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Mechanism of mustard oil-induced skin inflammation in mice

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Abstract

We examined the mechanism of the inflammatory response induced by topical application of mustard oil (0.5–20.0%/20 μl per ear) to the mouse ear compared to that of the response to capsaicin. The dose-dependent increases in plasma extravasation and ear thickness reached a maximum at approximately 30 min after mustard oil application. Topical pretreatment of ears with capsaicin (250 μg/ear) diminished mustard oil-induced plasma extravasation for up to day 7 but not at day 14 after treatment. However, desensitization of the exudative response was not evoked by reapplication of mustard oil to ears. The inflammatory response to mustard oil did not differ between the ears of mast cell-deficient mice and those of the congenic normal mice. Mustard oil-induced plasma extravasation was unaffected by pretreatment with histamine H_1 and 5-HT_2 receptor antagonists and the capsaicin-functional inhibitor, ruthenium red, which inhibit capsaicin-induced ear oedema. The endopeptidase inhibitor, phosphoramidon, enhanced the ability of mustard oil to increase dye leakage. The tachykinin NK_1 receptor antagonist, SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2.]octane, chloride), not only inhibited mustard oil-induced plasma extravasation but also blocked the enhancement by phosphoramidon of the response to mustard oil. In contrast, the tachykinin NK_2 receptor antagonist, SR 48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4,-dichlorophenyl)butyl]benzamide), and the tachykinin NK_3 receptor antagonist, SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide), had no effect on plasma extravasation. The present results demonstrated that mustard oil induces mouse skin inflammation through a mechanism different from that for capsaicin. Mediators such as histamine and 5-HT from mast cells appear to be minor factors in the response to mustard oil. In addition, evidence supports the assumption that the tachykinin NK_1 receptor is involved in this model. © 1997 Elsevier Science B.V.

Keywords: Mustard oil; Neurogenic inflammation; Plasma extravasation; Ear oedema; Capsaicin; SR 140333

1. Introduction

Neurogenic inflammatory responses comprising vasodilatation, plasma extravasation, and mast cell activation are induced by electrical and chemical stimulation of sensory neurones which contain neuropeptides including tachykinins and calcitonin gene-related peptide (CGRP) (Holzer, 1988; Lynn, 1988). Neuropeptides thus play a pathophysiological role in neurogenic inflammation in the skin and airways. Furthermore, the presence of nerve fibers containing substance P and CGRP has been demonstrated in the vasculature of the skin (Gibbins et al., 1987; Wallengren et al., 1987). Neurokinin A-immunoreactive fibers have also been observed in rat skin with a location similar to that of substance P (Dalsgaard et al., 1985). Substance P preferentially activates tachykinin NK_1 receptors, whereas neurokinin A and neurokinin B interact with tachykinin NK_2 and NK_3 receptors, respectively (Guard and Watson, 1991). Among these, tachykinin NK_1 receptors are predominantly involved in the neurogenic inflammatory response to tachykinins in the hamster cheek pouch (Gao et al., 1993), in the rat knee (Lam and Ferrell, 1991), and in the rat skin (Andrews et al., 1989). Recently, non-peptide tachykinin receptor antagonists, such as SR 140333 (Emonds-Alt et al., 1993) for tachykinin NK_1 receptors, SR 48968 (Emonds-Alt et al., 1992) for tachykinin NK_2 receptors, and SR 142801 (Emonds-Alt et al., 1995) for tachykinin NK_3 receptors, have proved to be useful for the investigation of the physiological and pathological role of tachykinin receptors.

Topical application of capsaicin to the mouse ear produces neurogenic skin inflammation (Mantione and Ro-

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drigues, 1990; Gábor and Rázga, 1992). Capsaicin-induced mouse ear oedema is inhibited by the capsaicin functional inhibitor, ruthenium red, by tachykinin NK_1 receptor antagonists and histamine H_1 and/or 5-HT_2 receptor antagonists, but not by inhibitors of the arachidonate pathway (Inoue et al., 1993, 1995a,b). Evidence from our previous studies suggests that tachykinins released from sensory nerves by capsaicin cause an inflammatory response to capsaicin by activation of tachykinin NK_1 receptors and by releasing vasoactive amines from mast cells. Mustard oil, as well as capsaicin, has also been used as a chemical algogen in the study of neurogenic inflammation, and has proved to increase blood flow and vascular permeability in rodent skin (Jancsó et al., 1967; Jancsó et al., 1977; Gamse et al., 1980; Lynn and Shakhanbeh, 1988). Furthermore, it has been considered that substance P and CGRP participate in these responses (Louis et al., 1989; Lembeck et al., 1992). In the present study, we examined the mechanism of mustard oil-induced inflammatory responses such as plasma extravasation and oedema formation in mouse skin in comparison with the response to capsaicin.

2. Materials and methods

Six-week-old male ddY mice weighing 30–35 g (Japan SLC, Japan) or nine-week-old mast cell-deficient male Slc:WBB6F_1-W/W^v(WB-W/+ × C57BL/6-W^v/+) mice and the congenic normal Slc:WBB6F_1-+/+ mice weighing 25–30 g (Japan SLC) were used for the experiments. The animals were kept in an environmentally controlled room (24 ± 1°C, 55 ± 10% humidity) with a light:dark cycle of 12 h and were allowed free access to food and water.

