Effects of Topically Applied Capsaicin Cream on Neurogenic Inflammation and Thermal Sensitivity in Rats

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ABSTRACT—The effects of capsaicin cream on neurogenic inflammation and thermal nociceptive threshold were investigated in rats. Firstly, for topical application of capsaicin cream to hind paw, we shaped boots from dental cement to prevent the animals from licking off the drug. Capsaicin cream (1%) led to significant increases in the amounts of Evans blue and substance P (SP) released into the perfusate, and the former response was significantly suppressed by pretreatment with RP67580, an NK1-receptor antagonist, but not by treatment with an NK2-receptor antagonist. Subsequent electrical stimulation of the sciatic nerve resulted in a significant reduction in Evans blue and SP extravasation 24 h after topical application of capsaicin cream. On the other hand, when capsaicin cream was repeatedly applied to both hind paws once a day, withdrawal latency for noxious heat stimulation decreased after 24 h, and this thermal hyperalgesia was reversed 3 days later. These results suggest that capsaicin cream initially affects neurogenic inflammation mechanisms and then blocks the pain transmission mechanism.

Keywords: Capsaicin cream, Analgesic, Neurogenic inflammation, Substance P

Capsaicin [(E)-N-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide] is the pungent ingredient in Capsicum peppers. Topical application of capsaicin initially evokes neurogenic responses as characterized by nociception, axon reflex vasodilation and plasma extravasation. These excitatory responses are blocked, however, by repetitive application of capsaicin (1–3). It is also known that capsaicin causes release of substance P (SP) and other neuropeptides from the peripheral terminals of the capsaicin-sensitive afferent C-fibers. Following the release of these neuropeptides from the sensory neurons in the periphery, excitation of nociceptive terminals is thought to be the cause of the transient hyperalgesia experienced immediately following the topical application of capsaicin. The blockade of axonal transport and reduction in intra-axonal levels of SP explains the ability of capsaicin to desensitize the nociceptive terminals of afferent C-fibers (4, 5), and this phenomenon is involved in the role of capsaicin as a topical analgesic in patients with post-herpetic neuralgia (6–9) and painful diabetic neuropathy (10, 11).

As mentioned, capsaicin is widely used as a topical analgesic for various cutaneous disorders, but animal studies of capsaicin in vivo have been mainly conducted by means of systemic administration. Since for topical application experiments, capsaicin compounds diluted by ethanol or dimethylsulfoxide (DMSO) have generally been used, there have been few animal studies of the pharmacological action of drugs containing capsaicin (capsaicin cream).

We therefore produced a capsaicin-containing cream to study the effect of the topical application of this cream on neurogenic inflammation and thermal nociception in rats.

MATERIALS AND METHODS

Experimental animals
Male Sprague-Dawley rats (approx. 200 g body weight) were used. Food and water were supplied ad libitum, and the animals were kept in a 12-h light-dark cycle.

Drug application
Cream was applied at the dose of 100 mg/animal once daily onto the lightly ether-anesthetized rat hind instep and sole. The area of application was covered with an adhesive bandage (Elastopore®; Nitciban Co., Ltd., Tokyo) and covered with the boots made of dental cement (GC-ostron II®; GC Co., Ltd., Tokyo) to prevent the animals from
licking off the drug. The experiment was started 1 h after wiping off the cream with cotton soaked in 10% ethanol.

**Neurogenic extravasation and SP release into the subcutaneous space**

The animals were anesthetized with urethane (780 mg/kg, i.p.), and the subcutaneous space of the hind in-step was perfused by the previously described method (12). Briefly, a double polyethylene tube about 5 cm in length (the 0.5-mm diameter inner tube was 5-mm longer than the 3-mm diameter external one) was introduced into the subcutaneous space of the instep. Through this double coaxial tube and with the aid of a peristaltic pump, perfusion was carried out with saline containing the aminopeptidase inhibitor bestatin (3 mg/100 ml) and dipeptidase inhibitor captopril (0.1 mM) at a rate of 0.1 ml/min. Fractions of the perfusate were collected through the outer tube for periods of 10 min with a fraction collector, and the tubes were then placed in an ice bath. Each 10-min fraction (1 ml volume) was lyophilized, and the SP content was measured by means of radioimmunoassay as described previously (12). For measurement of vascular permeability, Evans blue (50 mg/kg) was intravenously injected into the animals. The subcutaneous space of the instep was perfused in the same manner as the saline perfusion. The amount of dye released into the perfusate (collected in 10-min fractions) was measured at a wavelength of 620 nm with a spectrophotometer (model UV-240; Shimadzu Scientific Instruments, Kyoto).

