Participation of Serotonin in Capsaicin-Induced Mouse Ear Edema

Hideo Inoue¹, Nobuyuki Nagata¹ and Yasuko Koshihara²

¹Research Laboratory, Minophagen Pharmaceutical Co., 2–5233, Komatsubara, Zama, Kanagawa 228, Japan
²Department of Biosignal Research, Tokyo Metropolitan Institute of Gerontology, Sakaecho, Itabashi-ku, Tokyo 173, Japan

Received April 13, 1995 Accepted June 18, 1995

ABSTRACT—We investigated the involvement of serotonin (5-HT) in mouse ear edema induced by topical application of capsaicin (250 µg/ear). Application of capsaicin to the ear caused degranulation of mast cells in skin connective tissue. Capsaicin-induced ear edema was significantly inhibited by preadministration of 5-HT2 receptor antagonists such as ketanserin (2 mg/kg, i.v.) and LY 53857 (1 mg/kg, i.v.), but not 5-HT1-, 5-HT3- and 5-HT4-receptor antagonists. Intradermal injection of α-methyl 5-HT (5-HT2-receptor agonist) and 5-HT into ear skin produced edema formation more potently than 8-OH-DPAT (5-HT1A agonist) and 2-methyl 5-HT (5-HT3 agonist). 5-HT2 antagonists markedly suppressed the edema response to 5-HT and its receptor agonists, whereas any antagonist for 5-HT1, 5-HT3 and 5-HT4-receptors had no effect. Furthermore, 5-HT2-receptor antagonists partly prevented ear edema in response to substance P (SP), a putative mediator of capsaicin-induced edema, and compound 48/80, a releaser of vasoactive amines from mast cells. These results suggest that 5-HT released from mast cells is partly involved in the development of capsaicin-induced mouse ear edema via 5-HT2 receptors in the ear skin.

Keywords: Capsaicin, Serotonin (5-HT), Ear edema (mouse), 5-HT receptor, 5-HT-receptor antagonist

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent substance contained in many red peppers, evokes neurogenic inflammatory responses such as axon reflex vasodilation, plasma extravasation and painful sensitization (1). Topical application of capsaicin to the mouse ear produces neurogenic skin inflammation (2, 3), which may be mediated by neuropeptides including substance P (SP) released through activation of primary afferent sensory neurons. Virus and Gebhart (4) have suggested that in addition to SP, serotonin (5-HT) also plays a role in the pharmacological activity of capsaicin. Others have reported that treatment of newborn rats with capsaicin causes an increase in the concentrations of histamine and 5-HT in the rat skin as a result of changes of the mast cell system in response to the permanent degeneration of sensory nerves (5). In fact, evidence exists to indicate that peripheral nerve endings and mast cells are in close spatial and functional association (6–8). Previous studies have found that methysergide is effective in inhibiting capsaicin-induced mouse ear edema (3). However, clear evidence for the involvement of 5-HT in capsaicin-induced inflammation is lacking.

5-HT receptors have been classified into at least four subtypes: 5-HT1, 5-HT2, 5-HT3 and 5-HT4 (9, 10). Among them, 5-HT2 receptors participate in the increase of cutaneous vascular permeability induced by 5-HT in rats (11, 12). 5-HT3 receptors are located on neurons and elicit depolization which results in transmitter release from parasympathetic, sympathetic, sensory and enteric neurons (13). Recent studies have shown that neuropeptides are partly released by mediation of 5-HT3 receptor from capsaicin-sensitive afferent neurons (14). These evidence suggest the possibility that 5-HT receptors may mediate neurogenic inflammation.

In the present study, the authors examined the involvement of 5-HT and its receptor subtypes in capsaicin-induced mouse ear edema by using their respective receptor antagonists. For this, we also investigated the 5-HT receptors involved in ear skin inflammation evoked by intradermal injection of 5-HT receptor-agonists and SP.

