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# Recent Advances in Molecular Pharmacology of the Histamine Systems: Regulation of Histamine $H_1$ Receptor Signaling by Changing Its Expression Level

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**Abstract.** Histamine $H_1$ receptor (H1R) signaling is regulated by changing its expression level. Two mechanisms are involved in this regulation. One is down-regulation through receptor desensitization. Receptor phosphorylation seemed crucial because stimulation of the mutant H1R lacking five putative phosphorylation sites did not show down-regulation. The phosphorylation level of the mutant receptor was much smaller than that of the wild type ones by several protein kinases. The other is up-regulation through activation of receptor gene expression. Protein kinase C (PKC) signaling was suggested to be involved in this up-regulation. Regulation of H1R expression level was mediated not only through H1R but also autonomic nerve receptors. Stimulation of $M_3$ muscarinic receptors (M3R) induced both down-regulation and up-regulation of H1R. Down-regulation of M3R-mediated H1R seemed not to be mediated by PKC activation, although PKC activation induced H1R phosphorylation. Elevation of H1R expression was induced by the stimulation of M3Rs. PKC was suggested to be involved in this up-regulation. Stimulation of $\beta_2$-adrenergic receptors induced H1R down-regulation through several mechanisms. One of them is enhanced receptor degradation after desensitization and another is suppression of receptor synthesis that includes the suppression of receptor gene expression and enhanced degradation of the receptor mRNA. Protein kinase A was suggested to be involved in enhanced degradation and the activation of the receptor gene expression. Elevation of both H1R expression and its mRNA was observed in nasal mucosa of nasal hypersensitivity allergy model rat after toluene diisocyanate provocation. These results suggest that activation of H1R gene expression plays an important patho-physiological role in allergy. Elevation of the mRNA was partially but significantly suppressed by antihistamines.

***Keywords***: histamine $H_1$ receptor, down-regulation, up-regulation, $\beta_2$-adrenergic receptor, muscarinic receptor

## Introduction

Histamine $H_1$ receptors (H1Rs) mediate allergy in peripheral tissues and histaminergic neurotransmission in the central nervous system. Repetitive stimulation of H1Rs was extensively investigated to induce receptor desensitization by pharmacological studies in vivo. The mechanism of H1R desensitization has been studied at the molecular level (1 – 4) and classified into three steps (5). Down-regulation of H1Rs, the final step of desensitization, lasted for more than 24 h. This long-term mechanism is considered as depression of H1R-mediated histamine functions. Desensitization of H1Rs is induced not only by a homologous mechanism but also a heterologous mechanism. Heterologous desensitization of H1Rs induced by the stimulation of muscarinic receptors was reported. We observed the heterologous down-regulation of H1Rs through stimulation of $M_3$

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muscarinic receptors (M3Rs). On the other hand, H1Rs are synthesized by the activation of H1R gene expression. The H1R gene expression lasts for more than 24 h, and it is suggested to induce long-term activation of H1R-mediated functions. Heterologous up-regulation of H1Rs was induced by the stimulation of M3Rs. M3Rs mediate parasympathetic nervous functions in the airway system, while sympathetic functions are exerted through $\beta_2$-adrenergic receptors ($\beta$2Rs). We are describing mechanisms regulating H1R expression level in this mini-review.

## Methods

HeLa and U373 astrocytoma cells, naturally expressing human H1Rs, were used. CHO cells stably expressing recombinant human H1Rs (CHO-H1R cells) or recombinant human H1Rs lacking five putative phosphorylation sites (CHO-5sitesH1R cells) were used for H1R-mediated H1R down-regulation study. CHO-H1R cells expressing recombinant $M_3$ muscarinic receptors (CHO-H1R/M3R cells) or recombinant $\beta_2$-adrenergic receptors (CHO-H1R/$\beta$2R cells) were used for study of H1R level and H1R gene expression mediated by M3R and $\beta$2R, respectively. Radio-ligand binding assay for H1Rs, M3Rs, and $\beta$2Rs were performed with [$^3$H]mepyramine, [$^3$H]QNB, and [$^3$H]DHA, respectively. Inositol phosphates were determined by labeling cells with *myo*-[$^3$H]inositol. H1R mRNA was determined by real-time reverse transcription-PCR using a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Norwalk, CT, USA). The sequences of the primers and probe were as follows: forward primer for H1R, 5'-CAG AGG ATC AGA TGT TAG GTG ATA GC-3'; reverse primer for H1R, 5'-AGC GGA GCC TCT TCC AAG TAA-3'; and probe FAM-CTT CTC TCG AAC GGA CTC AGA TAC CAC C-TAMRA. H1R gene promoter assay was performed using pGL3-basic (Promega, Madison, WI, USA) encoding the H1R promoter region.

