The mutation at Thr 315 (but not that at Ser 316) abolished internalization. Taken together, these results suggest that the 51 C-terminal amino acids of the histamine H2 receptor are important for agonist-induced internalization, but not for either signaling or homologous desensitization of the receptor. This is consistent with a recent study demonstrating that the 51 C-terminal amino acids of the angiotensin II receptor inhibited agonist-induced internalization of this receptor, but not desensitization of the calcium response mediated via the receptor.

To evaluate the role of the histamine H2 receptor C terminus in signaling, desensitization, and agonist-induced internalization, canine H2 receptors with truncated C termini were generated. Wild-type (WT) and truncated receptors were tagged at their N termini with a hemagglutinin (HA) epitope and expressed in COS7 cells. Most of the C-terminal intracellular tail could be truncated (51 of 70 residues, termed T308 mutant) without loss of functions: cAMP production, tiotidine binding, and plasma membrane targeting. In fact, the T308 mutant produced more cAMP than the WT when cell-surface expression per cell was equivalent. Pretreatment of cells with 10^{-5} M histamine reduced cell-surface anti-HA antibody binding by approximately 30% (by 30 min, t_{1/2} = 15 min), but did not affect the K_{max} of tiotidine in membrane fractions, which represents total receptor amounts, suggesting that WT receptors were internalized from the cell surface. In contrast, no internalization was observed with T308 receptors following histamine treatment. A mutant with a deletion of the 30 C-terminal amino acids, termed T329, was functional but was as potent as the WT in terms of cAMP production. Apart from being desensitized by histamine, the internalization of the receptor was indistinguishable from that of the WT. Internalization was observed in the T320 but not in T315 mutant, narrowing the region involved in internalization to that between Glu314 and Asn320 (ETSLRSN). Of these seven residues, either Thr315, Ser316, or both, were replaced with Ala. Thr315 and Ser316 are conserved among species. The mutation at Thr315 (but not that at Ser316) abolished internalization. Taken together, these results demonstrate that Thr315 is involved in agonist-induced internalization. Furthermore, the finding that T308 receptors were desensitized in the absence of internalization suggests that internalization and desensitization are mediated by independent mechanisms.

As observed in a number of guanine nucleotide-binding protein-coupled receptors (GPCRs),1 cAMP responses occurring via the histamine H2 receptor are rapidly desensitized after agonist stimulation (1–5). The H2 receptor also exhibits an agonist-induced internalization from the cell surface (2, 6). However, the mechanisms underlying these phenomena have yet to be identified. Recently, a number of studies focusing on GPCRs have examined the role of the C terminus in agonist-induced desensitization and internalization, with varying results. For example, C-terminal truncation of the angiotensin II receptor inhibited agonist-induced internalization of this receptor, but not desensitization of the calcium response mediated via the receptor (7). On the other hand, C-terminal truncation of b2-adrenergic, a1b-adrenergic, lutropin/choriogonadotropin, platelet-activating factor, and neurokinin-2 receptors resulted in impairment of homologous desensitization (8–12). Thus, the C termini of GPCRs have functional importances, which may differ among receptors. The present study was designed to analyze the role of this important portion of the histamine H2 receptor in signaling, desensitization, and agonist-induced internalization. To this end, we constructed H2 receptor cDNAs, devoid of either the 71 or the 51 amino acids at the C terminus, and expressed these cDNAs in COS7 cells. Herein, we present evidence that the 51 C-terminal amino acids of the histamine H2 receptor are important for agonist-induced internalization, but not for either signaling or homologous desensitization of cAMP response, which occurs via this receptor. In addition, these amino acids exert inhibitory effects on cAMP production via the H2 receptor.

EXPERIMENTAL PROCEDURES

Materials—[3H]Tiotidine was purchased from NEN Life Science Products. Goat anti-mouse IgG was purchased from ICN (Costa Mesa, CA). Cimetidine was obtained from Sigma. Anti-hemagglutinin (anti-HA) monoclonal antibody 12CA5 was purchased from Boehringer Mannheim (Germany). DEAE-dextran was obtained from Pharmacia Biotech Inc. (Uppsala, Sweden).

