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Profile of capsaicin-induced mouse ear oedema as neurogenic inflammatory model: comparison with arachidonic acid-induced ear oedema

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1 We have investigated the mechanism of capsaicin-induced mouse ear oedema compared with that of arachidonic acid (AA)-induced ear oedema, and evaluated the possible involvement of neuropeptides in the development of capsaicin-induced oedema.

2 Topical application of capsaicin (0.1–1.0 mg per ear) to the ear of mice produced immediate vasodilatation and erythema followed by the development of oedema which was maximal at 30 min after the treatment. This oedema was of shorter duration with less swelling than AA-induced oedema (2.0 mg per ear).

3 Capsaicin-induced ear oedema was unaffected when inhibitors of arachidonate metabolites including platelet activating factor (PAF) were administered before capsaicin (250 μg per ear) application, while these agents significantly prevented AA-induced oedema. Dexamethasone, histamine H_1 and/or 5-hydroxytryptamine (5-HT) antagonists, and substance P (SP) antagonists were effective in inhibiting both models. Furthermore, a Ca^{2+}-channel blocker and the capsaicin inhibitor, ruthenium red, were effective inhibitors of capsaicin oedema but had no effect on AA-induced oedema.

4 Phosphoramidon (50 μg kg^{-1}, i.v.), an endopeptidase inhibitor, markedly ($P<0.001$) enhanced only capsaicin-induced ear oedema, but bestatin (0.5 mg kg^{-1}, i.v.), an aminopeptidase, failed to enhance oedema formation.

5 Neuropeptides (1–100 pmol per site) such as rat calcitonin gene-related peptide (CGRP), SP, neurokinin A (NKA), and vasoactive intestinal peptide (VIP), which are released from capsaicin-sensitive neurones, caused ear oedema by intradermal injection. Furthermore, a synergistic effect of CGRP (10 fmol per site) and SP (10 pmol per site) on oedema formation was observed.

6 The oedema induced by neuropeptides was significantly ($P<0.05$ or $P<0.001$) inhibited when cyproheptadine (20 mg kg^{-1}, p.o.), a histamine H_1 and 5-HT antagonist, was administered before injection. In contrast, nifedipine (50 mg kg^{-1}, p.o.), a Ca^{2+}-channel blocker, and indomethacin (10 mg kg^{-1}, p.o., except for NKA), a cyclo-oxygenase inhibitor, had little effect on neuropeptide-induced oedema.

7 These results suggest that the mechanism of capsaicin-induced ear oedema is different from that of AA-induced oedema and suggest that the development of capsaicin-induced ear oedema is primarily mediated by neuropeptides. The neuropeptides released after activation of sensory nerves cause an increase of vascular permeability by interactions with endothelial cells and by histamine (and 5-HT) release from mast cells.

Keywords: Capsaicin; ear oedema; ruthenium red; calcitonin gene-related peptide; substance P; vasoactive intestinal peptide; neurokinin A; phosphoramidon

Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the primary pungent ingredient of red peppers in the genus *Capsicum*, is well known to evoke neurogenic acute inflammatory responses such as axon reflex vasodilatation, plasma extravasation, coughing, and painful sensitization (see review by Holzer, 1991). With repeated administration, protective actions characterized by an inhibition of inflammation and antinociception have been found. Various actions of capsaicin appear as a result of selective stimuli of a subset of capsaicin-sensitive afferent neurones which contain neuropeptides as neurotransmitters (Holzer, 1988; Maggi & Meli, 1988 for reviews). Neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and neurokinin A (NKA), which are released by capsaicin from peripheral endings of afferent neurones, have been considered as the chemical mediators of skin inflammation (Holzer, 1988; 1991; Maggi & Meli, 1988; Saria *et al.*, 1988). Other studies have shown that inflammatory responses including oedema formation, vasodilatation and wheal are evoked in response to intradermal injection of neuropeptides (Williams, 1982; Devillier *et al.*, 1986; Brain & Williams, 1988). Further, neuropeptides have been found in the synovial fluid of patients with rheumatoid arthritis (Larsson *et al.*, 1989; Marshall *et al.*, 1990) and suggested to play a role in skin diseases including psoriasis (Farber *et al.*, 1986), blister, burn (Wallengren *et al.*, 1986), dermatographism and cold urticaria (Wallengren *et al.*, 1987).

Recently, it has been reported that capsaicin can induce acute inflammation in the mouse ear (Mantione & Rodriguez, 1990; Gábor & Rázga, 1992) and rat paw (De & Ghosh, 1990). However, direct evidence for the participation of mediators in this inflammatory response is lacking. In this paper the authors discuss the mechanism of capsaicin-induced mouse ear oedema compared with arachidonic acid (AA)-induced ear oedema which is predominantly mediated via the arachidonate pathway (Young *et al.*, 1984; Carlson *et al.*, 1985; Opas *et al.*, 1985; Chang *et al.*, 1986; Inoue *et al.*, 1988) and the possible involvement of neuropeptides in the capsaicin-induced ear oedema.

