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Heterologous up-regulation of the histamine H_1 receptor by M_3 muscarinic receptor-mediated activation of H_1-receptor gene transcription

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Abstract

Histamine H_1 receptor (H1R) level varies under various pathological conditions, and these changes may be responsible for some pathogenesis, such as allergic rhinitis. Previously, we showed that H1R was heterologously down-regulated (through degradation of H1R) by prolonged stimulation with muscarinic M_3 receptor (M3R) in Chinese hamster ovary (CHO) cells stably expressing H1R and M3R. However, this cell was inadequate for studying the effects on H1R gene regulation, because the cell expresses H1R, which is under the control of the SV40 promoter. Therefore, in this study, we have investigated the possible role of M3R stimulation in the H1R gene transcription and H1R mRNA stability by using U373 astrocytoma cells that express endogenous H1R and transfected M3R. Stimulation of M3R significantly increased H1R promoter activity and H1R mRNA level without alteration in H1R mRNA stability. The H1R level was also up-regulated by M3R activation (150% of control by treatment with carbachol for 24 h). These M3R-mediated events were almost completely blocked by the protein kinase C (PKC) inhibitor, Ro 31-8220, suggesting the involvement of PKC. These results indicated that M3R was involved in the up-regulation of H1R by activating H1R gene transcription through a PKC-dependent process.

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Introduction

The various actions of histamine are mediated through four histamine receptor subtypes, H_1, H_2, H_3 and H_4 receptors (Hill et al 1997; Nguyen et al 2001). All these four histamine receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), and histamine H_1 receptor (H1R) is coupled, via $G_{Q/11}$ family of G proteins, to phospholipase Cβ, which catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate to form inositol-1,4,5-trisphosphate and diacylglycerol. Inositol-1,4,5-trisphosphate causes the mobilization of calcium from intracellular stores, and diacylglycerol activates protein kinase C (PKC). H1Rs are distributed in a wide variety of tissues including: mammalian brain; smooth muscle from airways, gastrointestinal tract, genitourinary system, and the cardiovascular system; adrenal medulla; and endothelial cells and lymphocytes (Hill et al 1997).

The level of H1R in the brain and peripheral tissues is dynamically regulated under various physiological and pathological conditions: up-regulation of H1R or H1R mRNA in the electrical foci in the temporal cortex of epileptic patients (Iinuma et al 1993) and in the nasal mucosa of patients with allergic rhinitis (Iriyoshi et al 1996; Hamano et al 1998), down-regulation of H1R in the frontal cortex of patients with chronic schizophrenia (Nakai et al 1991) and in the frontal and temporal areas of patients with Alzheimer's disease (Higuchi et al 2000). The altered H1R level may be related to pathological conditions such as described above, and thus it will be important to delineate the regulatory mechanism of H1R expression.

The H1R level may be regulated by various processes just as indicated in the regulation of other types of G protein-coupled receptors (GPCRs) e.g. modulation of receptor gene transcription (Collins et al 1989), alteration of mRNA stability (Tholanikunnel et al 1995), and receptor degradation or down-regulation (Tsao et al 2001). Among these processes, receptor down-regulation is caused by prolonged receptor stimulation, and our previous

studies have shown that H1R stimulation down-regulates H1R in cultured Chinese hamster ovary (CHO) cells (Horio et al 2004). Moreover, stimulation of M_3 muscarinic receptor (M3R) also down-regulated H1R in the same type of cultured cells expressing H1R and M3R (Miyoshi et al 2004). However, this type of cell was inadequate for studying the effects on H1R gene regulation, because the cell expresses H1R, which is under the control of the SV40 promoter. Therefore, we have investigated whether M3R stimulation had any effect on H1R gene transcription and H1R mRNA stability by using U373 astrocytoma cells that express endogenous H1R and transfected M3R.