Cell Culture—COS7 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin sulfate, and 0.1 mg/ml gentamicin sulfate.

Construction of cDNAs for the H2 Receptors with C-terminal Truncation—cDNAs for mutant canine H2 receptors with mutations involving C-terminal truncation of 70, 51, 46, 39, 30, and 15 amino acids, respectively, were constructed by polymerase chain reactions. They were termed T325, T308, T313, T320, and T305, respectively. cDNAs for mutant receptors, in which Thr315, Ser316, or both, were

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1 The abbreviations used are: GPCR, guanine nucleotide-binding protein-coupled receptor; HA, hemagglutinin; WT, wild-type; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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results

Expression of Wild-type and C Terminus-truncated Histamine H2 Receptors—To investigate the role of the cytoplasmic tail of the histamine H2 receptor in signaling and desensitization, we generated C terminus-truncated receptor cDNAs. Fig. 1 presents a schematic representation of the 359-amino acid canine histamine H2 receptor. Consensus sites for post-translational modifications, N-glycosylation, and palmitoylation are also indicated (17). We chose to delete 51 and 70 amino acids from the C-terminal tail, yielding two distinct mutant receptors, termed T308 and T289, lacking 11 and 13 serine/threonine residues, respectively. The T308 receptor was truncated at the beginning of the cytoplasmic C terminus and the T289 receptor just distal to the putative palmitoylation site (Cys350). All receptor constructs were tagged at their N termini with a 9-amino acid HA epitope, as shown in Fig. 1. As the HA-epitope is located in the extracellular region, the cell-surface receptor amount can be estimated utilizing the antibody against the HA-epitope, 12CA5. The cDNA constructs were subcloned into an expression vector, pCAGGS and transfected into COS7 cells. COS7 cells do not have detectable endogenous histamine H2 receptors, as demonstrated by the absence of histamine-deendent cAMP production and the absence of specific [3H]tiotidine binding (data not shown), and are thus a suitable model system for these studies. At 48 h after transfection, the cells were subjected to immunocytochemistry using the anti-HA antibody or the anti-H2RCT antibody. Expression of the receptors is shown in Fig. 2. The WT, the HA-wild-type (HA-WT), and the HA-T308 receptor were distributed in the plasma membranes, while the HA-T289 receptor, the 70 C-terminal deleted amino acids of which include Cys350, a consensus site for palmitoylation, was distributed intracellularly as well as in the plasma membranes. Whether the different distribution of the HA-T289 receptor was due to the loss of palmitoylation or to lack of the proximal amino acid residues from 290 to 307 was not determined. In either case, it can be concluded that the 51 C-terminal amino acid residues of the histamine H2 receptor, a large part of the C terminus, are not involved in trafficking of the H2 receptor to the plasma membrane. Similar results were obtained when various amounts of plasmid DNAs were used for transfection of COS7 cells (data not shown), indicating that the different distribution of the receptors is not a function of receptor numbers.

Tiotidine Binding of the WT and Truncated Receptors—To examine the effect of C-terminal truncation on ligand binding, intact COS7 cells on 24-well plates were subjected to binding of tiotidine (15), an H2 receptor antagonist, at 48 h post-transfection. The affinities of the WT and truncated receptors for tiotidine were determined by Scatchard plot analysis. Kd values for tiotidine of the WT and T308 receptors were indistinguishable (Table 1). In addition, HA-tagging did not affect the affinity of these receptors for tiotidine (Table 1). However, irrespective of the HA-tagging, the T308 receptor showed no tiotidine binding. The third and fifth transmembrane regions are reportedly important for ligand binding of the H2 receptor (18). This finding might be accounted for by a conformational change in the T308 receptor. 

