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Yoshiro Tanizaki[*] Haruki Komagoe[†] Michiyasu Sudo[‡]
Jun Ohtani[**] Ikuro Kimura[††]
Katsumi Akagi[‡‡] Robert G. Townley[§]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,
[§]Greighton University,

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Abstract

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KEYWORDS: mast cells, Ca2+ uptake, histamine release, Ca2+ antagonist

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INHIBITORY EFFECT OF THE CA^{2+} ANTAGONIST NIFEDIPINE ON HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

Yoshiro TANIZAKI, Haruki KOMAGOE, Michiyasu SUDO, Jun OHTANI*, Ikuro KIMURA*, Katsumi AKAGI* and Robert G.TOWNLEY**

*Department of Medicine, Okayama University Medical School, Misasa Branch Hospital, Tottori 682-02; *Second Department of Medicine, Okayama University Medical School, Okayama 700, Japan: and **Department of Medicine, Creighton University School of Medicine, Omaha 68178, U.S.A.*

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Abstract. ^{45}Ca uptake and histamine release was examined in mast cells from rats sensitized with ovalbumin and Bordetella Bertussis as an adjuvant. The uptake of ^{45}Ca by the mast cells was significantly increased by stimulation with ovalbumin as was the release of histamine from the mast cells. Nifedipine, a calcium antagonist, inhibited the increase in both ^{45}Ca uptake and histamine release stimulated by ovalbumin, though the effect on ^{45}Ca uptake was stronger than that on histamine release.

Key words: mast cells, Ca^{2+} uptake, histamine release, Ca^{2+} antagonist.

It has been reported that extracellular calcium is required for the release of chemical mediators from mast cells and basophils (1), and may participate in a two-step antigen-antibody release reaction (2). Extracellular calcium may act in the early stage and be associated with permeability of the cell membrane (3). Intracellular calcium at the late stage is essential for all stimulations. Foreman *et al.* (4) have shown that stimulation of mast cells by an antigen-antibody reaction induces an increase in the uptake of ^{45}Ca, which is accompanied by histamine release, and that there is a close relationship between the amount of ^{45}Ca uptake and the magnitude of histamine release.

Recent studies by Hirata *et al.* (5) and Ishizaka *et al.* (6) have shown that con A and antigens cause stimulation of phospholipid methylation in rat mast cells. This process is followed by an influx of calcium into mast cells and release of histamine. When the influx of calcium into mast cells is inhibited, the release of histamine from the cells is also inhibited.

Calcium antagonists are popularly used for heart disease and hypertension, but not for allergic diseases. In this study, an inhibitory effects of Nifedipine, a calcium antagonist, on ^{45}Ca uptake and histamine release was examined in rat peritoneal mast cells.

MATERIALS AND METHODS

Sprague-Dawley rats weighing 200 to 250 gm were sensitized with ovalbumin according to the method described by Foreman *et al.* (4). Twelve to fourteen days after sensitization, rats were sacrified and peritoneal cells were removed. The peritoneal cells were washed once with physiological saline by low speed centrifugation at $50 \times g$ for 10 min. Then the cells were resuspended in physiological saline. Two milliliters of 56 % (w/v) bovine serum albumin (BSA) was put into the bottom of a test tube (15×105 mm) and 2 ml of 40 % (w/v) BSA on top. The cell suspension (2 ml) was added to the tube and allowed to settle for 20 min and then centrifuged at $300 \times g$ for 20 min. Mast cells in the 56 % w/v BSA layer were washed three times with physiological saline, and cell number was adjusted to $2.5\text{-}10 \times 10^4$/0.2 ml. The purity of the mast cells in this study was 95.4 ± 0.8 %.

