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Sensitization of the Histamine H_1 Receptor by Increased Ligand Affinity*

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Histamine regulates a variety of physiological processes including inflammation, gastric acid secretion, and neurotransmission. The cellular response to histamine is subject to dynamic control, and exaggerated histamine reactivity in response to cysteinyl leukotrienes and other stimuli is important in a variety of different pathological conditions. The molecular mechanisms controlling histamine responsiveness are still unresolved. In investigating histamine responses in embryonic stem (ES5) and F9 embryonic carcinoma cells, we encountered a novel mechanism controlling the cellular reaction to histamine. Unstimulated cells displayed neither [^{3}H]pyrilamine binding nor histamine-induced increases in cytosolic Ca^{2+} levels. Pretreatment of these cells, however, with leukotriene D_4, leukotriene E_4, serotonin, or fetal calf serum induced an immediate and transient ability of these cells to respond to histamine with an increase in cytosolic Ca^{2+} levels. This effect could be inhibited by pertussis toxin and was mimicked by GTP analogues. Importantly, the latter compounds also provoked immediate high affinity [^{3}H]pyrilamine binding. We conclude that in these cells histamine responsiveness is directly controlled by pertussis toxin-sensitive G protein-coupled receptors, whose activation enables the H_1 receptor to bind its ligand. These findings define a novel mechanism for regulating histamine H_1 receptor activity and provide for the first time molecular insight into the mechanism by which cysteinyl leukotrienes and other external stimuli can increase histamine responsiveness.

Histamine, a biogenic amine formed by decarboxylation of the amino acid L-histidine (1), is found in large quantities in most tissues, mainly in the granules of mast cells, although numerous other cell types are capable of histamine synthesis as well (2). Histamine controls a multitude of physiological functions by activating specific receptors on target cells. Three types of receptors for histamine have been described, denominated as the H_1, H_2, and H_3 receptor and are distinguished on the basis of their sensitivity to specific agonists and antagonists (3). In general, the H_3 receptor is implicated in autoinhibition of histamine synthesis and release and the H_2 receptor in gastric acid secretion, whereas the H_1 receptor is involved in inflammatory responses, mediating for instance blood vessel and bronchial constriction, vascular permeabilization, and synthesis of other inflammatory agents (4). Histamine receptors are subject to dynamic regulation, receptor activity being increased or diminished in response to various conditions (5–7), and exaggerated histamine reactivity is associated with a variety of pathological disorders.

Cysteinyl leukotrienes have been implicated in the stimulation of histamine reactivity. Inhalational challenge with these inflammatory eicosanoids increases histamine responsiveness of the airways (8–12), and cysteinyl leukotriene-induced histamine hypersensitivity is presumed to be important in asthmatic disease (13). Also, other signaling molecules stimulate histamine responsiveness. Especially serotonin, platelet-activating factor, and thromboxanes are known to enhance histamine reactivity (14, 15). The molecular mechanisms, however, by which such stimuli can provoke increased histamine responsiveness have remained obscure.

In the present study we describe a molecular mechanism by which external stimuli can enhance histamine reactivity by directly controlling the affinity of the histamine H_1 receptor for its ligand. We have reported earlier that the P19 embryo carcinoma (EC)[1] cell, a pluripotent cell type resembling the inner cell mass of the embryo, expresses functional histamine H_1 receptors (16), although its function with respect to embryogenesis is not clear. To obtain more insight into the function of histamine receptor expression in uncommitted cells, we decided to investigate the presence of cellular responses to histamine in other pluripotent cells. We observed that F9 EC cells and embryonic stem (ES5) cells displayed neither high affinity [^{3}H]pyrilamine binding nor histamine-induced increases in cytosolic Ca^{2+} levels. A pretreatment of these cells with cysteinyl leukotrienes, serotonin, or FCS, however, induced an immediate and transient ability of these cells to react to histamine. This effect was inhibited by pertussis toxin and was mimicked by GTP analogues. Importantly, induction of histamine responses coincided with the appearance of high affinity [^{3}H]pyrilamine binding sites on these cells. Apparently pertussis toxin-activating agents can regulate histamine responses by inducing high-affinity binding sites for histamine. These findings define for the first time a molecular mechanism by which cysteinyl leukotrienes and other external stimuli can increase histamine responsiveness and identify a novel mechanism for the regulation of G protein-coupled receptors.

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[1] The abbreviations used are: EC, embryo carcinoma; ES, embryonic stem; FCS, fetal calf serum; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); [^{3}H]pyrilamine, [pyridinyl-5-^{3}H]pyrilamine; DTT, dithiothreitol; GDPβS, guanosine-5′-*O*-(2-thiodiphosphate).

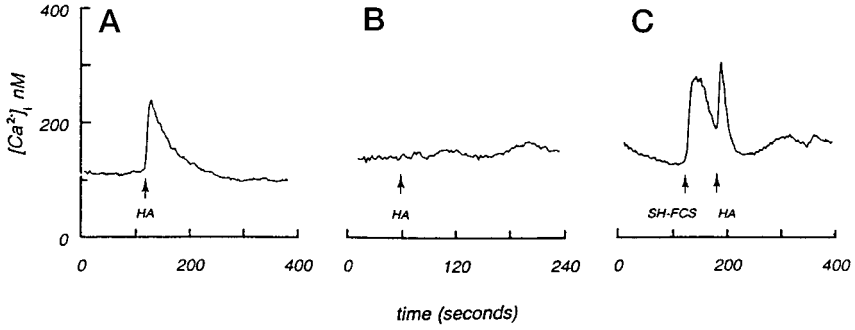

FIG. 1. **The effect of histamine (*HA*) on the intracellular Ca^{2+} concentration in P19 EC and F9 EC cells.** Representative traces of the intracellular Ca^{2+} concentration of Fura-2-loaded P19 EC (*A*) and F9 EC (*B* and *C*) are shown. The additions of the stimuli are indicated.