2.1. Materials

The following drugs were used: indomethacin, NDGA (nordihydroguaiaretic acid), chlorpheniramine maleate, ruthenium red, L-NAME (N^G-nitro-L-arginine methyl ester hydrochloride), phosphoramidon, bestatin, CGRP-(8–37), dexamethasone, and capsaicin (Sigma, St. Louis, MO, USA); LY 53857 maleate (6-methyl-1-(1-methylethyl)-ergoline-8-carboxylic acid (8β)-2-hydroxy-1-methylpropyl ester(Z)-2-butenedioate (1:1)) (Research Biochemicals International, Natick, MA, USA); allyl isothiocyanate, Evans blue (Wako, Osaka, Japan); mineral oil (Aldrich, Tokyo, Japan); SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2.]octane, chloride), SR 48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4,-dichlorophenyl)butyl]benzamide), SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide) (Sanofi Recherche, Montpellier, France). AC 5–1 (3,4,2′,4′-tetrahydroxy-2-geranyldihydrochalcone) was prepared by Dr. Y. Fujimoto, Faculty of Pharmaceutical Sciences, Nihon University. SR 140333, SR 48968, and SR 142801 were dissolved in saline by using Tween 80 (Tokyo Kasei, Tokyo, Japan; final concentration less than 0.2%) but indomethacin was suspended in saline. NDGA, AC 5–1, and dexamethasone were dissolved in ethanol for topical application. Other compounds were dissolved in saline.

2.2. Mustard oil- and capsaicin-induced inflammation in mouse ear skin

The animals were anaesthetized with pentobarbital sodium (50 mg/kg, i.p., Abbott, North Chicago, IL, USA). Allyl isothiocyanate was diluted with mineral oil to concentrations of 0.5–20.0% (v/v) and 20 μl was then applied topically to both surfaces of an ear of each mouse. Evans blue (30 mg/kg. i.v.) was administered as a marker for plasma extravasation just before application of mustard oil. In experiments designed to study the ability of mustard oil to induce an increase of vascular permeability, Evans blue was injected at different times (0 min) just before and (5, 15, 30, and 60 min) after application. Topical and oral treatments were given 30 min (but dexamethasone was applied 3 h) before application of mustard oil, respectively. Agents were also injected i.v. in a tail vein 15 min before application of mustard oil. Mice in the control group received the vehicle via the corresponding route. Twenty minutes after application of mustard oil, the animals were killed and an ear was removed by cutting at the base of the ear and then weighed. Evans blue dye in the skin was extracted with a mixture of 0.2 M phosphoric acid and acetone (5:13) after incubation in 1 M potassium hydroxide at 37°C for 18 h and was measured by spectrophotometry at 620 nm (Katayama et al., 1978). Plasma extravasation in ear skin was expressed as μg of Evans blue per 100 mg of wet tissue.

Ear oedema was induced with capsaicin according to the method described previously (Inoue et al., 1993). Capsaicin was dissolved in acetone at a concentration of 12.5 mg/ml and 20 μl (250 μg/ear) was then applied topically to ears of mice anaesthetized with pentobarbital sodium (50 mg/kg, i.p.). As Evans blue attenuates the effects of capsaicin in in vivo models (Bolser et al., 1995), the oedema response was examined by measuring the increase in the thickness at the edge of the ear before and 30 min after capsaicin application, in units of 0.001 mm with dial calipers (Ozaki Factory, Japan). The oedema response to mustard oil was also examined before and various times after treatment. Oedema was expressed as the increase in ear thickness.

2.3. Histological examination

Thirty minutes after application of mustard oil, ears were removed and fixed in 10% neutral buffered formal-

