Histamine H1 Receptor Induces Cytosolic Calcium Increase and Aquaporin Translocation in Human Salivary Gland Cells

Ji-Hyun Kim, Seong-Hae Park, Young Wha Moon, Sungmin Hwang, Donghoon Kim, Su-Hyun Jo, Seog Bae Oh, Joong Soo Kim, Jeong Won Jahng, Jong-Ho Lee, Sung Joong Lee, Se-Young Choi, and Kyungpyo Park

Departments of Physiology (J.-H.K., S.-H.P., S.H., D.K., S.B.O., J.S.K., S.J.L., S.-Y.C., K.P.) and Oral and Maxillofacial Surgery (J.W.J., J.-H.L.), Dental Research Institute, Seoul National University School of Dentistry, Seoul, Korea; Department of Biology, Catholic University of Korea College of Medicine, Seoul, Korea (Y.W.M.); and Department of Physiology, Kangwon National University School of Medicine, Chuncheon, Korea (J.-H.K., S.-H.J.)

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ABSTRACT

One of the common side effects of antihistamine medicines is xerostomia (dry mouth). The current consensus is that antihistamine-induced xerostomia comes from an antimuscarinic effect. Although the effect of antihistamines on salivary secretion is both obvious and significant, the cellular mechanism whereby this happens is still unclear because of the lack of knowledge of histamine signaling in human salivary glands. Here, we have studied histamine receptors and the effect of antihistamines on human submandibular acinar cells. In primary cultured human submandibular gland and a HSG cell line, histamine increased the intracellular Ca2+ concentration. The histamine-induced cytosolic free Ca2+ concentration ([Ca2+]i) rise without decreasing cytosolic Ca2+ concentration. The histamine-induced [Ca2+]i rise without "heterologous desensitization." Chlorpheniramine inhibited a carbachol-induced [Ca2+]i increase at a 100-fold greater concentration than histamine receptor antagonism, whereas astemizole and cetirizine showed more than 1000-fold difference, which in part explains the xerostomia-inducing potency among the antihistamines. Notably, histamine resulted in translocation of aquaporin-5 to the plasma membrane in human submandibular gland cells and green fluorescent protein-tagged aquaporin-5 expressing HSG cells. We found that histidine decarboxylase and the histamine H1 receptor are broadly distributed in submandibular gland cells, whereas choline acetyltransferase is localized only at the parasympathetic terminals. Our results suggest that human salivary gland cells express histamine H1 receptors and histamine-synthesizing enzymes, revealing the cellular mechanism of antihistamine-induced xerostomia.

The salivary gland is an exocrine gland that secretes saliva (Turner and Sugiya, 2002). The saliva secretion requires the coordinated activity of membrane transporters, receptors, and ion channels. Close communication between the salivary gland and neurons significantly modulates salivary secretion. Neuronal modulation of the salivary gland is mediated by neurotransmitter release from neurons and receptor activation in salivary gland cells. The most important neuronal modulation is muscarinic signaling from parasympathetic neurons (Turner and Sugiya, 2002; Li et al., 2006). In salivary gland acinar cells, acetylcholine activates muscarinic M3 receptors, the Gq/11 protein, and phospholipase C, increasing cytosolic Ca2+ levels, which is the most critical signal molecule for salivary secretion (Ambudkar, 2000). Animals lacking muscarinic M3 receptors (Matsui et al., 2000) or the inositol triphosphate receptor (Putatsugi et al., 2005) show severely decreased salivation.

Xerostomia (dry mouth) is a pathological condition characterized by decreased salivary secretion (Guggenheimer and Moore, 2003). Xerostomia usually results in the sensation of a burning mouth, halitosis, and oral infection. Prolonged xerostomia induces rampant caries, erosion, and, finally, tooth loss. Saliva is especially important in host defense of...
the oral cavity; thus, loss of the salivary fluid membrane can increase the amount of pathological bacteria, such as Streptococcus, and induce oral disease. For these reasons, modulation of salivary secretion has been studied intensively. A series of neuronal inputs, which are triggered through activation of the autonomic nervous system by external stimulation (acid or mechanical), delicately modulate salivary gland function.

Xerostomia can result from many conditions with salivary gland dysfunction, including radiation therapy of salivary glands or Sjögren’s syndrome, an autoimmune disease characterized by decreased salivary and lacrimal secretion. However, xerostomia is most frequently experienced as a side effect of medication (Sreebny and Schwartz, 1997; Guggenheimer and Moore, 2003). A series of extrinsic medicines decrease salivary secretion without any obvious pathological changes in the salivary gland. Currently, muscarinic agonists are one of the few xerostomia-causing extrinsic medicines for which the mechanism is fully understood. Anticholinergic drugs decrease salivation and induce xerostomia by modulating receptor-mediated Ca\(^{2+}\) signaling in salivary gland cells.