EXPERIMENTAL PROCEDURES

Chemicals—Histamine dihydrochloride, pyrilamine (maleate salt), leukotrienes, serotonin, and valinomycin were obtained from Sigma. Fura-2 acetoxymethyl ester was from Molecular Probes (Eugene, OR), streptolysin O was from Wellcome Diagnostics (Dartford, UK), GTPγS was from Boehringer Mannheim, okadaic acid was from Life Technologies, Inc., and [pyridinyl-5-^{3}H]pyrilamine ([^{3}H]pyrilamine) was from Amersham (Buckinghamshire, UK). The enantiomers of cicletanine were kind gifts from the Henri Beaufour Institute-IPSEN Laboratories, France. SH-FCS was prepared at our laboratory by DTT treatment of fetal calf serum. DTT hydrolyzes the protein S–S bridges and thereby inactivates most of the polypeptide growth factors in FCS. The thus-treated serum is dialyzed to remove traces of DTT.

Cell Culture—F9 EC and P19 EC cells were cultured at 7.5% CO_2 and 37 °C in bicarbonate-buffered DF-medium supplemented with 7.5% FCS. ES5 and D3 ES cells were maintained in conditioned minimal essential medium (Life Technologies, Inc.) supplemented with 10^{-4} M β-mercaptoethanol and 20% FCS. The cells were passaged three times a week using EDTA (0.2 mg/ml) for F9 EC and trypsin (0.05%), EDTA (0.2 mg/ml) for P19 EC and the ES cell lines. Two days before experimentation the cells were plated to yield subconfluent cultures for experiments.

Ca^{2+} Determinations—For Ca^{2+} measurements, cells were maintained in serum-free medium for 1 h and subsequently loaded with 10 μM Fura-2 acetoxymethylester for 30–45 min at 33 °C in a Hepes-buffered saline of the following composition: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose, 0.2% bovine serum albumin adjusted to pH 7.3 with NaOH. During experiments, cells were maintained in Hepes-buffered saline at 33 °C. For experiments with NaF and $AlCl_3$, use was made of a saline containing 120 mM NaCl, 20 mM NaF, 75 μM $AlCl_3$, 5 mM KCl, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose, pH 7.3. The measurements were carried out with a fluorescence microscope focusing on a group of 8–12 cells with a 50 × water immersion objective and a SPEX dual-wavelength fluorimeter. Emission fluorescence was digitally sampled at 340 and 380 nM and corrected for background fluorescence as determined from unlabeled cells. The intracellular Ca^{2+} concentration was calculated according to Grynkiewicz *et al*. (17). For digital image analysis, pictures were taken from a video recording of Fura-loaded cells (excited at 340/380 nM) and processed with the Crystal Particle Package Version 1.08 (Quantel).

Electrophysiology—For whole-cell patch clamp analysis, cells were measured in a saline solution of the following composition: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose adjusted to pH 7.3 with NaOH. During experiments, cells were maintained at 33 °C.The patch pipette contained 140 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, 10 mM Hepes adjusted to pH 7.1 with KOH. Using the fluorimetric method described by Civitelli *et al.* (18), we determined a resting membrane potential for the F9 EC cells of −51 mV, and cells were clamped at this potential. Currents were analyzed as described earlier (19).

[^{3}H]Pyrilamine Binding—Scatchard analysis on membrane preparations and intact cells was performed as described earlier (16). For Scatchard analysis, cells were serum-starved for 1 h, after which they were labeled for 1 h at 4 °C in Hepes-buffered Dulbecco's modified Eagle's medium containing 4 nM [^{3}H]pyrilamine and different concentrations of unlabeled pyrilamine, after which total binding reached plateau phase (not shown). Subsequently, cells were washed three times with phosphate-buffered saline, and cell protein was precipitated with 0.2 M NaOH. The bound radioactivity was determined by liquid scintillation counting. In each experiment, each condition was tested in triplicate. For experiments with NaF (20 mM) and $AlCl_3$ (75 μM), the same buffer was used as described for Ca^{2+} determinations and applied for 20 min at room temperature, after which the cells were placed on ice and the [^{3}H]pyrilamine binding assay was performed. For experiments with GTPγS, cells were first permeabilized for 5–10 min at 37 °C with 0.5 IU/ml streptolysin O in 100 mM KCl, 5.6 mM glucose, 1 mg/ml bovine serum albumin, 1.3 mM $CaCl_2$, 2 mM EGTA, 0.1 mM $MgCl_2$, 1 mM ATP, 10 mM Hepes, pH 7.2 (20) and washed once in the absence of streptolysin O. GTPγS was added to the permeabilized cells and incubated shortly (1–2 min) at room temperature to allow GTPγS to enter and sensitize the cells. Thereafter cells were placed on ice and labeled as described above. For permeabilized cells, the incubation buffer contained 150 mM KCl, 5 mM NaCl, 5.6 mM glucose, 1 mg/ml bovine serum albumin, 1 mM ATP, 1.3 mM $CaCl_2$, 0.1 mM $MgCl_2$, 2 mM EGTA, 10 mM Hepes, pH 7.2.

