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Short communication

Histamine H_4 receptors regulate ACTH release in AtT-20 cells

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ABSTRACT

The early research described that adrenocorticotropic hormone (ACTH) release from mouse pituitary tumor AtT-20 cells was regulated by histamine H_3 receptors. Here, we provide the evidence that histamine H_4 receptor was expressed in AtT-20 cell, and that the accelerated ACTH secretions from the cells by histamine and *R*-α-methylhistamine were blocked by JNJ7777120, a specific H_4 receptor antagonist, in concentration dependent manner, but not by the H_1 and H_2 receptor antagonists. The results indicate, for the first time, that histamine H_4 receptor, rather than histamine H_3 receptor, played a role in regulation of ACTH release from mouse pituitary AtT-20 cells.

1. Introduction

The histamine H_4 receptor is a relatively newly discovered member of histamine receptors. It has a distinct expression profile on immune cells, including mast cells, eosinophils, dendritic cells, and T cells, and appears to play a role in multiple functions of these cells, such as, activation, migration, cytokine and chemokine production (Zhang et al., 2007; de Esch et al., 2005; Dunford et al., 2006). Earlier researchers have identified histamine H_3 receptors in rat and guinea-pig pituitary glands and in the mouse pituitary tumor cell line, AtT-20. Histamine H_3 receptor agonists *N*-α-methylhistamine and *R*-α-methylhistamine are reported to bind to AtT-20 cell membranes, as well as to stimulate adrenocorticotropic hormone (ACTH) release from the cells. The effect was blocked by histamine H_3, but not H_1 or H_2 receptor antagonists. Therefore, it was concluded that high affinity histamine H_3 receptor regulated ACTH release from that cells (Clark et al., 1992; West et al., 1994).

However, it has been reported recently that histamine H_3 receptor agonist, *N*-α-methylhistamine bound specifically to histamine H_4 receptor with high affinity, while another H_3 agonist, *R*-α-methylhistamine, and the H_3 antagonist, thioperamide, competed with this binding. *N*-α-methylhistamine and *R*-α-methylhistamine also produced a reduction in forskolin-induced cAMP accumulation in histamine H_4 receptor-expressing cells (Nakamura et al., 2000). Moreover, functional studies demonstrate that histamine H_3 receptor either in central nervous system or in peripheral tissues mainly exerts "inhibitory effects", such as negative control on neurotransmitter release or gastric acid secretion (van der Werf and Timmerman, 1989; Levi and Smith, 2000; Coruzzi et al., 2001). Oppositely, the activation of histamine H_4 receptor has been shown to have "excitatory effects", such as chemotaxis and mediator release in various types of immune cells including mast cells, monocytes, eosinophils, dendritic cells and T cells (Hofstra et al., 2003; Ling et al., 2004; Zhang et al., 2006; Damaj et al., 2007). All these results imply that histamine receptor mediated ACTH secretion from AtT-20 may not be related to the actions H_3 subtype of histamine receptors. Here we provide definitive proof that histamine H_4 receptors are expressed in AtT-20 cells and involved in modulation of ACTH release from the cells.

2. Materials and methods

The mouse pituitary tumor AtT-20 cells were obtained from American Type Culture Collection (Bethesda, MD, USA). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml streptomycin, 100 U/ml penicillin at 37 °C in a 5% CO_2 atmosphere. Culture medium was changed every 3 days. Total RNA was isolated from AtT-20 cells and mouse spleens with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacture's instructions, and the RNA samples were treated with DNase I to remove any genomic DNA contamination. Two micrograms of total RNA was used for reverse transcription using random primers. The fragment of mouse histamine H_4 receptor gene (GenBank accession No. AF358859) from exon 5 to 8 was amplified from the resulting transcripts by PCR using the following gene-specific oligonucleotide primers, 5′-ATGTCGGAGTCTAACAGTACTGG-3′ (61-83) and 5′-AGAAGATACTGACTGGTTCTGTGA-3′ (1211–1233). The PCR was carried out for 35 cycles at 94 °C for 60 s on denaturing; 55 °C for 45 s on

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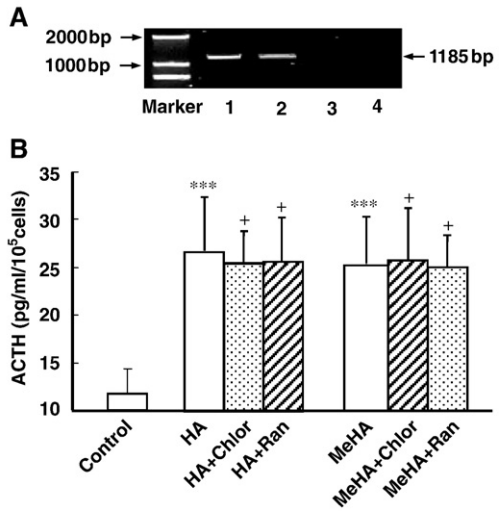