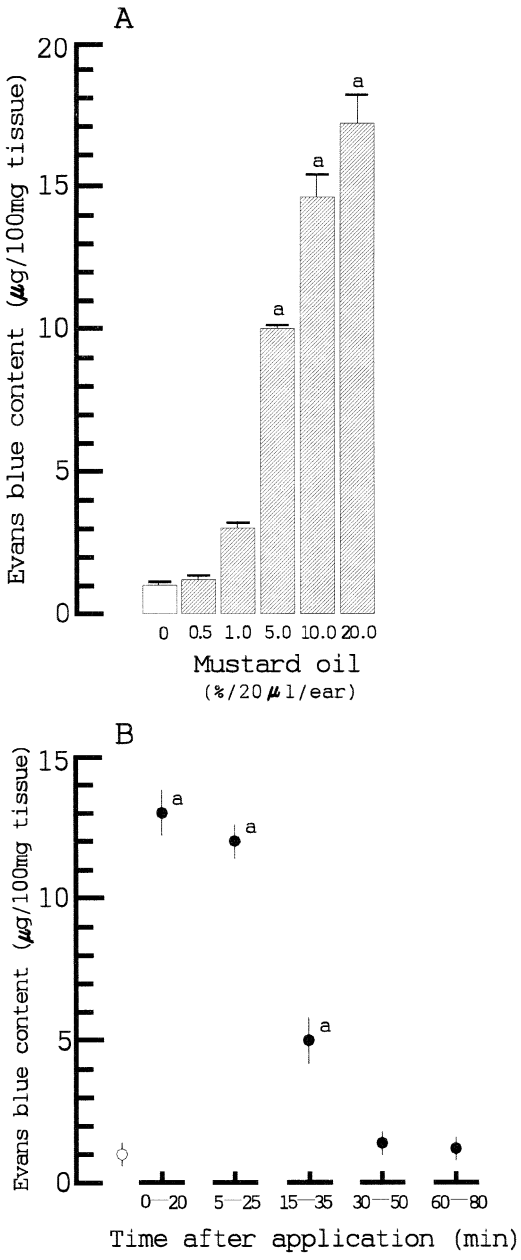

Fig. 1. Plasma extravasation induced in mouse ear skin by topical application of mustard oil at various concentrations (A) and by 5% mustard oil when Evans blue was injected at (0 min) just before or 5, 15, 30, and 60 min after application (B). Results are the means ± S.E.M. for 6–7 animals. [a] $P < 0.01$ compared with mineral oil (A: 0%, B: ○).

dehyde. Sections of the tissue were stained with hematoxylin/eosin for light microscopy and examined under a microscope at ×200 and ×400 magnification.

2.4. Pretreatment of mouse ears with capsaicin and mustard oil

Capsaicin (250 μg/20 μl acetone per ear) or 5% mustard oil was applied topically to mouse ears at 1, 3, 7 or 14 days before mustard oil-induced plasma extravasation. Control ears were treated with acetone or mineral oil.

2.5. Data analysis

Results are expressed as the means ± S.E.M. The statistical significance of differences between control and test groups was determined with the Turkey–Kramer multiple comparisons test after one-way analysis of variance.

3. Results

3.1. Dose-dependence and time-course of mustard oil-induced inflammation

Plasma extravasation was dose dependently induced in ear skin by topical application of mustard oil at concentrations of 0.5 to 20% (Fig. 1A). Significant ($P < 0.01$) increases in dye leakage were observed after treatment with mustard oil at concentrations above 5%. The ability of 5% mustard oil to induce plasma extravasation was tested further by injecting Evans blue dye at (0 min) just before or 5, 15, 30, and 60 min after application (Fig. 1B). The greatest dye content in ear skin occurred between 0 to 20 min when Evans blue was injected just before mustard oil application. The exudative response then rapidly decreased with time and had ended by 30 min after exposure. Oedema formation became detectable at 5 min and reached a maximum at approximately 30 min after application of mustard oil (Fig. 2). The oedema was sustained up to 1 h but had slightly decreased at 2 h. Dose-dependent enhancement of oedema development was observed with increased concentrations of mustard oil (1–20%), but the response to 1% mustard oil was not marked. Application of mineral oil had no effect on ear thickness (data not shown). In subse-

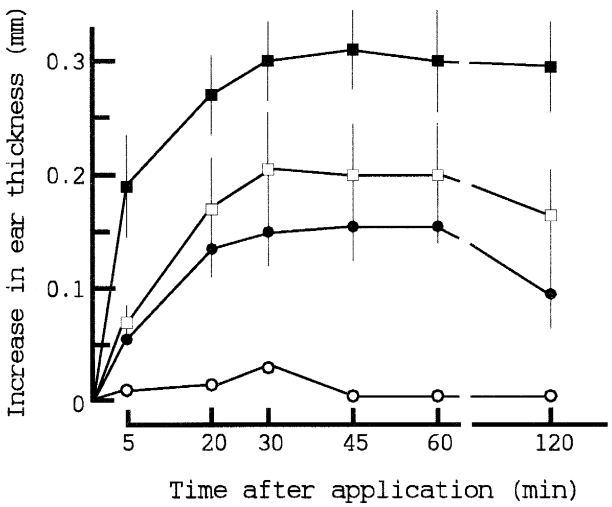

Fig. 2. Time-course of mouse ear oedema induced by topical application of mustard oil. Oedema was evaluated as the mean ear thickness of test animals minus that of animals before application. Each point represents the means ± S.E.M. for 6–7 animals. (○) 1%; (●) 5%; (□) 10%; (■) 20%.

