Forum Minireview

Recent Advances in Molecular Pharmacology of the Histamine Systems: Organic Cation Transporters as a Histamine Transporter and Histamine Metabolism

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Abstract. Histamine is inactivated by the histamine-metabolizing enzyme histamine N-methyltransferase (HNMT) in bronchus, kidney, and the central nervous system. HNMT seems to be localized in the cytoplasm, but histamine is unable to easily enter the intracellular space. Therefore, two hypotheses can be elicited: one is the plasma membrane hypothesis that HNMT can be translocated to the plasma membrane and function at the cell surface under growth factor stimulation and the other is the transporter hypothesis that organic cation transporter (OCT)-2 and -3 can function as a histamine transporter as well. To investigate the involvement of OCT2, HEK293 cells stably double transfected with C-terminal hemagglutinin (HA)-tagged HNMT cDNA and/or C-terminal myc-tagged rat OCT2 were prepared for analysis of HNMT activity associated with OCT2 function. After 60-min incubation of these cells with PBS including HA (100 µM), N\textsuperscript{-}\textgreek{tau}-methylhistamine (MHA) concentration of the supernatants was determined by the HPLC-fluorometry method. MHA from cells with HNMT plus OCT-2 was produced at about 3-fold higher level than that from cells with HNMT alone, suggesting that OCT-2 could function as a histamine transporter as well and that HNMT function could partly depend on OCT-2 transporter activity. Using OCT-3 knockout (OCT-3\textsuperscript{−/−}) mice, histamine content and survival rates were investigated in lipopolysaccharide (LPS)-induced endotoxemia model. Without LPS stimulation, histamine content was compared between OCT-3\textsuperscript{−/−} and wild mice. Histamine content in the spleen of OCT-3\textsuperscript{−/−} mice was higher than that of wild mice. With LPS stimulation, the survival rate of OCT-3\textsuperscript{−/−} mice was significantly decreased 12 h after LPS (20 mg/kg) stimulation, suggesting that before immunological stimulation, a higher content of histamine in spleen could stimulate histamine receptors in mast cells, macrophages, dendritic cells, as well as T lymphocytes and explaining the decreased survival rate in OCT-3\textsuperscript{−/−} mice possibly due to the functional changes of immunological cells.

Keywords: histamine N-methyltransferase, organic cation transporter

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**Introduction**

Histamine is synthesized and stored in the vesicles of mast cells and basophils (1 – 3). Recent studies have documented that histamine production from monocytes/macrophages, T-lymphocytes, and vascular endothelial cells was induced by lipopolysaccharide (LPS), interleukin-1β, TNF-α, or concanavalin-A (4 – 6). Histamine is involved in regulation and modulation of immune response through the stimulation of four distinct subtypes of receptors, H₁, H₂, H₃, and H₄, present on the target cells (7 – 10). The histamine released from storage or producing cells into the extracellular space must be inactivated to terminate the effect of histamine via histamine receptors on target cells. To inactivate histamine, it must be metabolized into inactive metabolites and/or must be transported into cells to remove an excess amount of histamine in extracellular space.

Comparatively higher affinity transporters for reuptake of monoamines, such as norepinephrine, dopamine, serotonin, and histamine, have been demonstrated (11, 12). These studies indicated that among these transporters organic cation transporter (OCT)-2 and -3 have the ability to transport histamine into cells in a potential-sensitive mode (13). OCT-2 was expressed exclusively in the kidney, whereas OCT-3 was expressed ubiquitously, although there is, to some extent, differences in quantities of OCT-3 expression among species (12). Recently, the OCT-3 gene has been targeted by homologous recombination in mouse embryonic stem cells, using a construction with deleted first exon (14). It is reported that utilizing OCT-3 gene knockout mice, an OCT-3 plays a role as uptake-2 transporter in extraneuronal tissues such as the kidney, heart, vascular system, and central nervous system (14 – 16).