## H1R-mediated H1R down-regulation

Five putative phosphorylation sites were identified by an in vitro phosphorylation assay. These sites included Thr-140, Thr-142, Ser-396, Ser-398, and Thr-478. Down-regulation of wild-type and mutant H1Rs in CHO-H1R cells and CHO-5sitesH1R cells, respectively, were examined. Histamine-stimulation of CHO-H1R cells induced down-regulation of H1Rs, while that of in CHO-5sitesH1R cells abolished the down-regulation (Fig. 1). Phosphorylation of wild-type and mutant H1Rs was examined using membranes from CHO-H1R cells and CHO-5sitesH1R cells, respectively. Levels of phosphorylation by protein kinase C (PKC), protein kinase A, calcium/calmodulin-dependent protein kinase II, and protein kinase G were significantly lower in CHO-5sitesH1R cells compared with CHO-H1R cells.

## M3R-mediated H1R down-regulation

M3R-mediated down-regulation of H1Rs was checked using CHO-H1R/M3R cells. Stimulation by carbachol of CHO-H1R/M3R cells induced down-regulation of H1Rs. Stimulation by the combination of carbachol and histamine induced more down-regulation of H1Rs compared with that of by carbachol or histamine alone. Phosphorylation of H1Rs were induced by carbachol in CHO-H1R/M3R cells and it was completely suppressed in the presence of Ro31-8220, an inhibitor of PKC. However, Ro31-8220 did not suppress M3R-mediated down-regulation of H1Rs.

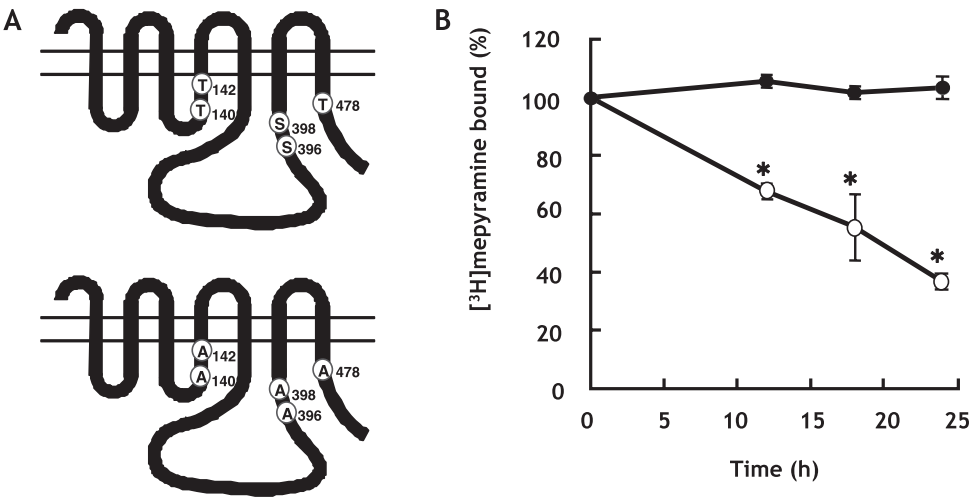


**Fig. 1.** Histamine $H_1$ receptor (H1R)-mediated H1R down-regulation. A: Structures of wild (upper) and mutant (lower) H1Rs. Mutant H1Rs lack putative phosphorylation sites by replacing serine and threonine residues by alanine residues. B: Time-course of H1R-mediated H1R down-regulation. CHO-H1R cells expressing wild H1Rs (open circle) and CHO-5sitesH1R cells expressing mutant H1Rs (closed circle) were stimulated with histamine (100 $\mu$M). * indicates significant difference *vs* mutant H1Rs ($P$<0.001) (n = 6).