Quantification of Cell-surface H2 Receptors—At 24 h post-transfection, COS7 cells expressing the WT and truncated H2 receptors with the HA epitope or parental COS7 cells were plated onto 24-well plates at a density of 105 cells/well. At 48 h post-transfection, cells were incubated in Hepes-Tyrode’s buffer (140 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl2, 0.49 mM MgCl2, 0.37 mM NaH2PO4, 5.6 mM glucose, 25 mM Hepes, pH 7.4) containing 0.1% bovine serum albumin (BSA) at 37 °C for 30 min. After incubation in the presence or absence of 10-5 m histamine or 37 °C for the indicated times, the cells were fixed with PBS containing 5% paraformaldehyde, washed again with PBS, and incubated with 5% skim milk/PBS for 1 h at room temperature. They were incubated with 200 μl of 12CA5 (5 μg/ml) in PBS for 2 h at room temperature, washed again with PBS, and incubated for another 1 h with 200 μl of 125I-labeled goat anti-mouse IgG (1:200 dilution) at room temperature. The wells were then washed twice with 5% skim milk/PBS, twice with PBS, and three times with 0.05% Tween 20/PBS. Bound [125I]labeled goat anti-mouse IgG was solubilized in 1% SDS, and radioactivities were determined in a γ-counter. Specific bindings were determined by subtraction of the nonspecific bindings observed in parental COS7 cells.

Tiotidine Binding Assay—Tiotidine binding assays involving intact cells were performed as described previously (14). COS7 cells, grown in 24-well plates, were assayed at a density of 1 x 105 cells/well. The cells were incubated for 2 h at 37 °C in 200 μl of Hepes-Tyrode’s buffer containing 1 nM [3H]tiotidine and various concentrations of unlabeled tiotidine (15). All samples were analyzed in triplicate. After incubation, cells were washed three times with ice-cold PBS, then removed from the wells in 0.1% SDS, and radioactivities were determined by liquid scintillation counting. Tiotidine bindings in the membranes were assessed as described previously, with some modifications (2). Membrane fractions (200 μg) from COS7 cells were incubated with 1 nM [3H]tiotidine and varying concentrations of unlabeled tiotidine in 25 mM Hepes, 0.1% BSA, pH 7.4, in a final volume of 200 μl at 37 °C for 2 h. The binding reaction was terminated by filtration over Whatman GF/C glass fiber filters, followed by 10-ml washes with ice-cold incubation buffer. Radioactivity on the filters was determined by liquid scintillation counting. In both experiments, specific bindings were calculated by subtraction of the nonspecific bindings determined in the presence of 10-4 μM cimetidine. No specific binding was observed in either parental COS7 cells or COS7 cells transfected with the expression vector alone.

Measurement of cAMP Production—COS7 cells, plated onto 24-well plates, were assayed at a density of 1 x 105 cells/well, as described previously (14). The cells were incubated for 30 min at 37 °C in 450 μl of Hepes-Tyrode’s buffer containing 0.1% BSA and 0.1 mM 3-isobutyl-1-methylxanthine, after which 50 μl of histamine solution was added to initiate the reaction. After 10 min of incubation at 37 °C, the reaction was terminated by the addition of 500 μl of 12% trichloroacetic acid. The samples were centrifuged for 5 min at 3000 x g at 4 °C. Following extraction of the supernatants three times with diethylether, cAMP contents in the samples were measured using a radioimmunoassay (16). Histamine-dependent and forskolin-dependent cAMP productions were determined by subtracting basal cAMP productions.
receptor induced by the deletion of amino acid residues from 290 to 307. Since tiotidine is membrane-permeable and is capable of binding to receptors distributed intracellularly, the inability of the T289 mutant to bind to tiotidine is not due to the difference in the intracellular distribution of the receptor shown in Fig. 2.

cAMP Production via the WT and Truncated Receptors—To examine the role of the C terminus in H2 receptor signaling, we measured histamine-dependent cAMP productions via the WT and truncated receptors in COS7 cells. cAMP productions mediated by these receptors can be compared only when the amounts of cell-surface receptors are equivalent. Iida-Klein et al. (19) reported that in a COS7 transfection system using the DEAE-dextran method, the amount of plasmid DNA used for transfection determined the number of cell-surface parathyroid hormone/parathyroid hormone-related protein receptors expressed per cell but not the percentage of transfected cells. We have obtained similar results, in that the amounts of plasmid DNA affected the number of H2 receptors expressed per cell and the percentage of transfected cells were constant even when the amount of plasmid DNA used was not equivalent (Table II). We transfected various amounts of plasmid DNA for HA-WT, HA-T308, and HA-T289 receptors and measured the number of receptors expressed per cell. As shown in Fig. 3A, the amounts of cell-surface receptors were dependent on the amounts of plasmid DNA used for transfection and, in addition, differed markedly among the WT and mutant receptors even when equivalent amounts (molar) of plasmid DNAs were used for transfection. The number of cell-surface HA-WT receptors expressed was larger than that of HA-T308 receptors when the equivalent amounts of plasmid DNAs were used for transfection. It is essential to study the functions of these receptors at similar expression levels.