**Electrical stimulation**

The sciatic and the saphenous nerves were sectioned proximally and then placed on bipolar enamel-insulated stimulating electrodes under liquid paraffin. The distal stumps of both nerves were stimulated simultaneously for 20 min with a 10-V square wave (frequency of 2 Hz and duration of 1 ms).

**Behavioral assessment (Hot plate test)**

Reaction times for withdrawal of hind limbs from a hot plate heated to 52°C were measured. Each rat was tested 3 times with 15-min intervals. The latencies thus obtained were averaged. A time limit of 60 s was employed to prevent thermal injury to the hind paw.

**Statistics**

The results obtained were expressed as means ± S.E.M. To determine the significance of differences among the groups, Student's t-test or Dunnett's multiple comparison test was used.

**Drugs**

Capsaicin creams labeled 0.1% (lot 33191), 0.3% (lot 33221) and 1% (lot 33231) contained 1, 3 and 10 mg capsaicin in 1 g of cream base, respectively. These creams and the cream base (lot 33251) were manufactured at the Central Research Laboratories of Maruishi Pharmaceutical Co., Ltd. Bestatin, captopril, diethylether and carbamyl chloride were purchased from Wako Pure Chemicals (Osaka). The NK1-receptor antagonist RP67580 and its stereoisomer RP68651 (Rhone-Poulenc Rorer, Paris, France) were dissolved in a small amount of 1 N HCl and then adjusted with saline to obtain 1 mg/ml solution. A 1 mg/ml solution of SR48968 (Sanofi Recherché, Montpellier, France) was prepared with 5%-ethanol-containing saline.

![Fig. 1. Effect of topical application of capsaicin cream and electrical stimulation of the sciatic nerve on release of Evans blue (open symbols) and SP release (solid symbols) into the subcutaneous perfusate. Cream containing 1% capsaicin was applied to the rat hind paw at the time indicated by the arrow, and electrical stimulation with a 10-V square wave (2 Hz, 1-ms duration) was performed at the time indicated by the solid line (stimulation). Values represent the mean ± S.E.M. of 5–7 experiments.]

<table>
<thead>
<tr>
<th>Concentration of capsaicin (%)</th>
<th>n</th>
<th>Evans blue content (μg/ml)</th>
<th>Capsaicin stimulus</th>
<th>Electrical stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>6</td>
<td>1.15 ± 0.6</td>
<td>3.15 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>6.13 ± 1.79*</td>
<td>4.73 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>6</td>
<td>9.47 ± 2.69*</td>
<td>5.09 ± 1.89</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>6</td>
<td>12.65 ± 2.07*</td>
<td>3.83 ± 0.48</td>
<td></td>
</tr>
</tbody>
</table>

Electrical stimulation was performed 140 min after topical application of 1% capsaicin cream. In the capsaicin application and nerve stimulation experiments, the amount of Evans blue indicates the total amount of Evans blue released for 140 min as a result of application of capsaicin (from immediately after application until before electrical stimulation; fractions No. 8–21 in Fig. 1) and for 30 min as a result of electrical stimulation (from immediately after electrical stimulation; fractions No. 22–24 in Fig. 1). *P<0.05, values for vehicle-treated animals (base) vs values obtained of the capsaicin stimulation (Dunnett's test).
RESULTS

Evans blue extravasation

When Evans blue (50 mg/kg) was injected intravenously, the amount of Evans blue in the perfusate was very low. Capsaicin cream topically applied to the hind paw produced a significant increase in the amount of Evans blue in the perfusate in a dose-dependent manner. Subsequent electrical stimulation of the sciatic nerve also led to a significant increase in the Evans blue extravasation, which then declined gradually to its pre-stimulation value (Fig. 1). This means that electrical stimulus-induced extravasation was not affected by pretreatment with capsaicin cream (0.3–1%) 2 h previously (Table 1). However, as shown in Fig. 2, capsaicin stimulus- and electrical stimulus-induced extravasation was significantly suppressed 24 h after the application of 1% capsaicin cream.