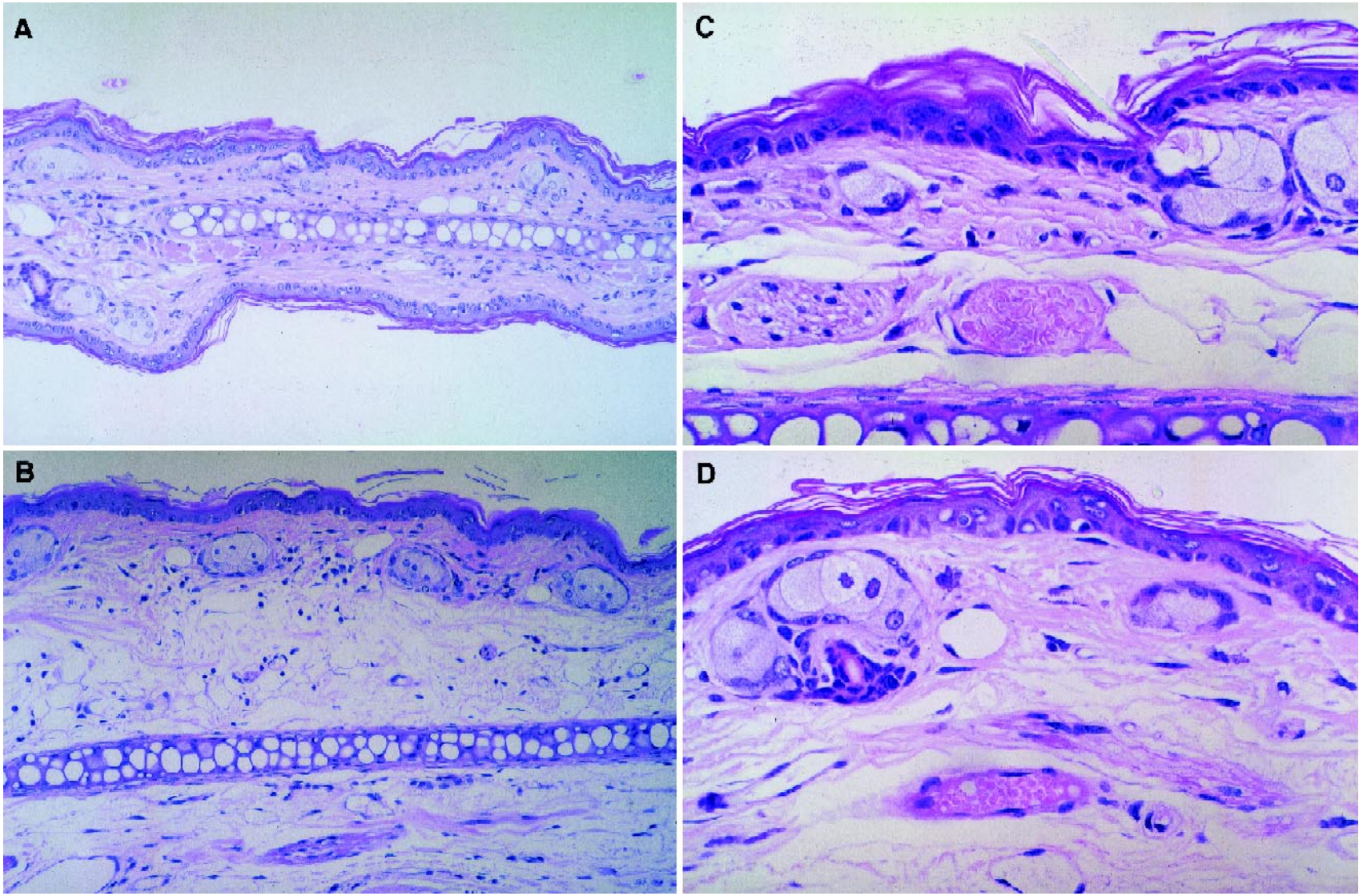

Fig. 3. Representative light microphotograph of mustard oil-induced mouse ear oedema. A thin section was obtained from ear tissue 30 min after application of mineral oil or 5% mustard oil (hematoxylin–eosin staining). (A) mineral oil×200; (B) mustard oil×200; (C) mineral oil×400; (D) mustard oil×400.

quent experiments, the inflammatory response was usually induced by application of 5% mustard oil.

3.2. Histological profile of mustard oil-induced ear inflammation

Histological examination clearly revealed the oedema response in the dermis and subcutaneous tissue following a single topical application of 5% mustard oil (Fig. 3A,B). However, there was no evidence of damage to the epidermis and auditory cartilage. In addition, blood cell leakage into the surrounding tissue was not observed (Fig. 3C,D).

3.3. Effect of capsaicin treatment on mustard oil-induced inflammation

Topical pretreatment of mouse ears with capsaicin (250 μg/ear) significantly ($P < 0.01$) reduced the plasma extravasation which was induced by application of 5% mustard oil on days 1, 3, and 7 after capsaicin treatment (Fig. 4). However, there were no differences between the response to mustard oil in capsaicin-treated and in control ears at day 14. Plasma extravasation evoked after reapplication of mustard oil on days 1, 7, and 14 was not different from that induced after an initial application.

3.4. Mustard oil-induced inflammatory response in ear skin of mast cell-deficient mice

Inflammatory responses evoked by topical application of 5% and 20% mustard oil were examined in the ear skin of mast cell-deficient $WBB6F_1$-W/W^v mice and of the congenic normal $WBB6F_1$-$+/+$ mice. No significant differences in the ability of mustard oil to induce plasma extravasation and oedema formation were observed with between mast cell-deficient and normal mice (Fig. 5A, B). However, the oedema response to capsaicin (250 μg/ear) in deficient mice was lower than that in normal mice (Fig. 5B).

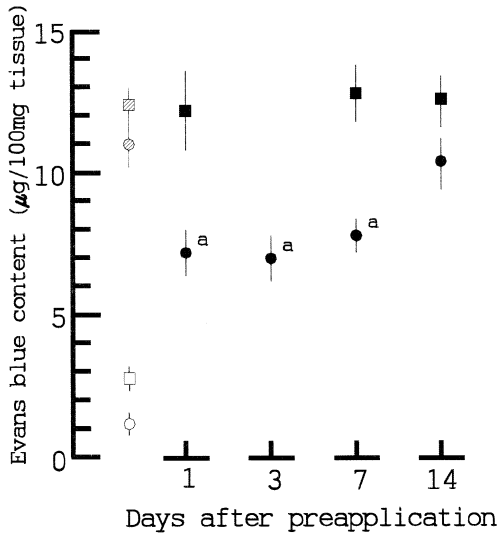

Fig. 4. Effect of capsaicin on mustard oil-induced plasma extravasation in mouse ear skin. Ears were topically treated with capsaicin (●, 250 μg/ear) or mustard oil (■, 5%) at 1, 3, 7 or 14 days before induction of plasma extravasation. Control mice received acetone (dashed circle) or mineral oil (dashed square) at 24 h before application of mustard oil. Evans blue extravasation in skin was examined 20 min after application. Each point represents the means ± S.E.M. for 6–7 animals. [a] $p < 0.01$ compared with the control. ○ and □: mineral oil.