In general, Scatchard plots made using intact cells show considerable nonspecific low affinity binding of [^{3}H]pyrilamine (16), more so in permeabilized cells when compared with nonpermeabilized cells. Therefore, Scatchard plots were fitted according to a one- or two-site model, using the formula, bound/free = 0.5 ($[B_{max1}$ − bound$]/K_{d1}$ + $[B_{max2}$ − bound$]/K_{d2}$) + 0.5 ($[\{B_{max1}$ − bound$\}/K_{d1}$ + $\{B_{max2}$ − bound$\}/K_{d2}]^2$ + 4 $[\{B_{max1}\ B_{max2}\}/K_{d1}\ K_{d2}]$]), in which B_{max1}, B_{max2}, K_{d1}, and K_{d2} are the respective maximal binding capacities and dissociation constants of the different affinities. The observed points of the Scatchard plot of unstimulated cells were satisfactorily fit with a one-site (low affinity) model, whereas two affinity binding sites could be distinguished in the sensitized cells. To determine best fit, we calculated the χ^2 distribution of the estimated curve relative to the observed values. We accepted the fit if the χ^2 did not exceed the probability value of 5%.

RESULTS

Induction of Histamine Signal Transduction in F9 EC and D3 ES Cells—Different EC and ES cell lines showed marked differences in their reaction toward histamine (1 μM). P19 EC cells and ES5 cells responded to histamine with a marked increase in cytosolic Ca^{2+} levels and transmembrane currents, as assayed with whole-cell patch clamp electrophysiology and fluorimetric Ca^{2+} determinations (Figs. 1 and 2). Such responses, however, were absent in the F9 EC and D3 ES cells (Figs. 1 and 2; Tables I and II). Even digital image analysis (which allows detection of small responses in single cells) of Fura-2-loaded F9 EC and ES5 cells did not reveal any response to histamine in these cells (Fig. 3). Importantly, we noted that stimulation of F9 EC and D3 ES cells with 5% (DTT-treated) fetal calf serum (SH-FCS), 1 μM leukotriene D_4, 1 μM leukotriene E_4, or 3 μM serotonin induced an ability in these cells to respond to histamine: after prestimulation with one of these compounds, both histamine-induced Ca^{2+} responses and transmembrane currents were easily detected (Figs. 1–3; Tables I and II). Control experiments consistently showed that F9 EC and D3 ES cells spontaneously reacted toward ATP (50 μM) and bradykinin (1 μM) but never did show uninduced histamine responses (n = 29). Furthermore, ATP and bradykinin were not able to induce histamine responsiveness. We were confident, therefore, to have encountered a novel form of regulation of histamine responsiveness, as the cellular reaction to histamine in the F9 EC and D3 ES lines requires sensitization by specific stimuli.

Histamine Responsiveness Induced by SH-FCS Is Transient

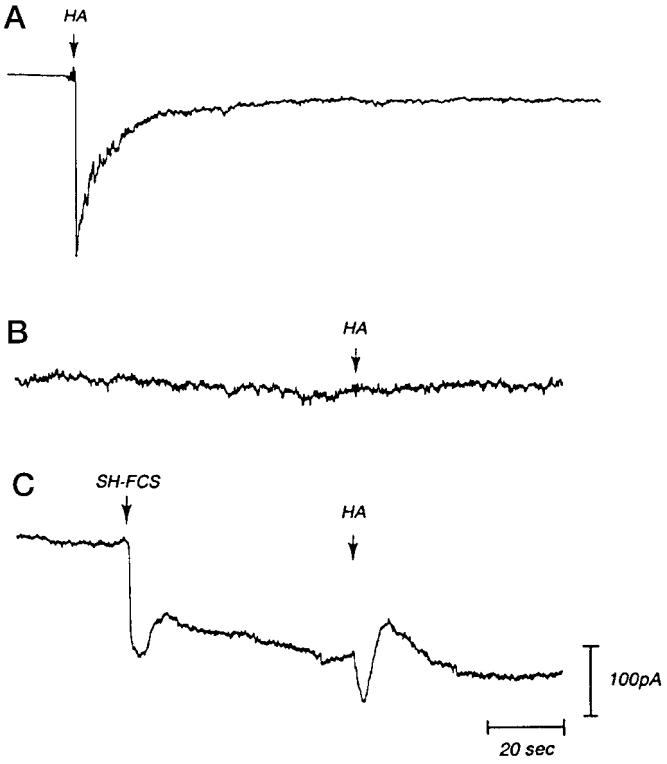

FIG. 2. **The effect of histamine on transmembrane currents in embryonal carcinoma cells.** Representative whole cell patch clamp tracings (outward currents down) of P19 EC (*A*) and F9 EC cells (*B* and *C*) are shown. The additions of the stimuli are indicated (*HA* = 0.1 mM histamine; SH-FCS = 5% DTT-treated fetal calf serum).

TABLE I
Ca^{2+} determinations

The number of histamine responses is shown (*i.e.* an increase in cytosolic Ca^{2+} in excess of 25 nM)/total number of experiments. ND, not determined.

Condition	F9 EC	ES5
0.1 mM histamine	0/29	0/12
5% SH-FCS + 0.1 mM histamine	66/84	20/20
100 ng/ml pertussis toxin + 5% SH-FCS + 0.1 mM histamine	0/7	ND
20 mM NaF + 75 μM AlF_3 + 0.1 mM Histamine	10/20	ND

TABLE II
Whole patch clamp experiments in F9 EC cells

The number of histamine responses is shown (*i.e.* an increase in transmembrane currents in excess of 10 pA)/total number of experiments and average histamine-induced current ± standard deviation.