The xerostomia-inducing mechanisms of many other medicines are unclear. One example is antihistamine medication: classically, the histamine H1 receptor inhibitors. Frequently prescribed for minor allergic reactions or the cold-like symptoms of allergic rhinitis, antihistamines are reported to cause xerostomia (Elad et al., 2006); however, the exact mechanism for an antihistamine-induced decrease in salivary secretion is still unknown. The biological action of histamines is mediated by their binding to histamine receptors, classified as H1, H2, H3, and H4 (Parsons and Ganellin, 2006). Even though the xerostomia-inducing effect of antihistamines strongly suggests that histaminergic Ca\(^{2+}\) signaling is functional in human salivary glands, there is no solid evidence for the expression of any histamine receptors. The current consensus is that antihistamine-induced xerostomia comes from an antimuscarinic effect; indeed, a number of studies have shown that first-generation histamine H1 receptor antagonists possess antimuscarinic activity (Yasuda and Yasuda, 1999; Liu et al., 2006).

We hypothesized that the xerostomic effects of antihistamines are mostly caused by histamine H1 receptor blocking. To test this hypothesis, we sought to elucidate the action of histamine and antihistamines in human salivary glands. Here, we report intracellular Ca\(^{2+}\) increase and subsequent aquaporin translocation by histamine H1 receptor activation in human submandibular glands.

**Materials and Methods**

Histamine, chlorpheniramine, astemizole, ranitidine, thioperamide, carbachol, dianabolizide, and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO). Fura-2-acetoxyethyl ester (fura-2-AM) was obtained from Invitrogen (Carlsbad, CA). Aquaporin-5 (AQP-5) antibody and secondary donkey anti-goat IgG-hors eradish peroxidase were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-rabbit Cy3 and normal donkey serum were obtained from Jackson Immunoresearch Laboratories (West Grove, PA). Biotinylated anti-goat IgG and Vectastain Elite ABC Kit were purchased from Vector Laboratories (Burlingame, CA). Anti-rabbit human histidine decarboxylase antibodies and anti-rabbit human histamine receptor H1 antibodies were purchased from Progen Biotechnik GmBH (Heidelberg, Germany) and Acris Antibodies GmBH (Herford, Germany), respectively. Goat anticholine acetyltransferase antibodies were purchased from Millipore Bioscience Research Reagents (Temecula, CA). Collagenase P was purchased from Roche Molecular Biochemicals (Indianapolis, IN). Modified Eagle’s medium, bovine calf serum, and penicillin/streptomycin were purchased from Invitrogen. \(^{3}H\)cAMP was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Lipofectamine 2000 was obtained from Invitrogen. Polyethylene glycol 400 diesterate was purchased from Polysciences (Warrington, PA).

**Cell Preparation.** The dissociation of human submandibular salivary gland cells was performed as described previously (Choi et al., 2006). Pieces of human submandibular glands were surgically removed from 12 patients with oral cancer, who had provided informed consent. The patients included males and females ranging from 38 to 69 years of age. The glands did not contain atypical cells when assessed histologically. The tissues were quickly removed and finely minced in a physiological salt solution (containing 135 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl\(_2\), 0.8 mM MgSO\(_4\), 0.33 mM NaH\(_2\)PO\(_4\), 0.4 mM KH\(_2\)PO\(_4\), 10 mM glucose, 2 mM glutamine, and 20 mM HEPES adjusted to pH 7.4 with NaOH), supplemented with 10 mM sodium pyruvate, 0.02% trypsin inhibitor, and 0.1% bovine serum albumin. The cells were then digested in the same solution containing collagenase P (0.3 mg/7.5 ml) at 37°C for 75 min with continuous agitation. The prepared acinar cells were resuspended in physiological salt solution containing 0.1% bovine serum albumin. This study was performed according to the guidelines for experimental procedures found in the Declaration of Helsinki, the World Medical Association, and this study was approved by the Institutional Review Board (CR06002) of Seoul National University Dental Hospital. The HSG cell line was grown in modified Eagle’s medium supplemented with 10% (v/v) heat-inactivated bovine calf serum and 1% (v/v) penicillin (5000 U/ml) + streptomycin (5000 µg/ml) solution. The cells were cultured in a humidified atmosphere of 95% air and 5% CO\(_2\). The culture medium was changed every 2 days, and the cells were subcultured weekly.