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Methods

Assay for capsaicin- and AA-induced mouse ear oedema

Six week old male ddY mice weighing 30–35 g (Japan SLC, Japan) were used for the experiments. Animals were conscious when tested. Induction of mouse ear oedema was based on the method of Mantione & Rodriguez (1990) for capsaicin and of Inoue *et al.* (1988) for AA. Capsaicin and AA were dissolved in acetone at a concentration of 12.5 mg ml^{-1} and 100 mg ml^{-1}, respectively and 20 μl (capsaicin; 250 μg per ear, AA; 2 mg per ear) was then applied topically to both surfaces of an ear of each mouse. The development of capsaicin-induced ear oedema was observed for 3 h following application. Pharmacological agents were dissolved in acetone or ethanol for topical application, and were suspended with 1% polyoxyethylene sorbitan monooleate (Tween 80, Tokyo Kasei Chemical Industry, Japan) in saline (0.9% NaCl) for oral and intravenous administration. Topical and oral administration were given 30 min and 60 min before capsaicin application, respectively. For AA-induced ear oedema, both treatments were given 30 min before AA application. Intravenous administration of agents was performed 15 min before the irritant treatment. Mice in the control group received the vehicle only. Ear thickness was measured 30 min and 60 min after capsaicin and AA treatment with dial calipers (Ozaki Factory, Japan) measuring to an accuracy of 0.01 mm. Ear oedema was expressed as an increase in ear thickness. Erythema was assessed with the naked eye compared with the untreated ear.

Intradermal injection of neuropeptides into the ear

Intradermal injections into the ear of male ddY mice (6 weeks old) were made with a hypodermic needle (0.28 × 18.0 mm) and a repeating dispenser (Hamilton Co., U.S.A.). The outer surface of the ear of mice anaesthetized with ether (Showa Ether Co., Japan) received an injection of 5 μl saline containing SP, CGRP, NKA, or VIP at various concentrations. Control mice received saline. Ear oedema was examined 30 min after peptide-treatment with dial calipers.

Materials

Pharmacological agents were purchased as follows: aspirin, Nakarai Chemical Co., Japan; AA, capsaicin, SP, αCGRP (rat), VIP (porcine), NKA, nordihydroguaiaretic acid (NDGA), indomethacin, pyrilamine, dexamethasone, cyproheptadine, chlorpheniramine, nifedipine, verapamil, diltiazen, nicardipine, cinnarizine, flunarizine, ruthenium red, [D-Pro^2,D-$Trp^{7,9}$]-SP, [D-Arg^1,D-$Trp^{7,9}$,Leu^{11}]-SP (spantide), [D-P-Cl-Phe^6,Leu^{17}]-VIP, CGRP fragment $_{8-37}$, bestatin, D-Arg,-[Hyp^3,D-Phe^7]-bradykinin (NPC 567), pimozide, Nα-adamantaneacetyl-D-Arg-[Hyp^3,$Thi^{5,8}$,D-Phe^7]-bradykinin, [$Thi^{5,8}$,D-Phe^7]-bradykinin, [Leu^8, des Arg^9]-bradykinin, tetracaine and phosphoramidon, Sigma Chemical Co., U.S.A.; gossypol and CV 3988 (rac-3-CN-n-octadecyl carbamoyloxy)-2-methoxypropyl-2-thiazolioethyl-phosphate), Biomol Research Laboratories, U.S.A.; methysergide, amiloride and loperamide, Research Biochemicals Incorporated, U.S.A.; PGAS 385 (H_2N-Lys-Pro-Arg-Arg-Pro-Tyr-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2, VIP antagonist) and [Ac-Tyr^1,D-Phe^2]-GRF (1–29) amide, Bachem Fine Chemicals Inc., U.S.A.; AA 861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone) was a generous gift from Takeda Chemical Industry, Japan. AC 5-1 (3,4,2′,4′-tetrahydroxy-2-geranyldihydrochalcone) was prepared by Dr Y. Fujimoto, Faculty of Pharmaceutical Sciences, Nihon University.

Statistics

The statistical analysis system (Meancomp) package (Institute of Endocrinology, Gunma University, Japan) was used for data analysis. Results are expressed as mean ± s.e.mean. Statistical significance of differences between control and test groups was determined by Student's *t* test for unpaired data or the Cochran-Cox test.

