Inhibitory Effect of Olopatadine Hydrochloride on the Sneezing Response Induced by Intranasal Capsaicin Challenge in Guinea Pigs

Toshihiko Kaise*, Yukino Akamatsu, Kenji Ohmori, Akio Ishii and Akira Karasawa
Department of Pharmacology, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagaizumi-cho, Santo-gun, Shizuoka 411-8731, Japan

Received December 22, 2000 Accepted April 6, 2001

ABSTRACT—To investigate the possible inhibitory effect of olopatadine hydrochloride (olopatadine), an antiallergic drug, on the tachykinin-mediated nasal responses, we examined the effect of olopatadine on the sneezing and the nasal rubbing responses induced by intranasal capsaicin challenge in guinea pigs. Olopatadine (10 mg/kg, p.o.) inhibited the sneezing response by 57% without affecting the nasal rubbing one. The antihistamines chlorpheniramine and clemastine did not affect the responses. Morphine caused the inhibition of both responses, which was antagonized by naloxone. These results suggest that olopatadine inhibits the sneezing response by the inhibition of the tachykinin release and not by its antihistaminic action.

Keywords: Olopatadine, Tachykinin release, Sneezing response

Olopatadine hydrochloride (\((Z)-11-(3\text{-dimethylamino}-\text{propylidene})-6,11\text{-dihydrobenz}[^{b,e}]\text{oxepin}-2\text{-acetic acid monohydrochloride, CAS 140462-76-6, KW-4679}\) (olopatadine) is a novel antiallergic drug with an antagonistic action against histamine H₁ receptor (1). The previous in vitro study (2) demonstrated that olopatadine, which did not affect the contraction induced by exogenous substance P (SP) or neurokinin A, inhibited the tachykininergic contraction induced by electrical field stimulation (EFS) in guinea pig bronchial muscles without affecting the cholinergic contraction. Moreover, the inhibitory effect of olopatadine on the tachykininergic contraction was suppressed by the small conductance Ca⁺-activated K⁺ (SKCa) channel blocker, apamin or scyllatoxin. From these results, olopatadine has been suggested to presynaptically inhibit the release of tachykinins from sensory nerves by activating SKCa channels. To investigate the possible inhibitory effect of olopatadine on the tachykinin release in vivo, we examined the effect of olopatadine on the sneezing and the nasal rubbing responses induced by the intranasal administration of capsaicin in conscious guinea pigs, in which the release of tachykinins from sensory nerves are involved (3).

Male Hartley guinea pigs, 4-week-old, were purchased from Japan SLC (Shizuoka), and after the acclimatization, they were used for the experiments at 5 – 6-week-old. Food and water were freely available. The present experiments were approved by the Animal Ethical Committee of Kyowa Hakko Kogyo Co., Ltd. (Shizuoka).

Capsaicin (Wako Pure Chemical Industries, Osaka) was dissolved in 5% ethanol and 5% Tween 80 and then diluted with saline to give a solution of 1 mmol/l. Olopatadine was synthesized at the Sakai Research Laboratories of Kyowa Hakko Kogyo (Osaka). LY303870 (\((R)-1\text{-}[N-(2\text{-methoxy-benzyl})\text{acetylamino}]\text{-3-}(1\text{H}\text{-indol-3-yl})\text{-2-}[N-(2\text{-4-(piperidin-1-yl)piperidin-1-yl})\text{acetyl}]\text{aminol-4-propane, an neurokinin-1 (NK₁)-receptor antagonist, was synthesized at the Analytical Research Center of Kyowa Hakko Kogyo (Shizuoka). Other reagents used were ketotifen fumarate (ketotifen; Sigma Chemicals, St. Louis, MO, USA), chlorpheniramine maleate (chlorpheniramine; Tokyo Kasei, Tokyo), clemastine maleate (clemastine; Kyowa Hakko Kogyo), codeine phosphate (codeine) and morphine hydrochloride (morphine) (Shionogi & Company, Osaka) and naloxone hydrochloride (naloxone) (Endo Laboratories, Tokyo). Olopatadine, ketotifen, chlorpheniramine and codeine were dissolved in distilled water. LY303870 was dissolved in acidified saline (4). Clemastine was suspended in 0.3 w/v% carboxymethylcellulose solution. Morphine and naloxone were dissolved in saline.