Materials and Methods

Materials

Plasmid DNA for human M3R was kindly provided by Dr Tom I. Bonner (National Institute of Mental Health, Bethesda, MD). [Pyridinyl-5–^{3}H]mepyramine ([^{3}H]mepyramine, 20 Ci mmol^{-1}) and quinuclidinyl [phenyl-4-^{3}H]benzilate ([^{3}H]QNB, 30 Ci mmol^{-1}) were obtained from Perkin Elmer (Boston, MA). PolyFect Transfection Reagent was from Qiagen K.K. (Tokyo, Japan). Dual-Luciferase Reporter Assay System, pRL-TK vector and pcDNA3.1/Zeo (+) vector were from Promega (Madison, WI). Pre-Developed TaqMan Assay Reagents of Human GAPDH were from Applied Biosystems (Foster City, CA). All other reagents, unless otherwise stated, were of analytical grade and were from Wako Pure Chemicals (Osaka, Japan) or Sigma (St Louis, MO).

Preparation of U373 astrocytoma cells stably expressing M3R

U373 astrocytoma cells stably expressing M3R were prepared according to the method described by Miyoshi et al (2004) with slight modification. In brief, pcDNA3.1/Zeo (+) vector encoding M3R was transfected into U373 astrocytoma cells by using PolyFect Transfection Reagent. U373 astrocytoma cells stably expressing M3R (U373-M3 cells) were then cloned from individual colonies cultured in Dulbecco's modified Eagle's medium plus 8% fetal bovine serum containing 100 μg mL^{-1} Zeocin. Cloned cells expressing M3R were cultured in the same medium without Zeocin, and the expression of M3R was measured by binding assay using [^{3}H]QNB.

Radioligand binding assay

Cultured cells grown to 70% confluency in 150-mm dishes were harvested with a cell scraper into 1.5 mL ice-cold 50 mM Na/K-phosphate buffer (37.8 mM Na_2HPO_4, 12.2 mM KH_2PO_4, pH 7.4). The cell suspension was homogenized with an ultrasonic disrupter, and the homogenate was centrifuged at 45 000 g for 30 min. The pelleted membranes were resuspended in 50 mM Na/K-phosphate buffer and used for the radioligand binding assay. [^{3}H]Mepyramine binding assay was carried out by incubation of the membranes with [^{3}H]mepyramine (0.1–4 nM) in the absence or presence of 10 μM triprolidine at 25°C for 60 min in a final volume of 500 μL. [^{3}H]QNB binding assay was performed by incubation of the membranes with [^{3}H]QNB (0.01–1 nM) in the absence or presence of 10 μM atropine at 37°C for 60 min in a final volume of 500 μL. Assays were terminated by rapid vacuum filtration through Whatman GF/B filters (Whatman, Maidstone, UK). In [^{3}H]mepyramine binding assay, filters were presoaked with 1% polyethyleneimine. Filters were soaked in 10 mL Aquasol II (Packard Instrument, Inc., Meriden, CT), and the radioactivity on the filter was counted in a liquid scintillation counter.

Assay of the H1R level

Membrane preparations from U373-M3 cells that had been treated with vehicle (control) or various drugs for appropriate times were subjected to radioligand binding assay using 4 nM [^{3}H]mepyramine. Changes in H1R level were expressed as the percentage of [^{3}H]mepyramine binding of the treated cells to that of vehicle-treated cells.

Real-time reverse transcription polymerase chain reaction (real-time RT-PCR) for the measurement of human H1R mRNA

