◎原 著

Antiallergic agent, azelastine, inhibits ^{45}Ca uptake and histamine release in rat mast cells stimulated by antigen

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Abstract : The effect of antiallergic agent, azelastine, was examined in immunogical secretory process of mast cells. 1. Azelastine significantly inhibited ^{45}Ca uptake by mast cells stimulated by antigen, and the maximum inhibition was attained at a concentration of 50 μg/mℓ showing approximately 30.5% inhibition. 2. Azelastine also inhibite the release of histamine from mast cells by antigen. The maximal inhibitory rate was 38.1%. The effect of azelastine on ^{45}Ca uptake and histamine release was dose-dependent, and compatible at employed cocentration of the agent. 3. Tachpylaxis to azelastine was not found in this experimental system.

Key words : Azelastine, Rat mast cells, ^{45}Ca uptake, Histamine release.

Introduction

Immunological secretory process of mast cells is a model of triggering events in immediate allergic reactions. In recent years many antiallergic agents have been developed for the purpose of inhibiting immunological release of chemical mediators from mast cells. The first developed antiallergic agent, disodium cromoglycate (DSCG) has been shown to act as a membrane stabilizer[1]. Earlier studies on DSCG demonstrated that the agent inhibited phosphodiesterase activity[2]. It is also shown that the agent inhibits an increased Ca^{2+} influx into mast cells following stimulation with antigen[3~5].

It has been reported that azelastine, an antiallergic agent, inhibits allergic and nonalleregic release of SRS－A[6] and histamine release[7~11]. In addition to these inhibitory effects, azelastine inhibits the production of leukotrienes B_4 and C_4[12]. The effect of azelastine on Ca^{2+} influx into mast cells by stimulation with antigen has not investigated.

In the present study inhibitory effect of azelastine on ^{45}Ca uptake and histamine release was examined in mast cells stimulated by antigen.

Materials and Methods

Azelastine (4-(p-chlorobenzyl)-2-(hexahydor-1-methyl-1H-azepine-4-yl)-1-(2H)-phthalazinone hydrochloride) was presented by Eisai Pharmaceutical Co. (Fig. 1).

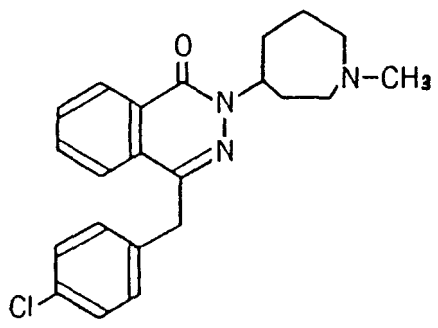

Fig. 1. Chemical structure of azelastin

Sprague Dawley rats were sensitized with an intramuscular injection of 0.5 mℓ saline containing ovalbumin, 50 mg/mℓ, and $8x10^8$ killed organisms (Bordetella pertussis) per milliliter, into each hind limb according to the method described by Foreman, et al[13]. 12-14 days after sensitization, the rats were sacrified, and mast cells were separated from abdominal cavity with 200 times gentle massage. The cells purified by the density gradient of BSA[14]. The purity of mast was more than 90%.

^{45}Ca uptake by mast cells induced by antigen was perfomed by the method[14] modified from that described by Ranadive, et al[15]. 3 μCi ^{45}Ca in 0.1 mℓ distilled water and 10 μg/mℓ ovalbumin in 0.7 mℓ of Tyrode's solution was added into each test tube. The test tube was kept in water bath at 37℃ for 30 min. After the number of mast cells was adjusted to 10^5 cells/0.1 mℓ, the cell suspension was added into the test tube containing ^{45}Ca and ovalbumin, and kept in water bath at 37℃. The mixed solution was incubated at 37℃ for 10 min. After the mast cells were washed twice with 5 mℓ of cold physiological saline, the residual free radioactive ^{45}Ca was removed through microfiber filter (Whatman, type GF/C, pore size 1.2 μm). The amount of ^{45}Ca in each cell suspension was determined by using a scintillation counter. All experiments for ^{45}Ca uptake was carried out in triplicate.