Therefore, we measured histamine-dependent cAMP productions via the WT and mutant receptors at each transfection level. This allowed comparison of the functions of these receptors. As shown in Fig. 3B, histamine-dependent cAMP productions were observed in HA-WT and HA-T308 cells but not in HA-T289 cells. Histamine-dependent productions of cAMP observed in HA-WT and HA-T308 were inhibited by cimetidine but not by diphenhydramine (data not shown). Interestingly, as compared with HA-WT receptors, HA-T308 receptors produced more cAMP than the WT receptor (Fig. 3B) if the numbers of cell-surface receptors per cell were equivalent. Therefore, the 51 C-terminal amino acids are not only essential for, but rather may actually inhibit, cAMP production mediated by the H2 receptor. It is also possible that inhibitory effects on the H2 receptor are exerted via the C-terminal region. Although less marked than those in HA-WT and HA-T308 cells, specific cell-surface anti-HA antibody bindings were also observed in HA-T289 cells (Fig. 3A). Thus, the absence of histamine-dependent cAMP production via T289 re-

![Fig. 2. Immunocytochemistry of the WT and truncated receptors expressed in COS7 cells. COS7 cells were transfected with each plasmid DNA. At 48 h post-transfection COS7 cells were fixed in PBS with 3% paraformaldehyde. Thin sections were prepared and immunostained with anti-HA antibody (a, b, c) or anti-H2R CT antibody (d). a, HA-T289; b, HA-T308; c, HA-WT; d, WT. Scale bar, 10 μm.](Image)

### Table I

| Dissociation constants (K_d) for tiotidine of wild-type and truncated H2 receptors |
|-------|
|       |
| WT    | 23.2 ± 1.1 |
| T308  | 25.2 ± 2.7 |
| T289  | No binding |
| HA-WT | 25.6 ± 0.8 |
| HA-T308 | 22.1 ± 1.5 |
| HA-T289 | No binding |

### Table II

| Transfection efficiency as assessed by immunostaining with the anti-HA antibody |
|------------------|
| [cDNA]           | HA-WT | HA-T308 | HA-T289 |
| μg               | %     | %       | %      |
| Exp. 1           | 5     | 17.3 ± 2.3 | 16.1 ± 2.7 | 17.6 ± 2.3 |
| Exp. 2           | 0.1   | 16.1 ± 1.2 | 17.2 ± 3.2 | 19.2 ± 3.2 |
| Exp. 3           | 0.1   | 19.4 ± 2.1 | 18.8 ± 4.7 | 16.1 ± 2.5 |
|                 | 0.1   | 21.1 ± 3.5 | 17.7 ± 5.8 | 17.9 ± 4.1 |
|                 | 0.1   | 18.5 ± 3.3 | 19.1 ± 3.6 | 16.9 ± 3.3 |
|                 | 0.1   | 19.3 ± 1.2 | 17.7 ± 2.4 | 18.1 ± 0.9 |