Cells cultured to 70% confluency in 35-mm dishes were cultured without serum for the next 24 h, and then treated with various drugs for appropriate times. After the treatment, the cells were scraped with 700 μL TRIzol Reagent (GIBCO-BRL, Carlsbad, CA), and 200 μL chloroform was added to the cell suspension. After centrifugation of the cell suspension at 15 000 g for 15 min at 4°C, the aqueous phase was collected and the RNA was precipitated by the addition of isopropanol. Total RNA was resolved in 20 μL diethylpyrocarbonate-treated water. Each RNA sample (5 μg) was reverse-transcripted to cDNA in 25 μL reaction buffer (375 mM KCl, 250 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 0.8 mM concentrations of each deoxyribonucleoside triphosphate (dNTPs), 40 μM oligo (dT) primers, 0.1 U RNase inhibitor, and 200 U reverse transcriptase). The transcripts were amplified by using the GeneAmp 5700 Sequence Detection System (Applied Biosystems) in 28 μL reaction mixture containing 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 3 mM $MgCl_2$, 200 μM dNTPs, 660 nM forward and reverse primer, 330 nM probe, 14 μL Platinum Quantitative PCR Super-Mix UDG (Invitrogen, Carlsbad, CA). The sequences of the primers and the 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′; probe, FAM-CTT CTC TCG AAC GGA CTC AGA TAC CAC C-TAMRA (human histamine receptor H1, HRH1, Entrez Gene ID: 3269). To standardize starting material, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used. Data were expressed by the ratio of H1R mRNA level to GAPDH mRNA level.

Luciferase reporter assay

Human H1R reporter plasmid (pH1R) was constructed as follows: a 2.1-kbp 5′-upstream fragment (–2029 to +64, +1 indicates the putative transcription initiation site of human H1R gene (De Backer et al 1998)) was amplified by PCR

using a forward primer (5′-GCTAGCCGAATGTGGGAA-GATCAGTAGTAG-3′) and a reverse primer (5′-AGATCT-GAAGGTCTTCTCCATGATGGGCTTC-3′), and ligated to pGEM-T Easy vector (Promega). After confirming the sequence, the fragment was then subcloned into the NheI-BglII site of the promoter-less luciferase reporter plasmid pGL3-Basic vector (Promega). Cells cultured in 12-well plates were co-transfected with pH1R and pRL-TK vector (Promega) by a ratio of 200:1. Plasmid-mixture (1 μg) was transfected into the cells using PolyFect Transfection Reagent or Lipofectamine plus Reagent according to the manufacturer's instructions. Following 5-h incubation, the medium was replaced with 1 mL DMEM without serum. After a further 24 h, the cells were stimulated with various drugs for the appropriate time in the same medium. Pretreatment with several drugs was performed 30 min before stimulation. After the treatment, the cells were washed with 500 μL cold phosphate-buffered saline twice and lysed with 100 μL passive lysis buffer (Promega). The lysates were frozen at −85 °C for at least 3 h and then thawed at room temperature. The lysates were analysed by using a Dual-Luciferase Assay System (Promega). Luminescence was measured by photoluminescence reader BLR 302 (ALOKA, Tokyo, Japan). The measurement was integrated over 20 s with no delay. In the case of luciferase reporter assay using the drug, Ro 31-8220, the data were standardized by the amount of protein, because the inhibition of PKC decreased luminescence from pRL-TK vector significantly. Data were expressed as fold increase of luminescence of treated cells over that of vehicle-treated cells.

Statistical analysis

Data are expressed as mean ± s.e.m. Statistical analysis was performed by Kruskal–Wallis test followed by Bonferroni's test, or by Mann–Whitney U-test. A value of $P < 0.05$ was considered statistically significant.

Results

Expression of H1R and M3R in U373 astrocytoma cells

U373 astrocytoma cells were transfected with pcDNA3.1 vector encoding human M3R gene. The stable expression of M3R in the cells (U373-M3 cells) was examined by radioligand binding assay using the muscarinic receptor antagonist [^{3}H]QNB. The K_d and B_{max} values obtained by Scatchard analysis were 0.09 ± 0.01 nM and 2.9 ± 0.3 pmol (mg protein)$^{-1}$, respectively, in U373-M3 cells (n = 4). The expression of H1R in U373-M3 cells was measured by using the specific H1R antagonist [^{3}H]mepyramine, and the K_d and B_{max} values were 7.8 ± 1.1 nM and 70.9 ± 10 fmol (mg protein)$^{-1}$, respectively (n = 4).