The sneezing and the nasal rubbing responses were induced by applying 50 µl of 1 mmol/l capsaicin solution into the right nostril of conscious guinea pigs, and the number of each response was counted for 30 min by observing with the naked eye. A single rubbing action with either front paw was regarded as one nasal rubbing. Olopatadine,
Morphine is reported to inhibit the tachykininergic contraction to eliminate the inhibitory action (4), chlorpheniramine and clemastine at doses sufficient to antagonize histamine H1 receptor (5), and codeine at 20 mg/kg, a dose reported to inhibit the cough response in guinea pigs (6).

Data are shown as means ± S.E.M. The Wilcoxon rank sum test was used for the analysis of the difference between two groups. A value of P less than 5% was considered to be significant.

The sneezing is assumed to be a nerve reflex involving the sneeze center in the central nervous system following the stimulation of sensory nerve endings (7). In this study, LY303870 tended to inhibit the capsaicin-induced sneezing response (P<0.10) (Table 1). The previous studies in guinea pigs demonstrated that the intranasal capsaicin challenge released SP (8), the intranasal application of SP caused the sneezing response (7), and the capsaicin-induced sneezing response was abolished by the systemic pretreatment with capsaicin (3). Collectively, the capsaicin-induced sneezing response is assumed to be mediated by the release of SP and/or other tachykinins.

The previous study in guinea pigs demonstrated that the intranasal capsaicin challenge released histamine (9), suggesting that histamine is involved in the capsaicin-induced sneezing. However, intranasal histamine caused sneezing responses at 30 mmol/l (7), which was much higher than the concentration of histamine released after the intranasal capsaicin challenge (9). Accordingly, histamine seems to play a minimal role in the capsaicin-induced sneezing response, which is in contrast to the antigen-antibody reaction-induced sneezing response involving histamine (10). In fact, histamine H1-receptor antagonists chlorpheniramine (30 mg/kg) and clemastine (5 mg/kg) did not affect the capsaicin-induced sneezing response in this study (Table 1).

The sneezing response is not clearly distinguished from the cough response in guinea pigs. Since inhaled capsaicin is reported to cause the cough response in guinea pigs (6), we examined the effect of codeine, an antitussive drug, on the sneezing response after the intranasal capsaicin challenge. As a result, codeine did not affect the number of sneezing and the nasal rubbing responses (Table 2). This result indicates that the capsaicin-induced sneezing response was not mixed up with the cough response in the present experimental condition.

In this study, morphine at 3 mg/kg significantly inhibited the number of the sneezing response, and naloxone tended to eliminate the inhibitory action (P<0.10) (Table 2). Morphine is reported to inhibit the tachykininergic contraction of guinea pig bronchi in vitro (11). The inhibition by morphine of the tachykininergic contraction was antagonized by naloxone (11). These observations suggest that there exists an opioid receptor regulating the tachykinin release in the peripheral sensory nerves. Thus morphine may have inhibited the capsaicin-induced sneezing response by suppressing the tachykinin release presynaptically through the activation of opioid receptors on nasal sensory nerves.