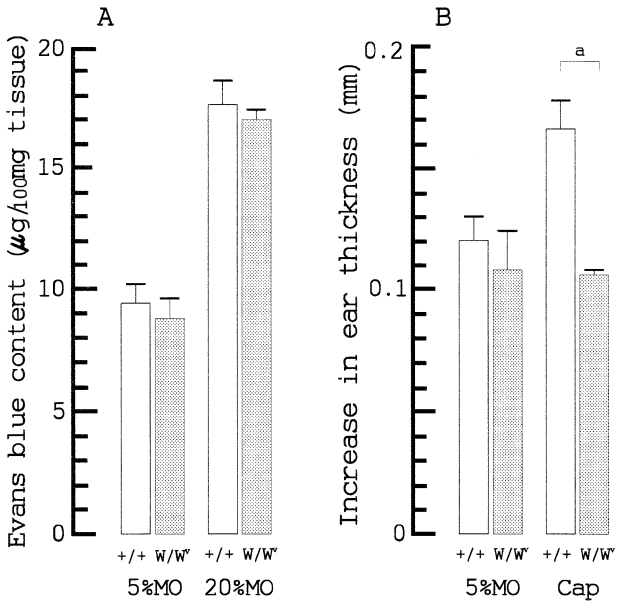

Fig. 5. Plasma extravasation (A) and oedema (B) induced by mustard oil (MO) and capsaicin (Cap) in mast cell-deficient $WBB6F_1$-W/W^v (W/W^v) mice. Evans blue extravasation in ear skin was estimated 20 min after application. Ear oedema was examined 30 min after application of 5% mustard oil or capsaicin (250 μg/ear). Each bar represents the means ± S.E.M. for 6–7 mice. [a] $p < 0.01$ compared with the congenic $WBB6F_1$-$+/+$ ($+/+$) mice.

3.5. Effect of pharmacological agents on mustard oil-induced inflammation

The influence of various agents on plasma extravasation induced in mouse ear skin by a single application of 5% mustard oil is summarized in Table 1. The response to mustard oil was 11.2 ± 0.5 μg (10 experiments, $n = 6$)/100 mg tissue whereas the basal Evans blue leakage in skin was 1.1 ± 0.8 μg (10 experiments, $n = 6$)/100 mg tissue. Mustard oil-induced plasma extravasation was unaffected when cyclooxygenase and 5-lipoxygenase inhibitors such as indomethacin (10 mg/kg, p.o., Calhoun et al., 1987), NDGA (1 mg/ear, Inoue et al., 1988) and AC 5–1 (1 mg/ear, Koshihara et al., 1988) were administered to the mice before the application. However, dexamethasone (50 μg/ear), a steroid anti-inflammatory drug, significantly ($P < 0.01$) suppressed the response to mustard oil. The histamine H_1 receptor antagonist, chlorpheniramine (5 mg/kg, i.v.), and the 5-HT_2 receptor antagonist, LY 53857 (5 mg/kg, i.v.), were ineffective in this model. The CGRP

Table 1
Effect of pharmacological agents on mustard oil-induced plasma extravasation in mouse ear skin

Drug	Dose (mg/kg or ear)	Route	Inhibition (%)
Indomethacin	10.0	p.o.	10 ± 13
NDGA	1.0	t.a.	−5 ± 2
AC 5–1	1.0	t.a.	1 ± 4
Dexamethasone	0.05	t.a.	63 ± 8 [a]
Chlorpheniramine	5.0	i.v.	−26 ± 8
LY 53857	5.0	i.v.	25 ± 6
L-NAME	50.0	i.v.	−13 ± 10
CGRP-(8–37)	1.0	i.v.	−12 ± 16
Ruthenium red	1.0	i.v.	18 ± 1

Compounds were administered orally (p.o.) and topically (t.a.) 30 min (but dexamethasone was given 3 h) before application of 5% mustard oil. Intravenous administration (i.v.) was performed 15 min before application. Plasma extravasation in skin was examined 20 min after application. Values are expressed as the means ± S.E.M. for 6 animals.
[a] $P < 0.01$ compared with control.

receptor antagonist, CGRP-(8–37) (1 mg/kg, i.v.), at a dose which inhibited plasma extravasation in rat skin following thermal injury (Siney and Brain, 1996), and the nitric oxide (NO) synthase inhibitor, L-NAME (30 mg/kg, i.v.), at a dose higher than that required to attenuate the hyperaemia evoked by mustard oil (Holzer and Jocič, 1994), had no effect on plasma extravasation. Ruthenium red (1.0 mg/kg, i.v.), a function inhibitor of capsaicin (Amann and Maggi, 1991), also failed to inhibit the increase in dye leakage and oedema formation (0.176 ± 0.031 mm vs. 0.194 ± 0.030 mm in the saline group, $n = 6$).