Effects of carbachol on the expression level of H1R

We examined the effect of M3R stimulation on the expression level of H1R in U373-M3 cells. As shown in Figure 1A, carbachol treatment (1 mM) increased the H1R level; the level

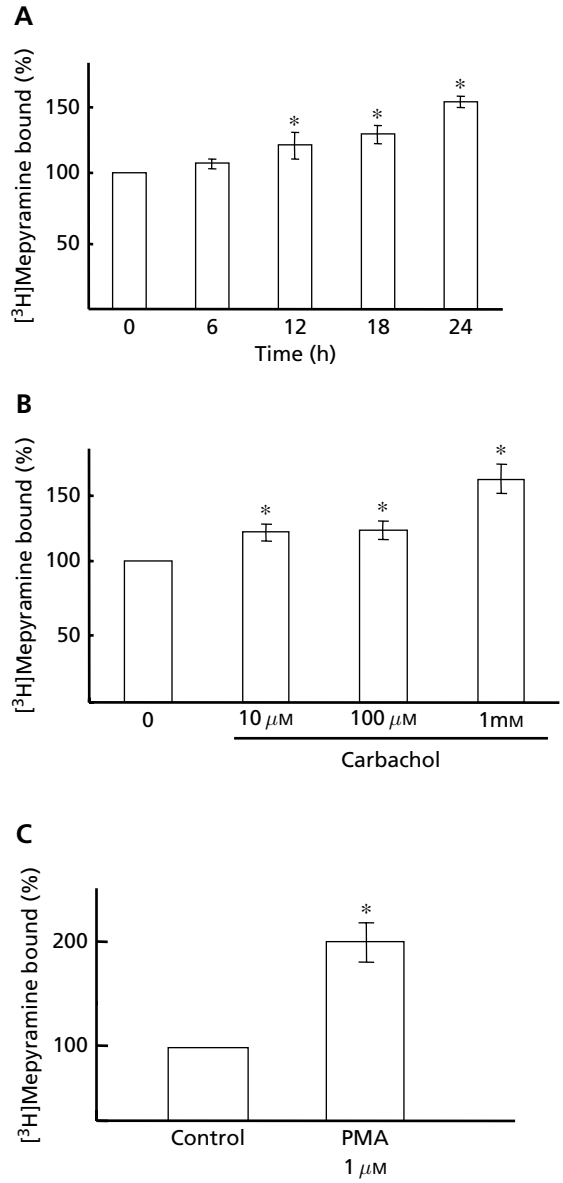

Figure 1 M_3 muscarinic receptor-mediated up-regulation of H1R in U373-M3 cells. [^{3}H]Mepyramine binding assay was used to measure H1R expression level. A. Time course of 1 mM carbachol-induced increase in H1R level. B. Dose–response study of carbachol-induced increase in H1R level. U373-M3 cells were treated with 10–1000 μM carbachol for 24 h. C. PMA-induced increase in H1R level. U373-M3 cells were treated with 1 μM PMA for 24 h. *$P < 0.05$ vs control (n = 4).

was significantly increased by 12-h treatment with carbachol and was approximately 150% of control after 24-h treatment. Carbachol in the concentration range of 10–1000 μM was effective in up-regulating H1R (Figure 1B). Up-regulation of H1R by 1 mM carbachol treatment for 24 h (160.4 ± 9.4% of control) was significantly inhibited by 10 μM atropine (103.8 ± 6.6%), indicating the involvement of muscarinic receptor stimulation in this process ($P < 0.05$, n = 4). M3R is coupled, via $G_{Q/11}$ family of G proteins, to phospholipase Cβ, which leads to the activation of PKC. Thus, we investigated

whether PKC was involved in M3R mediated up-regulation of H1R. As shown in Figure 1C, the treatment with the PKC activating phorbol ester, phorbol 12-myristate 13-acetate (PMA, 1 μM) for 24 h caused a marked increase in H1R level in U373-M3 cells, suggesting the involvement of PKC in this process. We examined the effect of the PKC inhibitor, Ro 31-8220 (Davies et al 2000) on carbachol-induced up-regulation of H1R. Ro 31-8220 (1 μM) completely inhibited carbachol (1 mM)-induced H1R up-regulation (95.5±6.8% vs 158.0±10.0%, $P<0.05$, n=3). This further suggested the involvement of PKC in this process.