Results

Development of mouse ear oedema induced by a topical application of capsaicin

The ear oedema induced by a single application of capsaicin at various doses was examined for 3 h by measuring ear thickness. Skin reactions such as erythema due to vasodilatation appeared immediately and oedema formation became detectable at 5 min after treatment (Figure 1). As previously found (Mantione & Rodriguez, 1990; Gábor & Rázga, 1992), the ear oedema developed rapidly and reached a maximum at 30 min after capsaicin treatment. Then oedema gradually decreased with time but erythema was attenuated at 2 h. A dose-dependent enhancement of oedema development was not observed with increased concentrations of capsaicin (100–1000 μg). In the subsequent experiments, induction of ear oedema was performed by treatment with capsaicin at a dose of 250 μg, which is the same dose as previously studied (Mantione & Rodriguez, 1990).

Effect of pharmacological agents on capsaicin- and AA-induced mouse ear oedema

The influence of various agents on both ear oedema induced by a single application of 250 μg capsaicin and 2 mg AA is summarized in Table 1a,b. AA-induced ear oedema reached a maximum level at 60 min after the treatment (Young *et al.*, 1984; Chang *et al.*, 1986; Inoue *et al.*, 1988). The capsaicin-induced ear oedema was less than AA-induced oedema, and an increase in ear thickness at 30 and 60 min after capsaicin and AA was 0.132 ± 0.023 and 0.308 ± 0.021 mm (66 experiments, $n = 6$), respectively. A PAF antagonist (0.5 or 1.0 mg per ear) and 5-lipoxygenase inhibitors (1.0 mg per ear) such as NDGA, AC-5, and AA 861 significantly inhibited AA-induced mouse ear oedema while these inhibitors did not suppress capsaicin-induced ear oedema. Dexamethasone (0.1 mg per ear), a steroid anti-inflammatory drug; chlorpheniramine (25 mg kg^{-1}, p.o.), a histamine H_1 blocker; methysergide (4 mg kg^{-1}, i.v.), a 5-HT antagonist; and cyproheptadine (25 mg kg^{-1}, p.o.), a histamine H_1 and 5-HT antagonist; were very effective in both models. The Ca^{2+}-channel blockers, nifedipine, verapamil, diltiazen, loperamide, nicardipine, and flunarizine significantly inhibited capsaicin-induced ear oedema at doses of less than 50 mg kg^{-1} (p.o.). However, these compounds had no effects on AA-induced

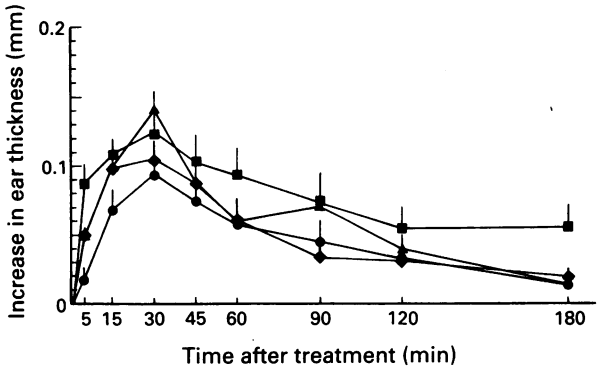

Figure 1 The time course of ear oedema formation induced by capsaicin. Mouse ears were treated by topical application of capsaicin at various doses. Ear oedema is the mean ear thickness of test animals minus that of animals before treatment. Each point represents the mean ± s.e.mean value from 7 animals; (●) 100 μg; (▲) 250 μg; (◆) 500 μg; (■) 1000 μg.

Table 1a Effects of pharmacological agents on capsaicin- and arachidonic acid (AA)-induced mouse ear oedema

Class	Dose (mg kg^{-1}, ear)	Route	Inhibition (%) Capsaicin oedema	AA oedema
Inhibitor of AA pathway				
Indomethacin	10	p.o.	− 20 ± 17	24 ± 6*
	1.0	t.a.	− 2 ± 16	57 ± 8***
Aspirin	200	p.o.	− 22 ± 25	19 ± 7
	1.0	t.a.	− 4 ± 20	− 12 ± 6
NDGA	1.0	t.a.	− 1 ± 20	44 ± 3***
AC-5	1.0	t.a.	− 5 ± 18	66 ± 6***
AA 861	1.0	t.a.	− 10 ± 13	53 ± 7***
Tetracaine	1.0	t.a.	33 ± 6	53 ± 8**
Dexamethasone	0.1	t.a.	76 ± 4***	56 ± 5***
PAF antagonist				
Gossypol	1.0	t.a.	2 ± 10	55 ± 7***
CV 3988	0.5	t.a.	− 38 ± 10	33 ± 8**
Histamine and 5-HT antagonist				
Cyproheptadine	12.5	p.o.	33 ± 14	
	25		35 ± 11*	64 ± 4***
	50		52 ± 4***	70 ± 3***
Histamine antagonist				
Chlorpheniramine	12.5	p.o.	7 ± 17	
	25		37 ± 7**	31 ± 9*
	50		54 ± 4**	26 ± 5*
Pyrilamine	50	p.o.	− 7 ± 14	13 ± 4*
5-HT antagonist				
Methysergide	2	i.v.	31 ± 13	35 ± 12*
	4		53 ± 6**	52 ± 11**
Ca^{2+}-channel blocker				
Nifedipine	12.5	p.o.	42 ± 5	
	25		64 ± 8*	− 2 ± 4
	50		71 ± 6**	3 ± 4
Verapamil	12.5	p.o.	44 ± 8	
	25		61 ± 5*	− 10 ± 3
	50		69 ± 3*	6 ± 6
Diltiazen	25	p.o.	13 ± 7	
	50		46 ± 6**	− 1 ± 1
Loperamide	25	p.o.	37 ± 12	− 1 ± 4
	50		56 ± 6***	7 ± 5
Nicardipine	25	p.o.	− 2 ± 18	− 7 ± 2
	50		58 ± 2**	6 ± 4
Cinnarizine	50	p.o.	19 ± 11	4 ± 4
Pimozide	50	p.o.	25 ± 14	18 ± 5
Amiloride	50	p.o.	21 ± 11	3 ± 4
Flunarizine	25	p.o.	2 ± 12	− 1 ± 3
	50		53 ± 4**	6 ± 5