The tachykinin NK_1 receptor antagonist, SR 140333 (50–500 μg/kg, i.v.), was effective ($P < 0.01$) to inhibit

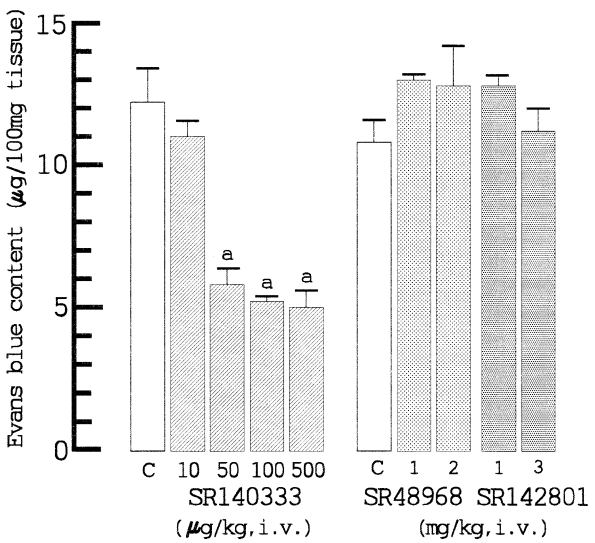

Fig. 6. Effect of tachykinin receptor antagonists on mustard oil-induced plasma extravasation in mouse ear skin. Antagonists were administered intravenously 15 min before application of 5% mustard oil. Evans blue extravasation in skin was examined 20 min after application. Each bar represents the means ± S.E.M. for 6–7 mice. The basal value for dye in skin was 1.24 ± 0.13 μg/100 mg tissue. [a] $p < 0.01$ compared with the vehicle control (C).

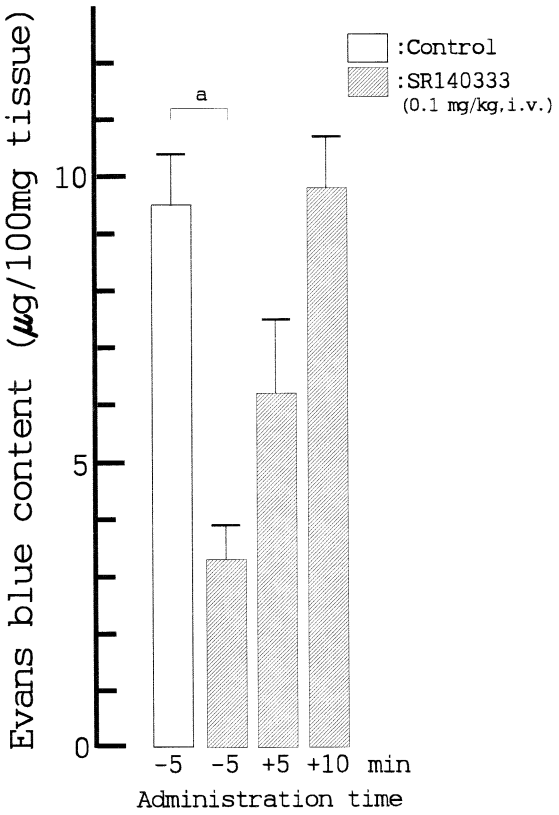

Fig. 7. Effect of SR 140333 (0.1 mg/kg, i.v.) on plasma extravasation in mouse ear skin when administered 5 min before or 5 and 10 min after application of 5% mustard oil. Control mice received the vehicle 5 min before mustard oil application. Evans blue extravasation in skin was examined 20 min after application. Each bar represents the means ± S.E.M. for 6–7 mice. The basal value for dye in skin was 1.62 ± 0.13 μg/100 mg tissue. [a] $P < 0.01$ compared with the vehicle control (C).

mustard oil-induced plasma extravasation in ear skin (Fig. 6). The inhibition caused by doses of 100 and 500 μg/kg SR 140333 was not significantly different from that caused by 50 μg/kg. The inhibitory effect was not observed with the tachykinin NK_2 receptor antagonist, SR 48968 (2 mg/kg, i.v.), and the tachykinin NK_3 receptor antagonist, SR 142801 (3 mg/kg, i.v.). Furthermore, SR 140333 (0.1

Table 2
Enhancement by phosphoramidon of mustard oil-induced plasma extravasation in mouse ear skin

	Dose (mg/kg, i.v.)	Evans blue content (μg/100 mg tissue)
Control		11.3 ± 0.3
Phosphoramidon	0.05	13.4 ± 0.3
	0.10	15.4 ± 0.4 [a]
	0.50	17.6 ± 0.5 [a]
Control		10.3 ± 0.3
Bestatin	0.50	9.8 ± 0.4
	1.00	10.5 ± 0.9

Compounds were administered 15 min before application of 5% mustard oil. Plasma extravasation in skin was examined 20 min after application. Values are expressed as the means ± S.E.M. for 6 animals.
[a] $P < 0.01$ compared with control.

