (c,d) alterations in C-polymodal nociceptors (C-PMN) afferent function after topical application of capsaicin in vehicle 3. (a,b) Average changes ($\pm$ s.e., vertical bars) in number of spikes fired to a constant mechanical stimulus (a) and in heat threshold (b) over 5–25 and 25–45 min after treatment with 3–100 mM capsaicin. Data from 11 units. Mean numbers of spikes in (a) are significantly below control levels at both times after capsaicin application ($P < 0.01$; Wilcoxon signed rank test), but values at the two times do not differ significantly. Heat thresholds (b) at 5–25 min are significantly less than control levels ($P = 0.006$, t test) and levels at 25–45 min ($P = 0.04$). Mean threshold at 25–45 min is not significantly different from control. (c,d) Effect of different concentrations on mechanically-evoked responses (c) and heat thresholds (d) 5–25 min after treatment.

values on day 1, to 50% ($\pm 5\%$, $n = 5$) on day 2 and to 51% ($\pm 8\%$, $n = 5$) on day 4.

Discussion

As has previously been reported, topical application of capsaicin to rat skin leads to excitation of afferents of the C-PMN class (Kenins, 1982) and to increases in skin blood flow (Jancso, 1968). In this study, vasodilatation was detectable with concentrations above 1 mM, and this was also the concentration above which excitation of C-PMNs was regularly seen. The C-fibre firing was low in frequency, with average rates typically in the range 0.1–0.3 Hz for 33 mM capsaicin. Vasodilatation, on the other hand, was large and 33 mM capsaicin caused increases averaging 171%. Note, however, that as has been described in other species (Celander & Folkow, 1953; Lynn & Shakhaneh, 1988b), substantial vasodilatation has been found to occur in rat skin with low frequency stimulation of cutaneous nerves (Lynn & Cotsell, 1992b). For example, the dilatation during a 10 min, 0.2 Hz stimulation of the saphenous nerve averaged 218% above baseline in 4 rats (Lynn & Cotsell, unpublished data).

Figure 4 Skin blood flow after topical capsaicin application to the skin. (a) Laser Doppler flux expressed as % pre-application levels (upper trace); systolic blood pressure (lower trace; 100% = 200 mmHg). Application at time zero of 33 mM capsaicin in vehicle 3. Note lack of effect of vehicle 3 alone (a, middle trace), although a small shift in signal level was registered, probably an artefact due to small movement of probe. (b) Average increases in blood flow 15–60 min after topical application of different concentrations of capsaicin. Data from 5 rats tested at 3 to 6 sites on each leg. Increases expressed as % of baseline flow. Numbers of repeats are given next to each upper error bar. Blood flow with 1 mM capsaicin is just significantly above vehicle and 0.3 mM ($P = 0.04$; Mann-Whitney test, 1-tailed); with > 1 mM blood flow is always significantly above vehicle ($P < 0.02$).

Figure 5 Antidromic vasodilatation before (a) and 15–60 min after (b) local capsaicin treatment (33 mM in vehicle 3). Antidromic vasodilatation produced by stimulation of saphenous nerve at maximal C-fibre strength and with 10 shocks at 1 Hz. Average of 3 trials.

Thus our examination of C-PMN firing and vasodilatation after topical capsaicin and of antidromic vasodilatation under the same conditions, fits well with the widely accepted

Figure 6 Firing thresholds ($\pm$ s.e., vertical bars) of C-polymodal nociceptor (C-PMN) units 1–2 days after topical 33 mM capsaicin (Caps) treatment. Numbers of units given next to error bars. (a) Heat thresholds for 1°C s⁻¹ ramp stimulus. No significant differences between groups. (b) Pressure thresholds for von Frey bristles. Note logarithmic force scale. Thresholds at 1 day are significantly greater than vehicle or 2 days ($P < 0.01$, Newman-Keuls test); there is no significant difference between thresholds at 2 days and those with vehicle (Veh).

Figure 7 Antidromic vasodilatation response up to 4 days after topical treatment with 33 mM capsaicin. In each preparation 12–18 sites were measured by laser-Doppler flowmetry on each leg. One leg was vehicle-treated, the other capsaicin-treated. The ratios of the average antidromic vasodilatation (ADV) (capsaicin side/vehicle side) for each preparation were in turn averaged over the 2–5 preparations studied at each time interval. Antidromic vasodilatation measure was response 'area' defined as the product of peak flow (% baseline) and time for 50% recovery ($t_{1/2}$, min). Reduction compared with contralateral control was significant at 4–9 h, 1 day and 2 days (t tests, 1-tailed, $P < 0.05$); there was no significant difference in the reductions at these times, but analysis of variance showed a significant 'linear' time trend from 4–9 h to 4 days ($P = 0.03$).

view that capsaicin-induced vasodilatation results from the release of vasodilator peptides from C-PMN afferent terminals in response to their excitation by capsaicin (e.g. see Holzer, 1988; 1991).