Test compounds were applied topically (t.a.) 30 min (but dexamethasone was given 3 h) before irritant treatment. Oral administration (p.o.) was performed 30 min for AA oedema and 60 min for capsaicin oedema before treatment. Intravenous administration was 15 min before treatment. Values are expressed as mean ± s.e.mean of 6–7 animals. Statistical significance from the control at $*P<0.05$, $**P<0.01$ and $***P<0.001$.

oedema. Ruthenium red, a reported capsaicin inhibitor (Amann & Maggi, 1991 for review), inhibited capsaicin-induced oedema dose-dependently with an ED_{50} value of 0.40 (95% limit; 0.27–0.61) mg kg^{-1} (i.v.) (Figure 2). Of the neuropeptide receptor antagonists, the SP antagonist, spantide and [D-Pro^2,D-Trp^{7,9}]-SP (0.25 or 0.50 mg kg^{-1}, i.v.), significantly inhibited both models. Three kinds of VIP antagonists showed a slight effect, but no significant suppression, of capsaicin-induced ear oedema. $CGRP_{8-37}$ (CGRP antagonist) had liitle effect in either model. Bradykinin (BK) antagonists of B_1 and B_2 receptors were ineffective in both types of ear oedema. The ear oedema induced by capsaicin was significantly ($P<0.001$) enhanced by the intravenous administration of phosphoramidon (endopeptidase inhibitor) at a dose of 50 μg kg^{-1}. This compound had no effect on AA-induced ear oedema at less than 500 μg kg^{-1} (Table 2). Phosphoramidon itself did not have any effect on the ear, and bestatin (500 μg kg^{-1}, i.v.), an aminopeptidase inhibitor, did not enhance the inflammatory response.

Direct effect of neuropeptides given by intradermal injection into mouse ear

As phosphoramidon enhanced the capsaicin-induced ear oedema, participation of neuropeptides such as CGRP, SP, NKA, and VIP on oedema formation was examined by their intradermal injection into the ear. The ear injected with CGRP, SP, NKA, and VIP (10 or 100 pmol per site) swelled significantly ($P<0.05$ or $P<0.01$) (Figure 3). NKA caused marked ear oedema ($P<0.01$) even when injected at a low concentration (1 pmol per site). Furthermore, we examined the possibility of interaction of neuropeptides in the development of ear oedema (Figure 4). CGRP (1 pmol per site) and SP (10 pmol per site) themselves did not produce significant oedema: there were no differences in ear thickness from the control. However, ear oedema was induced ($P<0.001$) when SP and CGRP were injected together into ear and additionally SP (10 pmol per site) promoted oedema formation ($P<0.001$) in the presence of CGRP (10 fmol per site). But

Table 1b Effects of pharmacological agents on capsaicin- and arachidonic acid (AA)-induced mouse ear oedema