Condition	No. of responses	Histamine-induced current
		pA
0.1 mM histamine	0/15	
5% SH-FCS + 0.1 mM histamine	9/9	90 ± 50
1 μM LTD_4 + 0.1 mM histamine	4/4	70 ± 10
1 μM LTE_4 + 0.1 mM histamine	6/7	80 ± 50
3 μM serotonine + 0.1 mM histamine	4/4	120 ± 80
100 ng/ml pertussis toxin + 5% SH-FCS + 0.1 mM histamine	0/5	
100 ng/ml pertussis toxin + 1 μM LTE_4 + 0.1 mM histamine	0/4	

and Is Mediated by the H_1 Receptor—To further characterize the induction of histamine responsiveness, we performed fluorimetric Ca^{2+} determinations in the F9 EC cell line using SH-FCS as a pre-stimulus. The induction of histamine responsiveness by SH-FCS is fast, as coapplication of SH-FCS and histamine had a supra-additive effect. The SH-FCS-induced

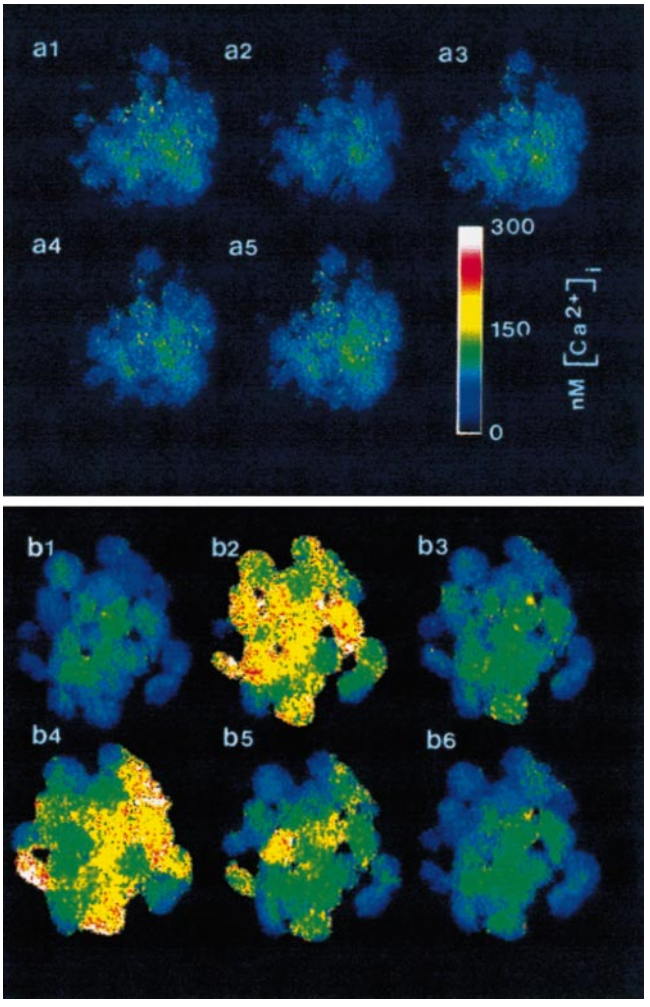

FIG. 3. **The effect of histamine on the intracellular Ca^{2+} concentration in F9 EC cells.** Pseudo-color images of video-recorded Fura-2/AM-loaded cells. *Upper panel*, absence of a response to 0.1 mM histamine (*a1*, 30 s before histamine addition; *a2*, 5 s before histamine addition; *a3*, 15 s; *a4*, 30 s; *a5*, 60 s after histamine addition). *Lower panel*, induction of histamine responsiveness by 5% SH-FCS (*b1*, 30 s before SH-FCS; *b2*, 20 s after SH-FCS; *b3*, 55 s after SH-FCS, 5 s before histamine addition; *b4*, 5 s after histamine; *b5*, 10 s after histamine addition; *b6*, 60 s after histamine addition).

responsiveness, however, is of a highly transient nature. Already, 10 min after application of serum, the sensitivity of these cells to histamine was lost (Fig. 4). Pharmacological studies carried out after induction of histamine responsiveness with 5% SH-FCS showed that the reaction to histamine in these cells was inhibited by the H_1 receptor antagonists pyrilamine (1 μM) and (−)cicletanine (15 μM) but not by the H_1-unspecific enantiomer (+)cicletanine (15 μM). Apparently, SH-FCS transiently enables H_1 receptor signaling in D3 ES and F9 EC cells. Subsequent experiments were performed to obtain insight into the mechanisms implicated in the regulation of this transient histamine H_1 receptor responsiveness.