Fig. 1. A: Expression of histamine H_4 receptor specific mRNA in AtT-20 cells. Total RNA was purified from AtT-20 cells or mouse spleen, and RT-PCR was performed to identify the transcription products of mouse histamine H_4 receptors. 1: RT-PCR product of AtT-20 cells; 2: RT-PCR product of mouse spleen; 3 and 4: no-RT control, which is the result of PCR using total RNA isolated from AtT-20 cells (3) or mouse spleen (4) as template. B: Effects of chlorpheniramine (Chlor, 10 μM) and ranitidine (Ran, 10 μM) on histamine (HA, 0.01 μM) and *R*-α-methylhistamine (MeHA, 0.1 μM) induced ACTH release from AtT-20 cells. Bars represent concentrations of released ACTH (mean±S.D.), $n=6$. $^{***}P<0.01$ vs control; $^{+}P>0.05$ vs corresponding value of HA or MeHA treated group respectively.

annealing, and 72 °C for 90 s on extension. Amplification ended with 7 min of incubation at 72 °C. The amplified products were analyzed on 1% agarose gel.

AtT-20 cells were grown in 24 well culture plates to a density of 2×10^5 per well. The culture media was then aspirated from the well and the wells were incubated with complete culture medium containing the indicated concentrations of histamine, H_3 agonist *R*-α-methylhistamine or H_4 receptor specific antagonist J1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120) respectively. At the indicated times, the supernatants were collected and centrifuged to eliminate any dislodged cells. Next, the cell free supernatant was assayed for ACTH by an enzyme-linked immunosorbent assay (ELISA) following the manufacture's instruction (Market Inc. San Jose, CA, USA). Briefly, 100 μl of the supernatant and mouse ACTH standard (0–750 pg/ml) together with 50 μl of a biotinylated anti-mouse ACTH antibody was added into the appropriate wells of ELISA plates immobilized with anti-mouse ACTH monoclone antibody for 180 min incubation at 37 °C. The plates were then washed 5 times with wash buffer followed by the addition of horseradish peroxidase conjugated streptavidin. After wash, a solution of substrate/chromogen is added and incubated for 12 min, resulting in the development of a blue color. The color development is terminated by the addition of 100 μl of 2 N HCl to each well and absorbance was read at 450 nm on a microplate reader (Bio-Rad Loboratories, Hercules, CA, USA). The concentration of ACTH for each sample was calculated from the standard curve and normalized by cell number of each well. Data are expressed as means±standard deviation (S.D.) of the mean. Significance of differences between mean values was calculated using Student's *t*-test. A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Histamine H_4 receptor expression in AtT-20 cells

To determine whether histamine H_4 receptor existed in AtT-20 cells, we performed RT-PCR to examine the gene expression of histamine H_4 receptor. RNA from mouse spleen was chosen as a positive control, as histamine H_4 receptor are dominantly expressed in mouse spleen. The segment of histamine H_4 receptor gene was detected in both AtT-20 cells and mouse spleen (Fig. 1A). The primer sequences used in the present study specifically bound to corresponding sequence of mouse histamine H_4 receptor cDNA without homologue with sequence of H_3 receptor cDNA.

3.2. JNJ7777120 antagonizes the histamine and R-α-methylhistamine induced ACTH release from AtT-20 cells

Application of histamine (10 nM) or *R*-α-methylhistamine (100 nM) to AtT-20 cells induced a prominent ACTH release. Chlopheniramine (10 μM), an H_1 receptor antagonist, or ranitidine (10 μM), an H_2 receptor antagonist, did not affect the effects of histamine and *R*-α-methylhistamine (Fig. 1B). However the selective histamine H_4 receptor antagonists JNJ 7777120 significantly antagonized the histamine or *R*-α-methylhistamine evoked ACTH secretion from AtT-20 cells in concentration dependent manner with IC_{50} values of 0.36 μM against histamine or 0.23 μM against *R*-α-methylhistamine respectively (Fig. 2).

4. Discussion

The recently identified histamine H_4 receptor is localized in the peripheral blood leukocytes, spleen, thymus, small intestine, colon, bone marrow and synovial cells. The tissue distribution of the histamine H_4 receptor and known physiological function of histamine lead us to speculate about its function as an immune modulator and a possible link to the pathology of allergy and asthma (Zhang et al., 2006; Dunford et al., 2006; de Esch et al., 2005). Based on homology analysis, the amino acid sequence of the recently cloned human histamine H_4 receptor exhibits a −60% amino acid homology in the transmembrane domains with the H_3 receptor and a much lower homology to H_1 and H_2 receptors (Oda et al., 2000;