Class	*Dose* (mg kg^{-1}, ear)	*Route*	*Inhibition* (%) *Capsaicin oedema*	*AA oedema*
Capsaicin antagonist				
Ruthenium red	0.5	i.v.	49 ± 7*	
	1.0		70 ± 8**	10 ± 5
Substance P antagonist				
[D-Pro^2,D-Trp^{7,9}]-SP	0.25	i.v.	− 9 ± 18	− 3 ± 7
	0.50		53 ± 7**	41 ± 2***
[D-Arg^1,D-Trp^{7,9},Leu^{11}]-SP (Spantide)	0.25	i.v.	19 ± 7	38 ± 7**
	0.50		40 ± 4*	59 ± 4***
VIP antagonist				
[D-P-Cl-Phe^6,Leu^{17}]-VIP	0.50	i.v.	32 ± 19	21 ± 7
[Ac-Tyr^1,D-Phe^2]-GRF (1-29) amide	0.50	i.v.	27 ± 4	not tested
PGAS 385	0.50	i.v.	25 ± 9	1 ± 5
CGRP antagonist				
CGRP fragment$_{8-37}$	0.50	i.v.	− 1 ± 14	3 ± 1
Bradykinin antagonist				
Nα-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK	0.50	i.v.	38 ± 8	− 4 ± 6
D-Arg,[Hyp^3,D-Phe^7]-BK	0.50	i.v.	21 ± 13	− 9 ± 4
[Thi^{5,8},D-Phe^7]-BK	0.50	i.v.	21 ± 12	− 19 ± 6
[Leu^8,desArg^9]-BK	0.50	i.v.	32 ± 6	2 ± 4

Test compounds were administered intravenously 15 min before irritant treatment. Values are expressed as mean ± s.e.mean of 6–7 animals. Statistical significance from the control at $*P<0.05$, $**P<0.01$ and $***P<0.001$.

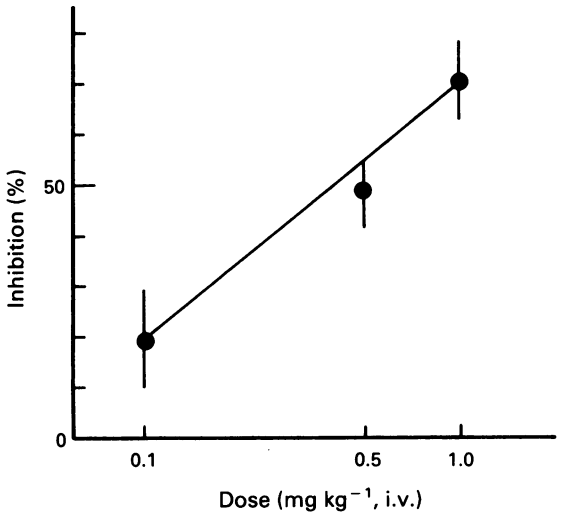

Figure 2 The inhibitory effect of ruthenium red on capsaicin-induced mouse ear oedema. The dye was administered 15 min (i.v.) before capsaicin treatment (250 μg per ear). Ear oedema was measured 30 min after treatment. Each point represents the mean ± s.e.mean value from 4–5 experiments ($n = 6$).

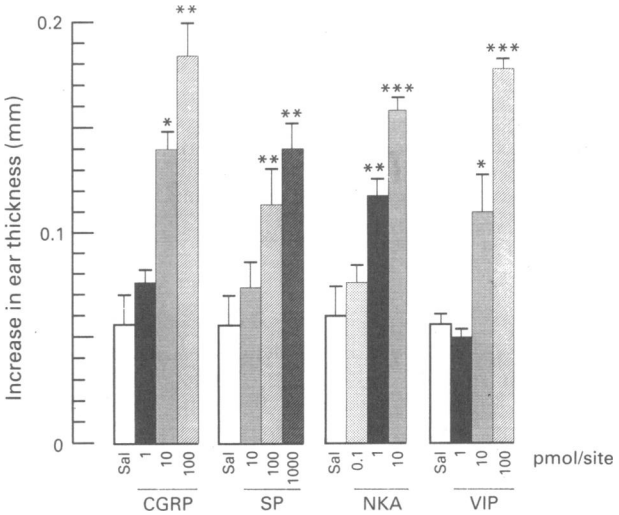

Figure 3 The effect of neuropeptides on oedema formation by intradermal injection. Ear oedema is expressed as increase in ear thickness of test animals at 30 min after treatment. Each column represents mean value (with s.e.mean) from 6 animals. Statistical significance at $*P<0.05$, $**P<0.01$, and $***P<0.001$.

Table 2 Enhancement by phosphoramidon of capsaicin-induced ear oedema

	Dose (μg kg^{-1})	*Increase in ear thickness* (mm) *Capsaicin oedema*	*AA oedema*
Control		0.134 ± 0.008	0.319 ± 0.021
Phosphoramidon	25	0.120 ± 0.013	
	50	0.203 ± 0.009***	
	100	0.330 ± 0.013***	
	500		0.307 ± 0.015
Control		0.107 ± 0.004	not tested
Bestatin	250	0.115 ± 0.005	
	500	0.104 ± 0.008	

Compounds were administered intravenously 15 min before irritant treatment. Values are expressed as mean ± s.e.mean of 6–7 animals. Statistical significance at $***P<0.001$.

concomitant injection of CGRP (1 pmol per site) together with SP, at a dose of less than 1 pmol per site, failed to produce oedema. This synergistic effect of CGRP and SP on oedema formation was observed at much lower doses than those previously reported in the rat (Gamse & Saria, 1985; Cruwys *et al.*, 1992).