Role of the Ca^{2+} Response in the Induction of Histamine Responsiveness—Although leukotrienes and serotonin activate only minor Ca^{2+} fluxes when compared with SH-FCS, histamine-induced Ca^{2+} responses were not different after induction with either leukotrienes, serotonin, or SH-FCS (Fig. 4*B*; Table II). Also, stimulation with FCS, which tends to yield bigger Ca^{2+} responses when compared with SH-FCS (not shown), did not produce different Ca^{2+} responses to histamine. These results suggest that the size of the Ca^{2+} response produced by the sensitizing stimulus is not indicative of the

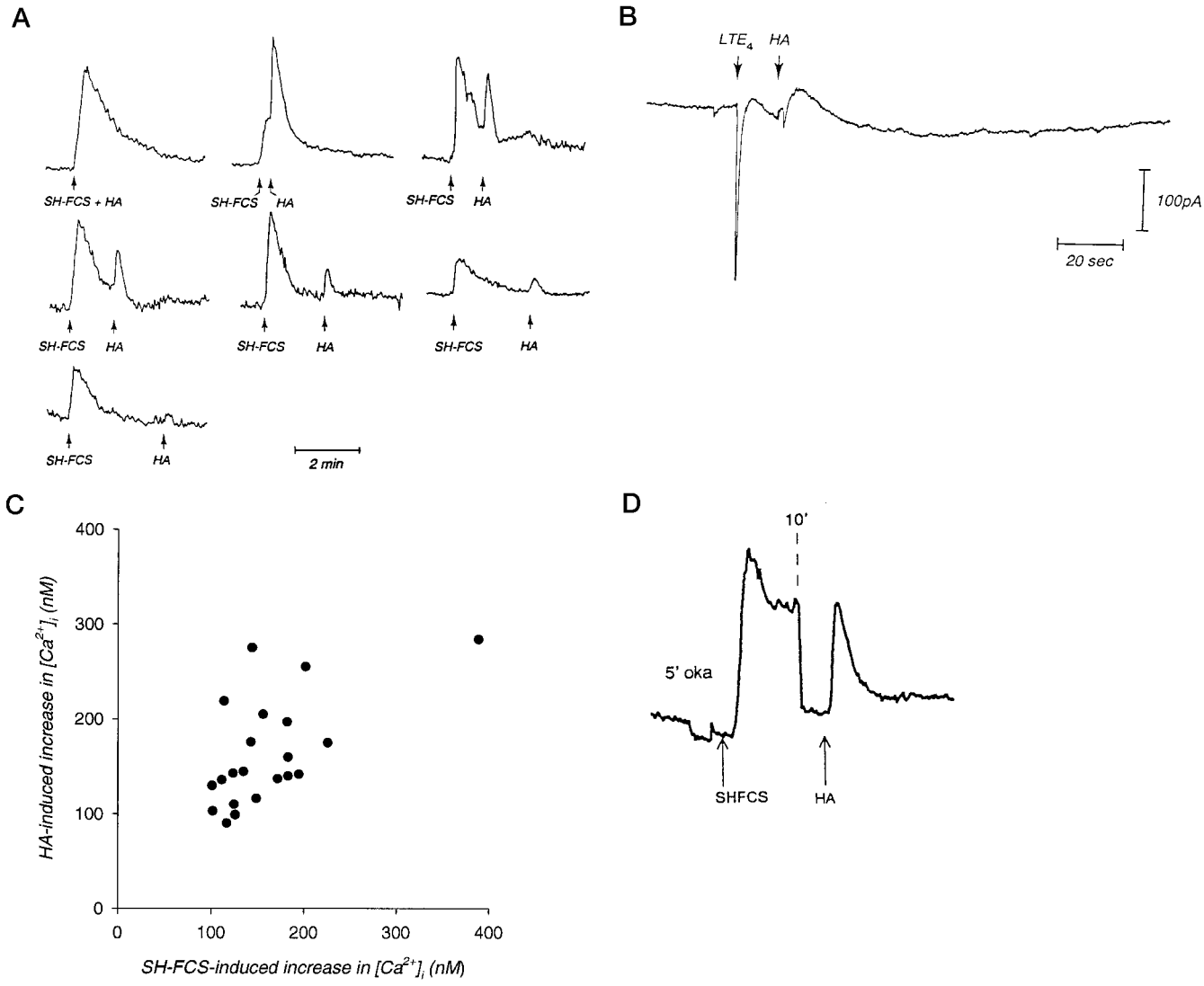

FIG. 4. **Application of SH-FCS to F9 EC cells yields a transient responsiveness to histamine.** *A*, the kinetics of the response to 5% SH-FCS followed by 0.1 mM histamine (*HA*) with increasing time intervals. *B*, example of a LTE_4-induced histamine response as assayed with whole cell patch clamp. *C*, relation between the response to histamine and SH-FCS, measured at the peak of the response, with a time interval of 50–70 s between the subsequent stimuli. *D*, effect of okadeic acid (*oka*) on histamine responsiveness induced by SH-FCS as analyzed with fluorimetric Ca^{2+} determinations.

amount of subsequently induced histamine responsiveness. To test this possibility, we initiated a series of experiments in which the size of the Ca^{2+} response to SH-FCS was compared with the Ca^{2+} response to histamine added 60 s later. As shown in Fig. 4*C*, no relationship between the two Ca^{2+} responses was detected. We concluded that the induction of histamine responsiveness is independent of the size of the prior Ca^{2+} response provoked by the sensitizing agent.

Induction of Histamine Responsiveness in F9 EC Cells Requires Pertussis Toxin-sensitive G Proteins—To further investigate the signal transduction pathways regulating this transient histamine responsiveness, we observed that an increase in intracellular Ca^{2+}, cAMP analogues or forskolin treatment, cGMP analogues, inhibitors of phospholipase A_2, arachidonic acid, or the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (acute or overnight treatment) neither induced nor prevented the sensitization of histamine responses in F9 EC cells (not shown). Because serum, leukotrienes, and serotonin are potent inducers of membrane hyperpolarization in this cell type (not shown), we also tested the effect of the K^+ ionophore valinomycin. Although this compound provoked strong hyperpolarization, no effect on the induction of histamine responsiveness was noted in either F9 EC cells or D3 ES cells (not shown). Treatment with the phosphatase inhibitor okadeic acid prolonged histamine responsiveness, suggesting an involvement of serine/threonine phosphorylation in the induction of histamine responsiveness (Fig. 4*D*).