### *β*2R-mediated H1R down-regulation

Down-regulation of H1Rs mediated by *β*2Rs was examined using CHO-H1R/*β*2R cells. Stimulation by fenoterol of CHO-H1R/*β*2R cells induced down-regulation of H1Rs. The down-regulation was partially inhibited by KT-5720, an inhibitor of protein kinase A.

### H1R-mediated H1R gene expression

Stimulation of HeLa cells by histamine induced up-regulation H1R mRNA (Fig. 2) and also H1Rs. Stability analysis showed that histamine has no effect on it. The promoter activity of the H1R was measured using luciferase reporter gene and the stimulation was induced by histamine (Fig. 3). The up-regulation was completely suppressed by Ro31-8220. Phorbol 12-myristate 13-acetate (PMA), a PKC activating phorbol ester, induced the elevation of H1R mRNA.

### M3R-mediated H1R gene expression

U373 astrocytoma cells expressing recombinant human M3Rs (U373-M3 cells) were established. Stimulation of U373-M3 cells by carbachol induced elevation of H1R mRNA and also H1Rs. The promoter of the H1R was also activated by carbachol. Carbachol showed no effect on stability of H1R mRNA. The up-regulation was completely suppressed by Ro31-8220.

### *β*2R-mediated suppression of H1R gene expression

U373 astrocytoma cells expressing recombinant human *β*2Rs (U373-*β*2 cells) were established. Stimulation of U373-*β*2 cells by fenoterol induced the declination of H1R mRNA expression. Down-regulation of H1Rs was also followed by this treatment. The activity of H1R gene promoter was decreased by fenoterol. Fenoterol showed a negative effect on stability of H1R mRNA. The reduction of H1R mRNA mediated by *β*2R was partially suppressed in the presence of KT7220, an inhibitor of protein kinase A.

### Concluding remarks

Histamine induced down-regulation of H1Rs through the receptor desensitization mechanism. The receptor phosphorylation was suggested to play crucial roles in the down-regulation because the mutant H1R lacking putative phosphorylation sites abolished the down-regulation and the phosphorylation of the mutant H1Rs by several protein kinases were also highly reduced (6 – 8). Histamine even induced up-regulation of H1Rs

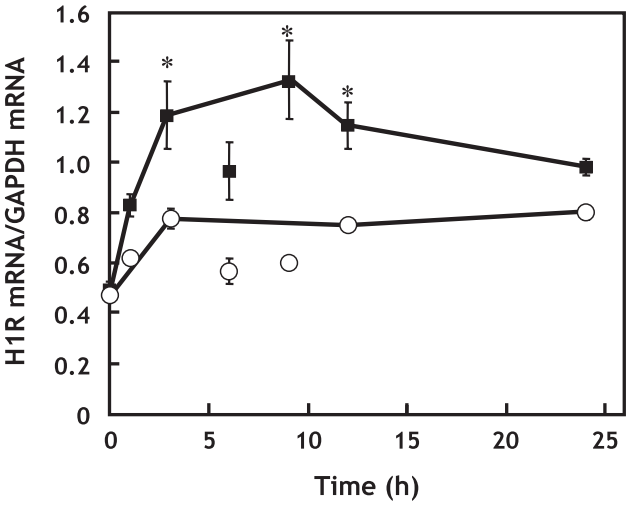


**Fig. 2.** Time-course of histamine $H_1$ receptor (H1R)-mediated H1R mRNA elevation. HeLa cells were stimulated with histamine (10 $\mu$M) (closed square). Control is shown as (open circle). * indicates significant difference *vs* control ($P$<0.05) (n = 3).