![Fig. 3. (A) Relationship between the amount of plasmid used to transfect COS7 cells and the cell-surface receptors expressed. COST cells were transfected with varying amounts of plasmid DNA containing cDNAs for the WT and truncated receptors. The cells were fixed in 3% paraformaldehyde/PBS and cell-surface anti-HA antibody bindings were determined as described under "Experimental Procedures." HA-WT; HA-T308; HA-T289. Data shown are means ± S.E. from three separate experiments, each performed in triplicate. When not shown, S.E. values were so small that they fell within the symbols. B. Relationship between the numbers of cell-surface receptors expressed and cAMP productions via WT and truncated receptors. At 48 h post-transfection, COST cells on 24-well plates at a density of 10^5 cells/well were preincubated in Heps-Tyrode's buffer containing 0.1% BSA and 0.1 mM 3-isobutyl-1-methylxanthine for 30 min, then incubated for an additional 10 min in the absence or presence of 10^-7 M (left panel) or 10^-8 M (right panel) histamine. Incubation was terminated by the addition of 500 μL of 12% trichloroacetic acid. cAMP contents were measured by a radioimmunossay.](Image)
Receptors was not due to a lack of cell-surface T<sub>289</sub> receptors, but rather to the inability of the receptor itself to couple to G<sub>s</sub>. It is not clear whether this is due to the absence of the amino acid residues from 289 to 307, or to a conformational change induced by the deletion. Furthermore, the possible contributions of these amino acid residues to cAMP production could not be examined.

**Desensitization of WT and T<sup>TGOS</sup> Receptor-mediated cAMP Responses**—The histamine H2 receptor reportedly shows homologous desensitization of the cAMP response following preincubation with histamine. We examined whether the C-terminal tail of the H2 receptor is involved in desensitization of the cAMP response, which occurs via this receptor. Since COS7 cells expressing the HA-T<sup>TGOS</sup> mutant did not mediate cAMP production in response to histamine (Fig. 3B), this mutant was omitted from the experiment. COS7 cells were transfected with plasmid DNAs containing HA-WT (0.4 µg/10-cm plate) or HA-T<sup>TGOS</sup> (1.0 µg/10-cm plate) receptors. Transfection with these DNA amounts resulted in similar expression levels of the HA-WT and HA-T<sup>TGOS</sup> receptors (Fig. 3A). Preincubation of transfected COS7 cells with 10<sup>-5</sup> M histamine for 30 min led to reduced cAMP production in both the HA-WT and HA-T<sup>TGOS</sup> cells as compared with untreated cells (Fig. 4A), indicating desensitization, while forskolin-dependent cAMP production was slightly increased (Fig. 4B). It is noteworthy that the transfection efficiency of the COS7 cell system is approximately 60% at best (Table II). Furthermore, the results shown in Fig. 4B do not necessarily mean that forskolin-dependent cAMP production in COS7 cells expressing the receptors was not altered. Therefore, we performed similar experiments in CHO cells stably expressing HA-WT or HA-T<sup>TGOS</sup> receptors and found that histamine-dependent cAMP production via these receptors was reduced by preincubation with 10<sup>-5</sup> M histamine, while forskolin-dependent cAMP production was not (data not shown). Similar results were obtained following a 15-min preincubation with 10<sup>-5</sup> M histamine (data not shown). Taken together, these results suggest that the 51 amino acids of the C terminus of the histamine H2 receptor are not essential for desensitization of the receptor-mediated cAMP response.

**Effect of C-terminal Truncation on Agonist-induced Internalization of the H2 Receptor**—A number of GPCRs have been reported to be internalized into compartments inaccessible to agonists acting at the plasma membrane. Agonist-induced internalizations of several other GPCRs have been detected by measuring the acid-resistant forms of radiolabeled agonists (7, 20–23). However, this experiment cannot be performed for the H2 receptor because of the high levels of nonspecific uptake of histamine by cells. To circumvent this difficulty, we utilized the HA-tag, which is located at the N-terminal extracellular region,
to quantify the cell-surface H2 receptor amount. Specific cell-surface bindings of anti-HA antibody were observed in COS7 cells expressing HA-WT and HA-T308 receptors (Fig. 3A), but not in parental or mock transfected COS7 cells (data not shown). We examined the effect of incubation with $10^{-5}$ M histamine on the cell-surface H2 receptor amount. As shown in Fig. 5A, in COS7 cells expressing HA-WT receptors the amount of cell-surface anti-HA antibody binding decreased by approximately 30% with a 30-min incubation with $10^{-5}$ M histamine and the $t_{1/2}$ was approximately 15 min. However, neither the binding maximum ($B_{\text{max}}$) of tiotidine in membrane fractions (Fig. 5B), which represents the total receptor amount, nor the $K_d$ for tiotidine (data not shown) was decreased. These findings indicate that HA-WT receptors were internalized from the cell surface. Immunocytochemical examination after a 30-min incubation with histamine revealed the HA-WT receptors to be on the plasma membrane, possibly indicating that the receptors were in coated pits or caveola. In contrast, incubation of COS7 cells expressing HA-T308 receptors with histamine affected neither cell-surface anti-HA antibody binding (Fig. 5A) nor the $B_{\text{max}}$ of tiotidine (Fig. 5B), indicating that the 51 C-terminal amino acids of the H2 receptor play a role in agonist-induced internalization of the receptor.