Histamine release from mast cells induced by antigen was examined under the same condition as the experiment for ^{45}Ca uptake except for the incubation with antigen for 15 min. The histamine content in the cells and supernatant fluid was measured by an automated spectrofluorometric histamine analysis system. The results were expressed as a percentage of total histamine content. All experiments for histamine release were performed in duplicate.

Tachyphylaxis[18] to azelastine was examined by the re-exposure following incubation with azelastine.

Results

Azelastine inhibited ^{45}Ca uptake by mast cells induced by antigen. The inhibitory effect of azelastine was dose-dependent, and the maximum inhibition was attained at a concentration of 50 μg/mℓ showing approximately 30.5% inhibition. The rate of azelas-tine on histamine release was 13.2% at 0.05 μg/mℓ, 15.2% at 0.5 μg/mℓ, 35.0% at 5 μg/mℓ and 38.1% at 50 μg/mℓ. The inhibitory effect on ^{45}Ca uptake and histamine release was significantly higher at 5.0 μg/mℓ and 50 μg/mℓ than the inhibition at 0.5 μg/mℓ of azelastine (significant difference between inhibitory effects at 0.5 μg/mℓ and at 5 μg/mℓ : Ca uptake ; $p<0.001$, histamine release ; $p<0.001$) (Fig. 2).

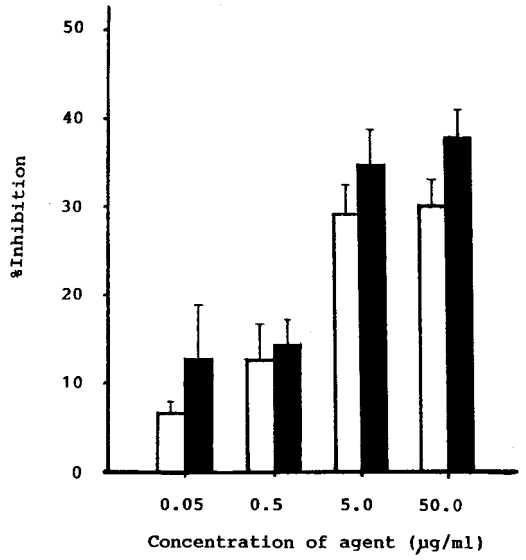

Fig. 2. Inhibitory effect of azelastin on ^{45}Ca uptake (□) and histamine release (■) in mast cells stimulated by antigen

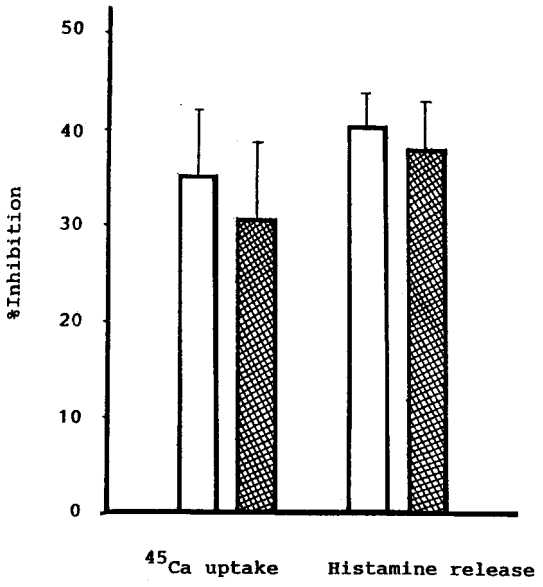

Fig. 3. Effect of re-exposure with azelastin (▨) following previous incubation with the agent (□) on the inhibition of ^{45}Ca uptake and histamine release in mast cells stimulated by antigen

Tachyphylaxis to azelastin was not observed in immunological secretory process of mast cells. The inhibition of azelastine was 35.1% for ^{45}Ca uptake and 40.2% for histamine release by single incubation with the agent. A similar inhibition was obtained at 50 μg/mℓ of azelastine, and 30.5% inhibition for ^{45}Ca uptake and 38.1% for histamine release when mast cells were re-exposed following previous exposure with the agent (Fig. 3).