The mechanism for the capsaicin-induced nasal rubbing response has not fully been elucidated. In this study, morphine at 3 mg/kg caused significant inhibition of the capsaicin-induced nasal rubbing response, which was antagonized by naloxone (Table 2). C sempasin not only stimulates the nasal C-fiber afferent nerves to release tachykinins but also transmits the information to the central nervous systems through the trigeminal dorsal horn in the medulla, leading to the sense of pain. In fact, the intranasal capsaicin challenge causes the sense of pain in humans (12). Aicher et al. (13) recently demonstrated that the µ-opioid receptor was often colocalized with the SP receptor, i.e., the tachykinin NK1 receptor, on the dendrites, which often existed in close contact with SP-containing axon terminals in the trigeminal dorsal horn of the rat. These results suggest that morphine inhibits the capsaicin-induced nasal rubbing response by the postsynaptic inhibition of SP-mediated nociceptive signals through the activation of µ-opioid receptor. Alternatively, the inhibitory effect of morphine on the rubbing response may be due to the analgesic action of this substance at the cerebral cortex. Thus the nasal rubbing response induced by capsaicin is likely to reflect the sense of pain.

In the present study, olopatadine at 10 mg/kg significantly inhibited the number of the capsaicin-induced sneezing response by 57%, and the drug tended to inhibit the response at 1 mg/kg (P<0.10) (Table 3). On the other hand, olopatadine did not affect the nasal rubbing response (Table 3), presumably because this response reflects the pain involving the central nervous system. The antihistaminic action of olopatadine is unlikely to be involved in the inhibition by this drug of the sneeze response, as the conventional antihistamines did not affect the response (Table 1). It is reported that the inhibition by olopatadine of the tachykininergic contraction of isolated guinea pig bronchi was diminished by the SKCa channel inhibitors and not by naloxone (2). These results suggest that olopatadine inhibits the sneezing response by suppressing the tachykinin release presynaptically through the activation of the SKCa channels and not the opioid receptors.

Ketotifen at 10 and 1 mg/kg inhibited the number of the capsaicin-induced sneezing response by 40% and 33%, respectively; however, these effects were not statistically significant (Table 3). Moreover, ketotifen did not affect
the nasal rubbing response (Table 3). Good et al. (14) reported that ketotifen inhibited the tachykinergic contraction of isolated guinea pig bronchi following the EFS at 100 μmol/l, while olopatadine suppressed the contraction at 10 μmol/l (2). The marginal inhibitory effect of ketotifen against the sneezing response seems to be due to its less

| Table 1. Effects of LY303870, chlorpheniramine and clemastine on the capsaicin-induced sneezing and nasal rubbing responses in guinea pigs |
|---------------------------------------------------------------|
| Treatment | N | Sneezing (times in 30 min) | Nasal rubbing (times in 30 min) |
|-----------|---|---------------------------|-------------------------------|
| Vehicle   | 10| 13.7 ± 2.6                | 21.3 ± 6.5                   |
| LY303870, 10 mg/kg (i.v.) | 10| 8.0 ± 1.6 (42)           | 20.1 ± 3.6 (6)               |
| Vehicle   | 10| 9.9 ± 2.1                 | 62.6 ± 12.4                  |
| Chlorpheniramine, 30 mg/kg (p.o.) | 10| 9.0 ± 1.4 (9)            | 58.6 ± 6.6 (6)               |
| Vehicle   | 8 | 11.6 ± 2.6                | 67.3 ± 11.4                  |
| Clemastine, 5 mg/kg (p.o.) | 8 | 9.0 ± 1.1 (22)           | 72.4 ± 14.0 (8)              |

N means the number of the animals. Data are shown as means ± S.E.M. The number in parentheses represents the inhibitory percentage as compared with the response in the vehicle group. LY303870 or its vehicle was intravenously administered 5 min before the capsaicin challenge. Clemastine, clemastine or the corresponding vehicle was orally administered 1 h before the capsaicin challenge.