Effects of Truncation of 15 and 30 C-terminal Amino Acids on H2 Receptor Signaling, Desensitization, and Internalization—To define more clearly the region involved in internalization, two mutant receptors, T329 and T344, with deletion of 30 and 15 C-terminal amino acids, respectively, were generated. These mutants were also tagged with the HA-epitope at their N termini and were expressed in COS7 cells. Both receptors were capable of binding tiotidine with affinities similar to that of the WT receptor (data not shown). In contrast to the finding in the HA-T308 mutant receptor, cAMP productions via both receptors were comparable to those mediated by the HA-WT receptor provided that the amounts of cell-surface receptors per cell were equivalent (Fig. 6A). These observations indicate that these 30 amino acids of the C terminus are not involved in inhibition of cAMP production. In addition, cAMP productions via these receptors were desensitized by $10^{-5}$ M histamine preincubation (data not shown). Finally, upon histamine exposure, HA-T329 and HA-T344 receptors showed internalization indistinguishable from that of the HA-WT receptor (Fig. 6B).

Identification of the Amino Acid Residues Involved in Internalization of the H2 Receptor—The results obtained indicate that the region responsible for agonist-induced internalization and inhibition of cAMP production resides between amino acid residues 308 and 328. To identify the amino acid residues involved in agonist-induced internalization, we generated additional mutants, HA-T313 and HA-T320 with deletions of 39 and 46 C-terminal amino acids, respectively. Interestingly, agonist-induced internalization was observed in the HA-T320, but not in the HA-T313 receptor (data not shown). Thus, the amino acid residues involved in agonist-induced internalization of the histamine H2 receptor are likely to reside between Glu314 and Asn320 (ETSLSRN). Of these seven amino acid residues, assuming that the hypothesis that serine and/or threonine phosphorylation is involved in agonist-induced internalization of the H2 receptor is correct, the likely candidates are Thr315 and Ser316. This is because these two residues are conserved among species (Table III). To determine whether these amino acid residues are involved in agonist-induced internalization, we generated three mutant receptors, HA-A315, HA-A316, and HA-A315,316 in which either Thr315 or Ser316, or both were replaced with Ala. As shown in Fig. 7, the HA-A316 receptor internalization was indistinguishable from that of the WT, whereas those of the HA-A315,316 and HA-A315 were not. This finding clearly demonstrates that Thr315 is involved in agonist-induced internalization of the histamine H2 receptor and that the inabilities of the HA-T308 and HA-T313 mutants to internalize were not due to nonspecific effects induced by the truncations.

![Figure 6. Effects of truncation of 15 and 30 C-terminal amino acids.](image)

**Table III**

| Truncation at | -46 (T313) | -39 (T320) | -30 (T329) |
|--------------|------------|------------|------------|
| Canine       | CRPASHNAQ  | ETSLSRN    | SSQLRNQSS  |
| Rat          | CKFASHSHK  | KSRLN    | NSLLPRQSQS | REPRQGEEKPLKLQWGSGETVTAPRGATGR |
| Human        | CRLLARNNSH | KTSRLSN  | ASQLRSTQSQS | REPRQGEEKPLKLQWGSGETVAPRGATGR |
| Guinea pig   | CRILASHNSH | ETSLSRLN | NSQLNRSQSC  | QEPRWQEDKPLNQWGSGETVAPRGATNR |
were incubated with 10−6 M histamine or vehicle for the indicated times and cell-surface anti-HA antibody bindings were determined. ○, HA-A315; □, HA-A316; ●, HA-A315,316. Shown are the means ± S.E. of two individual experiments, each performed in triplicate. When not shown, S.E. values were so small that they fell within the symbols.