Effects of carbachol on H1R mRNA level

We examined the effects of M3R stimulation on H1R mRNA level in U373-M3 cells by using real-time RT-PCR methods. Carbachol induced a transient increase in H1R mRNA level that was maximal by 4 h and returned to basal levels after 12-h treatment (Figure 2A). The treatment with 1 mM carbachol for 4 h increased H1R mRNA approximately 2-fold, and the treatment with 1 μM PMA for 4 h markedly increased H1R mRNA (Figure 2B). The PKC inhibitor, Ro 31-8220 (1 μM) completely inhibited carbachol-induced increase of H1R mRNA (0.87±0.10-fold vs 1.85±0.10-fold, $P<0.05$, n=6). The calcium calmodulin-dependent protein kinase II inhibitor KN93 (Sumi et al 1991) and the protein kinase G (PKG) inhibitor KT5823 (Hidaka & Kobayashi 1992) had no effect on carbachol-induced up-regulation of H1R mRNA (data not shown). The isozyme-specific PKC inhibitor Go 6976 (Davies et al 2000) significantly inhibited carbachol-induced up-regulation of H1R mRNA (0.98±0.12-fold vs 1.85±0.10-fold, $P<0.05$, n=6). To investigate whether carbachol treatment affected the degradation of H1R mRNA, U373-M3 cells were treated with and without carbachol in the presence of actinomycin D (10 μg mL^{-1}). The treatment with carbachol did not alter the degradation rate of H1R mRNA (Figure 2C).

Effects of carbachol on the H1R gene promoter activity

For H1R gene promoter assay, U373-M3 cells were co-transfected with pGL3 basic vector encoding promoter region of H1R and pRL-TK vector (Promega) for luciferase reporter assay. The H1R promoter activity was increased approximately 40% by treatment with 1 mM carbachol for 8 h and approximately 2-fold by treatment with 1 μM PMA for 8 h (Figure 3). The increase of H1R promoter activity by carbachol was significantly inhibited by 1 μM Ro 31-8220 (101.4±6.8% vs 148.0±14.2%, $P<0.05$, n=6) or 1 μM Go 6976 (101.1±7.9% vs 138.2±2.3%, $P<0.05$, n=6).

