（第３号様式）

学　位　論　文　要　旨

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論　文　名　　マスト細胞におけるトランスポーターを介するヒスタミンの
取り込み機構の解析

学位論文要旨

1. Introduction

The rat basophilic leukemia (RBL) cell line (RBL-2H3) is a mast cell line considered as a good and convenient model to study IgE-mediated degranulation of mast cells because of the expression of IgE receptors (FcεRI) and storage of mediators in granules. Although the mechanism of histamine transport from cytosol into granules has been already figured out, the mechanism of histamine transport from environment into the mast cell and vice versa is still not fully understood. In spite of its convenience for studying the exocytotic process in mast cells, the high amount of endogenous histamine hampered to observe the uptake process of histamine into the cells. In our laboratory, we identified the RBL-2H3 sub-clone cells, namely RBL-2H3 Sc98 cells, that have very low level of histamine compared to the wild type cells. With the very few amount of endogenous histamine content, RBL-2H3 Sc98 cells can be useful to monitor the uptak eprocess of histamine into the mast cells. Some transporters that belong to uptake-2 system including organic cation transporters (OCT 1-3) and plasma membrane monoamine transporter (PMAT) are known to have the ability to transport histamine, serotonin, and catecholamines. Since the uptake mechanism of histamine in mast cells is still not fully understood, in this study, using RBL-2H3 Sc98 cells, I show that mast cells took up histamine from environment mediated by uptake-2 transporters. I used various inhibitors of each transporter to see their suppressions against histamine uptake and provided the mRNA expression of these transporters in RBL-2H3 Sc98 cell to conclude which uptake-2 transporter that have a role as histamine transporter in mast cells.

2. Methods

a. Cells

RBL-2H3 and RBL-2H3 Sc98 cells were cultured in MEM containing 15% fetal calf serum and antibiotics in a flask in a humidified atmosphere (5% CO_2) at 37°C.

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b. Uptake of histamine and FFN206 (Fluorescent cation transporter indicator)

Histamine uptake was determined by measuring the total histamine content in the cells by HPLC-fluorometry. Decynium-22(nonspecific OCT inhibitor), desipramine (OCT1 inhibitor), cimetidine (OCT2 inhibitor), corticosterone (OCT3 inhibitor), and lopinavir (PMAT inhibitor) were used as inhibitor of uptake-2 transporters. Uptake of FFN206 in cells was monitored using a fluorometric imaging plate reader (FlexStation II).

c. mRNA Expression of uptake-2 transporters

Conversion of OCT1, OCT2, OCT3, and PMAT's mRNA to cDNA via RT-PCR followed by amplifying PCR were carried out using the Takara RNA PCR kit with the corresponding sense and antisense primers for each transporter. A housekeeping gene, GAPDH, was used as a positive control for the RT-PCR reaction.

3. Results

RBL-2H3 Sc98 cells possess low content of endogenous histamine. The activity of HDC in the RBL-2H3 Sc98 was very low without stimulation or after the stimulation with DNP-BSA as a ntigen or PMA. RBL-2H3 Sc98 cells have the ability to take up histamine from the environment into the cells following dose response and time-dependent manner of exogenous histamine loading, with calculated EC_{50} value is 111.4 ± 1.10μM. RBL-2H3 Sc98 cells also express the VMAT-2, suggesting that after the histamine is taken up into the cytosol, it will be transported and stored in the granules through VMAT-2. The histamine and *β*-hexosaminidase were released after stimulation with DNP-BSA in the dose-dependent manner, showing the exocytotic event was occurred. Decynium-22 (D-22) is well known as uptake-2 transporter inhibitor especially toOCT-3 and PMAT. showing that D-22 inhibited histamine uptake in RBL-2H3 Sc98 up to 30.07±11.43%. Desipramine, cimetidine, corticosteron and lopinavir as inhibitor for OCT1, OCT2, OCT3 and PMAT, respectively were used. Lopinavir gave high suppression (IC_{50} = 17.5 ± 3.3μM) to the histamine uptake of RBL-2H3 Sc98 cells, desipramine slightly inhibited, while cimetidine and corticosteron did not show inhibitory activities. Using uptake of FFN206, lopinavir and desipramine showed inhibitory effects with IC_{50} of 29.14 μM and 15.32 μM, respectively, but cimetidine and corticosterone have no inhibitory effects. The mRNA expression of the transporters confirmed that OCT1 and PMAT were expressed with high level in both cell lines.

4. Conclusion

The histamine uptake in mast cells was directly measured using histamine depleted mutant mast cell line (2H3Sc98) and the PMAT and OCT1 are the candidates of this uptake by confirming of expression of mRNA and using inhibitors pharmacologically.

キーワード（３～５）	ラット好塩基球性白血病細胞(RBL-2H3) plasma membrane monoamine transporter (PMAT) 有機カチオントランスポーター(OCT) ヒスタミン