| Table 2. Effects of codeine and morphine on the capsaicin-induced sneezing and nasal rubbing responses in guinea pigs |
|---------------------------------------------------------------|
| Treatment | N | Sneezing (times in 30 min) | Nasal rubbing (times in 30 min) |
|-----------|---|---------------------------|-------------------------------|
| Vehicle   | 10| 6.4 ± 1.2                 | 41.4 ± 14.8                   |
| Codeine, 20 mg/kg (p.o.) | 10| 6.5 ± 1.5 (–2)           | 46.3 ± 13.3 (–12)              |
| Vehicle   | 10| 6.1 ± 1.1                 | 50.3 ± 11.6                   |
| Morphine, 3 mg/kg (s.c.) | 10| 2.7 ± 0.5* (56)         | 14.1 ± 2.3 ** (72)             |
| Morphine, 3 mg/kg (s.c.) | 10| 5.0 ± 1.0 (18)         | 61.3 ± 11.0* (–22)             |
| + Naloxone, 0.5 mg/kg (s.c.) |         |                           |                               |

N means the number of the animals. Data are shown as means ± S.E.M. The number in parentheses represents the inhibitory percentage as compared with the response in the vehicle group. Codeine or its vehicle was orally administered 1 h before the capsaicin challenge. Morphine (or its vehicle) and naloxone (or its vehicle) was administered subcutaneously 30 and 15 min, respectively, before the challenge. *P<0.05 and **P<0.01 as compared with the number in the vehicle group. *P<0.001 as compared with the number in the morphine group.

| Table 3. Effects of olopatadine and ketotifen on the capsaicin-induced sneezing and nasal rubbing responses in guinea pigs |
|---------------------------------------------------------------|
| Treatment | N | Sneezing (times in 30 min) | Nasal rubbing (times in 30 min) |
|-----------|---|---------------------------|-------------------------------|
| Vehicle   | 10| 8.6 ± 1.8                 | 55.0 ± 8.4                   |
| Olopatadine, 10 mg/kg (p.o.) | 10| 3.7 ± 0.7* (57)          | 57.3 ± 9.5 (–4)               |
| Vehicle   | 10| 11.5 ± 2.2                | 52.3 ± 5.1                   |
| Olopatadine, 1 mg/kg (p.o.) | 10| 6.7 ± 1.4 (42)         | 66.9 ± 7.4 (–28)              |
| Vehicle   | 10| 9.9 ± 2.1                 | 62.6 ± 12.4                  |
| Ketonifin, 10 mg/kg (p.o.) | 10| 5.9 ± 1.3 (40)          | 60.0 ± 9.6 (4)                |
| Vehicle   | 10| 11.5 ± 2.2                | 52.3 ± 5.1                   |
| Ketonifin, 1 mg/kg (p.o.) | 10| 7.7 ± 1.6 (33)         | 62.5 ± 10.7 (–20)             |

N means the number of the animals. Data are shown as means ± S.E.M. The number in the parentheses represents the inhibitory percentage as compared with the response in the vehicle group. Olopatadine, ketotifen or their vehicle was orally administered 1 h before the capsaicin challenge. *P<0.05 as compared with the number in the vehicle group.
prominent inhibitory action on the tachykinin release, as compared with that of olopatadine.

The previous study demonstrated that intranasal capsaicin treatment ameliorated the nasal symptoms in pollinosis patients, suggesting that tachykinin release is involved in the pathogenesis of allergic rhinitis (15). Thus, olopatadine, exhibiting the inhibition of tachykinin release, may have some advantage over the conventional histamine H1-receptor antagonist in the treatment of allergic rhinitis. Further studies, however, are needed to confirm the inhibitory action of olopatadine on the tachykinin release in humans, since the effective dose (1, 10 mg/kg) for inhibiting the capsaicin-induced sneezing response in the present study was higher than the therapeutic dose in humans (5 mg, b.i.d.).

In conclusion, olopatadine inhibited the capsaicin-induced sneezing response in guinea pigs. This result suggests that olopatadine inhibits the release of tachykinins from sensory nerves in vivo. The nasal rubbing response seemed to be caused by the sense of pain, which thus olopatadine did not affect.