The ^{45}Ca uptake by mast cells was performed with a modified method described by Ranadive *et al.* (7). Each test tube, containing 3 μCi of ^{45}Ca in 0.1 ml distilled water and 10 μg/ml of ovalbumin in 0.7 ml of Tyrode solution was prepared in a water bath at 37℃. Then 0.2 ml cell suspensions containing 10^5 mast cells, which were preincubated with various concentrations of nifedipine for 30 min at room temperature, were added. After 10 min of incubation with ovalbumin at 37℃, the cell suspension was washed twice with 5 ml of cold physiological saline at $300 \times g$ for 10 min. The residual free radioactive ^{45}Ca was removed by passing through a glass microfiber filter (Gelman, Type A/E). The amount of ^{45}Ca in each cell suspension was determined with a liquid scintillation counter. All experiments for ^{45}Ca uptake were carried out in triplicate.

The release of histamine from mast cells was studied under the same conditions as for ^{45}Ca uptake. Histamine release was measured by an automated spectrofluorometric technique (Technicon) (8), and the results were expressed as percent inhibition of histamine release. The mean histamine release was 21.5 ± 0.7 % with stimulation by the antigen, and the spontaneous histamine release was 5.5 ± 0.3 %. All experiments for histamine release were performed twice.

RESULTS

^{45}Ca uptake of the ovalbumin-stimulated cells was significantly increased compared to that of the unstimulated cells ($p < 0.005$). The increase in ^{45}Ca uptake by the stimulated cells paralleled the increase in the number of the cells: 345 cpm by 2.5×10^4, 634 cpm by 5×10^4 and 1389 cpm by 10×10^4 mast cells. On the other hand, the uptake of ^{45}Ca by the unstimulated cells did not increase so: 182 cpm by 2.5×10^4, 280 cpm by 5×10^4 and 349 cpm by 10×10^4 mast cells. The results revealed that the increased uptake of ^{45}Ca by the stimulated cells was induced by an antigen-antibody reaction (Fig. 1).

Nifedipine inhibited the ^{45}Ca uptake of mast cells induced by ovalbumin. The inhibitory effect of nifedipine on the antigen-induced ^{45}Ca uptake peaked at a final concentration of 5 μg/ml, in which the percent inhibition was 63.5 % (Fig. 2). This effect was suppressed at a concentration of 50 μg/ml.

The release of histamine from the stimulated mast cells was slightly inhibited by nifedipine. This inhibitory effect peaked at a concentration of 5 μg/ml of nifedipine and decreased at 50 μg/ml. The maximum percent inhibition was 20.4 % (Fig. 3).

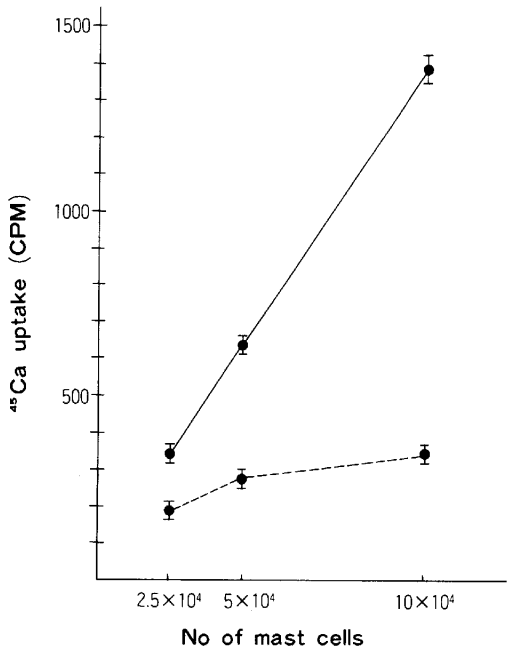

Fig. 1. Relationship between the ^{45}Ca uptake of mast cells and the number of cells. Stimulated cells (●—●), Unstimulated cells (●---●). Values are means ± SEM of three experiments.