Effect of indomethacin, nifedipine, and cyproheptadine on neuropeptide-induced ear oedema

We examined some pharmacological agents on mouse ear oedema induced by neuropeptides such as SP, CGRP, NKA, and VIP to clarify whether these peptides cause oedema directly or indirectly by promoting the release of chemical mediators leading to the inflammatory response. Active agents were given 30 min before peptide treatment. Indomethacin, a cylco-oxygenase inhibitor, significantly ($P<0.01$)

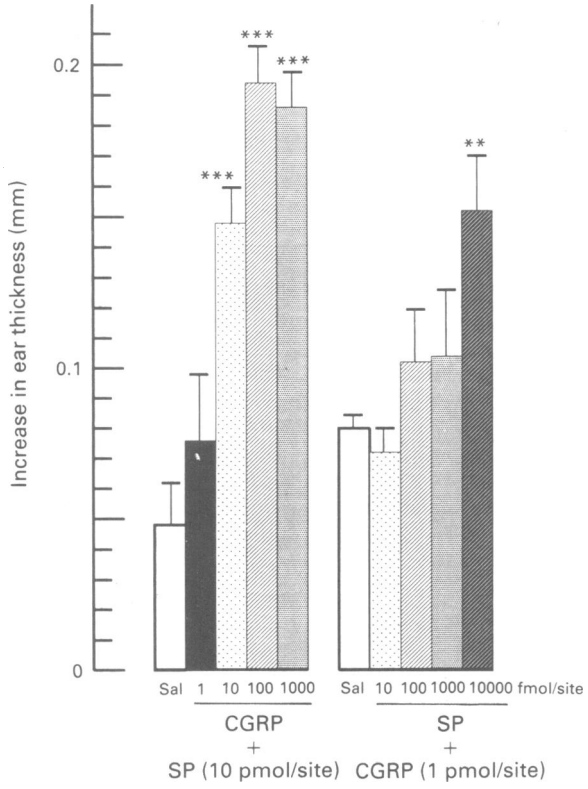

Figure 4 The synergistic effect of calcitonin gene-related peptide (CGRP) and substance P (SP) on oedema formation by concomitant injection. Oedema was induced by co-injected SP (10 pmol) with various concentrations of CGRP (1–1000 fmol) per site or co-injected CGRP (1 pmol) with various concentrations of SP (10–10000 fmol) per site. Ear oedema is expressed as increase in ear thickness of test animals at 30 min after treatment. Each column represents mean value with s.e.mean from 6 animals. Statistical significance from the control (Sal) at $^{**}P<0.01$ and $^{***}P<0.001$.

suppressed NKA-induced ear oedema at a dose of 10 mg kg^{-1} (p.o.) but CGRP-, SP-, and VIP-induced oedema were not affected (Figure 5). Nifedipine (50 mg kg^{-1}, p.o.), a Ca^{2+}-channel blocker, had little effect on any oedema. In contrast, cyproheptadine (20 mg kg^{-1}, p.o.), a histamine H_1 and 5-HT antagonist, strongly inhibited ear oedema induced by neuropeptides (1–100 pmol per site). Of the peptide antagonists (0.5 mg kg^{-1}, i.v.), spantide was a potent inhibitor ($P<0.001$) of SP oedema, and PGAS 385 also prevented ($P<0.05$) VIP-induced oedema. However, $CGRP_{8-37}$ did not suppress CGRP-induced oedema.

Discussion

The topical application of capsaicin to rat skin leads to excitation of afferent neurones (Kenins, 1982), increases in skin blood flow (Jancsó, 1968), and vasodilatation (Lynn *et al.*, 1992). In our experiment, the capsaicin-treated mouse ear became erythematous and oedematous, reaching a maximum effect at 30 min. On the other hand, a single application of AA (2 mg per ear) to the mouse ear resulted in a maximal peak of oedema formation 1 h after treatment. It is well known that the products of arachidonate pathway primarily participate in the AA-induced ear oedema, and the synthesis of prostaglandin E_2 (PGE_2), leukotriene C_4 (LTC_4) and LTD_4 is induced immediately after AA treatment (Young *et al.*, 1984; Opas *et al.*, 1985; Chang *et al.*, 1986; Inoue *et al.*, 1988).