Following the initial excitation of C-PMN units by capsaicin in 50% DMSO/ethanol there was a loss of sensitivity to mechanical and heat stimuli, as reported by Kenins (1982). With vehicle 3 (containing ethanol, propylene glycol and methyl laurate, but not DMSO) there was a dose-dependent reduction in mechanical sensitivity with effects reliably observed for concentrations above 0.1 mM. However, with vehicle 3, C-PMNs usually showed enhanced heat sensitivity. The more marked depressant effects of capsaicin in DMSO, along with the greater initial firing, may be because drug penetration through the skin is more rapid in this vehicle (Scheuplein & Bronaugh, 1983). In primates, capsaicin in DMSO has been found to cause heat sensitization of C-PMNs along with little change in mechanical thresholds

(Baumann *et al.*, 1991). In man over the first 24 h of topical capsaicin treatment of the skin, heat pain thresholds fall and skin heating becomes more painful (Carpenter & Lynn, 1981; Simone & Ochoa, 1991). In rats treated repeatedly with capsaicin cream, withdrawal latencies for noxious heating became shorter (McMahon *et al.*, 1991). Thus it appears that capsaicin has a widespread ability to potentiate the transducer mechanism underlying heat sensitivity, while not affecting, or even depressing, the operation of the transduction mechanism for pressure.

Afferent and efferent functions of C-fibres recovered at slightly different rates following a single treatment with 33 mM capsaicin. Afferent functions recovered first, with C-PMN heat sensitivity at normal levels on day 1 and mechanical thresholds showing full recovery by 48 h. There was also no increase at any time after topical capsaicin treatment in the proportion of C-fibres that had no cutaneous receptive fields. In this respect, skin treatment differs markedly from the effects of applying similar concentrations of capsaicin to the saphenous nerve itself when a large fall in the proportion of C-PMNs that can be excited from the skin and a corresponding increase in inexcitable C-fibres occurs (Pini & Lynn, 1991). Antidromic vasodilatation recovered more slowly than afferent sensitivity, remaining depressed at 2 days but showed complete recovery at 4 days. Other experiments with topical capsaicin have shown marked reductions in neuro-inflammatory reactions. For example, after repeated application of capsaicin, flare is absent or reduced in human skin (Carpenter & Lynn, 1981; Simone & Ochoa, 1991) and neurogenic plasma extravasation is reduced in the rat (McMahon *et al.*, 1991). This difference in rate of recovery between afferent C-PMN function and antidromic vasodilatation could be because the mechanisms involved in release of vasoactive agents from C-PMN terminals are affected more severely by capsaicin than those involved in sensory transduction. On the other hand, it may reflect the possibility that classes of C-fibre other than PMNs are involved in antidromic vasodilatation and other neurogenic inflammatory responses. For example it has been suggested that the lack of significant mechanically-evoked vasodilatation in normal rat skin indicates that most C-PMNs cannot cause antidromic vasodilatation (Lynn & Cotsell, 1992b). It has also been suggested that a special population of chemo-sensitive C-fibres trigger secondary hyperalgesia (LaMotte *et al.*, 1991). Note however, that if a special population of non-PMN C-fibres is involved in generating antidromic vasodilatation, then these would have to show the same sensitivity to capsaicin as do C-PMNs.

One possible mediator, the release of which might cause vasodilatation, is substance P (Lembeck & Holzer, 1979) and this peptide is known to be present in many afferent C-fibres (McCarthy & Lawson, 1989). Levels of substance P in skin showed little sign of recovery, even 4 days after topical 33 mM capsaicin. A similar situation of normal antidromic vasodilatation when substance P levels are still depressed occurs 2 weeks after intradermal injection of capsaicin into rat skin (Lynn, 1992). A lack of substance P might well reduce efferent functions of C-fibres more than afferent ones. Certainly, whatever the exact nature of the C-fibres that are involved in antidromic vasodilatation, our results show that afferent and efferent functions of C-fibres can be normal when, as at 4 days after topical capsaicin, total levels of substance P are only just over half of normal.

The ability of topical capsaicin concentrations of 0.1–1 mM to cause significant effects on C-PMN function is of particular interest in view of the finding that treatment of patients with low concentration (0.025%, approx 0.8 mM) capsaicin creams can give significant pain relief (Bernstein *et al.*, 1989). In the clinical situation, repeated application appears to be needed to achieve useful pain relief. The long duration of desensitization following topical capsaicin, at least after single applications at 33 mM, would in principle allow the effects of daily treatments to summate.

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