SP release

When 1% capsaicin cream was applied, the amount of SP in the perfusate significantly increased and then rapidly declined to its basal level. Subsequent electrical stimulation

![Graphs showing the effect of repetitive application of capsaicin on SP release and Evans blue extravasation](image-url)

**Fig. 2.** Effect of repetitive application of capsaicin cream on capsaicin stimulus- and electrical stimulus-induced Evans blue release. Values represent the mean ± S.E.M. of the number of experiments indicated in parentheses. * and * indicate differences with the significance level of $P<0.05$ between values for vehicle-treated animals (base), values obtained immediately after onset of application of capsaicin (0), and those obtained on days 1 and 3 (Dunnett’s test).

![Graphs showing the effect of repetitive application of capsaicin on SP release and Evans blue extravasation](image-url)

**Fig. 3.** Effect of repetitive application of capsaicin cream on capsaicin stimulus- and electrical stimulus-induced SP release. The columns represent the total amount of SP released for 20 min immediately after application of capsaicin cream (fractions No. 8 and 9), and for 20 min immediately after sciatic nerve stimulation (fractions No. 22 and 23). Values represent the mean ± S.E.M. of the number of experiments indicated in parentheses. * and * indicate differences with the significance level of $P<0.05$ between values for vehicle-treated animals (base), values obtained immediately after application of capsaicin (0), and those obtained on days 1 and 3 (Dunnett’s test).
Table 2. Effects of RP67580, RP68651 and SR48968 on Evans blue extravasation induced by capsaicin and electrical stimulus

<table>
<thead>
<tr>
<th></th>
<th>Evans blue content (μg/ml)</th>
<th>Capsaicin stimulus</th>
<th>Electrical stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>20.77 ± 5.8</td>
<td>3.69 ± 2.0</td>
</tr>
<tr>
<td>RP67580</td>
<td>6</td>
<td>12.73 ± 4.7*</td>
<td>4.11 ± 2.2*</td>
</tr>
<tr>
<td>RP68651</td>
<td>6</td>
<td>18.40 ± 4.0</td>
<td>8.67 ± 1.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>19.77 ± 4.7</td>
<td>9.50 ± 2.0</td>
</tr>
<tr>
<td>SR48968</td>
<td>6</td>
<td>20.10 ± 6.2</td>
<td>8.95 ± 2.3</td>
</tr>
</tbody>
</table>

Drugs were injected through the tail vein 10 min before topical application of capsaicin cream. In the capsaicin application and nerve stimulation experiments, the amount of Evans blue indicates the total amount of Evans blue released for 140 min as a result of application of capsaicin (from immediately after application until before electrical stimulation: fractions No. 8–21 in Fig. 1) and for 30 min as a result of electrical stimulation (from immediately after electrical stimulation: fractions No. 22–24 in Fig. 1). * and † indicate differences with the significance level of P<0.05 in values for vehicle-treated animals (Dunnnett’s test).

of the sciatic nerve also led to the release of SP, which then declined to its pre-stimulation level immediately after the end of stimulation (Fig. 1). As shown in Fig. 3, 24 h after onset of application of 1% capsaicin cream, capsaicin stimulus- and electrical stimulus-induced SP release were significantly suppressed.

Effect of NK1 receptor antagonist on plasma extravasation

Pretreatment with RP67580, an NK1 antagonist, at a dose of 1 mg/kg (i.v.) suppressed capsaicin stimulus- and antidromic stimulus-induced extravasation to 49% and 51%, respectively (Table 2). Neither RP68651, an inactive isomer of RP67580 nor SR48968, an NK2 antagonist, produced any effect up to 5 mg/kg (i.v.).

Behavioral assessment (Hot plate test)

Prior to application of either the cream base or 1% capsaicin cream, withdrawal latencies for thermal stimulation (52°C) were 22.6 ± 1.1 s (mean ± S.E.M., n = 25) and 18.2 ± 0.8 s (mean ± S.E.M., n = 33), respectively, with no significant difference between the two groups. On days 1 and 3 after application of the cream base, withdrawal latencies were 19.0 ± 1.2 s and 20.9 ± 2.2 s, respectively. On the other hand, on day 1 after application of 1% capsaicin cream, withdrawal latency became significantly shorter compared with that before treatment (14.1 ± 1.1 s, n = 33), but on day 3, after repetitive applications of capsaicin, it was significantly prolonged (34.2 ± 2.1 s, n = 33) (Fig. 4).