**[Ca\(^{2+}\)]\(_i\) Measurement.** Cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) was determined with the fluorescent Ca\(^{2+}\) indicator fura-2-AM as described previously (Choi et al., 2001). In brief, a cell suspension was incubated with fresh modified Eagle’s medium containing fura-2-AM (4 µM) for 40 min at 37°C with continuous stirring. The cells were then washed with a HEPES-buffered solution (140 mM NaCl, 5 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH) or Locke’s solution (154 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl\(_2\), 2.2 mM CaCl\(_2\), 5.0 mM HEPES, 10 mM glucose, pH 7.4 with NaOH) and left at room temperature until use. Sulfinpyrazone (250 µM) was added to all solutions to prevent dye leakage. For the dissociated human salivary gland, cells were transferred to poly(l-lysine)-coated 25-mm coverslips for cell attachment for 40 min in an incubator, and the coverslips were mounted onto the chamber. Changes in fluorescence ratio were monitored by a Ca\(^{2+}\)-imaging machine with MetaFluor software (Molecular Devices, Sunnyvale, CA) with dual excitation at 340 and 380 nm and emission at 500 nm. Experiments with suspended HSG cells were performed with the spectrofluorometer. In this case, calibration of the fluorescent signal in terms of [Ca\(^{2+}\)]\(_i\) was performed by use of the following equation:

\[
[Ca^{2+}]_i = K_0(R - R_{\min})/R_{\max} - R \times (S_{12}/S_{02})
\]

where \(R\) is the ratio of fluorescence emitted by excitation at 340 and 380 nm, \(S_{12}\) and \(S_{02}\) are the proportionality coefficients at 380 nm excitation of Ca\(^{2+}\)-free fura-2 and Ca\(^{2+}\)-saturated fura-2, respectively. To obtain \(R_{\min}\), the fluorescence ratios were measured after adding 4 mM EGTA, 30 mM Tris base, and 0.1% Triton X-100 to the cell suspension. To obtain \(R_{\max}\), the cell suspension was then treated with CaCl\(_2\) at a final concentration of 4 mM, and the fluorescence ratio was measured.
[3H]cAMP Measurement. The cAMP concentration in the cells was determined by [3H]cAMP competition assay for binding to the cAMP-binding protein (Choi et al., 2001). To determine cAMP production, cells were harvested and washed with Locke’s solution (154 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl2, 2.2 mM CaCl2, 5 mM HEPES, and 10 mM glucose, pH 7.4). The cells were then preincubated with Locke’s solution containing 1 mM isobutylmethyl xanthine to inhibit phosphodiesterase. Isobutylmethyl xanthine was also added to the stimulating buffer with agonists. After stimulation for 15 min at 37°C, the reaction was terminated by the addition of twice the volume of ice-cold absolute ethanol. Cells were then incubated for 2 h at −20°C to extract the cAMP. The cells in ethanol were centrifuged for 10 min at 4°C at 10,000g. The supernatant was evaporated, and the residues were dissolved in TE buffer (0.2 ml of 20°C to extract the cAMP. The cells in ethanol were centrifuged for 10 min at 4°C at 10,000g. The supernatant was evaporated, and the residues were dissolved in TE buffer (0.2 ml of Tris-HCl, 4 mM EDTA, pH 7.5). Fifty microliters of sample solution was used in the cAMP assay. This assay was based on competition between [3H]cAMP and unlabeled cAMP in the sample for a crude cAMP-binding protein prepared from bovine adrenal gland. Free [3H]cAMP was adsorbed onto charcoal and removed by centrifugation, and bound [3H]cAMP in the supernatant was determined by liquid scintillation counting. Each unknown sample was incubated with 50 μl of [3H]cAMP (5 μCi) and 100 μl of binding protein for 2 h at 4°C. The protein-bound cAMP and unbound cAMP were separated by adsorbing free cAMP onto charcoal (100 μl), followed by centrifugation at 12,000g at 4°C. Two hundred microliters of supernatant was placed in an Eppendorf tube containing 1.2 ml of scintillation cocktail to measure the radioactivity. The cAMP concentration in the sample was determined based on a standard curve and expressed as picomoles per cell number.