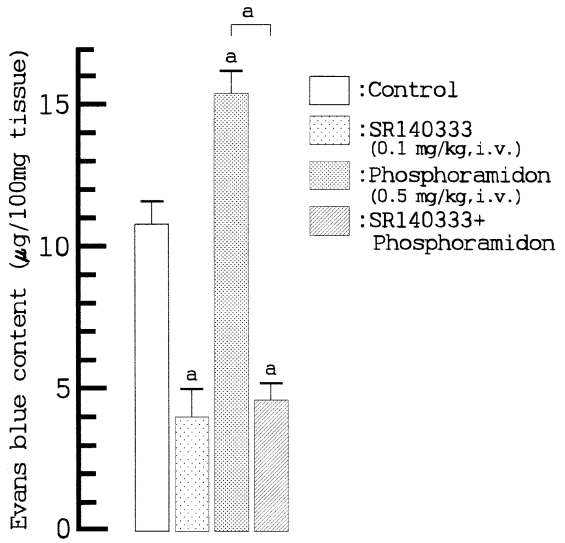

Fig. 8. Inhibition by SR 140333 of the exudative response to mustard oil with phosphoramidon in mouse ear skin. SR 140333 and phosphoramidon were administered 15 and 20 min before application of 5% mustard oil. Evans blue extravasation in skin was examined 20 min after application. Each bar represents the means ± S.E.M. for 6–7 mice. The basal value for dye in skin was 1.08 ± 0.06 μg/100 mg tissue. [a] $p < 0.01$ compared with the vehicle control or phosphoramidon.

mg/kg, i.v.) significantly ($P < 0.05$) reduced dye leakage from 9.00 ± 0.15 μg/100 mg tissue to 4.51 ± 0.12 μg/100 mg tissue ($n = 5$) in mast cell-deficient WBB6F$_1$-W/W^v mice. The administration of SR 140333 (0.1 mg/kg, i.v.) after exposure to mustard oil did not cause a significant inhibition of plasma extravasation, although there was a trend towards inhibition at 5 min after application (Fig. 7). The endopeptidase inhibitor, phosphoramidon (0.05–0.50 mg/kg, i.v.), enhanced plasma extravasation dose dependently, but the aminopeptidase inhibitor, bestatin (1.0 mg/kg, i.v.), failed to enhance the response to mustard oil (Table 2). This enhancement by phosphoramidon (0.5 mg/kg, i.v.) was completely blocked by SR 140333 (0.1 mg/kg, i.v.) (Fig. 8).

4. Discussion

The present results showed that topical application of mustard oil to the mouse ear causes acute inflammatory responses such as plasma extravasation and oedema formation, reaching a maximum effect at up to 30 min. However, infiltration of blood cells into the connective tissue of ear skin was not observed 30 min after mustard oil application whereas there was a marked oedema response in the dermis and subcutaneous tissue. This histological profile is different from that of mouse ear oedema induced by arachidonic acid and 12-O-tetradecanoylphorbol-13-acetate which produce arachidonate metabolites in ear tissue (Young and De Young, 1989). The inflammatory response to mustard oil in the rat skin has been considered to arise from the release of mediators from capsaicin-sensitive afferent nerve endings (Jancsó et al., 1967, 1977; Gamse et al., 1980). Capsaicin is also known to induce neurogenic inflammation through the release of neuropeptides from primary afferent neurones (Holzer, 1991). Reapplication of capsaicin (250 μg/ear) produces desensitization of ear oedema in mice, and this may be due to a reduction of neuropeptides such as substance P (Inoue et al., 1995a). In fact, the level of substance P in ear skin falls to 50–60% of the normal level 24 h after capsaicin treatment (unpublished). We observed that mustard oil-induced plasma extravasation was reduced by pretreatment of ears with capsaicin in this study. This is consistent with other findings showing that pretreatment of adult animals with capsaicin reduces vascular permeability (Jancsó et al., 1977; Lynn and Shakhanbeh, 1988) and hyperaemia (Holzer and Jocič, 1994) induced by mustard oil in rodent skin. Taken together, our data support the hypothesis that mediators released by mustard oil through the activation of capsaicin-sensitive neurones are involved in skin inflammation. Furthermore, the ability of mustard oil to evoke plasma extravasation in ear skin recovered at 14 days after capsaicin treatment. This finding indicates that replacement of mediators involved in the response to mustard oil demands 14 days after capsaicin treatment.

The administration of phosphoramidon, a specific inhibitor of endopeptidase-24,11, which hydrolyzes a variety of neuropeptides (Matsas et al., 1984), resulted in a significant enhancement of mustard oil-evoked extravasation. This is evidence for the involvement of neuropeptides in this model. Previous studies have shown that phosphoramidon enhances the pharmacological activity of tachykinins in in vivo models (Sakurada et al., 1990; Brokaw and White, 1994; Inoue et al., 1996). It is, therefore, likely that phosphoramidon potentiated the response to mustard oil by inhibiting endogenous endopeptidase in ear skin.