DISCUSSION

Cutaneous application of chemical irritants such as capsaicin or mustard oil causes stimulation of chemosensitive pain receptors and plasma extravasation. This plasma extravasation can also be evoked by antidromic stimulation of capsaicin-sensitive afferent C-fibers (2). Furthermore, there is some evidence that such stimulus-induced plasma extravasation is closely related to the release of SP from the peripheral terminal of capsaicin-sensitive afferent C-fibers (13–16). In the present study, topical application of capsaicin cream and electrical stimulation of the sciatic nerve resulted in a marked increase in Evans blue and SP release. These stimulus-induced increases were significantly suppressed by pretreatment with RP67580 (an NK1 antagonist), but not by RP68651 (an inactive isomer) or SR48968 (an NK2 antagonist). Our results thus raise the

![Fig. 4. Effect of repetitive application of 1% capsaicin cream on withdrawal latency from the hot plate (52°C). Values represent the mean ± S.E.M. of 25 (base) and 33 (capsaicin cream) experiments. * indicates a difference with the significance level of P<0.05 compared with values obtained before treatment with capsaicin cream (before) (Dunnnett’s test).](image-url)
possibility that the extravasation induced through the released SP was mediated through NK-1R.

Concerning the effect of capsaicin, Jancsó et al. reported that capsaicin-induced and sciotic nerve stimulus induced extravasation were inhibited by severing of the sciotic nerve and that these responses were also suppressed by the systematic pre-administration of capsaicin (2). In the present study, when the sciotic nerve was electrically stimulated 140 min after the first topical application of capsaicin, electrical stimulus induced extravasation and SP release were not affected by the pretreatment with capsaicin. One day after the topical application of capsaicin cream, however, the stimulus-induced releases of both Evans blue and SP were significantly attenuated. These results suggest that at least one day may be necessary for capsaicin cream to block the mediation of neurogenic inflammation via an axon reflex.

As for the effect of capsaicin on pain transmission, there is some evidence that systemic administration or direct application onto the sensory nerve axons produces an analgesic effect (17–19), while topical application of capsaicin onto the skin does not have any effect. In this connection, McMahon et al. observed that capsaicin cream (0.075% or 0.75%) applied twice daily to rat hind paws for continuous periods of 10 weeks did not prevent paw withdrawal to heat (20). Furthermore, Carter and Francis also reported that capsaicin (1% or 5%) applied three times daily to rat hindpaw for continuous periods of 4 days did not produce an increase in withdrawal latencies over the 4-day treatment period or during the 3-day period subsequent to drug treatment. However, intracutaneous injection of 0.1% capsaicin (100 μl) into the hindpaw caused significant increases in response latencies for up to 6 h following injection (21). In the current study, when 1% capsaicin cream was applied to the rat hindpaw once daily for continuous periods of 3 days, thermal withdrawal latency was significantly reduced 24 h after onset of application (hyperalgesia), while a few more applications were needed to produce significant increase in withdrawal latency to noxious heat stimulation (hyperalgesia). Results similar to the ones obtained in animal experiments were observed in a human study where it was easy to manage application of the drug. In that case, 10 days of topical application (1%) produced a significant increase in thermal pain threshold (22). In view of these findings, our results indicate the importance of the ability of transcutaneous drug delivery or of bioavailability for capsaicin cream to show its effect. In the present study, the region of cream application was first covered with an adhesive bandage and then with boots made of dental cement to prevent the animals from licking off the drug, so that capsaicin cream and skin were in close contact for a long time. This resulted in adequate absorption of the cream from the skin and good bioavailability.

In the current study, the time-course of decrease in

stimulus-induced SP release did not correlate with that of thermal analgesia, suggesting that more than one mechanism may be involved in the ability of capsaicin to produce thermal analgesia (23). In this connection, recently it has been observed that capsaicin elicits burning sensations through the activation of a capsaicin receptor (vanilloid receptor) containing a heat-gated ion channel that is likely to contribute to the detection of painful thermal stimuli in vivo, and desensitization or tachyphylaxis of this receptor also easily occurs (24). Therefore, it is thought that the inhibition of Ca²⁺-dependent capsaicin-activated current (25) may be involved as one of the anti-nociceptive mechanisms of capsaicin cream in addition to depletion of neuropeptides existing in capsaicin-sensitive afferent C-fibers such as SP.

In conclusion, our present study suggests that capsaicin blocks neurogenic inflammation mechanisms more easily than pain transmission mechanisms and that in addition, longer-term repetitive application of capsaicin cream has a therapeutic effect on subjects with noxious pain.

REFERENCES