REFERENCES

1 Nonaka H, Otaki S, Ohshima E, Kono M, Kase H, Ohta K, Fukui H and Ichimura M: Unique binding pocket for KW-4679 in the histamine H1 receptor. Eur J Pharmacol 345, 111 – 117 (1998)
2 Ikemura T, Okamura K, Sasaki Y, Ishii H and Ohmori K: KW-4679-induced inhibition of tachykininergic contraction in the guinea-pig bronchi by prejunctional inhibition of peripheral sensory nerves. Br J Pharmacol 117, 967 – 973 (1996)
3 Lundblad L, Lundberg JM and Anggård A: Local and systemic capsaicin pretreatment inhibits sneezing and the increase in nasal vascular permeability induced by certain chemical irritants. Naunyn Schmiedebergs Arch Pharmacol 326, 254 – 261 (1984)
4 Gitter BD, Bruns RF, Howbert J, Waters DC, Thr elkeld PG, Cox LM, Nixon JA, Lobb KL, Mason NR, Stengel PW, Cockerham SL, Silbaugh SA, Gehlert DR, Sch ober DA, Iyengar S, Calligaro DO, Regoli D and Hip skind PA: Pharmacological characterization of LY303870: a novel, potent and selective nonpeptide substance P (neurokinin-1) receptor antagonist. J Pharmacol Exp Ther 275, 737 – 744 (1995)
5 Fujimura H, Tsurumi K, Yanagihara M, Hiramatsu Y, Tamura Y, Shimizu Y, Hojo M, Yoshida Y and Akimoto Y: Pharmacological study of mequitazine (LM-209) (II): anti-allergic action. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) 78, 291 – 303 (1981) (text in Japanese with English abstract)
6 Gallico L, Borghi A, Dalla Rosa C, Ceserani R and Tognella S: Moguisteine: a novel peripheral non-narcotic antitussive drug. Br J Pharmacol 112, 795 – 800 (1994)
7 Imamura T and Kambara T: Substance P as a potent stimulator of sneeze responses in experimental allergic rhinitis of guinea pigs. Agents Actions 37, 245 – 249 (1992)
8 Kitajiri M, Kubo N, Ikeda H, Sato K and Kamazawa T: Effects of topical capsaicin on autonomic nerves in experimentally-induced nasal hypersensitivity. An immunocytochemical study. Acta Otolaryngol Suppl 500, 88 – 91 (1993)
9 Asakura K, Shirasaki H, Narita S, Koijima T and K ataura A: Study on the dye leakage response of nasal mucosa following topical, capsaicin challenge in guinea pigs. Acta Otolaryngol 112, 545 – 551 (1992)
10 Kawasaki H, Kaise T, Akamatsu Y, Nishikawa H and Ohmori K: Inhibitory effect of oxatomide, an antiallergic drug, on the allergic rhinitis model of guinea pigs. Allergology and Immunology (Arerugi Meneki) 7, 90 – 97 (2000) (text in Japanese with English abstract)
11 Kamikawa Y and Shimo Y: Morphine and opioid peptides selectively inhibit the non-cholinergically mediated neurogenic contraction of guinea-pig isolated bronchial muscle. J Pharm Pharmacol 42, 214 – 216 (1990)
12 Geppetti P, Fusco BM, Marabini S, Maggi CA, Fanciullacci M and Sicutieri F: Secretion, pain and sneezing induced by the application of capsaicin to the nasal mucosa in man. Br J Pharmacol 93, 509 – 514 (1988)
13 Aicher SA, Punnosse A and Goldberg A: μ-Opioid receptors often colocalize with the substance P receptor (NK1) in the trigeminal dorsal horn. J Neurosci 20, 4345 – 4345 (2000)
14 Good DM, Clapham JC and Hamilton TC: Effects of BRL38227 on neurally-mediated responses in the guinea-pig isolated bronchus. Br J Pharmacol 105, 933 – 940 (1992)
15 Stjärne P, Rinder J, Hedén-Bloquist E, Cardell LO, Lundberg J, Zetterström O and Anggård A: Capsaicin desensitization of the nasal mucosa reduces symptoms upon allergen challenge in patients with allergic rhinitis. Acta Otolaryngol 118, 235 – 239 (1998)