Reverse Transcription–Polymerase Chain Reaction. Total RNA was isolated from primary culture human submandibular cells. The CDNA was synthesized from 3 μg of total RNA by incubating the RNA for 1 h at 37°C in a reaction mixture containing 0.5 μg of oligo-(dT)15, 0.5 mM dNTP mix, 1× first-strand buffer, RNase inhibitor (20 units), 5 mM dithiothreitol, and Moloney murine leukemia virus reverse transcriptase (200 units). Histamine receptor mRNA expression was measured with the following polymerase chain reaction (PCR) reaction conditions (Matsubara et al., 2005): initial denaturing at 94°C for 3 min, denaturing at 94°C for 30 s, and extension at 72°C for 30 s. A total of 35 reaction cycles were performed. The amplified DNA products were separated by electrophoresis on a 1.5% agarose gel. PCR primers used for reverse transcription–polymerase chain reaction (RT-PCR) analysis of a salivary gland tissues were as follows: human GAPDH, 5′-AAG ACA GTC CAT GAC ATC AC-3′ and 5′-TCC ACC CTG TGT CTG TA-3′; human H1 receptor, 5′-GAC TGT GTA GCC GTC AAC CGG A-3′ and 5′-TAA CTA TAA AAC C-3′; human H2 receptor, 5′-TCA CGC GGC CTC TCA GTG CCC C-3′ and 5′-CTTG TGC TGT CCT GCT TCC T-3′; human H3 receptor, 5′-TCA GGT ACC GCT TCC TGT CGG TCA C-3′ and 5′-TGT AGT GAG CGC GCC TGC TCA GTG CCC C-3′; and human H4 receptor, 5′-GA A TTG TCT TGG GGC GCG GCC CTC TCA GTG CCC C-3′ and 5′-AAG AAT GAT GTG ATG GCA AGC ATG TAC C-3′.

Construction of AQP-5 and Western Blotting. In brief, total RNA was isolated from primary culture human submandibular saliva glands, and full-length cDNA extracted from it was amplified with RT-PCR. PCR utilized 5′-GAATTCATATGGAAGAAGAGGGTTGCTGCCC as the upstream primer and 5′-GGTAACGGGGTTGTACCGTAGCTATGGT as the downstream primer (the introduced EcoRI and KpnI restriction sites are underlined). The 798-bp human AQP-5 gene was then cloned into pEGFP-C1. HSG cells were transiently transfected with the AQP-5 gene by use of Lipofectamine 2000 (Invitrogen). AQP-5-transfected HSG cells were grown in a 60-mm dish and incubated with carbachol or histamine for 15 min at 37°C. The apical membrane fraction was obtained as described previously (Ishikawa et al., 2000). After electrophoresis in an SDS–12% polyacrylamide gel, the protein was transferred to a nitrocellulose membrane. After blocking, the membrane was incubated with anti-AQP-5 as the primary antibody. The membrane was then washed and incubated with donkey anti-goat IgG-horseradish peroxidase. The membrane was finally subjected to an electrochemiluminescence assay.

Immunofluorescence and Confocal Microscopy. GFP-tagged, AQP-5-transfected cells were plated on glass coverslips before experiments were performed. Cells were treated with carbachol and histamine for 15 min. Cells were then visualized for fluorescence with use of a differential interference fluorescence microscope (Carl Zeiss Inc., Thornwood, NY).

Immunohistochemistry. Human submandibular gland tissue samples were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 4 to 5 h, and then embedded in polyethylene glycol 400 diesterate wax after dehydration in graded alcohols. Eight-micrometer-thick tissue sections were obtained by use of a microtome (Leica RM 2135; Leica Microsystems, Inc., Deerfield, IL), deposited on gelatin-coated slides, and air-dried. After dewaxing with xylene followed by hydration, the tissue sections were treated with 1.5% hydrogen peroxide for 30 min and washed twice for 15 min in 0.1 M sodium phosphate-buffered saline. Tissue sections were treated with normal donkey serum (1:50 dilution) for 30 min and incubated with anti-rabbit human histidine decarboxylase antibody (1:100 dilution), anti-rabbit human histamine receptor H1 antibodies (1:100 dilution), or goat anticholine acetyltransferase antibodies (1:500 dilution) overnight. After washing twice in phosphate-buffered saline, the sections incubated with antihistidine decarboxylase or antihistamine H1 receptor antibodies were treated with α-rabbit Cy3 (1:200 dilution for antihistidine decarboxylase, 1:1000 dilution for antihistamine H1 receptor) for 1 h and then observed under a confocal laser-scanning microscope (LSM510 META; Carl Zeiss Inc.). Sections incubated with anticholine acetyltransferase antibodies were treated with biotinylated anti-goat IgG (1:100 dilution) for 1 h, and then bound secondary antibodies were amplified with the Vectastain Elite ABC Kit (Vector Laboratories). Antibody complexes were visualized with 0.05% diaminobenzidine for 3 min. Sections were washed twice with phosphate-buffered saline, counterstained with hematoxylin, dehydrated, and coverslipped with Permount slide mounting fluid.

Data Analysis. All quantitative data are expressed as mean ± S.E.M. We calculated the half-maximal effective concentration (EC50) and half-maximal inhibitory concentration (IC50) with the Microlab Origin program (OriginLab Corp., Northampton, MA). Differences were determined by one-way analysis of variance and considered to be significant only when P < 0.05.