In the present study, inhibitors of arachidonate metabolites were very effective in suppressing AA-induced ear oedema. These compounds, however, had little effect on capsaicin-induced mouse ear oedema. In addition, indomethacin (cyclo-oxygenase inhibitor) failed to prevent oedema induced by neuropeptides, except for NKA. These findings suggest that prostaglandins and leukotrienes of arachidonate metabolites cannot primarily mediate the development of capsaicin-induced ear oedema. It is also unlikely that the participation of PAF is essential for the development of capsaicin-induced ear oedema, although PAF is a candidate for mediation of AA-induced oedema (Merlos *et al.*, 1991). In contrast, histamine and 5-HT are involved in capsaicin- and AA-induced ear oedema, since their antagonists were inhibitors of both. Others have demonstrated that antagonists of histamine H_1 and SP can inhibit capsaicin-induced rat paw oedema (De & Ghosh, 1990). Similarly, SP antagonists such as [D-Pro^2,D-Trp^{7,9}]-SP and spantide are effective inhibitors of oedema induced by both agents suggesting that SP release is contributing to inflammatory response in both models.

Ruthenium red, an inorganic dye, suppressed capsaicin-induced ear oedema dose-dependently, while AA-induced oedema was unaffected. Other studies have shown that the dye significantly inhibits capsaicin-evoked release of neuropeptides (Maggi *et al.*, 1988; Amann *et al.*, 1989) by blocking the opening of the cation channel coupled to the capsaicin receptor (Maggi *et al.*, 1989a; Dray *et al.*, 1990). It is established that capsaicin evokes an influx of extracellular Ca^{2+} (Jancsó *et al.*, 1984; Zernig *et al.*, 1984) and acts by stimulating sensory C-fibre afferents through an effect on cation channels (Marsh *et al.*, 1987; review by Maggi & Meli, 1988). We have also found that some Ca^{2+}-channel blockers are able to inhibit capsaicin-induced oedema. Release of neurotransmitter is generally considered to require the presence of extracellular Ca^{2+}, but capsaicin-evoked release of neuropeptides was not reduced by blockade of voltage-sensitive Ca^{2+}-channels (Marsh *et al.*, 1987; Wood *et al.*, 1988; Maggi *et al.*, 1989b). Nevertheless, in our study, ruthenium red and Ca^{2+}-channel blockers with L-type effects such as nifedipine and verapamil were very effective in preventing the development of capsaicin oedema. In addition to this, nifedipine did not affect the oedema induced by intradermal administration of neuropeptides. Thus our data suggest that the inhibitory actions of the dye and Ca^{2+}-channel blockers on capsaicin oedema would either interrupt the coupling of the capsaicin recognition site or an increase in membrane permeability to cations, or some other way with the activation of the channels. Furthermore, it is possible that Ca^{2+} plays an important role as a second messenger in the initial stage of capsaicin-induced ear oedema, although this is a subject of debate.

The administration of phosphoramidon, a specific inhibitor of endopeptidase-24,11, resulted in a remarkable enhancement of the capsaicin-induced ear oedema. This enzyme can hydrolyze a variety of neuropeptides such as SP and NKA (Matsas *et al.*, 1984). Previous studies have shown that phosphoramidon enhanced tachykinin-induced behavioural responses of mice (Sakurada *et al.*, 1990) and that levels of SP and NKA released by capsaicin increased in guinea-pig lungs superfused with endopeptidase inhibitor (Martins *et al.*, 1991). This evidence suggests that phosphoramidon enhances the inflammatory response of capsaicin oedema by inhibiting endogenous endopeptidase in mouse ear, and the short duration of ear oedema is due to the rapid metabolism of neuropeptides by endopeptidase(s) in the ear.

We have shown that SP, CGRP, NKA, and VIP in pmol doses can produce ear oedema which is substantially reduced by cyproheptadine (a histamine H_1 and 5-HT antagonist) and certain specific neuropeptide antagonists. However, the CGRP antagonist, $CGRP_{8-37}$, was ineffective in inhibiting both capsaicin- and CGRP-induced ear oedema. This result suggests that CGRP-induced development of mouse ear oedema is dependent on CGRP-induced mast cell activation and independent of the $CGRP_1$ receptor. Furthermore, our