MATERIALS AND METHODS

Animals

Six-week-old male ddY mice weighing 30–35 g (Japan SLC, Hamamatsu) were used for the experiments. The animals were kept in an environmentally controlled room (24±1°C, 55±10% humidity) and allowed free access to food and water.
Capsaicin-induced mouse ear edema

Induction of mouse ear edema was performed as reported previously (3). Animals were conscious when tested. Capsaicin was dissolved in acetone at a concentration of 12.5 mg/ml, and 20 µl (250 µg/ear) was then applied topically to both surfaces of an ear of each mouse. Test compounds were intravenously administered to the tail vein 15 min before capsaicin treatment. GR 113808 was given 5 min before the capsaicin treatment because this drug would be rapidly hydrolyzed in vivo (a communication from Glaxo Research and Development, Ltd.). The control mice received the vehicle. The magnitude of edema was assessed by measuring the thickness at the edge of the ear before and 30 min after capsaicin treatment in units of 0.001 mm with dial calipers (Ozaki Factory, Tokyo). Ear edema was expressed as an increase in ear thickness.

Histological examination of mast cells in mouse ear skin

At 30 min after capsaicin treatment, ears were removed by cutting horizontally across the indentation at the base of the ear and fixed in 10% neutral buffered formaldehyde. Sections of the tissue were stained with toluidine blue for microscopic observation of mast cells.

Mouse ear edema induced by intradermal injection of 5-HT, 5-HT agonists, substance P and compound 48/80

Mice were anesthetized with animal ether (Showa Ether Co., Tokyo). Intradermal injections were made into the central site of the ear with a hypodermic needle (0.28 x 18.00 mm) and a repeating dispenser (Hamilton Co., Reno, NV, USA). A blister was formed in the outer surface of the ear by injection of 5 µl of saline containing 5-HT (0.01 - 10 nmol/site), 5-HT agonists (0.01 - 10 nmol/site), SP (100 pmol/site) and compound 48/80 (5 µg/site). Test compounds were intravenously administered 15 min, but GR 113808 was given 5 min, before intradermal injection. The control mice received the vehicle. Ear thickness was measured at the edge of the ear with dial calipers before and 30 min after injections.

Drugs

The following drugs were used: spiroxatrine, R(+)-8-hydroxy-DPAT hydrobromide, methysergide maleate, LY 278584 maleate (1-methyl-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1H-indazole-3-carboxamide maleate), ICS 205-930 (3-tropanyl-indole-3-carboxylate), LY 53857 maleate (6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid (8β)-2-hydroxy-1-methylpropyl ester (Z)-2-butenedioate (1 : 1), MDL 72222 (3-tropanyl-3,5-dichlorobenzoate), α-methyl-5-hydroxytryptamine maleate and (±)-2-methyl-5-hydroxytryptamine maleate (Research Biochemicals Incorporated, Natic, MA, USA); spantide II (H-D-Lys(nicotinoyl)-Pro-[3-(3-pyridyl)-Ala]- Pro-D-Phe(3,4-Cl)-Asn-D-Trp-Phe-D-Trp-Leu-Nle-NH2) (Nova Biochem, Läufelfingen, Switzerland); 5-hydroxytryptamine creatine sulfate (5-HT), ketanserin, substance P, chlorpheniramine maleate salt and (S)(−)-propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO, USA). GR 113808 (1-[2-(methylsulphonylamino)-ethyl]-4-piperidinyl)methyl 1-methyl-1H-indole-3-carboxylate maleate salt) was a generous gift from Glaxo Research and Development, Ltd., Middlesex, UK. Spantide II, spiroxatrine, methysergide, ketanserin and MDL 72222 were dissolved in DMSO (dimethyl sulfoxide; Wako Pure Chemical Industries, Osaka) and diluted with saline (final DMSO concentration, less than 1%). All other drugs were dissolved in saline.

Data analyses

Results are expressed as the mean ± S.E.M. Statistical significance of differences between the control and test groups was determined by Student’s t-test for unpaired data or the Cochran-Cox test after one-way analysis of variance.