Results

Functional Histamine H1 Receptor in Human Salivary Gland Cells and HSG Cells. We examined histamine-induced cellular responses in human submandibular gland cells. Throughout our experiments, we used primary cultured or dissociated human submandibular gland cells and the HSG cell line because of the limited supply of human normal submandibular tissue. Histamine (1 and 100 μM) triggered a [Ca2+]i rise in fura-2-loaded primary cultured human submandibular gland cells (Fig. 1A) and HSG cells (Fig. 1C). Histamine elevated [Ca2+]i in a concentration-dependent manner proportional to the histamine concentration between 10−7 M and 10−4 M (supramaximal concentration). The half-maximal effective concentrations (EC50) were 38.5 ± 3.0 μM in primary cultured human submandibular gland cells (Fig. 1C) and 11.8 ± 0.6 μM in HSG cells (Fig. 1D). It reached a peak level within a few seconds and fell back to the basal level in 200 s.

To identify the histamine receptor subtype expressed in HSG cells, subtype-specific antagonists were tested. In gen-
eral, it is accepted that chlorpheniramine, cetirizine, astemizole, and loratadine inhibit the histamine H1 receptor, ranitidine blocks the histamine H2, and tiotropamide inhibits H3 and H4 receptors (Liu et al., 2001; Parsons and Ganellin, 2006). Chlorpheniramine inhibited the histamine-induced [Ca\(^{2+}\)] increase (Fig. 2A) in a concentration-dependent manner with an IC\(_{50}\) of 128 ± 16 nM (Fig. 2B). Cetirizine also inhibuited the histamine-evoked Ca\(^{2+}\) response (Fig. 2E) in a concentration-dependent manner with an IC\(_{50}\) of 141 ± 17 nM (Fig. 2F). Astemizole and loratadine also inhibited histamine-induced [Ca\(^{2+}\)] increase with IC\(_{50}\) of 73.6 ± 4.5 nM (Fig. 2, C and D) and 64.2 ± 5.3 nM (Fig. 2, G and H), respectively. Ranitidine (30 µM) and tiotropamide (1 µM) did not affect the histamine-induced [Ca\(^{2+}\)] increase (Fig. 3A), despite application at concentrations known to be sufficient for receptor-specific blockade. We monitored histamine-induced cAMP production to investigate the existence of adenyl cyclase-linked histamine receptors (Kim et al., 2006). Forskolin (direct adenyl cyclase activator) and isoprotenerol (β-adrenergic agonist) successfully increased cAMP levels; however, histamine failed to elevate cAMP in HSG cells (Fig. 3B). To detect histamine receptor subtype mRNA expression in the human submandibular gland, we designed specific primers based on histamine receptor subtype sequences. The specificity of the primers was confirmed by sequencing the gene products and by amplifying full-length human H1, H2, H3, and H4 receptor cDNAs. Only the H1 receptor was expressed in the dissociated human salivary gland cells (Fig. 3C) and HSG cells (Fig. 3D).

**Histamine Receptor Signaling Is Independent from Muscarinic Receptor Signaling.** Increases in cytosolic Ca\(^{2+}\) play a critical role in saliva secretion (Ambudkar, 2000). Intracellular Ca\(^{2+}\) activates a series of membrane transporters, ion channels, and aquaporins in salivary gland acinar cells, which transport water and electrolytes to make primary saliva. Salivary gland cells have cytosolic Ca\(^{2+}\)-mobilizing muscarinic M3 receptors in their plasma membrane and receive signals from parasympathetic neurons (Nakamura et al., 2004). We confirmed these inputs by showing a carbachol-induced [Ca\(^{2+}\)] increase (Fig. 4). We examined the possibility that histamine and muscarinic receptors affect each other in terms of Ca\(^{2+}\) signaling. Interestingly, pretreatment with histamine did not dramatically decrease the carbachol-evoked [Ca\(^{2+}\)] increase in the dissociated human submandibular gland cells (Fig. 4A). Similar results were seen in HSG cells (Fig. 4B). In addition, pretreatment with carbachol did not inhibit the histamine-evoked [Ca\(^{2+}\)] increase (Fig. 4C). These data suggest that histamine and muscarinic receptors function independently of one another with no overlapping Ca\(^{2+}\) signaling pathway. We compared the carbachol- and histamine-evoked [Ca\(^{2+}\)] increases at the subcellular level but could not find a difference in localized [Ca\(^{2+}\)] increase (Supplementary Fig. 1).