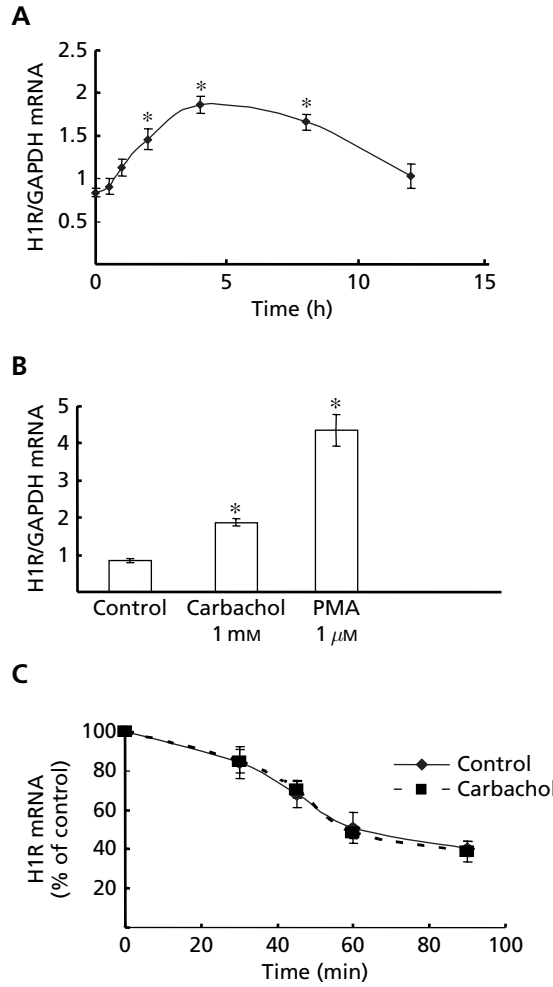

Figure 2 M_3 muscarinic receptor-mediated up-regulation of H1R mRNA in U373-M3 cells. The levels of H1R mRNA were determined by real-time RT-PCR. A. Time course of 1 mM carbachol-induced increase in H1R mRNA level. B. Increase in H1R mRNA level by 1 mM carbachol or 1 μM PMA. U373-M3 cells were treated with each drug for 4 h. C. Stability assay of H1R mRNA. U373-M3 cells were incubated with 1 mM carbachol or vehicle in the presence of 10 μg mL^{-1} actinomycin D. *$P<0.05$ vs control (n=6).

Discussion

Stimulation of M3R by carbachol in U373-M3 cells increased the H1R level in a time-dependent manner; the increase became significant after 12-h treatment. Atropine blocked carbachol-induced up-regulation of H1R, confirming the muscarinic receptor-mediated effect. Carbachol treatment led to a rapid increase in H1R mRNA level that was maximal by 4 h and returned to basal levels after 12-h treatment. The increase in H1R mRNA occurred well before the increase of H1R level, indicating that increased H1R mRNA led to an increase of receptor translation and thus receptor expression. Stimulation of M3R for only 2–4 h may have been sufficient to up-regulate H1R level, since such treatments increased H1R mRNA significantly. Activation of M3R increased H1R promoter activity, but had little effect on H1R mRNA stability, indicating that M3R-mediated up-regulation of H1R mRNA was a consequence of an increase of H1R mRNA synthesis. The increase in the levels of H1R and H1R mRNA after M3R stimulation was mimicked by the PKC activating phorbol ester, PMA, suggesting that a PKC-dependent process was involved in the up-regulation of H1R mRNA. This was confirmed by the result that M3R-mediated up-regulation of H1R and H1R mRNA was completely inhibited by the

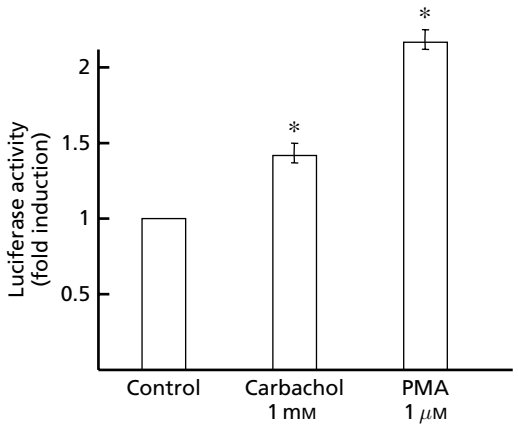

Figure 3 Effects of M_3 muscarinic receptor activation on H1R promoter activity in U373-M3 cells. Luciferase reporter assay was used. U373-M3 cells were treated with 1 mM carbachol or 1 μM PMA for 8 h. *$P < 0.05$ vs control (n = 6).

PKC inhibitor, Ro 31-8220. Furthermore, the subtype-specific PKC inhibitor, Go 6976, blocked H1R mRNA up-regulation. Go 6976 is a relatively specific antagonist of PKCα and PKCβ, and U373 astrocytoma cells express PKCα but not PKCβ (our private observation). Thus, PKCα was probably involved in M3R-mediated up-regulation of H1R mRNA. The H1R gene promoter activity was increased by PMA treatment, and Ro 31-8220 inhibited carbachol-induced increase of H1R gene promoter activity. These results were consistent with the effects of activator and inhibitor of PKC on the levels of H1R mRNA described above. PKC is known to activate activating protein-1 (AP-1), and human H1R promoter region contains AP-1 binding sites (De Backer et al 1998), and so it was possible that H1R gene promoter activity was increased by the activation of AP-1. However, M3R-mediated activation of AP-1 remains to be confirmed, and the AP-1 binding site involved in this process needs to be identified. The protein kinase inhibitors of CaMKII (KN-93) and PKG (KT5823) did not affect carbachol-induced H1R mRNA up-regulation, indicating that neither CaMK II nor PKG were involved in this process.