RESULTS

Histological change in capsaicin-induced mouse ear edema

Ear edema in mice developed rapidly and reached a maximum at 30 min after topical application of capsaicin, as previously reported (3). Influence of mast cells in mouse ears treated with capsaicin was histologically examined by staining with toluidine blue (Fig. 1). Only a small number of neutrophils in the connective tissue of ear skin was observed 30 min after capsaicin treatment (H. Inoue, unpublished data). Toluidine blue sections showed degranulation of mast cells and mast-cell distribution in connective tissue of ears treated with capsaicin. Application of the vehicle (acetone) caused no detectable mast cell degranulation.

Effects of 5-HT receptor antagonists on capsaicin- and 5-HT-induced mouse ear edema

5-HT-induced edema was fully developed at 30 min after injection. The increase in ear thickness at 30 min after capsaicin (250 µg/ear) and 5-HT (1 nmol/site) treatment was 0.163 ± 0.011 mm and 0.236 ± 0.007 mm (17 experiments, n = 6 - 7), respectively. In addition, the basal ear thickness (treated with saline without 5-HT) was 0.063 ± 0.004 mm (8 experiments, n = 6). Acetone had no effect on ear thickness. 5-HT2-receptor antagonists such as ketanserin and LY 53857 at an i.v. dose of 1 mg or 2 mg/kg significantly inhibited capsaicin-induced ear edema (Table 1). Methysergide (2 mg/kg, i.v.), a 5-HT1A- and
5-HT2-receptor antagonist, also inhibited capsaicin-induced edema. Further increases in the dose of ketanserin, LY 53857 and methysergide were unable to inhibit capsaicin edema (H. Inoue, unpublished data). Furthermore, these 5-HT2-receptor antagonists at less than 0.5 mg/kg, i.v. showed a significant inhibition of ear edema induced by intradermal injection of 5-HT. The ID50 values of ketanserin, LY 53857 and methysergide on 5-HT-induced edema were 0.64 (95% limit: 0.49–0.83) mg/kg, i.v.; 0.54 (95% limit: 0.37–0.71) mg/kg, i.v.; and 0.63 (95% limit: 0.39–1.01) mg/kg, i.v., respectively. In contrast, antagonists for 5-HT1, 5-HT3 and 5-HT4 receptors at a dose of 4 mg/kg had little effect on edema formation induced by both capsaicin and 5-HT.

Development of mouse ear edema induced by 5-HT-receptor agonists

Ear edema was dose-dependently induced by intradermal injection of 5-HT and its receptor agonists such as 8-OH-DPAT (5-HT1A), a-methyl 5-HT (5-HT2) and
2-methyl 5-HT (5-HT$_3$) at doses of 0.01 to 10 nmol/site (Fig. 2). Among them, α-methyl 5-HT and 5-HT at a low dose of 0.1 nmol/site produced potent ear edema more than 8-OH-DPAT and 2-methyl 5-HT: the rank order of potency for inducing edema was 5-HT > α-methyl 5-HT > 2-methyl 5-HT > 8-OH-DPAT.

### Effects of 5-HT receptor antagonists on mouse ear edema induced by 5-HT$_1$ agonists

Ear edema was evoked in response to intradermal injection of 8-OH-DPAT (10 nmol/site), α-methyl 5-HT (1 nmol/site) or 2-methyl 5-HT (10 nmol/site). LY 53857 (0.5 mg/kg, i.v.) or ketanserin (0.5 mg/kg, i.v.), the 5-HT$_1$-receptor antagonist, significantly inhibited ear edema induced by all 5-HT receptor agonists including α-methyl 5-HT (a 5-HT$_1$ agonist) (Table 2). In contrast, spiroxatrine and (S)(-)propranolol, the 5-HT$_1$-receptor antagonist, at a dose of 4 mg/kg, i.v. had no effect on edema induced by 8-OH-DPAT (a 5-HT$_1$ agonist). 5-HT$_3$-receptor antagonists such as LY 278584 (4 mg/kg, i.v.) were also ineffective in inhibiting α-methyl 5-HT- and 2-methyl 5-HT (a 5-HT$_3$ agonist)-induced ear edema.