Interestingly, antihistamine-induced xerostomia has been explained by blocking muscarinic rather than histamine receptors in human salivary glands (Sreebny and Schwartz, 1997; Guggenheimer and Moore, 2003). To clarify this possibility, we tested the effect of histamine H1 inhibitors on the carbachol-induced [Ca\(^{2+}\)] increase in HSG cells. Chlorpheniramine—one of the first-generation antihistamines— inhibited the carbachol-induced [Ca\(^{2+}\)] increase at high concentrations (Fig. 5A). Inhibition occurred in a concentration-dependent manner with an IC\(_{50}\) of 43.9 ± 9.2 µM: 100-fold greater than the IC\(_{50}\) at histamine H1 receptors (Fig. 5B). We next monitored the carbachol-induced [Ca\(^{2+}\)] response in the presence of three second-generation antihistamines. Cetirizine (300 µM; Fig. 5C) and astemizole (1 µM; Fig. 5E) did not affect, or only mildly inhibited, the muscarinic Ca\(^{2+}\) response. Loratadine (1 µM) showed more potent inhibition (Fig. 4G). Astemizole and cetirizine at higher concentrations showed a continuous increase in cytosolic Ca\(^{2+}\) level without any receptor-mediated responses (data not shown). Their predicted IC\(_{50}\) at muscarinic receptors were >100 µM and therefore 1000-fold higher than the inhibitory concentration at the histamine H1 receptor.
Histamine H1 Receptor Induces the Translocation of Aquaporin-5. AQP-5 is an important channel protein that regulates water movement in the salivary gland (Delporte and Steinfeld, 2006). To determine the role of histamine H1 receptors in salivary secretion, we examined whether histamine causes GFP-tagged AQP-5 to translocate to the plasma membrane in transfected HSG cells. Cells treated with carbachol showed higher fluorescence at the plasma membrane than in the cytosol (Fig. 6A, b and e), whereas cells treated with vehicle showed similar fluorescence intensities in both the cytosol and plasma membrane (Fig. 6A, a and d). Notably, we identified that histamine also caused higher fluorescence intensity in the plasma membrane (Fig. 6A, c and f). These results were confirmed by use of Western blotting for AQP-5 in the apical plasma membrane and intracellular membranes of HSG and primary cultured human submandibular gland cells. Both carbachol and histamine caused AQP-5 to translocate to the apical plasma membrane in HSG cells (Fig. 6B) and human submandibular glands (Fig. 6C). These results suggest that the histamine H1 receptor may also mediate AQP-5 translocation to the plasma membrane in human submandibular glands.

Histamine H1 Receptors and Histamine Histidine Decarboxylase Are Colocalized in Human Salivary Gland Cells. To elucidate the site(s) of histamine secretion and action, we labeled histamine H1 receptors with an H1 receptor-specific antibody. As shown in Fig. 7, the histamine H1 receptor was seen distributed across most of the human submandibular gland including the acinar and ducts. Interestingly, histidine decarboxylase (histamine-synthesizing enzyme) showed a distribution similar to labeled H1 receptors. In contrast, the distribution of choline acetyltransferase (ace-
Histamine-induced Ca\(^{2+}\) elevation was monitored. Each point represents mean ± S.E.M. The experiments were performed three or more times independently. B, cells were stimulated with 3 μM forskolin, 100 μM histamine, or 1 μM isoproterenol in the presence of 1 mM isobutylmethyl xanthine for 15 min; cAMP production was then monitored. The cAMP levels were measured as described under Materials and Methods. The relative cAMP productions are depicted as the percentage of forskolin-induced cAMP production with the mean ± S.E.M. of triplicate assays. The data are representative of three separate experiments. C, RNA extracted from HSG cells (C) and primary cultured human submandibular gland cells (D) were reverse-transcribed into cDNA, and amplification reactions were performed using histamine H1, H2, H3, and H4 receptor-specific primers and Pfu polymerases described under Materials and Methods. GAPDH was used as an internal loading control. All results were reproducible. ** P < 0.01.

**Discussion**

In this study, we have shown for the first time that histamine H1 receptors mediate Ca\(^{2+}\) signaling in human submandibular gland cells, and we suggest that the histamine H1 receptor signaling is a novel target mechanism in addition to the classical mechanism of antihistamine-induced xerostomia. We report that a) the histamine H1 receptor, expressed exclusively in human submandibular gland cells, increases cytosolic Ca\(^{2+}\) without cAMP production; b) histamine stimulation results in aquaporin translocation; and c) histamine receptors coexist with histidine decarboxylase in gland cells.

