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 immunological 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 inhibits 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 concentration of the agent. 3. Tachypylaxis 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. Earlier studies on DSCG demonstrated that the agent inhibited phosphodiesterase activity. It is also shown that the agent inhibits an increased Ca$^{2+}$ influx into mast cells following stimulation with antigen.

It has been reported that azelastine, an antiallergic agent, inhibits allergic and non-allergic release of SRS−A$^{6}$ and histamine release$^{6-10}$. In addition to these inhibitory effects, azelastine inhibits the production of leukotrienes B$_4$ and C$_4$. 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)-phthalazino-ne hydrochloride) was presented by Eisai Pharmaceutical Co. (Fig. 1).
Sprague Dawley rats were sensitized with an intramuscular injection of 0.5 ml saline containing ovalbumin, 50 mg/ml, and 8x10^6 killed organisms (Bordetella pertussis) per milliliter, into each hind limb according to the method described by Foreman, et al. 12-14 days after sensitization, the rats were sacrificed, and mast cells were separated from abdominal cavity with 200 times gentle massage. The cells purified by the density gradient of BSA. The purity of mast was more than 90%.

^45Ca uptake by mast cells induced by antigen was performed by the method modified from that described by Ranadive, et al. 3 μCi ^45Ca 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°C for 30 min. After the number of mast cells was adjusted to 10^6 cells/0.1 ml, the cell suspension was added into the test tube containing ^45Ca and ovalbumin, and kept in water bath at 37°C. The mixed solution was incubated at 37°C for 10 min. After the mast cells were washed twice with 5 ml of cold physiological saline, the residual free radioactive ^45Ca was removed through microfiber filter (Whatman, type GF/C, pore size 1.2 μm). The amount of ^45Ca in each cell suspension was determined by using a scintillation counter. All experiments for ^45Ca uptake was carried out in triplicate.

Histamine release from mast cells induced by antigen was examined under the same condition as the experiment for ^45Ca 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 to azelastine was examined by the re-exposure following incubation with azelastine.

**Results**

Azelastine inhibited ^45Ca 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 azelastine 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 ^45Ca 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).
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 stimulation with antigen play an important role in the release of chemical mediators through major routes. 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 A₂ and promote synthesis of arachidonic acid mainly from phosphatidylycerine. Prostaglandins and leukotrienes

Discussion

Tachyphylaxis to azelastin was not observed in immunological secretory process of mast cells. The inhibition of azelastine was 35.1% for ⁴⁵Ca 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 ⁴⁵Ca uptake and 38.1% for histamine release when mast cells were re-exposed following previous exposure with the agent (Fig. 3).
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(newly generated mediators) are synthesized and released by this route.

Our previous studies have shown that a calcium channel antagonists, nifedipine and nicardipine, inhibit \textsuperscript{45}Ca uptake by antigen-stimulated mast cells, and consequently reduce histamine release\textsuperscript{19,20}. Similar inhibitory effect was observed in an antiallergic drug, DSCG, suggesting that the agent also prevents the influx of Ca\textsuperscript{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\textsuperscript{21,22} or re-exposure with the agent (tachyphylaxis)\textsuperscript{23}.

Azelastrine possesses potent histamine H\textsubscript{1} receptor blocking properties\textsuperscript{7}. It has been reported that azelastrine strikingly inhibits the production of leukotrienes B\textsubscript{1} and C\textsubscript{1}\textsuperscript{22}. Thus, azelastrine inhibits allergic and non-allergic release of SRS-A\textsuperscript{20} and histamine\textsuperscript{23}. In the present study, the effect of an antiallergic agent, azelastrine, was examined in immunological secretory process of mast cells. The results from this study show that azelastrine significantly inhibits both \textsuperscript{45}Ca uptake and histamine release in mast cells stimulated by antigen. The inhibition of azelastrine on Ca\textsuperscript{2+} influx into mast cells might be related to the inhibition on the production of leukotrienes, and it might be suggested that azelastrine inhibits the release of leukotrienes and histamine by preventing an increase in activity of phospholipase A\textsubscript{2} stimulated by Ca\textsuperscript{2+}.

References


Azelastine and Ca uptake


ラット腹腔肥溝細胞の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、ラット腹腔肥溝細胞の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溝細胞の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，抗原刺激時の肥溝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，ラット肥溝細胞 Ca$^{2+}$ uptake，ヒスタミン遊離