Because in contrast to the H_1 receptor (16, 21), serotonin, leukotriene D_4, leukotriene E_4, and serum activate pertussis toxin-sensitive G proteins, we investigated the effect of a 4-h pretreatment with pertussis toxin (100 ng/ml). It appeared that the serum-, leukotriene- and serotonin-provoked inductions of histamine responsiveness were abolished by this procedure (as determined either by fluorimetric Ca^{2+} determinations or patch clamp electrophysiology; Tables I and II). Therefore, the sensitization of the histamine response appears to be dependent on the activation of pertussis toxin-sensitive G protein-coupled receptors. To test whether activation of G proteins is sufficient for induction of histamine responsiveness, F9 EC cells were injected with 10 μM GTPγS. This procedure was indeed sufficient for inducing histamine responsiveness, as assayed with whole-cell patch clamp (Fig. 5*B*), whereas GDPβS injection did not produce this effect. The GTPγS-induced histamine responsiveness was, however, of a highly transient

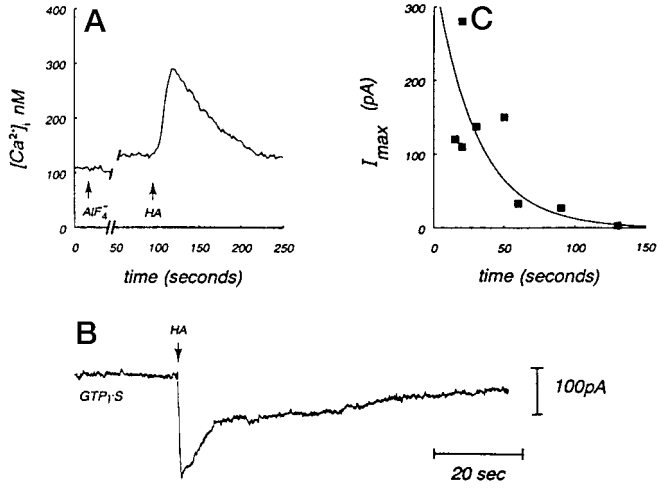

FIG. 5. **Induction of histamine responsiveness in F9 EC cells by AlF_4^- and GTPγS. *A* shows a representative Ca^{2+} tracing of the effect of 20 mM NaF and 75 μM $AlCl_3$ on the responsiveness to histamine (*HA*) added 6 min later.** Pretreatment with NaF and $AlCl_3$ permitted a Ca^{2+} response to histamine in 10 out of 20 experiments. *B* shows the response to 0.1 mM histamine on transmembrane currents (whole cell patch clamp) when 10 μM GTPγS was included in the patch pipette solution. Histamine was added 40 s after impalement. The relation between the response to histamine and the time interval after impalement is plotted in *C*.

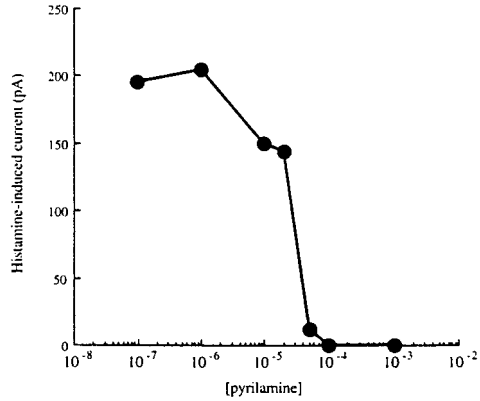

FIG. 6. **Effect of pyrilamine on histamine-induced transmembrane currents in GTPγS-injected F9 EC cells.** Cells were impaled with 20 μM GTPγS containing whole cell electrodes for 40 s in the presence of several concentrations of extracellular pyrilamine and subsequently stimulated with 0.1 mM histamine.

nature (Fig. 5*C*), maybe due to GTPγS-dependent activation of histamine signaling elements, making further stimulation of these signaling elements by the receptor impossible. In agreement, impalement of cells with GTPγS-containing pipettes provoked strong currents, indicating activation of such signaling elements by GTPγS. GTPγS-induced histamine responsiveness was eliminated by the H_1 receptor antagonist pyrilamine (Fig. 6) but not by cimetidine (a H_2 receptor antagonist), demonstrating that this histamine responsiveness is mediated by the H_1 receptor. Treatment of cells with 20 mM NaF and 75 μM $AlCl_3$ (which potently activates G proteins) led within 5 min after application to a slow but sustained increase in intracellular Ca^{2+} (Fig. 5*A*), probably due to activation of Ca^{2+}-mobilizing G protein-dependent signaling elements. Importantly, such a treatment also induced histamine-dependent Ca^{2+} responses on top of the aforementioned sustained increase in intracellular Ca^{2+} levels within 20 min after application of AlF_3 (Fig. 5*A*; Table I). We concluded that activation that activation of pertussis toxin-sensitive G proteins is implicated in the induction of histamine responsiveness.