Histamine has been reported to modulate salivary flow in humans and dogs (Shimizu and Taira, 1980); however, there has been no evidence concerning histamine H1 receptors in human salivary glands, and the currently accepted hypothesis is that antihistamine-induced xerostomia is caused by muscarinic receptor antagonism. This hypothesis, in general, was applied to classical antihistamines (for instance, chlorpheniramine and promethazine) that have been shown to inhibit muscarinic receptors (Brown and Eckberg, 1997; Yasuda and Yasuda, 1999; Shelton and McCarthy, 2000; Liu et al., 2006). However, recently developed “second-generation” antihistamines, such as astemizole and fexofenadine, also induce xerostomia (Wilson et al., 1987; Sreebny and Schwartz, 1997; Elad et al., 2006), despite their extremely low affinity for muscarinic receptors (Laduron et al., 1982; Liu et al., 2006). These observations suggest that xerostomia is not related solely to muscarinic receptor inhibition.

To address this question, we attempted to clarify the relationship between muscarinic and histaminergic signaling in human submandibular gland cells. First, we checked for cross-talk between the histamine and muscarinic receptors. If salivary gland cells express histamine receptors, both histamine and muscarinic signaling may modulate cytosolic [Ca\(^{2+}\)], and salivary secretion. In general, “heterologous desensitization” refers to a decrease in signaling of a certain receptor by another neurotransmitter that shares the same downstream signal transduction pathway. In our report, the carbachol-induced [Ca\(^{2+}\)] increase was not affected by histamine-induced [Ca\(^{2+}\)] increase, thereby ruling out heterologous desensitization between muscarinic- and histamine-mediated Ca\(^{2+}\) signaling (Fig. 4). These results suggest that histamine can independently induce Ca\(^{2+}\) signaling, and possibly induce salivary secretion, in the absence of muscarinic stimulation.

Second, we tested the affinity of antihistamines for muscarinic receptors. We monitored the effect of chlorpheniramine, a typical first-generation antihistamine, on muscarinic signaling. As in a previous report (Shelton and McCarthy, 2000), we confirmed that chlorpheniramine significantly inhibited the carbachol-induced [Ca\(^{2+}\)] increase (Fig. 5). The inhibitory effect, however, was obtained with a very high chlorpheniramine concentration: approximately 300-fold greater than the effective concentration against histamine H1 receptors. In clinical applications, the chlorpheniramine serum level reaches approximately 700 nM; this concentration is enough to inhibit histamine H1 receptors but would
Histamine and muscarinic receptors do not share Ca\(^{2+}\) signaling in HSG cells. A, fura-2-loaded primary cultured human submandibular gland cells treated with 100 \(\mu\)M histamine with (black trace) or without (gray trace) the pretreatment of 100 \(\mu\)M carbachol. B, fura-2-loaded HSG cells were treated 100 \(\mu\)M carbachol with (black trace) or without (gray trace) the pretreatment of 100 \(\mu\)M histamine. C, fura-2-loaded HSG cells were treated 100 \(\mu\)M histamine with (black trace) or without (gray trace) the pretreatment of 100 \(\mu\)M carbachol. All presented results are typical Ca\(^{2+}\) transients from three or more separate experiments.

hardly be effective against muscarinic M3 receptors (Fig. 5B). We suggest that the salivary histamine H1 receptor is a more probable and feasible candidate for antihistamine-induced xerostomia. Most first-generation histamine H1 receptor antagonists exhibit serious xerostomia, whereas some of the second-generation histamine H1 receptor antagonists show only a mild xerostomic side effect. Even though our results successfully showed a primary xerostomic mechanism of antihistamine, we could not clearly answer the reason for the difference in the xerostomic potency among H1 receptor antagonists. Theoretically, there should be no difference between the two generations of antihistamines if histamine signaling is the only xerostomic mechanism at play. Conversely, if muscarinic signaling is the only xerostomic target for the antihistamines, it would be unlikely for the second-generation histamine H1 receptors blockers (such as astemizole) to cause xerostomia. According to our results in human salivary glands, neither hypothesis is completely true. We found a relatively larger difference between inhibitory concentration for histamine receptors and muscarinic receptors for astemizole, loratadine, and cetirizine than the inhibitory concentrations recorded for chlorpheniramine. We found a large difference between inhibitory concentration for histamine receptors and muscarinic receptors for astemizole and cetirizine. The IC\(_{50}\) values for chlorpheniramine, however, revealed a relatively closer affinity for both receptors. From this we can conclude that the difference in xerostomia induction potency between first- and second-generation histamine H1 receptor antagonists may in part be because of their different affinities for muscarinic receptors. In fact, we believe that the difference in xerostomic potency might be caused not only by inhibition of muscarinic signaling but also by the multiple effects of blocking histamine H1 receptors, such as histaminergic signaling inhibition, sedation, and so on.