Previously we showed that activation of M3R caused down-regulation of H1R in Chinese hamster ovary (CHO) cells (Miyoshi et al 2004). In those studies, we used CHO cells in which H1R and M3R were expressed by stable transfection, and the expression of H1R was under the control of the SV40 promoter and not of the endogenous promoter. Therefore, those results indicated that M3R stimulation decreased H1R expression by accelerating H1R breakdown. Our present results obtained in U373-M3 cells indicated that M3R stimulation activated H1R mRNA synthesis through its endogenous promoter. These data suggested that M3R stimulation regulated H1R level mainly through two mechanisms; one was to activate H1R mRNA synthesis and up-regulate H1R, and the other was to accelerate the degradation of H1R leading to H1R down-regulation. Thus, H1R expression may depend on the balance between these two processes.

Previously we reported that stimulation of H1R regulated H1R level; it up-regulated H1R by accelerating H1R mRNA synthesis (Yoshimura et al 2005), and down-regulated H1R by accelerating H1R degradation (Horio et al 2004). Platelet-activating factor (PAF) has been reported to increase H1R mRNA level in rat trigeminal nerve ganglion cells (Nakasaki et al 1999), and β_2-adrenoceptor stimulation up-regulated H1R mRNA in airway smooth muscle (Mak et al 2000). To add to these data, we have provided evidence that M3R stimulation regulated H1R level by activating H1R mRNA synthesis. Thus, it is possible that many types of receptor including H1R, M3R, β_2-adrenoceptor and possibly others are involved in the regulation of H1R level. In these cases, the effect of each stimulant on H1R expression may be small, but under the conditions where theses effects are combined, it is possible that the H1R level may be altered well to affect various physiological functions. The H1R level should be maintained at a steady level in the normal condition, but in some diseases, H1R levels become abnormal; up-regulation of H1R is observed in the temporal cortex of epileptic patients (Iinuma et al 1993) and in the nasal mucosa of patients with allergic rhinitis (Iriyoshi et al 1996; Hamano et al 1998), and down-regulation of H1R is observed in the frontal cortex of patients with chronic schizophrenia (Nakai et al 1991), and in the frontal and temporal areas of patients with Alzheimer's disease (Higuchi et al 2000). Although the precise mechanism for the changes in H1R level which occurred in these conditions is unclear, it is possible that heterologous regulation of H1R level by M3R may have played a role in these processes.

Conclusions

We have investigated the possible role of M3R stimulation in the H1R gene transcription and H1R mRNA stability by using U373 astrocytoma cells that express endogenous H1R and transfected M3R. Stimulation of M3R significantly increased H1R promoter activity, H1R mRNA level and the H1R level, but had no effect on H1R mRNA stability. These M3R-mediated events were almost completely blocked by PKC inhibitors, suggesting that M3R stimulation up-regulated H1R by activating H1R gene transcription through a PKC-dependent process. Such heterologous regulation of H1R may play an important role in regulating the H1R level in the central nervous system and in the peripheral tissues.

References

Collins, S., Bouvier, M., Bolanowski, M. A., Caron, M. G., Lefkowitz, R. J. (1989) cAMP stimulates transcription of the beta 2-adrenergic receptor gene in response to short-term agonist exposure. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 4853–4857

Davies, S. P., Reddy, H., Caivano, M., Cohen, P. (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **351**: 95–105

De Backer, M. D., Loonen, I., Verhasselt, P., Neefs, J. M., Luyten, W. H. (1998) Structure of the human histamine H1 receptor gene. *Biochem. J.* **335**: 663–670

Hamano, N., Terada, N., Maesako, K., Ikeda, T., Fukuda, S., Wakita, J., Yamashita, T., Konno, A. (1998) Expression of histamine receptors in nasal epithelial cells and endothelial cells: the effects of sex hormones. *Int. Arch. Allergy Immunol.* **115**: 220–227

Hidaka, H., Kobayashi, R. (1992) Pharmacology of protein kinase inhibitors. *Annu. Rev. Pharmacol. Toxicol.* **32**: 377–397