The tachykinin NK$_1$ receptor antagonist, SR 140333, inhibited plasma extravasation by up to 64% (0.5 mg/kg). In contrast, the tachykinin NK$_2$ antagonist, SR 48968, and the tachykinin NK$_3$ antagonist, SR 142801, had no effect. Others have demonstrated that substance P binding sites, but not neurokinin A and neurokinin B, are localized in rat footpad skin over the dermal papillae, arterioles and postcapillary venules (O'Flynn et al., 1989). In addition, NK$_1$ receptor mRNA is distributed in the rat skin (Tsuchida et al., 1990). Thus, substance P released from sensory nerve endings by mustard oil seems to induce the inflammatory response to mustard oil via the tachykinin NK$_1$ receptor in mouse skin. This is supported by the finding that SR 140333 completely blocked the enhancement by phosphoramidon of mustard oil-induced plasma extravasation. Nevertheless, it is unlikely that tachykinin NK$_2$ and NK$_3$ receptors participate in the response to mustard oil. We also found that the administration of SR 140333 prior to application of mustard oil inhibited plasma extravasation but did not significantly affect the accumulation at the later

times (5 min and 10 min). In addition to this, mustard oil was more effective to evoke extravasation at the first 20 min than at the periods following (5–25 min and 15–35 min). Thus, it is conceivable that the tachykinin NK_1 receptor is involved principally in the first 5 min of the exudative response to mustard oil.

In the time-course experiment, the fact that mustard oil-induced extravasation was observed even at 15–35 min after application suggests that, in addition to neuropeptides, other mediators contribute to the inflammatory response. NO derived from endothelium is known to play a mediator role in the vasodilator component but not in the exudative response to mustard oil in the rat skin (Lippe et al., 1993). This can be partly confirmed by our finding that the NO synthase inhibitor, L-NAME, did not inhibit plasma extravasation induced by mustard oil in mouse ear. Others have reported that CGRP, as well as substance P, is the primary neurogenic mediators of mustard oil-induced vasodilatation (Louis et al., 1989). CGRP alone, however, does not cause plasma extravasation (Brain and Williams, 1985). Thus, the lack of inhibition by the CGRP antagonist, CGRP-(8–37), of mustard oil-induced dye leakage indicates that CGRP is unlikely to be the major mediator in the induction of the increase in vascular permeability. Similarly, prostaglandins and leukotrienes cannot primarily mediate the dye leakage evoked by mustard oil, since inhibitors of arachidonate metabolites such as indomethacin and NDGA, except for dexamethasone, had little effect. It has been proposed that the anti-inflammatory effect of dexamethasone is due to the induction of de novo synthesis of the proteins (Sautebin et al., 1992). Hence it seems likely that the inhibition by dexamethasone of plasma extravasation is dependent on de novo synthesis of proteins which act directly at the endothelial cell to inhibit plasma leakage (Peers and Flower, 1991).

Chlorpheniramine and LY 53857 are effective to inhibit capsaicin-induced mouse ear oedema (Inoue et al., 1995b), but failed to prevent the response to mustard oil in the present study. It has been accepted that mast cells located in close proximity to perivascular spaces play an important part in acute inflammatory responses (Yano et al., 1989; Ramos et al., 1991). In addition to the activation of tachykinin NK_1 receptors, substance P enhances the inflammatory response by increasing vascular permeability through the release of vasoactive amines from mast cells (Erjavec et al., 1981; Fewtrell et al., 1982). Therefore, histamine H_1 receptor antagonists, as well as tachykinin receptor antagonists, are expected to have inhibitory effects on neurogenic inflammation (Foreman, 1987; Donnerer and Amann, 1993). However, evidence exists to indicate that mast cell activation is not essential for plasma extravasation of neurogenic inflammation (Jancsó et al., 1967; Kowalski and Kaliner, 1988; Kowalski et al., 1990). The present results also suggest that mast cell-derived mediators such as histamine and 5-HT do not participate in mustard oil-induced skin inflammation. The most likely explanation for this is that substance P released by mustard oil activated the tachykinin NK_1 receptor but that its level was insufficient to degranulate mast cells in ear skin. With regard to this, it has already been proposed that, at high doses, tachykinins activate mast cells whereas lower doses act directly on tachykinin receptors to cause plasma protein extravasation in rodent skin (Kowalski et al., 1990; Ahluwalia et al., 1995).

Reapplication of mustard oil did not induce desensitization of the exudative response. Furthermore, pretreatment (24 h before capsaicin application) of ears with mustard oil caused only 25% reduction of capsaicin-induced oedema (data not shown). Thus it may be possible that the stimulation by mustard oil at the concentration used in this study was less potent as a noxious agent than that by capsaicin on sensory neurones in the mouse skin, but this problem needs more research. In the present study we also found that the response to mustard oil was insensitive to ruthenium red. In contrast to this, capsaicin-induced mouse ear oedema is prevented dose dependently by this dye (Inoue et al., 1993). Ruthenium red is suggested to inhibit capsaicin-evoked release of neuropeptides (Maggi et al., 1988) by blocking the opening of the cation channel coupled to the capsaicin binding site (Maggi et al., 1989; Dray et al., 1990). This implies that mustard oil may elicit the activation of sensory nerve endings with a mechanism distinct from that of capsaicin.

In conclusion, we demonstrated that topical application of mustard oil to the mouse ear produces acute skin inflammation differently from capsaicin. Mediators derived from mast cells, such as histamine and 5-HT, are unlikely to participate in this model. In addition, the present data suggest that the tachykinin NK_1 receptor is involved principally during the first 5 min of the inflammatory response to mustard oil.

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