The other ways of histamine inactivation are to metabolize histamine by two main catabolic pathways: methylation by histamine N-methyltransferase (HNMT, EC 2.1.1.8) and oxidative deamination by diamine oxidase (DAO, EC 1.4.3.6) (3). The HNMT enzyme from mouse, rat, and human has been cloned. It is suggested that the HNMT is a critical enzyme for degradation of histamine in the airway as well as the central nervous system (17, 18). Although these enzymes have been characterized by biochemical analysis, little information of intracellular localization of them has been reported. Because histamine is unable to easily enter the intracellular space, higher affinity transporters for histamine have been considered to be required for histamine metabolism. In the present review, we documented intracellular localization and translocation of HNMT and the role of organic cation transporters for reuptake of histamine.

**Membrane hypothesis and transporter hypothesis**

Mechanism of histamine methylation by HNMT in vivo has not been fully characterized from cell biological points of view, although intracellular localization of HNMT enzyme has been considered to be in the cytoplasm. In order to explain about how histamine can be metabolized, we hypothesized two models, the membrane hypothesis and transporter hypothesis (Fig. 1). The membrane hypothesis explained that HNMT protein can be induced to translocate or integrate into plasma membrane and interact with histamine. The transporter hypothesis explained that although HNMT protein can translocate to the plasma membrane, where there is little chance for histamine to access to HNMT enzyme, thus assuming the requirement of histamine transporter and the transporter could function bidirectionally for histamine and histamine metabolite, N°-
methylhistamine in the plasma membrane. Namely, the transporter hypothesis proposes that HNMT should mainly localize and metabolize histamine in the cytoplasm. Barnes et al. reported that membrane-bound HNMT activity in mouse brain was found in the synaptosome fractions and functionally metabolize histamine to N\(^{\tau}\)-methylhistamine (19). However they did not unveil the molecular identity of the membrane-bound HNMT activity. Furthermore, as the data they presented did not deny the histamine uptake mechanism, it is possible to assume histamine transporter involved in metabolism of histamine.

To elucidate the molecular mechanism of an access of HNMT to histamine, we established stably transformed HEK 293 cells with C-terminal HA-tagged human HNMT cDNA and investigated the intracellular localization of HNMT enzyme without any stimulation. HNMT enzyme could be detected to localize in the cytoplasm of the cells by confocal immunofluorescence analysis with a rabbit polyclonal anti HA antibodies followed by secondary antibodies of Alexa488-labeled IgG (Fig. 2: A and B). Thus, it seems reasonable to hypothesize two models for the histamine methylation reaction.

**Translocation of HNMT molecule**

To test whether HNMT enzyme is translocated to the plasma membrane upon receptor mediated stimulation, such as epidermal growth factor (EGF) receptor or adrenergic receptor, we utilized the stably transformed HEK 293 cells with human HNMT cDNA. Taking advantage of endogenously expressed EGF receptor and \(\beta\)-adrenergic receptors, the stably transformed cells were stimulated with EGF (10 ng/ml) or the \(\beta\)-adrenergic receptor agonist, isoproterenol (10 \(\mu\)M). While HNMT enzyme localized in cytoplasm without stimulation, after stimulation with EGF, the enzyme translocated to the plasma membrane within 30 min (Fig. 2A). In contrast, with stimulation of isoproterenol, the enzyme could start to move to the plasma membrane as well as the nucleus within 3 min (Fig. 2B). The response of translocation to the plasma membrane with \(\beta\)-adrenergic receptor stimulation is faster than that with EGF receptor stimulation (Fig. 2C). To clarify which type of receptors in the \(\beta\)-adrenergic response are involved in translocation of HNMT enzyme, atenolol as a \(\beta_1\)-selective inhibitor or butoxamine as a \(\beta_2\)-selective inhibitor were pretreated 10 min before isoproterenol stimulation. Butoxamine inhibited membrane translocation more effectively than atenolol did (Fig. 2D), suggesting that membrane translocation of HNMT enzyme seems to predominantly depend on \(\beta_2\)-adrenergic receptor stimulation although quantification of \(\beta_1\)- or \(\beta_2\)-adrenergic receptor expression was not fully investigated.