Higuchi, M., Yanai, K., Okamura, N., Meguro, K., Arai, H., Itoh, M., Iwata, R., Ido, T., Watanabe, T., Sasaki, H. (2000) Histamine H1 receptors in patients with Alzheimer's disease assessed by positron emission tomography. *Neuroscience* **99**: 721–729

Hill, S. J., Ganellin, C. R., Timmerman, H., Schwartz, J. C., Shankley, N. P., Young, J. M., Schunack, W., Levi, R., Haas, H. L. (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev*. **49**: 253–278

Horio, S., Ogawa, M., Kawakami, N., Fujimoto, K., Fukui, H. (2004) Identification of amino acid residues responsible for agonist-induced down-regulation of histamine H_1 receptors. *J. Pharmacol. Sci*. **94**: 410–419

Iinuma, K., Yokoyama, H., Otsuki, T., Yanai, K., Watanabe, T., Ido, T., Itoh, M. (1993) Histamine H1 receptors in complex partial seizures. *Lancet* **341**: 238

Iriyoshi, N., Takeuchi, K., Yuta, A., Ukai, K., Sakakura, Y. (1996) Increased expression of histamine H_1 receptor mRNA in allergic rhinitis. *Clin. Exp. Allergy* **26**: 379–385

Mak, J. C. W., Roffel, A. D. F., Katsunuma, T., Elzinga, C. R. S., Zaagsma, J., Barnes, P. J. (2000) Up-regulation of airway smooth muscle histamine H_1 receptor mRNA, protein and function by β_2-adrenoceptor activation. *Mol. Pharmacol*. **57**: 857–864

Miyoshi, K., Kawakami, N., Wakayama, Y., Izumi, N., Horio, S., Fukui, H. (2004) Histamine H1 receptor down-regulation mediated by M3 muscarinic receptor subtype. *J. Pharmacol. Sci*. **95**: 426–434

Nakai, T., Kitamura, N., Hashimoto, T., Kajimoto, Y., Nishino, N., Mita, T., Tanaka, C. (1991) Decreased histamine H1 receptors in the frontal cortex of brains from patients with chronic schizophrenia. *Biol. Psychiatry* **30**: 349–356

Nakasaki, T., Masuyama, K., Fukui, H., Ogino, S., Eura, M., Samejima, Y., Ishikawa, T., Yumoto, E. (1999) Effects of PAF on histamine H1 receptor mRNA expression in rat trigeminal ganglia. *Prostaglandins Other Lipid Mediat*. **58**: 29–41

Nguyen, T., Shapiro, D. A., George, S. R., Setola, V., Lee, D. K., Cheng, R., Rauser, L., Lee, S. P., Lynch, K. R., Roth, B. L., O'Dowd, B. F. (2001) Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol*. **59**: 427–433

Sumi, M., Kiuchi, K., Ishikawa, T., Ishii, A., Hagiwara, M., Nagatsu, T., Hidaka, H. (1991) The newly synthesized selective Ca2+/calmodulin dependent protein kinase II inhibitor KN-93 reduces dopamine contents in PC12h cells. *Biochem. Biophys. Res. Commun*. **181**: 968–975

Tholanikunnel, B. G., Granneman, J. G., Malbon, C. C. (1995) The M(r) 35,000 beta-adrenergic receptor mRNA-binding protein binds transcripts of G-protein-linked receptors which undergo agonist-induced destabilization. *J. Biol. Chem*. **270**: 12787–12793

Tsao, P., Cao, T., von Zastrow, M. (2001) Role of endocytosis in mediating downregulation of G-protein-coupled receptors. *Trends Pharmacol. Sci*. **22**: 91–96

Yoshimura, S., Mishima, R., Miyoshi, K., Fujimoto, K., Murata, Y., Kitamura, Y., Takeda, N., Fukui, H. (2005) Histamine H1 receptor-mediated histamine H1 receptor gene expression. *Inflamm. Res*. **54**: S42–43