DISCUSSION

The histamine H2 receptor plays a central role in acid production in gastric parietal cells via production of cAMP (24). Inappropriate gastric acid production can lead to the generation or exacerbation of peptic ulcer disorders. Tight control of signaling via the H2 receptor is thus vital for normal gastric function. It has been accepted that the histamine H2 receptor undergoes rapid desensitization of the cAMP response upon exposure to an agonist (1–5). Although homologous desensitization of agonist-induced signaling via GPCRs is an essentially ubiquitous phenomenon, the functional roles of the C terminus vary among receptors. In a number of GPCRs, agonist-induced serine/threonine phosphorylation of the C terminus has been implicated in receptor desensitization (8–10), while in others the C terminus has been suggested to be involved in agonist-induced internalization, but not in desensitization (20, 25, 26). To understand the as yet unidentified roles of the C terminus in the histamine H2 receptor, we generated two mutant receptors, T308 and T308*, which were truncated at the beginning of the C-terminal amino acids of the histamine H2 receptor. Recently, Barak et al. (27) reported that a tyrosine residue highly conserved in G-protein-coupled receptors and in the NPXY motif is involved in sequestration of the β3-adrenergic receptor. This tyrosine residue is conserved in the histamine H2 receptor (Tyr288) (17, 28, 29). The T308 receptor contains this tyrosine, such that the tyrosine residue Tyr288 appears to be necessary for, but is not by itself sufficient to produce, internalization of the histamine H2 receptor. Furthermore, desensitization of the cAMP response occurring via the T308 receptor, which was not internalized, indicates that internalization per se is not essential for agonist-induced desensitization of the cAMP response and that desensitization and internalization are mediated by independent mechanisms.

Second, the WT receptor was internalized upon histamine exposure while the T308 mutant was not. In addition, the T329 mutant with a deletion of 30 amino acids was internalized. Thus, the region involved in internalization of the histamine H2 receptor is present within the area including amino acid residues from 308 to 328. The observation that the T329 mutant, but not the T313 receptor, was internalized further confirmed the amino acid residues involved in agonist-induced internalization. Finally, the finding that internalization occurred in the HA-A316 receptor, but in neither the HA-A315 nor the HA-A315,316 receptor, identified the amino acid residue involved in agonist-induced internalization of the H2 receptor as Thr315. Our preliminary observations indicated that both the HA-WT and the HA-T308 receptors were phosphorylated after histamine stimulation (data not shown). Thus, although we can speculate that phosphorylation does actually play a part in desensitization, we cannot determine whether it is involved in agonist-induced internalization of the H2 receptor. Recently, Barak et al. (27) reported that a tyrosine residue highly conserved in G-protein-coupled receptors and in the NPXY motif is involved in sequestration of the β3-adrenergic receptor. This tyrosine residue is conserved in the histamine H2 receptor (Tyr288) (17, 28, 29). The T308 receptor contains this tyrosine, such that the tyrosine residue Tyr288 appears to be necessary for, but is not by itself sufficient to produce, internalization of the histamine H2 receptor. Furthermore, desensitization of the cAMP response occurring via the T308 receptor, which was not internalized, indicates that internalization per se is not essential for agonist-induced desensitization of the cAMP response and that desensitization and internalization are mediated by independent mechanisms.

In conclusion, we have shown in COS7 cells that the C terminus of the histamine H2 receptor is involved in agonist-induced internalization, but not in desensitization. Furthermore, the C terminus is likely to exert an inhibitory effect on cAMP production via the receptor. Since these experiments were performed using fibroblast cell lines transfected with histamine H2 receptor cDNAs, the results presented show that the processes involved in histamine-induced uncoupling of the histamine H2 receptor are not specific to gastric parietal cells. However, the extent of internalization observed in this system is minimal compared with those of other receptors. We cannot rule out the possibility that some other cofactor, which is not present in COS7, might function in histamine H2 receptor internalization. Future studies must be designed to identify the specific amino acid residues involved in agonist-induced desensitization.

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