### Test compounds were intravenously administered 15 min before treatment of 8-OH-DPAT (10 nmol/site), α-methyl 5-HT (1 nmol/site) or 2-methyl 5-HT (10 nmol/site). Ear edema was examined 30 min after agonist injection. Values represent the means ± S.E.M. of 6 animals. Statistical significance compared with the control: *P<0.05, **P<0.01 and ***P<0.001.

### Effects of 5-HT-receptor antagonists on mouse ear edema induced by substance P

The 5-HT$_2$-receptor antagonists, ketanserin (2 mg/kg, i.v.) and LY 53857 (1 mg/kg, i.v.), significantly inhibited the ear edema induced by intradermal injection of SP (100 pmol/site) by approximately 30% (Table 3). In contrast, no significant inhibition of the edema formation was observed by pretreatment with antagonists for 5-HT$_1$, 5-HT$_3$ and 5-HT$_4$ receptors. Spantide II, a peptide tachykinin NK$_1$-receptor antagonist, at a dose of 0.5 mg/kg, i.v produced a strong inhibition of the SP-induced ear edema. Moreover, chlorpheniramine (4 mg/kg, i.v.), a histamine H$_1$-receptor antagonist, significantly (P<0.01, Student's t-test) reduced ear edema from 0.193±0.008 mm to 0.160±0.008 mm (n=6).

### Effects of ketanserin and LY 53857 on mouse ear edema induced by compound 48/80

Chlorpheniramine (4 mg/kg, i.v.) significantly (P<0.01,
Student's t-test) inhibited up to 30% of compound 48/80 (5 μg/site)-induced ear edema (0.137 ± 0.013 mm vs 0.195 ± 0.008 mm in the saline group, n=6). Ketanserin (1 mg/kg, i.v.) and LY 53857 (1 mg/kg, i.v.), the 5-HT2-receptor antagonist, produced a significant inhibition of ear edema induced by compound 48/80 (Fig. 3).

**DISCUSSION**

Capsaicin-induced mouse ear edema is inhibited by a Ca2+-channel blocker and histamine H1 antagonists, but not by inhibitors of arachidonate metabolites including platelet activating factor, such as indomethacin, AA 861 and CV 3988 (3). In the present study, we confirmed that the application of capsaicin to mouse ears produces degranulation of mast cells in skin connective tissue. However, capsaicin itself does not induce histamine release from mast cells directly (3). This implies that capsaicin indirectly causes mast cell degranulation in the development of edema. Furthermore, we demonstrated that ketanserin (15) and LY 53857 (16), the 5-HT2-receptor antagonist, prevent capsaicin-induced mouse ear edema, as well as methysergide (17), a 5-HT1- and 5-HT2-receptor antagonist. However, the effects of 5-HT2-receptor antagonists were characterized by only partial inhibition. (S)(-)Propranolol (18) and spiroxatrine (19), the 5-HT1-receptor antagonist, and 5-HT3-receptor antagonists including ICS 205-930 (20), MDL 72222 (21) and LY 278584 (22) had little effect on capsaicin-induced edema. GR 113808 (23), a 5-HT4-receptor antagonist, at doses that prevented 5-hydroxytryptophan-induced diarrhea in mice (24), did not inhibit the edema. These results clearly indicate that 5-HT partly participates in the development of capsaicin-induced mouse ear edema via 5-HT2 recep-
5-HT is known to induce plasma extravasation by a direct action on the microvasculature in rats (25, 26) and to produce vasodilation via 5-HT1 receptors on peripheral blood vessels (27). A recent report (28) suggested that in addition to 5-HT receptors, endogenous nitric oxide is involved in 5-HT-induced increase in vascular permeability in mouse skin. We also showed that ketanserin, LY 53857 and methysergide dose-dependently inhibited ear edema in response to intradermal injection of 5-HT, whereas antagonists for 5-HT1, 5-HT3 and 5-HT4 receptors did not block the edema formation. Moreover, α-methyl 5-HT (29), a 5-HT2-receptor agonist, induced ear edema more potently than 8-OH-DPAT (30), a 5-HT1A agonist, and 2-methyl 5-HT (29), a 5-HT3 agonist. In addition to this, 5-HT agonist-induced edema was inhibited by 5-HT2-receptor antagonists. Thus, it is likely that the edema response to 8-OH-DPAT and 2-methyl 5-HT is due to cross-reactivity of 5-HT2 receptors. Furthermore, this result supports our suggestion that 5-HT2 receptors are predominantly involved in 5-HT-mediated mouse skin inflammation.