Third, we performed experiments to determine whether histamine activates cytosolic machinery related to salivary secretion in a manner similar to muscarinic receptors. In the salivary gland, AQP-5, 1 of 13 mammalian AQPs modulates water secretion to produce primary saliva (Ishikawa et al., 2005). Sjögren’s syndrome is characterized by a xerostomia that results from defective AQP-5 translocation to the luminal side (Tsubota et al., 2001). Therefore, translocation of AQP protein because of Ca\(^{2+}\) signaling is a critical step in fluid secretion. In our experiments, histamine stimulation resulted in translocation of AQP-5 to the luminal membrane, similar to the effect of carbachol (Fig. 6). It must be emphasized that altered AQP-5 translocation provides only indirect evidence for a role in salivary gland water secretion in vivo; however, an in vitro approach using human cells is preferable to the treatment of humans with histamine receptor agonists, which would inevitably induce severe allergy and hypersensitivity responses. Our results suggest that both histamine and parasympathetic acetylcholine can potentially control salivation.

Among histamine receptors, H2 receptor activation is known to produce cAMP, and H1 receptors are linked to Ca\(^{2+}\) mobilization. In rat parotid tissues, histamine is reported to stimulate H2 receptors and induce amylase secretion (Eguchi et al., 1998). Salivary secretion in cats and rats is inhibited by histamine H2 receptor antagonists (Stanovnik and Erjavec, 1983; Sim and Ang, 1985). Adenylyl cyclase-linked G protein-coupled receptor activation serves an important role in salivary protein secretion via intracellular cAMP production (Yamada et al., 2006). In other tissues, such as collecting duct of kidney, the increased cAMP could feasibly translocate AQP and trigger water secretion (Valenti et al., 2005). Our results in human submandibular gland cells, however, do not support the existence of histamine H2 receptors (Fig. 3), a finding perhaps explained by species differences. Previous studies have shown differences in the composition of saliva between humans and other species, and in the membrane transporter profiles of saliva components (Bardow et al., 2000). Because of the difficulties in fitting animal models to human physiology for certain features of salivary function, our results serve to enhance our understanding of the human salivation mechanism and enable better xerostomia treatment.

We next examined the histamine source and target to determine the origin of salivary modulation. During inflammation, histamine may be produced by factors circulating in the blood stream, such as, platelets. In “normal” conditions, however, neurons are likely to be the principal source of...
Histamine (Haas and Panula, 2003). Histamine was reported to be released together with acetylcholine from cardiac parasympathetic neurons, which contain for histidine decarboxylase and choline acetyltransferase (Singh et al., 1999). To address histamine secretion from parasympathetic terminals, we compared the choline acetyltransferase and histidine decarboxylase distributions in the human submandibular gland. Our results showed comparable distributions of histidine decarboxylase and histamine H1 receptor but no overlap with choline acetyltransferase. These results strongly suggest that the parasympathetic terminals in human submandibular glands are unlikely to release histamine. Instead, histamine may be released from the salivary gland itself and act in an autocrine manner.

Acetylcholine is secreted from parasympathetic terminals and diffuses approximately 100 nm before stimulating receptors on the salivary acinar cell membrane (Konttinen et al., 1996). Sympathetic neurons, with limited axon terminals to modulate a target organ, possess varicosities to increase the number of synaptic contacts (Konttinen et al., 1996); however, such varicosities are relatively less developed in parasympathetic neurons (Arvidsson et al., 1997). In general, synthesized histamine is stored in cytosolic vesicles and secreted by intracellular Ca\(^{2+}\)-triggered vesicular membrane fusion (Puri and Roche, 2008). Salivary gland cells also have the machinery for secretory vesicle fusion (Wang et al., 2007). We suggest the hypothesis that cholinergic release from parasympathetic terminals activates muscarinic Ca\(^{2+}\)-signaling in salivary gland cells. This triggers the release of histamine, which activates neighboring cells expressing H1 receptors. The cascade will spread the signal over the entire gland. Although further study is necessary to confirm this hypothesis, our data provide a possible role for histamine in the propagation of parasympathetic signals to the salivary gland despite its localized and limited cholinergic input.

Currently, the significance of H1 receptor expression to
However, taken together, our results show that histamine is a possible secretory signal and suggest that antihistamines directly inhibit histamine H1 receptors in human submandibular glands. Because salivary Ca\(^{2+}\) signaling is correlated with saliva secretion, these results may contribute to understanding the cellular mechanism of antihistamine-evoked xerostomia.

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References

Elad S, Hei


**Address correspondence to:** Dr. Se-Young Choi, Department of Physiology, Seoul National University School of Dentistry, 28 Yeongun, Jongno, Seoul, 110-749, Korea. E-mail: sychoi@snu.ac.kr