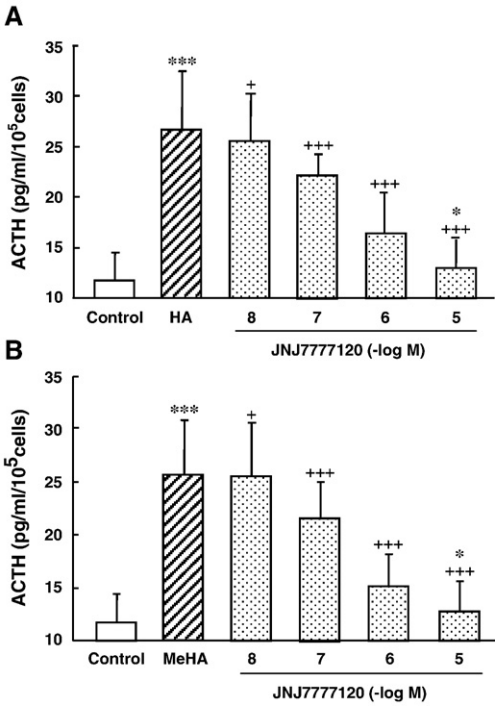

Fig. 2. Effects of JNJ7777120 on HA (0.01 μM) or MeHA (0.1 μM) induced ACTH secretion from AtT-20 cells. Bars represent concentrations of released ACTH (mean±S.D.), $n=6$. $^{*}P>0.05$, $^{***}P<0.01$ vs corresponding control values, $^{+}P>0.05$, $^{+++}P<0.01$ vs corresponding value of HA or MeHA treated group respectively.

Zhu et al., 2001). This is reflected in the pharmacological profile of known histamine receptor ligands, where most H_1 and H_2 receptor agonists and antagonists do not bind to the histamine H_4 receptor. However, histamine H_3 receptor agonists (*N*-α-methylhistamine and *R*-α-methylhistamine) and a histamine H_3 receptor antagonist (thioperamide) were found to compete out the binding of [^{3}H]-*N*-α-methylhistamine to histamine H_4 receptor and histamine H_3 receptor. The IC_{50} values of *N*-α-methylhistamine and *R*-α-methylhistamine for histamine H_4 receptor were markedly different from those for histamine H_3 receptor. Moreover, *N*-α-methylhistamine and *R*-α-methylhistamine produced a reduction in forskolin-induced cAMP accumulation in H_4 receptor-expressing cells, and the EC_{50} values of *N*-α-methylhistamine and *R*-α-methylhistamine for H_4 receptor were markedly different from those for H_3 receptor (Nakamura et al., 2000). Therefore, *N*-α-methylhistamine and *R*-α-methylhistamine are recognized as H_3/H_4 agonists. Both of them could mediate the effects through activation of either histamine H_3 or H_4 receptors.

Since the original description of the histamine H_3 receptor as an autoreceptor or heteroreceptor that mainly mediates the inhibitory effects on neurotransmitters release, H_3 receptor has been recognized as a presynaptic inhibitory receptor (Schwartz et al., 2003; Leurs et al., 2005). To date there are only two reports to demonstrate that histamine H_3 receptor activation mediates the "stimulatory effects", e.g. enhancing ACTH secretion from AtT-20 cell (Clark et al., 1992; West et al., 1994). The present study demonstrated that the histamine H_4 receptor specific mRNA was identified in AtT-20. Both histamine and *R*-α-methylhistamine evoked ACTH releases were antagonized by JNJ7777120 concentration-dependently. The IC_{50} values of JNJ7777120 on AtT-20 cells were consistent with that obtained in eosinophil cells (Ling et al., 2004). Since JNJ 7777120 has an affinity for the mouse and rat H_4 receptor that is similar to human H_4 receptor (Liu et al., 2001) and JNJ7777120 has a selectivity for the histamine H_4 receptor greater than 300–1000-fold over the histamine H_1, H_2 and H_3 receptors (Jablonowski et al., 2004; Terzioglu et al., 2004), as well as with little or no affinity for over 50 other targets (Thurmond et al., 2004), it can be used to verify that histamine and *R*-α-methylhistamine evoked ACTH releases are indeed due to activation of the H_4 receptor. The present results indicate that histamine H_4 receptor, rather than previous indicated histamine H_3 receptor, plays a role in regulation of ACTH release.

The abundant expression of histamine H_4 receptor in immune system constituents such as the spleen, thymus, leukocytes and dendritic cells indicates that H_4 receptors have a role in mediating either immune or inflammatory in peripheral tissues. The selective ligands for the H_4 receptor have enabled the elucidation of receptor function in mast cells and eosinophils, and histamine H_4 receptor antagonists have anti-inflammatory properties and antinociceptive effects in vivo (Thurmond et al., 2004; Coruzzi et al., 2007). The present study further reveals that histamine H_4 receptor plays a regulatory role by increasing the rate of release of ACTH from the anterior pituitary to enhance the circulating levels of corticosteroids, which, in turn, may modulate the inflammatory response. Our findings suggest that histamine H_4 receptor in central nervous system also plays a role in mediating immune and inflammatory response. The identification of histamine H_4 receptor expression and function in AtT-20 cells rectifies a previously made conclusion and mode of histamine action. This seminal finding introduces pertinent, new avenues to explore and to study the new mechanisms on the regulation of ACTH secretion.

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