**Role of OCTs in histamine metabolism in cell culture system**

Taken together, the intracellular localization of HNMT enzyme was shown to be located in the cytoplasm without stimulation and translocated to the plasma membrane after stimulation by EGF or isoproterenol, except for nuclear localization detected as well in the case of \(\beta\) adrenergic receptor stimulation. Based on the evidence that HNMT enzyme could be translocated to the plasma membrane, in order to constitutively locate HNMT enzyme to the plasma membrane, we have established cells with additional myristolation tag sequence (N-GSSKSKPKNPSQRSC-C) in the amino terminal of HNMT (Myr/HNMT). After treatment of the cells with 100 \(\mu\)M histamine for 1 h, \(N^{\tau}\)-methylhistamine concentration of cultured medium and HNMT activity in the cells were measured followed by calculation of the ratio (hereafter called “in vivo / in vitro assay”) of \(N^{\tau}\)-methylhistamine concentration in medium to HNMT activity (Fig. 3A). Cells with membrane-associated form of HNMT, cells with Myr/HNMT cDNA, revealed a lower rate of histamine metabolism in the in vivo / in vitro assay, suggesting that membrane translocation of HNMT could not participate in the enhancement of histamine methylation reaction, but rather take part in the inhibition of histamine metabolism. Therefore, it is plausible that the histamine transporter hypothesis is more likely than the membrane hypothesis.

To elucidate the role of OCT as a histamine transporter, rat OCT-2 which has been cloned and characterized by Inui et al. (20, 21), was introduced into stably transformed cells with HNMT cDNA and established stably transformed cells with OCT-2/HNMT double cDNA transfection. We tested the role of OCT-2 in histamine methylation reaction perfoming the in vivo / in vitro assay. After treatment of the cells with 100 \(\mu\)M histamine for 1 h, \(N^{\tau}\)-methylhistamine concentration of cultured medium and HNMT activity in the cells were measured by HPLC fluorometry. Overall transporter activity for histamine and \(N^{\tau}\)-methylhistamine was expressed as a ratio by the in vivo / in vitro assay. Compared to cells with HNMT activity alone, the other cells with HNMT/OCT-2 cDNA showed enhanced influx and outflow of \(N^{\tau}\)-methylhistamine and showed an increase in the ratio of the in vivo / in vitro assay (Fig. 3C). From these two experiments: 1) epidermal growth factor receptor or \(\beta\) adrenergic receptor stimulation and 2) utilization of OCT-2/HNMT double...
Fig. 2. Intracellular localization of histamine N-methyltransferase. A: HEK 293 cells with carboxy-terminal HA tagged HNMT cDNA were stimulated by epidermal growth factor (EGF) (10 ng/ml). Localization of HNMT enzyme was detected by confocal immunological fluorescence analysis with rabbit polyclonal primary anti HA antibodies and Alexa-488 labeled secondary antibodies. B: β-Adrenergic receptor stimulation of the HEK 293 cells was performed by isoproterenol (10 µM) for 3 min. The detection of HNMT enzyme was done with the same procedure as EGF stimulation. C: Biochemical detection of membrane associated HNMT enzyme was performed. Pretreatment of biotin labeling of plasma membrane proteins in the HEK 293 cells was performed with an NHS labeling kit (Pierce, Rockford, IL, USA). The HEK 293 cells were lysed with TNE solution followed by isolation of biotinylated proteins with streptavidin-agarose. D: Effect of selective β antagonist on membrane translocation. Atenolol as a β₁-selective antagonist and butoxamine as a β₂-selective antagonist were used in the NHS labeled membrane protein experiment, employing HEK293 cells with HNMT cDNA. Butoxamine inhibited membrane translocation more effectively than atenolol did. ISO, isoproterenol; CTL, control.
transfected cells, we concluded that the transporter hypothesis is a more preferable model for histamine methylation reaction than membrane hypothesis.