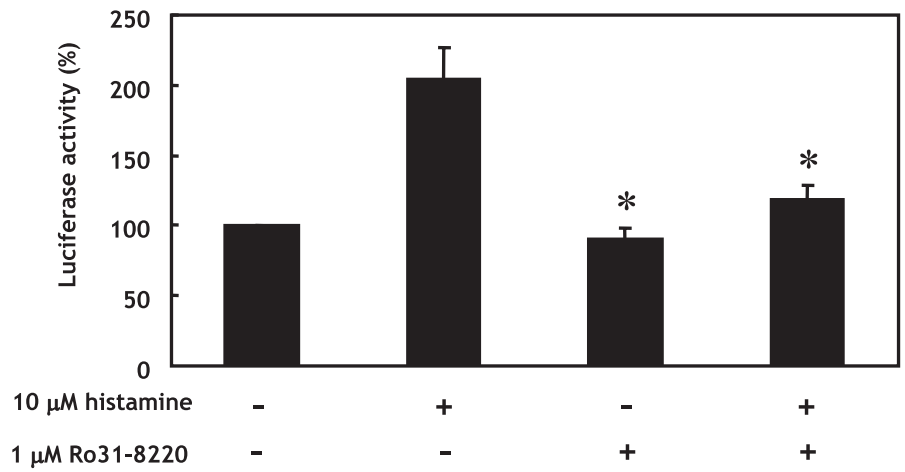


**Fig. 3.** Promoter assay of histamine $H_1$ receptor (H1R) gene. Assay system was described in Methods. Transfected HeLa cells were stimulated with and without histamine (10 $\mu$M) and Ro31-8220 (1 $\mu$M). * indicates significant difference *vs* histamine ($P$<0.05) (n = 3).

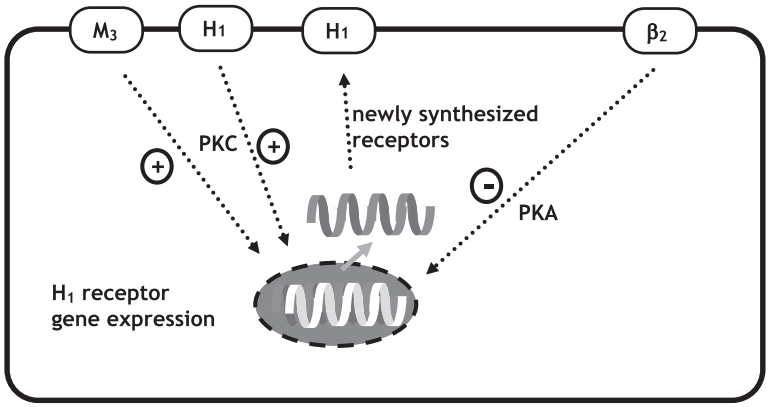


**Fig. 4.** Regulation of histamine $H_1$ receptor ($H_1$) gene expression. Histamine $H_1$ receptor gene expression was increased by the stimulation of $H_1$ receptor itself and $M_3$ muscarinic acetylcholine receptor ($M_3$). In contrast, $H_1$ receptor gene expression was decreased by the stimulation of $\beta_2$ adrenergic receptor ($\beta_2$).

through the receptor gene expression mechanism (Fig. 4). PKC signaling was suggested to mediate the up-regulation because PMA induced the up-regulation and was completely suppressed by Ro31-8220.

In addition to the homologous down and up-regulations by histamine, heterologous regulation of H1R expression was demonstrated (9 – 11). Stimulation of M3Rs induced down-regulation of H1Rs through the receptor desensitization mechanism and up-regulation of H1Rs through the gene expression mechanism (Fig. 4). The mechanism of M3R-mediated H1R down-regulation in which the receptor phosphorylation was not involved seemed quite different from that of H1R-mediated H1R down-regulation. However, the mechanism of H1R up-regulation by M3Rs was similar to that of by H1R. Up-regulation of H1Rs was observed in nasal mucosa of nasal hypersensitive allergic rats upon allergy provocation with toluene diisocyanate (12). This result indicated the dominance of H1R up-regulation. H1R up-regulation in nasal mucosa was partially (about 50%) mediated through H1Rs. Some unidentified signals are also involved in this up-regulation. M3R signaling may be a strong candidate.

Autonomic nerves have been recognized to play important roles in symptoms of allergic diseases. In current therapeutics for allergic diseases, $\beta$2R agonists and M3R antagonists are used. M3Rs and $\beta$2R induced up- and down-regulations of H1Rs, respectively, through an H1R gene expression mechanism. These results strongly suggested that the H1R gene expression mechanism is a target of therapeutics for allergic diseases including $\beta$2R agonists and M3R antagonists.

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