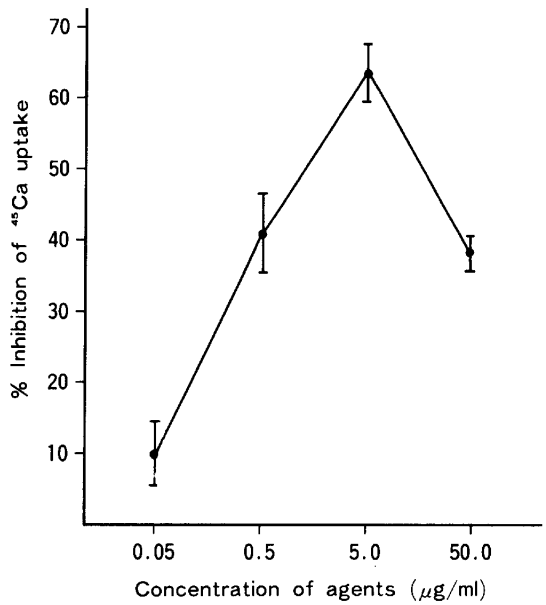

Fig. 2. Inhibitory effects of nifedipine on ^{45}Ca uptake of the stimulated mast cells. Values are means ± SEM of three experiments.

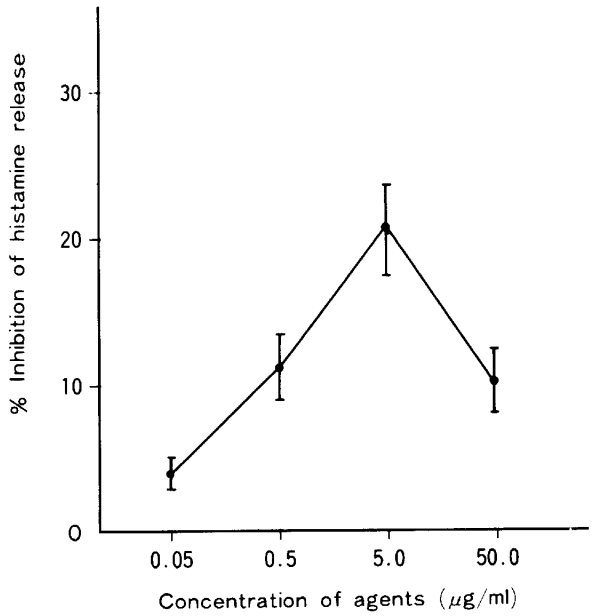

Fig. 3. Inhibitory effects of nifedipine on histamine release from the stimulated mast cells. Values are means ± SEM of three experiments.

DISCUSSION

It is well known that extracellular calcium participates in histamine release from mast cells induced by an antigen-IgE interaction at the membrane. An antigen stimulates phospholipid methylation of the cell membrane (6), which is related to the opening of the calcium gate. Foreman *et al.* (4) divided the uptake of ^{45}Ca by non-stimulated mast cells into two stages, rapid and slow. The rapid stage of ^{45}Ca uptake represents the exchange between the label and Ca bound to the surface of the mast cells. The binding sites equilibrate rapidly with the labelled Ca, and the exchange does not appear to become saturated as the external Ca concentration is raised. The slower stage of ^{45}Ca uptake represents the entry of calcium into the cells across the resting membrane.

When mast cells are stimulated by an antigen, an increase in the uptake of ^{45}Ca by the cells is observed. In this study, ^{45}Ca uptake by the antigen-stimulated mast cells was significantly increased (1389 ± 36 cpm/10^5 mast cells) compared to that by the unstimulated cells (348 ± 28 cpm).

Generally, the rates of histamine release paralleled the calcium uptake, though the inhibitory effect of nifedipine on ^{45}Ca uptake was stronger than that on histamine release. The maximal percent inhibition by nifedipine was 63.5 % of the uptake of ^{45}Ca and 20.4 % of the release of histamine.

The results of this study showed that nifedipine, a calcium antagonist, inhibits ^{45}Ca uptake by antigen-stimulated mast cells, followed by the inhibition of histamine release. These results suggest that a calcium antagonist may be useful as a drug for bronchial asthma and other allergic diseases.

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