Discussion

Calcium ions are considered to be indispensable for the process of the release of chemical mediators from mast cells. Calcium ions that enter cells from the extracellular fluid upon stimlation with antigen play an important role in the release of chemical mediators through major routs. Firstly, Calcium ions cause phosphorylation of proteins and induce release of chemical mediators with granules extruded from the cells. Histamine and heparin (preformed mediators) contained in granules are released in this fashion. Secondly, calcium ions enhance the activity of phospholipase A_2 and promote synthesis of arachidonic acid mainly from phosphatidylcholine. Prostaglandins and leukotrienes

(newly genarated mediators) are synthesized and released by this rout.

Our previous studies have shown that a calcium channel antagonists, nifedipine and nicardipine, inhibit ^{45}Ca uptake by antigen-stimulated mast cells, and consequently reduce histamine release[19,20]. Similar inhibitory effect was observed in an antiallergic drug, DSCG, suggesting that the agent also prevents the influx of Ca^{2+} into mast cells upon stimulation with antigen, thus inhibiting the release of chemical mediators. The inhibitory effect of DSCG, however, decreases by prolonged preincubation[21~23] or re-exposure with the agent (tachyphylaxis)[24].

Azelastine possesses potent histamine H1 receptor blocking properties[7]. It has been reported that azelastine strikingly inhibits the production of leukotrienes B_4 and C_4[12]. Thus, azelastine inhibits allergic and non-allergic release of SRS-A[7,8] and histamine[9~11]. In the present study, the effect of an antiallergic agent, azelastine, was ezamined in immunological secretory process of mast cells. The results from this study show that azelastine significantly inhibits both ^{45}Ca uptake and histamine reiease in mast cells stimulated by antigen. The inhibition of azelastine on Ca^{2+} influx into mast cells might be related to the inhibition on the production of leukotrienes, and it might be seggested that azelastin inhibits the release of leukotrienes and histamine by preventing an increase in activity of phopholipase A_2 stimulated by Ca^{2+}.

References

1. Cox JSG. Disodium cromoglycate (FPL 670) ('Intal") ; a sprcific inhibitor of reaginic antibody-antigen mechanisms. Nature 216 : 1328−1336, 1967.

2. Taylor WA, Francis DH, Sheldon D, Roitt IM. Antiallergic actions of disodium cromoglycate and other drugs known to inhibit 3', 5'-nucleotide phosphodiesterase. Int Archs Allergy Appl Immunol. 47 : 175−193, 1974.

3. Ishizaka T, Foreman JC, Sterk AR, Ishizaka K. Induction of calcium flux across the rat mast cell membrane by bridging IgE receptors. Proc Natl Sci USA 76 : 5858−5862, 1979.

4. Ishizaka T, Hirata F, Ishizaka K, Axelrod J. Stimulation of phospholipid methylation, Ca^{2+} influx and histamine release by bridging of IgE receptors on rat mast cells. Proc Natl Sci USA 77 : 1903−1908, 1980.

5. Enis M, Ind PW, Pearce FL, Dollery CT. Colcium antagonists and histamine release from rat peritoneal mast cells. Agents Actions 13 : 144−148, 1983.

6. Chand N, Diamantis W, Sofia RD, Antagonism of leukotrienes, calcium and histamine by azelastine. Pharmacologist 26 : 152−152, 1984.

7. Diamantis W, Chand N, Harison JE, Pillar J, Perhach JL, Sofia RD, Inhibition of SRS-A and its antagonism by azelastine (A),an H_1 antagonist-antiallergic agent. Pharmacologist 24 : 200−200, 1982.

8. Chand N, Pillar J, Diamantis W, Perhach JL Jr, Sofia RD. Inhibition of calcium ionophore (A23187)-stimulated histamine release from rat peritoneal mast cells by azelastine. Implications for its mode of action. Eur J Pharmacol. 96 : 227−233, 1983.