It has been reported that 5-HT excites nociceptive sensory neurons, and application of 5-HT to a blister base gives rise to the sensation of pain (29). This activation of nociceptive neurons is mimicked by 2-methyl 5-HT, while the response to 5-HT is inhibited by ICS 205-930. In addition, local administration of ICS 205-930 and MDL 72222 can reduce formalin- and Freund’s adjuvant-induced inflammatory pain in rat hindpaw (31). Similarly, ICS 205-930 has been shown to block carrageenan-induced hyperalgesia, which is thought to be dependent on 5-HT-induced sensitization of peripheral nociceptors (32). Nevertheless, 5-HT3 antagonists such as ICS 205-930, MDL 72222 and LY 278584 did not inhibit 5-HT-induced edema in this study. Hence, it seems likely that the action of 5-HT in hyperalgesia and edema formation is mediated by different 5-HT receptors, as others have previously reported (32). On the other hand, mediators such as SP, bradykinin and prostaglandins can release tachykinins from primaryafferent terminals (1). This indicates a possibility that in addition to activation of 5-HT3 receptors, 5-HT plays a role of releasing neuropeptides including SP as the second mediator of increased vascular permeability at inflammatory sites. However, RP 67580 (33), a non-peptide NK1-receptor antagonist, at doses of 0.1-1.0 mg/kg (i.v.) had no effect on 5-HT-induced edema (H. Inoue, unpublished data), suggest-

| Drugs               | Dose (mg/kg) | Increase in ear thickness (mm) | Inhibition (%) |
|---------------------|-------------|--------------------------------|---------------|
| 5-HT1 antagonist    |             |                                |               |
| Control             | 0.191±0.005 | 4                              |               |
| (S)(-)Propranolol   | 4.0         | 0.183±0.009                     | 3             |
| Spirodotatin        | 4.0         | 0.186±0.009                     | 3             |
| 5-HT2 antagonist    |             |                                |               |
| Control             | 0.181±0.025 | 29                             |               |
| Ketanserin          | 2.0         | 0.128±0.006**                   | 23            |
| LY 53857            | 1.0         | 0.140±0.005**                   |               |
| 5-HT3 antagonist    |             |                                |               |
| Control             | 0.180±0.003 | 1                              |               |
| ICS 205-930         | 4.0         | 0.178±0.009                     | 1             |
| MDL 72222           | 4.0         | 0.174±0.011                     | 3             |
| 5-HT4 antagonist    |             |                                |               |
| Control             | 0.221±0.010 | 75                             |               |
| GR 113808           | 4.0         | 0.231±0.011                     |               |
| NK1 antagonist      |             |                                |               |
| Control             | 0.186±0.012 | 20                             |               |
| SP 3707            | 0.1         | 0.149±0.022                     |               |
| Spantide II         | 0.5         | 0.047±0.006***                  |               |

Test compounds were intravenously administered 15 min before compound 48/80 injection. Each bar represents the mean±S.E.M. of 6 animals. Statistical significance compared with the control: **P<0.01 and ***P<0.001.