Induction of Histamine Responsiveness Coincides with the Appearance of High Affinity [^{3}H]Pyrilamine Binding—To further explore the processes underlying the induction of histamine responsiveness, Scatchard analysis was performed. Surprisingly, in unstimulated F9 EC cells, no high affinity [^{3}H]pyrilamine binding was observed (n = 7; Fig. 7*A*), in contrast to P19 EC cells (which react unconditionally to histamine), which exhibited high affinity binding of [^{3}H]pyrilamine ($k_d \approx 7$ nM; n = 2). In accordance, whole cell membrane preparations of P19 EC cells displayed high affinity binding of [^{3}H]pyrilamine, but no such binding could be detected in F9 EC cells. These results suggest that the failure of F9 EC cells to react to histamine under uninduced conditions is due to the absence of high affinity histamine binding activity, and that induction of histamine responsiveness is caused by a rapid increase of high affinity histamine binding sites on the plasma membrane. Indeed, introduction of GTPγS into the cells rapidly induces [^{3}H]pyrilamine binding with a K_d of 19 ± 4 nM and a B_{max} of 0.15 ± 0.02 pmol/10^6 cells (± S.E.; n = 3; Fig. 7). Also, treatment of nonpermeabilized cells with 20 mM NaF and 75 μM $AlCl_3$ induces high affinity [^{3}H]pyrilamine binding with an apparent K_d of 24 ± 3 nM and a B_{max} of 0.31 ± 0.12 pmol/10^6 cells (n = 4; Fig. 7*B*). As our observations with regard to the transient nature of the induced histamine responsiveness predicted a transient induction of [^{3}H]pyrilamine by external sensitizing stimuli like leukotrienes and AlF_3, we also tested whether such transient [^{3}H]pyrilamine binding to cells could be demonstrated. At 37 °C, these experiments yielded rather variable results, probably because only after 1 h of labeling, [^{3}H]pyrilamine binding to cells reaches equilibrium, whereas induction of histamine responsiveness at this temperature by such stimuli is short-lived. When experiments were performed at lower temperatures, however, transient induction of [^{3}H]pyrilamine binding became apparent (Fig. 8). Together our observations strongly suggest that a pre-stimulus-induced change in receptor conformation, resulting in a highly increased affinity for histamine, underlies the observed regulation of H_1 receptor action in F9 EC cells. Therefore, these results define a hitherto undescribed mechanism controlling H_1 receptor function.

DISCUSSION

In the present study we show that H_1 receptor signaling in F9 EC cells and ES5 cells required prestimulation of the cells with either fetal calf serum, serotonin, leukotriene D_4, or leukotriene E_4, whereas bradykinin and ATP did not produce this effect. This sensitizing effect was inhibited by pertussis toxin, whereas it was mimicked by GTPγS and AlF_4^-. Therefore, pertussis toxin-sensitive G proteins are probably mediating this regulation of H1 receptor action. The molecular basis for the induction H_1 receptor responsiveness appears to be the appearance of high affinity ligand binding sites, as control cells did not display high affinity [^{3}H]pyrilamine binding, but introduction of GTPγS or treatment with AlF_4^- immediately provoked such high affinity [^{3}H]pyrilamide binding sites. These findings strongly suggest that histamine responses in these cells are controlled by agents that induce high affinity binding sites for histamine and define a molecular mechanism by which external stimuli can control histamine H_1 receptor action.

The molecular details, however, by which external stimuli enable the H_1 receptor to interact with its ligand, remain unclear. An explanation for the impaired H_1 receptor function in unstimulated cells may be a physical impossibility for histamine to interact with its receptor. Generally, receptors may be continuously recycled between plasma membrane and endosomes. Although this process has not been reported for H_1 receptors, it has been found to occur with several other G

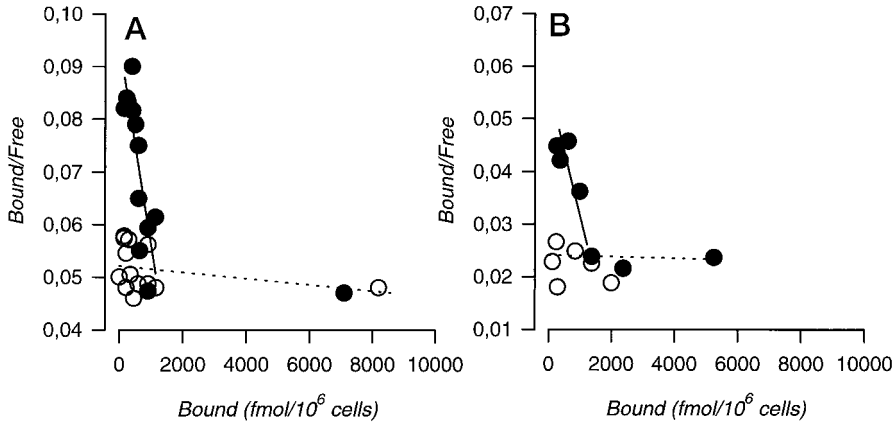

FIG. 7. **Induction of high affinity [^{3}H]pyrilamine binding sites by GTPγS (*A*) and AlF_4^- (*B*).** *A* shows a Scatchard plot representing the binding of [^{3}H]pyrilamine to streptolysin O (0.5 IU/ml in 100 mM KCl, 5.6 mM glucose, 1 mg/ml bovine serum albumin, 1.3 mM $CaCl_2$, 2 mM EGTA, 0.1 mM $MgCl_2$, 1 mM ATP, 10 mM Hepes, pH 7.2) permeabilized F9 EC cells (*open circles*) and permeabilized F9 EC cells treated with 20 μM GTPγS (*filled circles*). The *solid line* indicates the induced high affinity binding, whereas the *dotted line* indicates the constitutive nonspecific low affinity binding (16). *B*, displays a Scatchard plot of [^{3}H]pyrilamine binding to unpermeabilized cells (*open circles*) and to unpermeabilized cells treated with NaF and $AlCl_3$ (*filled circles*). The *solid line* indicates the induced high affinity binding, whereas the *dotted line* indicates the constitutive nonspecific low affinity binding. Scatchard analysis and fitting was performed as described earlier (16).