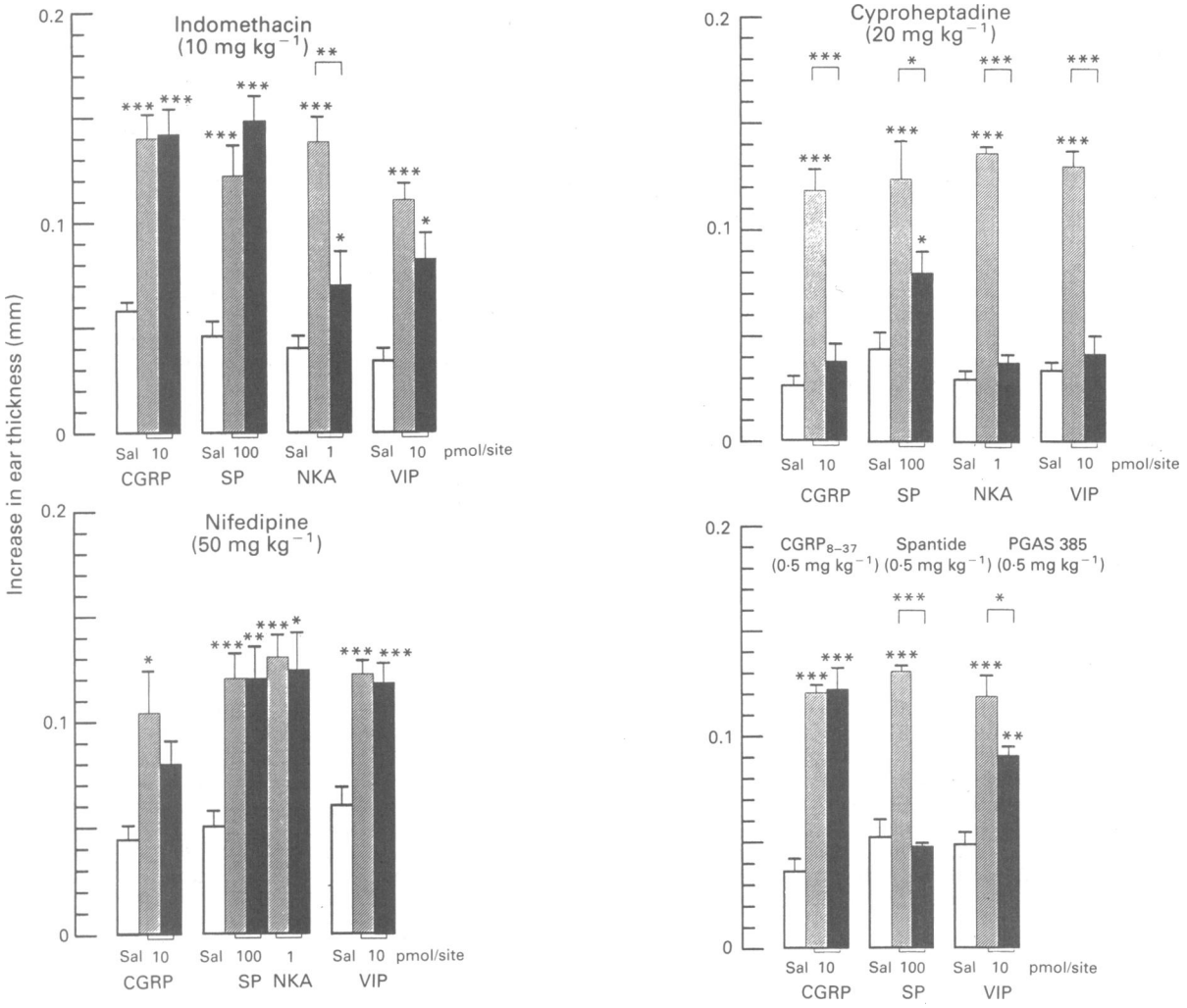

Figure 5 The effect of indomethacin, nifedipine, and cyproheptadine and peptide antagonists on neuropeptide-induced ear oedema. Ear oedema is expressed as increase in ear thickness of test animals at 30 min after intradermal injection. Test compounds were administered (p.o.) 30 min before injection except for peptide antagonists which were given at 15 min (0.5 mg kg^{-1}, i.v.) before injection. Open columns: saline (control); hatched columns: neuropeptide alone; solid columns: neuropeptide plus test compound treatment. Each column represents mean value (with s.e.mean) from 6 animals. Statistical significance at $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

studies confirmed that the synergistic effect of neuropeptides such as CGRP and SP in fmol could be participating in the development of ear oedema. Neuropeptides have been reported to release histamine from mast cells (Piotrowski & Foreman, 1985; 1986; Devillier *et al.*, 1986), but capsaicin did not cause histamine release from mast cells directly (data not shown). Additionally, mast cells are numerous around blood vessels and nerves (Nilsson *et al.*, 1990); this is further support for the suggestion that the neuropeptides evoked by capsaicin through activation of sensory nerves are able to release histamine (and 5-HT) from mast cells as the chemical mediator, and this response is an essential pathway in the development of capsaicin-induced ear oedema. In addition to this, it is very likely that the neuropeptides caused oedema formation by increasing vascular permeability through the activation of endothelial cells, as reported by others (Iwamoto & Nadel, 1989).

In conclusion, our studies demonstrate that the mechanism of capsaicin-induced mouse ear oedema is quite different from that of AA-induced oedema and that neuropeptides such as CGRP, SP, VIP, and NKA can induce oedema formation, and suggest that neuropeptides play important roles of releasing histamine (and 5-HT) as the secondary mediator of increased vascular permeability at inflammatory sites. Thus it is likely that the capsaicin-induced ear oedema is primarily mediated by neuropeptides evoked by capsaicin through activation of sensory nerves in the mouse skin. However, it is also possible that other peptides and inflammatory mediators are involved in the development of capsaicin-induced oedema.

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