Fig. 3. Effects of ketanserin and LY 53857 on compound 48/80 induced mouse ear edema. Test compounds were intravenously administered 15 min before compound 48/80 treatment (5 μg/site). Ear edema was examined 30 min after compound 48/80 injection. Each bar represents the mean±S.E.M. of 6 animals. *P<0.05, **P<0.01, statistically significant compared with the control group (0) by Student’s t-test.

Table 3. Effects of 5-HT receptor antagonists on substance P-induced mouse ear edema
ing that the release of SP from sensory nerves is not an important factor in the development of 5-HT-induced ear edema.

Neuropeptides such as SP and calcitonin-gene related peptide (CGRP) are released by capsaicin from peripheral endings of afferent neurons (1, 34). We have previously suggested that SP is an important mediator in capsaicin-induced mouse ear edema, since SP antagonists such as spantide and [d-Pro², d-Trp⁷,⁹]-SP inhibit the edema, whereas CGRP₈-₃₇, a CGRP antagonist, has little effect (3). SP is considered to induce the inflammatory response by increasing vascular permeability through the release of vasoactive amines from mast cells (35, 36) and activation of NK₁ receptors (33, 37). Therefore, a histamine H₁ blocker and tachykinin antagonists are expected to have inhibitory effects on neurogenic inflammation (38, 39). Spantide II (40), a SP antagonist, strongly inhibited SP-induced ear edema and chlorpheniramine, a histamine H₁ antagonist, was also an inhibitor of the edema. Wang et al. (41) have reported that mouse ear edema evoked by SP is suppressed by diphenhydramine combined with methysgeride. Methysgeride is more potent than histamine antagonists in reducing SP-induced plasma extravasation in rat knee joint (42). In this study, ketanserin and LY 53857 induced a partial inhibition of SP-induced ear edema, whereas any antagonist for 5-HT₁, 5-HT₃ and 5-HT₄ receptors had no effect. From these results, we consider that 5-HT released from mast cells plays a role in SP-induced ear edema via 5-HT₂ receptors. This is supported by the finding that ketanserin and LY 53857 inhibited ear edema induced by compound 48/80, a mast cell degranulating agent.

In conclusion, 5-HT₂-receptor antagonists not only inhibited capsaicin-induced mouse ear edema but also blocked edema in response to 5-HT agonists, SP and compound 48/80. Thus, it seems likely that 5-HT released from mast cells partly participates in capsaicin-induced ear edema via 5-HT₂ receptors in mouse skin. Furthermore, it is possible that 5-HT enhances other mediators such as SP and histamine involved in capsaicin-induced edema via 5-HT₂ receptors.

Acknowledgments

The authors wish to thank Drs. JD Gale and MB Bain, Glaxo Research and Development, Ltd., for the kind gift of GR 113808. We are also grateful to Emeritus Prof. M. Ostuka, Tokyo Medical and Dental University, for his invaluable advice, and to Emeritus Prof. S. Shibata, University of Tokyo, for giving us great encouragement. We also acknowledge Dr. H. Kajigaya, Nippon Professional School of Medical Technology, for helpful advice on the pathological studies.