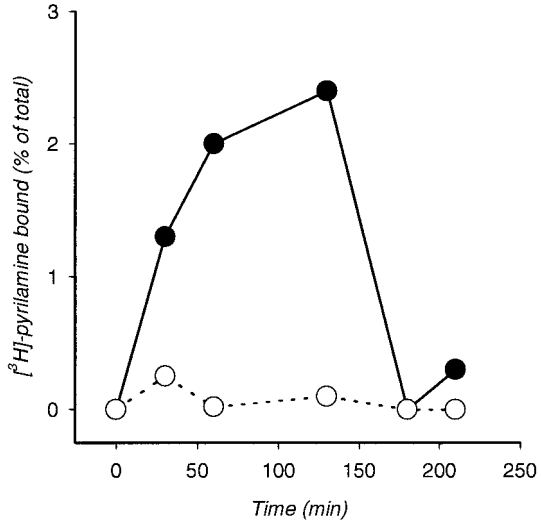

FIG. 8. **Transient induction of high affinity [^{3}H]pyrilamine binding in intact F9 EC cells by sensitizing stimuli.** Our observations with regard to the transient nature of the induced histamine responsiveness predicted a transient induction of [^{3}H]pyrilamine by external sensitizing stimuli like leukotrienes and AlF_3, without the need for cell permeabilization. We tested whether such transient [^{3}H]pyrilamine binding to intact cells could be demonstrated. Cells incubated with 4 nM [^{3}H]pyrilamine in the presence (*filled circles, solid line*) or absence (*open circles, dotted line*) of the sensitizing agent at 4 °C for the time periods indicated. To determine high affinity [^{3}H]pyrilamine binding, the amount of label nondisplaceable with 100 μM cold pyrilamine was subtracted. Under these conditions, transient induction of high affinity [^{3}H]pyrilamine binding becomes visible. [^{3}H]Pyrilamine bound at each time point is the total specifically bound expressed as a percentage of total unbound and bound at the same time.

protein-coupled receptors (*e.g.* Ref. 22), including the H_2 receptor (7), and some of the signaling elements involved have been identified (*e.g.* Refs. 23 and 24). It is conceivable that in cell types that require sensitization for histamine responsiveness, the balance between endosome and plasma membrane localization is shifted to the endosomal state, and that sensitization releases this shift. Such a scheme would imply that in unstimulated F9 EC and ES5 cells the large majority of histamine receptors has an endosomal location. In other cells types, however, which show unconditional histamine responses, the balance between endosomal and plasma membrane-localized receptors should be shifted in favor of a plasma membrane location. In this context it is interesting to note that pertussis toxin-sensitive G proteins have been implicated in the stimulation of vesicle fusion (*e.g.* Refs. 25–27) and that rab3-mediated exocytosis from mast cells is pertussis toxin-sensitive (28). Such a mechanism, however, can not explain the absence of high affinity binding of [^{3}H]pyrilamine in whole cell membrane preparations of uninduced F9 EC cells, prompting alternative explanations for induction of histamine responses in these cells.

Therefore, control of the histamine response by SH-FCS, leukotrienes, and serotonin may be mediated by the induction of a conformational change of the histamine H_1 receptor, resulting in increased ligand affinity. Our experiments using the phosphatase inhibitor okadaic acid in F9 EC cells implicate a serine/threonine phosphorylation event in the stimulus-dependent sensitization of the histamine response, opening the possibility that a phosphorylation of the receptor underlies this conformational change. In agreement, mutational analysis of the H_2 receptor has shown that relatively small changes in the intracellular domain can have profound influences on ligand affinity (7), and the primary sequence of the H_1 receptor contains a number of serine and threonine residues that may serve as potential phosphorylation sites for an affinity controlling kinase (Yamashita *et al.* (30)). Such a mechanism would contrast the proposed regulation of rhodopsin and the a_2-adrenergic receptor, where receptor phosphorylation is associated with deactivation (29). Further biochemical and mutational characterization of the H_1 receptor is required to determine whether specific phosphorylation sites are involved in the regulation of the receptor affinity and activity.

H_1 receptor activation has been implicated in processes like inflammation and anaphylaxis, and therefore H_1 receptor action must be carefully regulated. It is to be expected that mechanisms have evolved for controlling the signaling by the H_1 receptor. The regulation of histamine responsiveness as described in the present study provides such a control mechanism because for stimulation of the H_1 receptor in these cells, both a pre-stimulus as well as histamine are necessary. Interestingly, in pathological conditions like asthma and allergy, exaggerated histamine reactivity is associated with the formation of cysteinyl leukotrienes (13) and serotonin (15), but no molecular details are known. The findings described in this study for inducing histamine responsiveness define for the first time a molecular mechanism by which such control of hista-

mine reactivity may be exerted, but further studies are required to assess the importance of this mechanism in these pathological conditions. These studies are currently under progress.

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