**Role of organic cation transporter-3 as a histamine transporter in vivo**

To characterize the OCT-3 as a histamine transporter, we utilized the OCT-3 knockout (OCT-3−/−) mice generated by mouse OCT-3 gene targeting (14). It is reported that the human homolog of OCT-3, EMT, also functions as a histamine transporter (13). We have compared histamine contents in heart, lung, kidney, and spleen between wild and OCT-3−/− mice. We found that there is significant increase in histamine content in the spleen of OCT-3−/− mice, although there is no significant difference between them in other tissues (Fig. 4A). Histamine has a wide variety of functions in immune regulation including vascular endothelial growth factor (VEGF) production via H2 receptor stimulation, mast cell chemotaxis via H4 receptor stimulation, T-cell proliferation, and dendritic cell maturation (22 – 25). We speculated that higher content of histamine in spleen could attenuate the immunological response through histamine receptors in T lymphocytes, macrophages, and mast cells. Thus, we tested the survival rate in the endotoxaemia model using LPS injection (20 mg/kg) into the peritoneal cavity. The significant decrease in survival rate was found in OCT-3−/− mice. Histamine content in spleen tissue of OCT-3−/− mice in LPS induced endotoxemia model was higher than that of wild type mice (Fig. 4B). We speculated that the higher content of histamine at the constitutional level may possibly change Th1/Th2 balance through histamine receptors in T lymphocytes. Moreover, in this model of endotoxemia, inducible histamine production in macrophages would increase and inhibition of histamine clearance in the microenvironment would participate in the increase of histamine content due to OCT-3 deficiency. Hori et al. reported that in the peritonitis model with inoculation of *E. coli* into the peritoneal cavity employing histamine-deficiency (HDC−/−) mice, elimination of *E. coli* from the peritoneal cavity is more efficient than that in HDC+/+ mice, explaining the clearance of bacteria from the peritoneal cavity. They also reported that pretreatment with an H1 or H2 antagonist impaired the clearance of *E. coli* from the peritoneal cavity in the HDC−/− peritonitis model, but in contrast, pretreatment with an H1 or H2 antagonist prompted the clearance of *E. coli* from the peritoneal cavity (26).
This study demonstrated that histamine was an important mediator in the peritonitis model. From the point of regulation of histamine concentration in tissues, OCT-3 is a critical transporter for immunological modulation in endotoxemia or peritonitis model.

**Conclusion**

In the current review, we demonstrated that the transporter hypothesis is a more suitable model for histamine transport and metabolism associated with HNMT, although intracellular translocation to the plasma membrane may have a potential role. The intracellular localization analysis of HNMT enzyme revealed that HNMT could localize in the cytoplasm without stimulation and that with stimulation of EGF or β-adrenergic receptor, HNMT could translocate to the plasma membrane. In addition, in the case of β-adrenergic receptor stimulation, HNMT could also localize into nucleus, although the functional significance remains to be determined. In comparison with the in vitro/in vivo assay of cells with Myr/HNMT, the in vitro/in vivo assay of cells expressing OCT-2/HNMT revealed a higher amount of histamine metabolites in the medium, suggesting that OCT-2 functions as a histamine transporter and participates in enhancement of histamine metabolism. Moreover, we confirmed the role of OCT as a histamine transporter using genetically deleted OCT-3−/− mice. When we analyzed the histamine contents of tissues between WT and OCT-3−/− mice, we detected a higher amount of histamine in the spleen of OCT-3−/− mice. In addition, in the endotoxemia model with LPS administration, OCT-3−/− mice revealed significantly decreased survival rate and higher amount of histamine. It is supposed that due to OCT-3 deficiency, histamine clearance in tissues is affected, resulting in increased histamine content and decreased survival rate.

**References**

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