REFERENCES

1 Holzer P: Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 43, 143 – 201 (1991)
2 Mantione CR and Rodriguez R: A bradykinin (BK) receptor antagonist blocks capsaicin-induced ear inflammation in mice. Br J Pharmacol 99, 516 – 518 (1990)
3 Inoue H, Nagata N and Koshihara Y: Profile of capsaicin-induced mouse ear oedema as neurogenic inflammatory model: comparison with arachidonic acid-induced ear oedema. Br J Pharmacol 110, 1614 – 1620 (1993)
4 Virus RM and Gebhart GF: Pharmacological actions of capsaicin: apparent involvement of substance P and serotonin. Life Sci 25, 1273 – 1283 (1979)
5 Holzer P, Saria A, Skofocht G and Lembeck F: Increase in tissue concentrations of histamine and 5-hydroxytryptamine following capsaicin treatment of newborn rats. Life Sci 29, 1099 – 1105 (1981)
6 Kiernan JA: Degranulation of mast cells following antidromic stimulation of cutaneous nerves. J Anat 111, 349 – 350 (1971)
7 Wiener-Menzel L, Schulz B, Vakilzadeh F and Czarnetzki BM: Electron microscopic evidence for a direct contact between nerves fibres and mast cells. Acta Dermatol Venereol 61, 465 – 469 (1981)
8 Leon A, Burlani A, Dal Toso R, Fabris M, Romanello S, Aloe L and Levin-Montalcini R: Mast cells synthesis, store, and release nerve growth factor. Proc Natl Acad Sci USA 91, 3739 – 3743 (1994)
9 Humphrey PPA, Harting P and Hoyer H: A proposed new nomenclature for 5-HT receptors. Trends Pharmacol Sci 14, 233 – 236 (1993)
10 Martin GR and Humphrey PPA: Classification Review. Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. Neuropharmacology 33, 261 – 273 (1994)
11 De Clerck F, Van Gorp L, Beetens J and Reneman RS: Platelet-mediated vascular permeability in the rat: a predominant role for 5-hydroxytryptamine. Thromb Res 38, 321 – 339 (1985)
12 Cohen ML and Schenck K: Effect of LY53857, a selective 5-HT₂ receptor antagonist, on 5-HT-induced increases in cutaneous vascular permeability in rats. Life Sci 44, 957 – 961 (1989)
13 Fozard JR: Neuronal 5-HT receptors in the periphery. Neuropharmacology 23, 1473 – 1486 (1984)
14 Tramontana M, Giuliani S, Del Bianco E, Lecci A, Maggi CA, Evangelista S and Geppetti P: Effects of capsaicin and 5-HT₃ antagonists on 5-hydroxytryptamine-evoked release of calcitonin gene-related peptide in the guinea pig heart. Br J Pharmacol 108, 431 – 435 (1993)
15 Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J and Janssen PAJ: Receptor binding profile of R 41 468, a novel antagonist at 5-HT₇ receptors. Life Sci 28, 1015 – 1022 (1981)
16 Cohen ML, Fuller RW and Kurz KD: LY53857, a selective and potent serotoninergic (5-HT₇) receptor antagonist, does not lower blood pressure in the spontaneously hypertensive rat. J Pharmacol Exp Ther 227, 327 – 332 (1983)
17 Bradley PB, Engel G, Fenik W, Fozard JR, Humphrey PPA, Middlemiss DN, Mylecharane EJ, Richardson BP and Saxena PR: Proposals for the classification and nomenclature of func-
tional receptors for 5-hydroxytryptamine. Neuropharmacology 25, 563–576 (1986)
18 Middlemiss DN: Stereoselective blockade at [3H]-5HT binding sites and at the 5-HT autoreceptor by propranolol. Eur J Pharmacol 101, 207–208 (1984)
19 Nelson DL and Taylor EW: Spiroxatrine: a selective serotonin receptor antagonist. Eur J Pharmacol 124, 207–208 (1986)
20 Donatsch P, Engel G, Richardson BP and Stadler PA: ICS 205-930: a highly selective and potent antagonist at peripheral neuronal 5-hydroxytryptamine (5-HT) receptors. Br J Pharmacol 81, 34P (1984)
21 Fozard JR: MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn Schmiedebergs Arch Pharmacol 326, 36–44 (1984)