9. Fields DAS, Pillar J. Diamantis W, Perhach JL Jr, Sofia RD, Chand N. Inhibi tion by azelastine of nonallergic histamine release from rat peritoneal cells. J Allergy

clin Immunol. 73 : 400−403, 1984.
10. Fisher B, Schmutzler W. Inhibition by azelastine of the immunologically-induced histamine release from isolated guinea pig mast cells. Arzneimittel-Forsch. 31 : 1193−1195, 1981.
11. Chand N, Pillar J, Diamantis W, Sofia RD. Inhibition of allergic histamine release by azelastine and selected antiallergic drugs from leukocytes. Int Archs Allergy Appl Immunol. 77 : 451−455, 1985.
12. Nishihira J, Hayakawa T, Suzuki K, Kato K, Ishibashi T. Effect of azelastine on leukotriene synthesis in murine peritoneal cells and on thromboxane synthesis in human platelets. Int Archs Allergy Appl Immunol. 90 : 285−290, 1989.
13. Foreman JC, Hallet MB, Mongar JL. The relationship between histamine secretion and ^{45}Ca uptake by mast cells. J. Physiol. 271 : 193−214, 1977.
14. Tanizaki Y, Townley RG. Effect of BSA on Ca^{2+} influx in mast cells stimulated by ovalbumin. Int Archs Allergy Appl Immunol. 70 : 143−145, 1983.
15. Ranadive NS, Dahnari N. Movement of calcium ions and release of histamine from rat mast cells. Int Archs Allergy Appl Immunol. 70 : 143−145, 1980.
16. Tanizaki Y, Komagoe H, Morinaga M, Kitani H, Goda Y, Kimura I. Allergen- and anti-IgE-induced histamine release from whole blood. Int Archs Allergy Appl Immunol. 73 : 141−145, 1984.
17. Tanizaki Y, Komagoe H, Sudo M, Kitani H, Nakagawa S, Tada S, Takahashi K, Kimura I. Basophil histamine release induced by Candida albicans. Relationship to specific IgE and IgG antibodies. Jpn J Allergol. 34 : 422−427, 1985.
18. Sung CP, Saunders HL, Lenhardt E, Chaknin LW. Further studies on the tachphylaxis to DSCG. The effect of concentration and temperature. Int Archs Allergy Appl Immunol. 55 : 385−394, 1977.
19. Tanizaki Y, Akagi K, Lee KN, Townley RG. Inhibitory effect of nifedipine and cromolyn sodium on skin reactions and ^{45}Ca uptake and histamine release in rat mast cells induced by various stimulating agents. Int Archs Allergy Appl Immunol. 72 : 102−109, 1983.
20. Tanizaki Y, Komagoe H, Ohtani H, Tada S, Takahashi K, Kimura I. Inhibitory effect of nicardipine on ^{45}Ca uptake and histamine release in mast cells stimulated by antigen. Jpn J Allergol. 134 : 204−209, 1985.
21. Kusner EJ, Dubnick B, Herzig DJ. The inhibition by disodium cromoglycate in vitro of anaphylactically-induced histamine release from rat peritoneal mast cells. J Pharmac Exp Ther. 184 : 41−46, 1973.
22. Thomson DS, Evans DP. Inhibition of immediate hypersesitivity reactions by sodium cromoglycate. Requirement for activity in two laboratory models. Clin Exp Immunol. 13 : 537−544, 1973.
23. Tanizaki Y, Komagoe H, Ohtani J, Maeda M, Kitani H, Takahashi K, Kimura I. Effect of DSCG on immunolgical secretory process of mast cells. Relationship to the tachyphylaxis. 7 : 202−207, 1983.
24. Evans DP, Marshall PW, Thomson DS. Inhibition of immediate hypersensitivity reactions in the rat by ICI 74 717 and disodium cromoglycate. Tachyphylaxis and cross reactivity in vivo and in vitro. Int Archs Allergy Appl Immunol. 49 : 417−427, 1975.

ラット腹腔肥満細胞のCa^{2+} influxおよびヒスタミン遊離に対する抗アレルギー薬アゼラスチンの抑制効果について

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抗原刺激時の，ラット腹腔肥満細胞の Ca^{2+} uptake およびヒスタミン遊離に対する，抗アレルギー薬アゼラスチンの抑制効果について検討を加えた。その結果，1. アゼラスチンは抗原刺激時の肥満細胞の Ca^{2+} uptake に対して濃度依存性の抑制効果を示した。2. 同様に，抗原刺激時の肥満細胞からのヒスタミン遊離に対しても，濃度依存性の抑制効果を示した。3. アゼラスチンの再暴露による抑制効果の減弱傾向は見られず，アゼラスチンではtachyphylaxisは観察されなかった。

キーワード：アゼラスチン，　ラット肥満細胞，
Ca^{2+}uptake，　ヒスタミン遊離