22 Fludzinski P, Evrard DA, Bloomquist WE, Lacefield WB, Pfeifer W, Jones ND, Deeter JB and Cohen ML: Indazoles as indole bioisosteres: synthesis and evaluation of the tropanyl ester and amide of indazole-3-carboxylate as antagonists at the serotonin 5HT3 receptor. J Med Chem 30, 1535–1537 (1987)
23 Gale JD, Grossman CJ, Whitehead JWF, Oxford AW, Bunce KT and Humphrey PPA: GR113808: a novel, selective antagonist with high affinity at the 5-HT4 receptor. Br J Pharmacol 111, 332–338 (1994)
24 Hegde SS, Moy TM, Perry MR, Loeb M and Eglen RM: Evidence for the involvement of 5-hydroxytryptamine 4 receptors in 5-hydroxytryptophan-induced diarrhea in mice. J Pharmacol Exp Ther 271, 741–747 (1994)
25 Leme JG, Hamamura L, Migliorini RH and Leite MP: Influence of diabetes upon the inflammatory response of the rat. A pharmacological analysis. Eur J Pharmacol 23, 74–81 (1973)
26 Arvier PT, Chahl LA and Ladd RJ: Modification by capsaicin and compound 48/80 of dye leakage induced by irritants in the rat. Br J Pharmacol 59, 61–68 (1977)
27 Jazayeri A, Meyer WJ and Kent TA: 5-HT1B and 5-HT1D serotonin binding sites in cultures Wistar-Kyoto aortic smooth muscle cells. Eur J Pharmacol 169, 183–187 (1989)
28 Fujii E, Irie K, Uchida Y, Tsukahara F and Muraki T: Possible role of nitric oxide in 5-hydroxytryptamine-induced increase in vascular permeability in mouse skin. Naunyn Schmiedebergs Arch Pharmacol 350, 361–364 (1994)
29 Richardson BP, Engel G, Donatsch P and Stadler PA: Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316, 126–131 (1985)
30 Hoyer D: Functional correlates of serotonin 5-HT1, recognition sites. J Recept Res 8, 59–81 (1988)
31 Giordano J and Rogers LV: Peripherally administered serotonin 5-HT3 receptor antagonists reduce inflammatory pain in rats. Eur J Pharmacol 170, 83–86 (1989)
32 Eschalier A, Kayser V and Guilbaud G: Influence of a specific 5-HT3 antagonist on carrageenan-induced hyperalgesia in rats. Pain 36, 249–255 (1989)
33 Garret C, Carruette A, Fardin V, Moussaoui S, Peyronel J-F, Blanchard J-C and Laduron PM: Pharmacological properties of a potent and selective nonpeptide substance P antagonist. Proc Natl Acad Sci USA 88, 10208–10212 (1991)
34 Holzer P: Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24, 739–768 (1988)
35 Erjavec F, Lembeck F, Florjanc-Irman T, Skofitsch G, Donnerer J, Saria A and Holzer P: Release of histamine by substance P. Naunyn Schmiedebergs Arch Pharmacol 317, 67–70 (1981)
36 Fewtrell CMS, Foreman JC, Jordan CC, Oehme P, Renner H and Stewart JM: The effect of substance P on histamine and 5-hydroxytryptamine release in the rat. J Physiol (Lond) 330, 393–412 (1982)
37 Lembeck F and Holzer P: Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn Schmiedebergs Arch Pharmacol 310, 175–183 (1979)
38 Foreman JC: Peptides and neurogenic inflammation. Br Med Bull 43, 386–400 (1987)
39 Donnerer J and Amann R: The inhibition of neurogenic inflammation. Gen Pharmacol 24, 519–529 (1993)
40 Xu X-J, Hao J-X, Wiesenfeld-Hallin Z, Håkanson R, Folkers K and Häkfelt T: Spantide II, a novel tachykinin antagonist, and galanin inhibit plasma extravasation induced by antidromic C-fiber stimulation in rat hindpaw. Neuroscience 42, 731–737 (1991)
41 Wang J-P, Raung S-L, Lin C-N and Teng C-M: Inhibitory effect of norathyriol, a xanthone from Tripterospermum lanceolatum, on cutaneous plasma extravasation. Eur J Pharmacol 251, 35–42 (1994)
42 Lam FY and Ferrell WR: Mediators of substance P-induced inflammation in the rat knee joint. Agents Actions 31, 298–307 (1990)